XV FISV CONGRESS
Sapienza University of Rome, Italy
September 18-21, 2018

Programme & Abstracts

AAI ABCD AGI SIB SIBBM SIBE SIBV SICA SIF SIGA SIMAG SIMGBM SIP MeT SIPaV

FISV - Federazione Italiana Scienze della Vita

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CONTENTS

WELCOME LETTER

I am pleased to announce the XV edition of the Meeting of the Italian Federation of Life Sciences (FISV), which will take place from 18th to 21st September 2018 in Rome at the University "La Sapienza".

Fourteen Scientific Societies (AAI, ABCD, AGI, SIB, SIBBM, SIBE, SIBV, SICA, SIF, SIGA, SIMAG, SIMGBM, SIP MeT, SIPaV), with a total of more than 7.000 researchers, are active members of our Federation. I would like to thank all the Scientific Societies which have contributed to organize what promises to be an exceptional scientific event for our Country and have defined an exciting program. The past editions of this meeting have all been very successful and this edition is expected to provide an efficient platform for scientific and interdisciplinary exchange at the forefront of the life sciences. Internationally renowned scientist form Italy and abroad will present their most recent data; in addition, the meeting will also allow several young Italian and foreign scientists to present their research in a highly stimulating scientific environment.

For this edition of the meeting, topics will range, just to mention some, from biological clocks to the emergence of archaic and modern humans, from epigenetic reprogramming to mechanisms at the basis of inflammation of disease, from oxidative stress in health and disease to genetic engineering in plants. Furthermore, a round table will take place on some of the most current aspects and societal implications of the Life Sciences.

We are sure that you will enjoy very much this exciting Congress and anxiously look forward to meeting you in Rome.

Gennaro Ciliberto
FISV President

FISV MEMBER SOCIETIES

AAI	**Associazione Antropologia Italiana**
ABCD	**Associazione di Biologia Cellulare e del differenziamento**
AGI	**Associazione Genetica Italiana**
SIB	**Società Italiana di Biochimica e Biologia Molecolare**
SIBBM	**Società Italiana di Biofisica e Biologia Molecolare**
SIBE	**Società Italiana di Biologia Evoluzionistica**
SIBV	**Società Italiana di Biologia Vegetale**
SICA	**Società Italiana di Chimica Agraria**
SIF	**Società Italiana di Farmacologia**
SIGA	**Società Italiana di Genetica Agraria**
SIMAG	**Società Italiana di Mutagenesi Ambientale e Genomica**
SIMGBM	**Società Italiana di Microbiologia Generale**
SIP MeT	**Società Italiana di Patologia e Medicina Traslazionale**
SIPaV	**Società Italiana di Patologia Vegetale**

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SESSION ORGANISERS

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Bianca Colonna
Michele Morgante
Mario Pezzotti
Telmo Pievani
Valeria Poli
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Plenary Symposia

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Alessandro Achilli
Fiorentina Ascenzioni
Daniela Barilà
Cinzia Bertea
Francesco Bonomi
Sara Cabodi
Rita Casadio
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Gabriele Stocco
Nicola Tomasi
Anita Zamboni
Michela Zottini

PROGRAMME

Tuesday, September 18

11:00 - 13:00 **Registration**

13:30 - 13:50 **Opening & Welcome**
Eugenio Gaudio *(Rettore – Sapienza, University of Rome)*
Gennaro Ciliberto *(FISV President)*

13:50 - 14:00 **Tribute to Luigi Luca Cavalli-Sforza: a Pioneering Geneticist**
Lucio Luzzatto *(Muhimbili University of Health and Allied Sciences, Tanzania)*

14:00 - 15:00 **The EMBO Keynote Lecture**
FISV Lecture Riccardo Cortese
Chair: Valeria Poli *(Turin)*

Wolf Reik *(The Babraham Institute, Cambridge and University of Cambridge, UK)*
Single cell epigenome landscape of development and ageing

15:00 - 17:00 **Plenary Symposium**
The Circadian Clock. A Living Organism's Best Friend in a Spinning World
Chair: Alex Costa *(Rome),* Rodolfo Costa *(Padua)*

Amita Sehgal *(Howard Hughes Medical Institute and University of Pennsylvania, USA)*
How do clocks control behavior and physiology: insights from a small animal model

Jay C. Dunlap *(Geisel School of Medicine at Dartmouth, Hanover, NH, USA)*
The regulatory network governing global responses to changes in light and time

Alex A. R. Webb *(University of Cambridge, UK)*
Dynamic plasticity of circadian oscillators

17:00 - 17:30 **Coffee Break**

17:30 - 19:30 **Round table** (This session will be in italian)
Which future for the smart biotechnologies
Chair: Anna Meldolesi *(Corriere della sera, CRISPeR Mania)*

Anna Cereseto *(Università di Trento)*
Gilberto Corbellini *(Sapienza Università di Roma)*
Andrea Lenzi *(Sapienza Università di Roma e CNBBSV)*
Massimo Losito *(Ateneo Pontificio Regina Apostolorum, Roma)*
Michele Morgante *(Università di Udine)*
Luigi Naldini *(San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Milano)*
Sandro Vitale *(Istituto di Biologia e Biotecnologia Agraria, CNR, Milano)*

19:30 - 20:30 **Welcome Cocktail**

Wednesday, September 19

8:30 - 11:30 Short talks by participants (Session I)

PSI.1

8:30 - 10:00 Environmental Microbiology and Biotechnology (Topic 1)

Chairs: Duccio Cavalieri, Teresa Rinaldi

Annamaria Bevivino *(Rome)*
Environmental microbial signatures revealed by metagenomic analysis of the airways of cystic fibrosis patients

Bruno Casciaro *(Rome)*
Frog-skin esculentin-1a derived peptides against *Pseudomonas aeruginosa* keratitis: anti-biofilm activity and immobilization to soft contact lenses

Simona Crognale *(Rome)*
Exploitation of biological As(III)-oxidation in water treatment systems: potentialities and microbiome profiling in bioreactors

Giovanni Bacci *(Sesto Fiorentino, FI)*
Flying to Mars: the human microbiome under isolated conditions

Francesca D'Angelo *(Rome)*
Biotechnological exploitation of bacterial communication processes: from quorum sensing inhibition to the generation of synthetic cells interfacing with natural cells

10:00 - 11:30 Genetics of Microorganisms (Topic 7)

Chairs: Francesco Imperi, Renato Fani

Sarah Hijazi *(Rome)*
Hijacking bacterial iron metabolism using the transition metal gallium

Alice Checcucci *(Sesto Fiorentino, FI)*
Playing with the rhizobial Mega-Apps: creation and multi-omics characterization of a genomically hybrid strain in the nitrogen-fixing symbiotic bacterium *Sinorhizobium meliloti*

Olivier Jousson *(Trento)*
The role of Type VI Secretion System on the competitiveness of *P. aeruginosa* clinical isolates

Alessandra Polissi *(Milan)*
Characterization of an *E. coli* suppressor mutant that can survive and assemble a functional LPS transport machinery in the absence of the essential inner membrane-tethered LptC

Alessandra Fortuna *(Rome)*
DksA-dependent regulation of quorum sensing in *Pseudomonas aeruginosa*

PSI.2

8:30 - 10:00 Genomics, Proteomics and Systems Biology (Topic 2)

Chairs: Rita Casadio, Paolo Provero

Federico Martinelli *(Palermo)*
Meta-analysis of RNA-seq data to gain insight into crop responses to environmental stresses

Marco Russo *(Bologna)*
Analyses of genomic R-loop maps induced by G-quadruplex ligands in human cancer cells

Federica Zinghirino *(Catania)*
VDAC (voltage dependent anion selective channel) promoters elements and their involvement in metabolic stress

Elisa Margherita Maffioli *(Milan)*
Nanostructured surfaces promote differentiation and maturation events in different cell types

Marcello Manfredi *(Alessandria)*
Integrating serum proteomics, metabolomics and lipidomics to study the effect of sport activity

PSI.3

8:30 - 10:00 **Chromosome Biology, Cell Division and Cell Cycle (Topic 3)**

Chairs: Giovanni Cenci, Giulia Guarguaglini

Valeria Palumbo *(Rome)*
Morgana/CHP-1 is required for oogenesis and early embryonic divisions in *Drosophila*

Patrizia Lavia *(Rome)*
RANBP2: a nucleoporin with key roles in protein SUMOylation

Ferdinando Di Cunto *(Orbassano, TO)*
Citron kinase inactivation inhibits medulloblastoma progression by inducing apoptosis and cell senescence

Patrizia Sarogni *(Pisa)*
Cohesin over-expression promotes colorectal cancer development

Veronica Ferrucci *(Naples)*
A genotype/ phenotype correlation study to dissect the clinical features of the PRUNE-1 syndrome (#617481)

10:00 - 11:30 **DNA replication, Repair and Recombination (Topic 8)**

Chairs: Antonio Musio, Ennio Prosperi

Serena Montalbano *(Parma)*
Identification of the biological target of new platinum, copper and nickel complexes

Anita Palma *(Rome)*
MUS81 endonuclease activity is regulated by CHK2 for replication forks rescue upon replication stress and BRCA2 depletion

Antonella Porrazzo *(Rome)*
Low doses of gamma irradiation render *Drosophila melanogaster* resistant to the DNA damage

Elisa Palumbo *(Padua)*
Identification of molecular biomarkers useful for predicting the likelihood of radiation-induced side effects in oncological patients undergoing radiotherapy

Giulio Ticli *(Pavia)*
Live cell imaging analysis of the influence of p21CDKN1A on PCNA-partners turnover at UV-induced DNA damage sites

PSI.4

8:30 - 10:00 **Oncogenes and Tumor Suppressors (Topic 5)**

Chairs: Sara Cabodi, Miriam Martini

Giulia Bon *(Rome)*
HMGA1/E2F1 axis and NFkB pathways regulate LPS progression and trabectedin resistance

Amani Bouzidi *(Rome)*
Impact of the inhibition of the mitochondrial Serine hydroxymethyltransferase enzyme in mitochondrial respiration and cancer cell growth

Viola Calabrò *(Naples)*
Secreted YB-1 regulates NFkB signaling pathway in receiving cells

Lidia Chellini *(Rome)*
Regulation of ovarian cancer progression by IQGAP1 through endothelin-1 receptor signalling

Silvia Soddu *(Rome)*
The p53 mitotic centrosomal localization contributes to mitotic surveillance pathway

10:00 - 11:30 **Cellular Stress, Apoptosis and Autophagy (Topic 11)**

Chairs: Daniela Barilà, Lucia Morbidelli

Romina Burla *(Rome)*
A new telomeric and ESCRT complex associated factor

Andrea Guarino *(Naples)*
YB-1 recruitment to stress granules in Zebrafish reveals a differential adaptive response

Alberto Inga *(Trento)*
An RBP complex containing DHX30 and PCBP2 operates translation control acting as gatekeeper for p53-dependent apoptosis

Marialuisa Piccolo *(Naples)*
Nucleolipidic-based Ru(III) nanosystems induce multiple cell death pathways activation in preclinical models of human breast cancer

Gaetana Paolella *(Fisciano, SA)*
Involvement of mitochondria and endoplasmic reticulum in stress response to the environmental pollutant 4-Nonylphenol in a human liver cell line

PSI.5

8:30 - 10:00 **Transcriptional Mechanisms and Epigenetic Modifications (Topic 4)**

Chairs: Giacomo Donati, Aymone Gurtner

Michele Menotta *(Urbino)*
ATM unconventional splicing modulation by glucocorticoids

Veronica Ruta *(Rome)*
Strategies to inhibit PRC2 methyltransferase activity in *Arabidopsis* seedlings

Annalisa Roberti *(Pavia)*
Epigenetic marks at satellite-free and satellite-based centromeres

Elisa Coluzzi *(Rome)*
How oxidative stress affects telomere structure and telomeric epigenetic modifications?

10:00 - 11:30 **Non-coding RNA (Topic 9)**

Chairs: Alessandro Fatica, Francesca Orso

Silvia Biscarini *(Rome)*
LncRNAs in motor neuron differentiation and physiopathology

Neri Mercatelli *(Rome)*
HULC/miR-186/Twist: a novel molecular axis in the resistance of Ewing Sarcoma to YK-4-279

Simone Detassis *(Trento)*
An innovative technology for the direct detection of microRNAs in biofluids

Giorgio Dieci *(Parma)*
SINE-encoded ncRNA profiling unveils a new layer of epigenetic dysregulation in cancer

Lidia Villanova *(Rome)*
MiR-663 sustains NSCLC by inhibiting mitochondrial outer membrane permeabilization (MOMP) through PUMA/BBC3 and BTG2

8:30 - 10:00

PSI.6

Plant Nutrition and Biofortification (Topic 10)

Chairs: Laura De Gara, Anita Zamboni

Francesca Cardinale *(Grugliasco, TO)*
Strigolactones effects on root acidification ability

Sara Cimini *(Rome)*
Lead exposure differentially affect growth, antioxidative network and nutrient uptake in metallicolous and non-metallicolous *Zygophyllum fabago* populations

Flaviana Marzano *(Bari)*
Scouting cross-kingdom transfer: effect of plant microRNA on the expression of human genes involved in cell proliferation

Pasqualina Woodrow *(Caserta)*
Tvv1 retrotransposon family is activated by seaweed extracts in Micro-Tom genome

Sezen Yilmaz-Sarialtin *(Ankara, Turkey)*
In vitro antioxidant and anti-inflammatory activities of some *viola L.* species in Turkey

10:00 - 11:30

Photosynthesis, Metabolism and Environmental Stress (Topic 6)

Chairs: Cinzia Bertea, Nicola Tomasi

Giulia Russo *(Grugliasco, TO)*
Strigolactones and abiotic stress in plants: modulation of abscisic acid transport

Cecilia Brunetti *(Sesto Fiorentino, FI)*
Abscisic acid dynamics in leaves, shoot and roots of Populus nigra exposed to drought: relationships with water relations and carbohydrate status

Libero Gurrieri *(Bologna)*
Rearrangements in carbon metabolism under osmotic stress: from central metabolism to stress response

Davide Marzi *(Rome)*
COP1 mediates light-controlled stamen growth in Arabidopsis thaliana

Alberta Pinnola *(Verona)*
Archeology of stress-resistance genes: a novel strategy to improve photosynthesis and productivity in crops

11:30 - 12:00 **Coffee Break**

12:00 - 13:00

Plenary Lecture

Chair: Omar Rota-Stabelli *(Trento)*

Alexei Maklakov *(University of East Anglia, Norwich, UK)*
Why do we age: disposable soma of functional trade-offs?

13:00 - 13:15 **SARTORIUS Short Talk**

T. Claudio Bencivenga *(Milan)*
IncuCyte® S3: live-cell analysis system

13:15 - 14:30 **Lunch time and Posters Viewing**
(Session I: Topics 1, 2, 3, 4, 6, 7, 8, 9, 10, 11)

14:30 - 16:30

Plenary Symposium

Oxidative Stress, Protein Damage and Repair: Implications in Health and Disease

Chairs: Alessandra Polissi *(Milan)*, Roberto Sitia *(Milan)*

Frédéric Barras *(Institut Pasteur, Paris, France)*
Protein homeostasis control: fighting ROS damages by repairing oxidized proteins

Roberto Sitia *(Università Vita-Salute San Raffaele, Milan)*
Tuning redox signaling in and between cells

Alessandro Giuffrè *(CNR-IBPM, Rome)*
Energy, redox status and gasotransmitters: poisons at work!

Anna Teresa Palamara *(Sapienza University of Rome)*
Recurrent Herpes Simplex Virus -1 (HSV-1) infection causes neurodegenerative signs and cognitive deficits in mice. Role of protein oxidation

16:30 - 17:00 **Coffee Break**

17:00 - 19:00

Parallel Symposia

1. Emergence and Spread of Archaic and Modern Humans: News from Bones and Genomes

Chairs: Antonio Torroni *(Pavia)*, Luca Sineo *(Palermo)*

Giorgio Manzi *(Sapienza University of Rome)*
The evolution of the genus *Homo* has been driven by a series of geographical dispersals

Robert Foley *(University of Cambridge, UK)*
Evolution of *Homo sapiens* in Africa: integrating genes, fossils and behaviour

Carles Lalueza-Fox *(CSIC-University Pompeu Fabra, Barcelona, Spain)*
Genomics of the bell beaker phenomenon and the transformation of Western Europe

Cristian Capelli *(University of Oxford, UK)*
Recent, ancient and archaic genomic signatures in the Italian population

17:00 - 19:00

2. Genetic and Epigenetic Mechanisms Regulating Transgenerational Inheritance

Chairs: Michele Morgante *(Udine)*, Antonella Russo *(Padua)*

Marco Barchi *(University of Rome Tor Vergata)*
Molecular mechanisms underlying X-Y chromosomes segregation errors during meiosis

EMBO Young Investigator Lecture

Nicola Iovino *(Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany)*
Epigenetic inheritance through the germline in *Drosophila*

James Hackett *(EMBL, Monterotondo, Rome)*
Genetic and epigenetic regulation of the mammalian germline

EMBO Young Investigator Lecture

Isabel Bäurle *(University of Potsdam, Germany)*
Chromatin regulation of heat stress memory in plants

17:00 - 19:00 **3. Proteins as Drug Target and Drugs, and Protein Degradation as a Therapeutic Strategy**

Chairs: Alberto Boffi *(Rome)*, Andrea Mozzarelli *(Parma)*

Giuseppe Zanotti *(University of Padua)*
Structural determination of proteins of *Helicobacter pylori* relevant for bacterial survival and pathogenicity

Antonella Isacchi *(Nerviano Medical Sciences, Milan)*
Kinases as drug targets: state of the art and future perspectives

Cecilia Weigelt *(Biotech Research & Product Development, Chiesi Group, Lidingo, Sweden)*
The CMC and biological development path of an enzyme replacement therapeutic drug - from idea to EMA approval

Alessio Ciulli *(University of Dundee, Scotland, UK)*
Drug design for targeted protein degradation

17:00 - 19:00 **4. Crossing Biological Barriers, in Health and Disease**

Chairs: Massimo Masserini *(Milan)*, Vito De Pinto *(Catania)*

Francesca Re *(University of Milano-Bicocca)*
Modeling the blood-brain barrier (BBB) in healthy and diseased states

Sara Nicoli *(University of Parma)*
Innovative strategies to overcome ocular barriers

Valeria Dall'Asta *(University of Parma)*
Cationic amino acid transport: physiology and pathology

Krisztina Herédi-Szabó *(SOLVO Biotechnology, Budaörs, Hungary)*
Challenges and opportunities to develop drugs traversing biological barriers

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19:00 - 21:00 **Societies' time**

AGI

19:00 Premio AGI "Ferruccio Ritossa" 2018 e presentazione del lavoro premiato
19:20 Premio Dottorato AGI/Zanichelli 2018 e presentazione del lavoro premiato
19:40 Assemblea dei Soci AGI

SIBV

Premi 2018:
- SIBV Seed Grants
- Giovani Biologi Vegetali

Assemblea Sociale

SIMAG

Assemblea dei Soci

Thursday, September 20

8:30 - 11:30 Short talks by participants (Session II)

PSII.1

8:30 - 10:00 Development, Differentiation and Ageing (Topic 12)

Chairs: Daniele Condorelli, Fulvio Della Ragione

Adriana Borriello *(Naples)*
Studies on cancer-associated *CDKN1B* mutations: unravelling a novel mechanism for tumor suppressor loss of function

Marina Bruno *(Florence)*
Role of sphingosine 1-phosphate signaling axis in the differentiation of epithelial cochlear progenitors

Roberta Cascella *(Florence)*
The mechanism of neuronal toxicity by oligomers and fibrils of a-synuclein

Stefania Martucciello *(Fisciano, SA)*
Tbx1 interacts genetically with Vegfr3 to regulate lymphangiogenesis in mice

Rebecca Piccarducci *(Pisa)*
Influence of APOε polymorphism and physical activity on the well-being of erythrocytes: antioxidant capability and cellular membrane assessment

10:00 - 11:30 Stem Cells, iPS, Cancer Stem Cells (Topic 18)

Chairs: Gianni Cuda, Gabriele Stocco

Maria Giovanna Garone *(Rome)*
Development of new cranial motor neuron differentiation protocol from human iPS cells carrying ALS mutations

Chiara Giacomelli *(Pisa)*
New insights on the human bone-marrow and gingival mesenchymal stem cell responses to senescence induction

Giusj Pugliese *(Rome)*
Generation of an iPSC model to investigate replicative stress as the possible molecular mechanism underlying Schimke immune-osseous dysplasia

Alessandro Rosa *(Rome)*
Dysregulation of RNA metabolism in iPSC-derived FUS mutant human motor neurons, an in vitro model system for Amyotophic Lateral Sclerosis

Federico Salaris *(Rome)*
Transcriptome and proteome analysis of FXS patient-derived iPSC lines to investigate FMRP role and its interactors during neural development

PSII.2

8:30 - 10:00 **Metabolism and its Regulation in Health and Diseases (Topic 13)**

Chairs: Mauro Magnani, Luigi Palmieri

Giulia Guiducci *(Rome)*
Beyond intermediary metabolism: characterization of the moonlighting function of human serine hydroxymethyltransferase

Alessio Menga *(Bari)*
Blockade of glutamine synthetase enhances inflammatory response in microglial cells

Vito Porcelli *(Bari)*
Molecular identification and functional characterization of a novel glutamate transporter in yeast and plant mitochondria

Karim Zuhra *(Rome)*
Synthesis and breakdown of H2S, the third gasotransmitter

Gianluca Mattei *(Florence)*
Metabolism in breast cancer: a systems biology approach

PSII.3

8:30 - 10:00 **Human Genetics and Genomic Diversity (Topic 14)**

Chairs: Alessandro Achilli, Luca Pagani

Michela Leonardi *(Cambridge, UK)*
Human genetic diversity and the effects of subsistence strategies

Angelica Macauda *(Pisa)*
Inherited variation in the xenobiotic transporter patwhay and survival of multiple myeloma (MM) patients: an IMMEnSE study

Manuel Gentiluomo *(Pisa)*
A Mendelian randomization approach to predict the risk to develop pancreatic ductal adenocarcinoma

Marco Capodiferro *(Pavia)*
Exploring the genetic history of Panamanians through ancient genomes

Linda Ongaro *(Tartu, Estonia)*
The european heritage of american populations

10.00 - 11.30 **Evolutionary Biology (Topic 20)**

Chairs: Giorgio Manzi, Marco Oliverio

Valeria Russini *(Rome)*
A biogeographic analysis of loss of planktotrophy in caenogastropod molluscs (Gastropoda, Nassariidae)

Omar Rota Stabelli *(San Michele all'Adige, TN)*
The genome of *Drosophila subpulchrella* helps polarising the evolution of pest traits in its sister species *Drosophila suzukii*

Roberto Feuda *(Bristol, UK)*
A common regulatory neurogenic toolkit in Bilateria

Costantino Buzi *(Rome)*
On the shoulder of the past. The scapula of the Neanderthal from Altamura, Italy

PSII.4

8:30 - 10:00 **Neurobiology and Neuropharmacology (Topic 15)**

Chairs: Giulia Piaggio, Giorgio Racagni

Carlo Brighi *(Rome)*
Modeling Fragile X syndrome with iPSC-derived neurons as a purpose of deciphering the molecular mechanisms and the neurobiological phenotypes associated with the pathology

Anna Maria Lucianò *(Rome)*
M2 muscarinic receptors show inhibitory effects on drug resistance in human neuroblastoma cells

Claudia Colussi *(Rome)*
Nitro-oxidative stress dependent Nup153 alteration affects neural stem cell function in a mouse model of Alzheimer's disease

Serena Faggiano *(Parma)*
Human serine racemase, a drug target for neuropathologies

Ciro Iaccarino *(Sassari)*
Role of LRRK2 in the regulation of dopamine receptor trafficking

10:00 - 11:30 **Nutrition Biochemistry (Topic 19)**

Chairs: Francesco Bonomi, Silvana Hrelia

Paola Antonia Corsetto *(Milan)*
Nutrient deprivation alters lipid phenotype in triple-negative breast cancer

Simone Luti *(Florence)*
Investigating the nutraceutical properties of peptides extracted from Tuscany sourdoughs

Laura Giusti *(Pisa)*
A proteomic approach to study the neuroprotective effect of oleocanthal in SH-SY5Y cells

Stefania Iametti *(Milan)*
Structure-dependent biological activities of food-related stilbene derivatives isomers

Pasquale Marrazzo *(Rimini)*
Enhancing stem cell functionality through antioxidant supplementation

PSII.5

8:30 - 10:00 Immunology and Host-Pathogen Interaction (Topic 16)

Chairs: Fiorentina Ascenzioni, Cristina Parolin

Monica Di Paola *(Sesto Fiorentino, FI)*
Strain level analysis of intestinal fungi and immune mediated host-yeast interaction

Marta Kinga Lemieszek *(Lublin, Poland)*
Beneficial effect of cathelicidin on treatment of hypersensitivity pneumonitis induced by *Pantoea agglomerans* – in vivo studies

Maria Luisa Mangoni *(Rome)*
How to control *Pseudomonas aeruginosa*-induced pneumonia? A lesson from derivatives of the amphibian skin peptide esculentin-1a

Luca Cavinato *(Rome)*
Escaping the oxidative burst: dual role of the *P. aeruginosa* superoxide dismutases

Federica Runci *(Rome)*
The contribution of iron uptake to *Acinetobacter baumannii* pathogenicity

10:00 - 11:30 Plant Development and Disease (Topic 22)

Chairs: Franco Faoro, Michela Zottini

Alex Costa *(Milan)*
Looking for aminoacid-gated calcium channels in plants: functional, biochemical and structural characterization of one GLR isoform

Cristina Ruberti *(Muenster, Germany)*
Fine-tuning the unfolded protein response in plants

Anna Fiorillo *(Rome)*
Biochemical characterization of Salt tolerance-related protein (STRP): a new protein involved in cold stress in *A. thaliana*

Federica Locci *(Rome)*
Oxidation of Damage-Associated Molecular Patterns as a homeostatic mechanism in plant immunity and development

Vincenzo Lionetti *(Rome)*
Pectin methylesterases in plant immunity: activators or susceptibility factors?

PSII.6

8:30 - 10:00 **Biotransformations (Topic 17)**

Chairs: Loredano Pollegioni, Raffaele Porta

Paola Branduardi *(Milan)*
Optimizing the biotransformation of residual biomasses by ameliorating the effectiveness of cell factories

Mohammed Sabbah *(Naples)*
Innovative chitosan-based biodegradable plastics as food coating and wrapping

Valeria Vecchi *(Verona)*
Identification of two novel hemicellulosolytic enzymes from the hyperthermophilic bacterium *Thermotoga neapolitana* and their exploitation for lignocellulose degradation

Andrea Franzetti *(Milan)*
Removal and biodegradation of air pollutants by plant-bacteria interactions in urban areas

Sara Ragucci *(Caserta)*
Purification and characterization of a novel peroxidase from seeds of *Araujia sericifera* Brot

10.00 - 11.30 **Glycoconjugates (Topic 21)**

Chairs: Alberto Passi, Alessandro Prinetti

Livia Cabitta *(Milan)*
Identification of the antigen recognized by rHIgM22, a remyelination-promoting human monoclonal antibody and his effect on glial cells

Ilaria Caon *(Varese)*
The long non coding RNA HAS2-AS1 regulates breast cancer cells aggressiveness acting as a competing endogenous RNA

Elena Caravà *(Varese)*
Role of TNFα on endothelial glycocalyx

Michela Pucci *(Bologna)*
Glycosylation in colorectal cancer: role of B4GALNTII and Sialyl Lewis x antigen

11:30 - 12:00 **Coffee Break**

12:00 - 13:00 **Plenary Lecture**

Chair: Mario Pezzotti *(Verona)*

Luis Herrera Estrella *(LANGEBIO, Mexico)*
Genetically modified plants: past, present and future

13:00 - 13:15 **SENECO Short Talk** (This talk will be in Italian)

Ettore Senes *(Milan)*
Ultrasound-assisted emerging technologies for applied and basic research

13:15 - 14:30 **Lunch time and Posters Viewing (Session II: Topics 5, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22)**

14:30 - 16:30

Plenary Symposium

Life is Also a Matter of Taste and Smell

Chairs: Roberto Barale *(Pisa)*, Guglielmina Nadia Ranzani *(Pavia)*

Wolfgang Meyerhof *(Saarland University, Homburg, Germany)*
Different tongues for different people? - Genetics of bitter taste receptors and perceptual differences in individuals and the population

Catia Sternini *(UCLA, Los Angeles, CA, USA)*
Gut bitter taste receptors as sensors of luminal content

Paolo Gasparini *(University of Trieste)*
Genes and food preferences and their role in personal choices, lifestyle and health

Darren Logan *(Wellcome Trust Sanger Institute, Cambridge, UK)*
Do you smell what I smell? The genetics of olfaction

16:30 - 17:00 **Coffee Break**

17:00 - 19:00

Parallel Symposia

1. RNA Biology

Chairs: Irene Bozzoni *(Rome)*, Salvatore Oliviero *(Turin)*

Dan Dominissini *(Sheba Medical Center & Tel-Aviv University, Israel)*
Concepts and controversies in epitranscriptomics

Danny Incarnato *(IIGM, Turin)*
Dissecting the RNA structurome to decipher in vivo RNA folding dynamics

Gian Gaetano Tartaglia *(Centre for Genomic Regulation and ICREA, Barcelona, Spain)*
Aggregation, neurodegeneration and RNA networks

Julie Martone *(Sapienza University of Rome)*
Lnc-049, a cytoplasmiclLong non-coding RNA involved in skeletal muscle differentiation

17:00 - 19:00

2. Inflammation and Disease

Chairs: Carlo Riccardi *(Perugia)*, Paolo Pinton *(Ferrara)*

Cecilia Garlanda *(Humanitas University, Milan)*
IL-1R8, a negative regulator of inflammation and checkpoint molecule

Francesco Di Virgilio *(University of Ferrara)*
ATP: the quintessential DAMP

Maria Lina Bernardini *(Sapienza University of Rome)*
PAMPs: linking bacterial recognition and inflammatory response

Stefano Bruscoli *(University of Perugia)*
Glucocorticoid and B cell functions: role of Glucocorticoid-Induced Leucine Zipper (GILZ)

17:00 - 19:00 **3. Carbon Cycle and Climate Change: the Future**

Chairs: Luca Espen *(Milano)*, Sergio Esposito *(Napoli)*

Maria De Nobili *(University of Udine)*
Soil, the second active planet scale interface: effects and perspectives of climate change

Nick Ostle *(Lancaster University, UK)*
Climate change and soil biotic carbon cycling

Mauro Centritto *(IVALSA, CNR, Florence)*
Evolution of stomatal and mesophyll conductance: implications for carbon and water exchange in drylands

Rémi Lemoine *(CNRS and University of Poitiers, France)*
Flux of carbon from leaves to roots in plants under environmental stress

21:00 **Social Dinner**

Friday, September 21

8:30 - 10:30 **Plenary Symposium**

Farming for Pharming: Plants as Biofactories (in the Production of Vaccines, Antibiotics, Anticancer)

Chairs: Eugenio Benvenuto *(Rome)*, Franco Faoro *(Milan)*

George P. Lomonossoff *(John Innes Centre, Norwich, UK)*
Plant-based expression of virus-like particles and their use in biomedicine and bionanotechnology

Emanuela Noris *(CNR, Torino)*
Transient expression of anticancer and antiviral antigens in plants

Julian K-C. Ma *(St. George's, University of London, UK)*
Molecular pharming in tobacco to provide antibody based solutions for global health

Selene Baschieri *(ENEA Centro Ricerca Casaccia, Rome)*
Plant-derived vaccines and smart drugs

Linda Avesani *(University of Verona)*
Plant-made nanomaterials for diagnosis and therapy

10:30 - 11:30 **Plenary Lecture**

Chair: Bianca Colonna *(Rome)*

Josep Casadesús *(University of Seville, Spain)*
Waddington's landscapes in the microbial world

11:30 - 11:45 **SEQUENTIA BIOTECH Short Talk**

Walter Sanseverino *(Barcelona, Spain)*
When computer science meets life science: novel bioinformatics approaches to improve NGS data interpretation

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11:45 - 12:00 **Coffee Break**

12:00 - 14:00 **Plenary Symposium**

Regulatory Network Dynamics: from Interaction to Function

Chairs: Matteo Barberis *(Amsterdam, The Netherlands)*, Pier Luigi Martelli *(Bologna)*

FEBS National Lecturer
Luis Serrano Pubul *(Centre for Genomic Regulation, Barcelona, Spain)*
Comprehensive systems analysis of the transcriptome and its regulation of a genome reduced bacterium

Matteo Barberis *(University of Amsterdam, The Netherlands)*
Deciphering yeast physiology by a multi-scale framework integrating cell cycle and metabolism

Davide Cacchiarelli *(TIGEM, Napoli)*
Understanding transcription factors biology through genomics

Adil Mardinoglu *(KTH Royal Institute of Technology, Stockholm, Sweden)*
The employment of systems biology in treatment of liver diseases

Poster Prizes and Closing Remarks

15:00 - 17:00 **Societies' Time**

SIBV

Elevator Pitch Session
Premio Assunta Baccarini Melandri

SIMGBM

Consegna Premi Franco Tatò e Mario Campa 2018
Assemblea Generale dei Soci

ABSTRACTS

THE EMBO KEYNOTE LECTURE

Single cell epigenome landscape of development and ageing

Wolf Reik[1,2,3], F von Meyenn[1], M Eckersley-Maslin[1], S Clark[1], T Stubbs[1], H Mohammed[1], R Berrens[1], F Santos[1], W Dean[1]

[1]*Epigenetics Programme, Babraham Institute, Cambridge CB22 3AT;*
[2]*Centre for Trophoblast Research, University of Cambridge, CB2 3EG;*
[3]*Wellcome Trust Sanger Institute, Cambridge CB10 1SA*

Epigenetic information is relatively stable in somatic cells but is reprogrammed on a genome wide level in germ cells and early embryos. Epigenetic reprogramming appears to be conserved in mammals including humans. This reprogramming is essential for imprinting, and important for the return to naïve pluripotency including the generation of iPS cells, the erasure of epimutations, and perhaps for the control of transposons in the germ line. Following reprogramming, epigenetic marking occurs during lineage commitment in the embryo in order to ensure the stability of the differentiated state in adult tissues. Signalling and cell interactions that occur during these sensitive periods in development may have an impact on the epigenome with potentially long lasting effects. The epigenome changes in a potentially programmed fashion during the ageing process; this epigenetic ageing clock seems to be conserved in mammals. Using single cell multi-epigenomics techniques, we are beginning to chart the epigenetic and transcriptional dynamics and heterogeneity during development and ageing.

PLENARY LECTURE

Genetically modified plants: past, present and future

Luis Herrera-Estrella
Laboratorio Nacional de Genómica para la Biodiversidad del Centro de Investigación y de Estudios Avanzados del IPN, Ciudad de México, MX

In 1983, the first example of the functional transfer of foreign genes into plant cells was reported. One year later two papers reported the feasibility of producing transgenic plants and soon after, the first examples of virus and insect resistant transgenic plants were published. Ten years later the first commercial transgenic lines enter the market, becoming one of the fastest technologies to have a global impact in the history of our society. Gene transfer technology became one of the most powerful and versatile tools for plant biology; thousands of papers using the *Agrobacterium* as gene transfer vector were published. GM plant varieties became a multibillion dollar industry, and despite the active opposition of environmentalist groups, over 120 million hectares of GM crops are cultivated worldwide and directly or indirectly consumed in most countries. Today with great advances on plant functional genomics and the development of genome editing technologies a new kind of genetically modified plants are being produced. In this paper, I will review the development of GM plants from the early days and the new developments using genome editing.

Why Do We Age: Disposable Soma or Functional Trade-Offs?

Alexei A. Maklakov
University of East Anglia, Norwich, UK

Despite tremendous progress in recent years, we are still far from understanding why do organisms age. The long-standing paradigm, the "disposable soma" theory of ageing, maintains that ageing evolves because of competing energy demands between reproduction and somatic maintenance. This paradigm has been increasingly challenged in recent years both theoretically and empirically. Many studies in different model organisms managed to uncouple reduced reproduction and increased longevity, thereby questioning the central importance of reproduction-soma trade-off for the evolution of ageing. A new theory has been put forward, which proposes that ageing is a direct consequence of the development program running at suboptimally high levels (i.e. at "hyperfunction") in adulthood because it is unchecked by weak natural selection in late-life. I will discuss the existing evidence and suggest that both theories are mutually non-exclusive proximate hypotheses rooted in the classic antagonistic pleiotropy theory of ageing. I will propose ways to distinguish between these two mechanims of ageing.

Waddington's landscapes in the microbial world

Josep Casadesús
Departamento de Genética, Universidad de Sevilla, Sevilla, Spain

Conrad Waddington's epigenetic landscape, a visual metaphor for the development of a multicellular organism, can be extended outside eukaryotes: formation of epigenetic lineages occurs also in microbial populations. In bacteria, examples of differentiation resulting in morphological change have been known for decades (spores of *Bacillus*, fruiting bodies of *Myxococcus*, etc.). However, phenotypic differences that do not entail morphological change occur also in microbial populations, and the advent of single cell analysis and fluorescent proteins has unveiled scores of examples. Formation of microbial lineages can have adaptative value either as a division of labor or as a bet-hedging strategy, and facilitates adaptation to

hostile and/or unpredictable environments. Lineage formation is often under epigenetic control, thus avoiding the burden of mutation. In bacteria, epigenetic lineages are formed whenever a cell-to-cell phenotypic difference is propagated by a heritable feedback loop. In other cases, the mechanism of lineage formation is more complex, and involves the formation of heritable DNA methylation patterns.

PLENARY SYMPOSIUM

The Circadian Clock. A Living Organism's Best Friend in a Spinning World

How Do Clocks Control Behavior and Physiology: Insights from a Small Animal Model

Amita Sehgal
Neuroscience, The University of Pennsylvania, Philadelphia, PA, USA

We are interested in the mechanisms that generate daily cycles of physiology and behavior, especially sleep. In addition to endogenous circadian clocks, sleep is driven by a homeostatic process that ensures sufficient amounts of sleep. Our studies of circadian rhythms and sleep use primarily a Drosophila model, which was invaluable for dissecting molecular mechanisms of the clock conserved all the way to humans. We are interested in the control of behavior by brain clocks and in the control of physiology by brain and peripheral clocks. We recently identified a clock in the blood brain barrier (BBB) that confers a 24 hour rhythm on to permeability of the BBB. In other work, we have identified circuits that conduct time-of-day signals from the clock to produce rhythms of locomotor activity. Current research in the laboratory is linking the circadian circuitry to sleep homeostasis circuits, thereby illuminating how these systems interact to control sleep and wake.

The regulatory network governing global responses to changes in light and time

Jay C. Dunlap[1], J. J. Loros[2]
[1]Department of Molecular & Systems biology; [2]Department of Biochemistry and Cell Biology The Audrey and Theodor Geisel School of Medicine at Dartmouth Hanover, NH, 03755

Circadian systems are adaptive, allowing cells to coordinate their metabolism as well as to anticipate recurring changes in their environment. Such clocks arose at least three times in evolution, in cyanobacteria, in higher plants, and in the common ancestor of fungi and animals. The fungus Neurospora is a tractable model for understanding the proteins and networks governing circadian regulation and has also pioneered the understanding of photobiology in the fungi. The clock comprises a negative feedback loop wherein a complex of the transcription factors WC-1 and WC-2 (the WCC), in the dark, drives expression of *frq*. FRQ protein nucleates a complex including FRH and casein kinase 1, and after phosphorylation-mediated delays, this complex downregulates the ability of the WCC to drive transcription. The clock is reset when blue light, detected by FAD stably bound by WC-1, elicits a conformational change in the WCC resulting in activation of *frq* gene expression. The clock drives circadian output when WCC activates *ccg*s, genes whose products do not impact the oscillator. (Dunlap & Loros, 2017, Making Time: Conservation of Biological Clocks from Fungi to Animals. Microbiol Spectr)

Dynamic plasticity of circadian oscillators

Alex A R Webb
Department of Plant Sciences, University of Cambridge, Cambridge, UK

Circadian rhythms are defined by their free running period of near 24 hours in constant conditions. However, circadian period is variable dependent on environmental conditions. Light intensity, duration and quality, temperature, ions, hormones and sugars can adjust circadian period. Our laboratory is investigating the mechanisms by which metabolites such as sugars and nicotinamide regulate circadian period, and why circadian period is plastic. New data

will be presented that describe the molecular mechanisms by which sugars and nicotinamide adjust circadian period and phase. Studies in mutant plants with altered responses to sugars and nicotinamide suggest that metabolites dynamically adjust circadian period to regulate entrainment, the process by which circadian oscillators synchronise to environmental signals. Our combined experimental and theoretical studies suggest that dynamic plasticity of circadian period contributes also to carbon homeostasis through the regulation of starch turnover. Considering the circadian oscillator in dynamic adjustment suggests that the the system is less a clock, and more a molecular signalling pathway integrated in to the biology of the cell.

Oxidative Stress, Protein Damage and Repair: Implications in Health and Disease

Protein homeostasis control: fighting ROS damages by repairing oxidized proteins

C. Henry[1,2], M. M. Cox[2], B. Ezraty[1], **Frédéric Barras**[1,3]
[1]Aix-Marseille Université, CNRS, Laboratoire de Chimie Bactérienne, UMR 7283, Marseille, France; [2]Departement of Biochemistry, University of Wisconsin-Madison, USA; [3]Institut Pasteur, Paris, France

DNA is continuously attacked by reactive oxygen species (ROS) and living cells evolved enzymes that continuously protect DNA such as the protein RecA. In this study, we found that RecA itself is targeted by ROS. We found Met residues of RecA to be targeted by ROS that convert them to methionine sulfoxide (Met-O). *In vitro*, RecA containing Met-O residues loses its ability to form filaments on DNA, to hydrolyse ATP and to perform DNA strand exchange. Moreover, we show that the methionine sulfoxide reductases MsrA and MsrB, which reduce Met-O residues back to Met are able to repair oxidized RecA. Consistently, we found *E. coli msrA msrB* mutants to exhibit defects in RecA-dependent process. Two Met residues were identified that are targeted by ROS. Oxidation of one such Met residues leads to loss of function of RecA. In contrast, oxidation of the other Met residue seems to allow constitutive expression of the SOS regulon. We propose that RecA is under redox control and that MsrA/B plays a key role in allowing ROS to act on RecA activity.

Tuning redox signaling in and between cells

S. Bestetti, M. Galli, I. Medraño-Fernandez, A. Rubartelli, **Roberto Sitia**
Division of Genetics and Cell Biology Università Vita-Salute San Raffaele Hospital, Milan, Italy
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The talk will focus on the mechanisms that regulate H_2O_2 production and transport to integrate redoxstasis and calcium signaling across organelles. H_2O_2 molecules produced by NADPH oxidases in the extracellular space or in the lumen of the endoplasmic reticulum amplify tyrosine kinase receptor signaling inhibiting PTP1B, PTEN and other phosphatases. H_2O_2 require proteinaceous channels like aquaporin 8 (AQP8) to cross membranes and reach its targets in the cytosol (Bertolotti et al., 2013; 2016). We have recently shown that the peroxiporin activity of AQP8 is reversibly gated by sequential sulfenylation- persulfidation of a conserved cysteine (Medraño-Fernandez et al., 2016; Bestetti et al., 2018). Moreover, several H_2O_2 producers and scavengers (Ero1, Prx4, Gpx8) are enriched at MAMs, the contact sites between the endoplasmic reticulum and mitochondria, coupling oxidative folding and Ca^{2+} signaling (Yoboue et al., 2017). A precise topographic organization of molecules and organelles seems to be essential to dynamically regulate redox signaling.

Energy, redox status and gasotransmitters: poisons at work!

Alessandro Giuffrè
CNR Institute of Molecular Biology and Pathology, Rome, Italy

Merely considered as toxic gases in the past, more recently NO, CO and H_2S were found to be endogenously produced also in higher organisms where they play a key signaling role. In such organisms, including humans, these gaseous molecules (also known as 'gasotransmitters') are implicated not only in the regulation of several crucial physiological functions, such as

vasodilation, neurotransmission, immune response and tissue homeostasis, but also in the control of cell redox and energy status. It is thus not surprising that gasotransmitters have been recognized to play a key role in human health and disease. A functional crosstalk between the three molecules has been assessed, yet the molecular mechanisms underlying such an interplay are only partly understood. This talk will focus on the role of cystathionine beta-synthase, a major source of H_2S, in the crosstalk between gasotransmitters, and on the effects on cellular respiration of H_2S, as compared to NO and CO. It will also be provided a possible explanation for how bacteria can respire O_2 in sulfide-rich environments and discussed the potential impact of these findings on human health, environmental science and evolution.

Recurrent Herpes Simplex Virus -1 (HSV-1) infection causes neurodegenerative signs and cognitive deficits in mice. Role of protein oxidation

G. De Chiara[1], M. Fabiani[2], R. Piacentini[3], A. Mastrodonato[3], A. Tramutola[4], M.E. Marcocci[2], G. Napoletani[2], F. Di Domenico[4], M. Perluigi[4], C. Grassi[3], **Anna Teresa Palamara**[2,5]

[1]Institute. of Translational Pharmacology., National. Research Council, Rome Italy; [2]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia - Fondazione Cenci Bolognetti; [3]Institute of Human Physiology, Università Cattolica, Rome, Italy; [4]Department of Biochemical Sciences, Sapienza University, Rome, Italy; [5]San Raffaele Pisana, IRCCS, Telematic University, Rome, Italy

HSV is a DNA virus causing life-long latent infection with multiple reactivations. The role of HSV-1 infection in the pathogenesis of Alzheimer's disease (AD) has been suggested by several data but a cause-effect relationship between virus reactivations and this disorder has yet to be definitely proved. For this reason, we investigated a mouse model of recurrent HSV-1 infection for the appearance over time of AD markers, including brain accumulation of amyloid-β and hyperphosphorylated tau proteins, oxidative damages and neuroinflammation. Biochemical analysis of mouse brains revealed that multiple HSV-1 reactivations induced all these hallmarks. Specific oxidative damages were: increased levels of 4-hydroxynonenal (HNE, marker of lipid peroxidation), protein nytrosylation and carboxylation; alteration in the level of 13 HNE-modified proteins involved in important intracellular processes, suggesting that their oxidation may affect brain physiology. Finally, behavioral tests evidenced cognitive deficits that increased with multiple virus reactivations. Overall, our data suggest that recurrent HSV-1 infections concur to AD neurodegeneration also through oxidative damages.

Life is Also a Matter of Taste and Smell

Different tongues for different people? - Genetics of bitter taste receptors and perceptual differences in individuals and the population

Wolfgang Meyerhof
Center for Integrative Physiology and Molecular Medicine, Saarland University, Homburg, Germany

Bitterness is crucial for forming food preferences and aversions. It is mediated by ~25 taste receptor family 2 (*TAS2R*) genes encoded by the human genome. The *TAS2R* genes are characterized by numerous genetic polymorphisms which predict extensive differences in bitterness perception which could in turn affect ingestive behavior.TAS2Rs recognize specific sets of bitter substances, yet their compound spectra overlap. Moreover, many bitter chemicals activate several receptors. We investigated, in samples of the Caucasian population, taste responses to bitter substances that differ in their number of cognate receptors. We sequenced the TAS2R loci, inferred long-range haplotypes, mapped their effects on phenotype variation and characterized functionally causal allelic receptor variants. Our findings illustrate that genetic influences on bitter taste stem from both receptor activation patterns and linkage structure among *TAS2R* genes. The findings also suggest that genetically determined perceptual differences in bitter taste perception may be less frequent than predicted by the high number of genetic polymorphisms in *TAS2R* genes.

Gut bitter taste receptors as sensors of luminal content

Catia Sternini[1-3], F. Caremoli[1,2], J. Huynh[1,2], J.E. Rozengurt[1,2], R. De Giorgio[4]
[1]CURE:Digestive Diseases Research Center, Departments of [2]Medicine and [3]Neurobiology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA; [4]Department of Medical Sciences, Nuovo Arcispedale S.Anna at Cona (Ferrara), University of Ferrara, Ferrara, Italy

Gut chemosensory processes are critical for nutrients digestion and absorption, and for protection from harmful substances by activating sensory receptors on the gut mucosa. Bitter taste receptors (TAS2Rs) are G protein-coupled receptors (GPCRs) that in the tongue detect the bitter taste, a warning signal against outside threats. TAS2Rs are expressed by the gut epithelial lining including enteroendocrine (EEC) cells and their activation leads to release of Ca^{2+} and peptides involved in gut chemosensing. High fat diet that alters gut microbiome composition, upregulates TAS2R subtypes in the large intestine in a microbiota-dependent manner. Antibiotics that decrease bacteria species diversity and abundance, suppress high-fat diet induced TAS2R upregulation, which is partly restored by fecal transplantation that reverses antibiotic-induced decrease in species diversity. TAS2Rs are significantly correlated with different bacteria and bacteria products activate a GPCR cascade associated with TAS2R transduction in EEC cell lines. TAS2Rs might serve as sensors of luminal content and mediate host functional responses to changes in the gut microbiota by sensing bacterial products.

Genes and food preferences and their role in personal choices, lifestyle and health

Paolo Gasparini
Genetica Medica, IRCCS-Burlo Garofolo, Università di Trieste

Food preferences are influenced by physiological, nutritional, environmental and sociocultural factors. Twin studies showed that also genetics factors might play a role. Moreover, it has been

demonstrated that self-reported food preferences may be determinants of food intake and thus good predictors of health outcomes. We developed a large database (4000 Italian samples) for which a large collection of lifestyle, clinical, taste, food preferences and genetic data are available, to investigate factors (including genetic ones) influencing food liking as well as the effect of food liking on metabolic phenotypes. Genetics studies revealed different loci for food liking, but also gender and food adventurousness showed a strong effect. As regards to food liking and health, different associations were found (i.e. high meat liking and increased systolic blood pressure, BMI and fat mass or decreased HDL cholesterol levels, etc.). These results represent a first step towards understanding the genetic bases of food liking and their possible use, in combination with other factors such as gender, age or eating behaviour, as an effective and feasible dietary measure in nutritional studies.

Do you smell what I smell? The genetics of olfaction

Darren Logan[1,2]
[1]Welcome Trust Sanger Institute, Hinxton, Cambridge, UK; [2]Waltham Centre, Mars Petcare, Waltham-on-the-Wolds, UK

Our sense of smell is mediated by the detection of chemical odours by olfactory receptors (ORs) expressed in the olfactory sensory neurons (OSNs) of the nose. This initiates a neural percept of the odour in the brain. Some individuals report a different percept of specific odours to others, in terms of intensity, valence or detection threshold. This is due, in some cases, to variability in OR gene sequences between individuals. Here I will introduce another mechanism that individualises the sense of smell: variance in the distribution of OSN subtypes. Using an RNA-seq based method to quantify the abundance of all OSN subtypes in mice, I'll demonstrate that genetically diverse individuals have stereotypically different proportions of OSN subtypes in their nose. These differences are independent of OR gene sequence, but are genetically instructed in *cis* to the OR gene expressed in each OSN subtype. In addition, I'll explain how odour experience subtly influences the OSN subtype distribution in an odour-specific manner. Taken together, I'll conclude that variation in OSN repertoires is genetically controlled but environmentally modulated to individualise the sense of smell.

Farming for Pharming: Plants as Biofactories (in the Production of Vaccines, Antibiotics, Anticancer)

Plant-based expression of virus-like particles and their use in biomedicine and bionanotechnology

George P. Lomonossoff[1], J.F.C. Steele[1], M. Walden[1], Y. Meshcheriakova[1], J. Marsian[1], K. Saunders[1], E.C. Thuenemann[1], H. Peyret[1], E.L. Hesketh[2], R.F. Thompson[2], D. Hurdiss[2], N.A. Ranson[2]

[1]Dept. of Biological Chemistry, John Innes Centre, Norwich Research Park, UK;
[2]Astbury Centre for Structural Molecular Biology, University of Leeds, UK

Viral capsids play an essential role in the replication cycle of viruses. Not only do they protect the labile genetic material from degradation but they play a vital role in the transmission of infections both within and between host organisms. Furthermore, it is usually the capsid protein to which a host initially reacts when mounting a defence response, be it through adaptive or innate immunity. For this reason, many vaccines are based either on inactivated virus particles or on recombinant virus-like particles (VLPs). In addition, VLPs have been exploited in bionanotechnology for such purposes as bioimaging and drug delivery.

Recent developments in next-generation sequencing, bioinformatics, plant-based transient expression and cryo-electron microscopy make it possible to produce a virus capsid and determine its structure without ever needing to propagate the parent virus. To demonstrate the feasibility of this approach we have expressed the coat proteins of several viruses of both plants and animals, in plants, isolated the VLPs and determined their structures by cryo-EM. Furthermore, we have used the VLPs to develop novel diagnostic reagents and candidate vaccines.

Transient expression of anticancer and antiviral antigens in plants

Emanuela Noris

Istituto per la Protezione Sostenibile delle Piante, CNR, Torino, Italy

Plants have been explored in the last years as inexpensive and versatile platforms to obtain vaccines and other biopharmaceuticals. Efficient and high yielding transient expression systems are available to produce recombinant proteins. By these systems, structural proteins of viruses which can self-assemble and form virus-like particles have been obtained with the purpose to use them as vaccine candidates on their own or as carriers of immunogenic sequences. A number of antitumor proteins have also been produced and their efficacy has been evaluated in model systems, providing interesting perspectives for further immunological testing.

Molecular Pharming in tobacco to provide antibody based solutions for global health

Julian Ma

St. George's, University of London, UK

The first products of molecular pharming (the manufacture of recombinant pharmaceutical biologics using plant biotechnology) are already on the market and others are in the pipeline. In Europe, approval of a GMP compliant manufacturing process for monoclonal antibody production in tobacco plants dispelled concerns around quality control of plant-derived biologics. Elsewhere, transient expression technologies are being used for Phase III studies of an influenza vaccine and the anti-Ebola ZMapp antibodies entered clinical trials in Africa. Regulatory hurdles were not the major barrier that many feared and whole plant manufacturing systems have been shown to match conventional cell culture based production for quality and reproducibility. This

talk will explore how molecular pharming can provide a manufacturing route for novel uses of antibody in the control of infectious diseases, and enhance the presentation of vaccines to the immune system. It will identify key technological selling points of molecular pharming, discuss how plant-derived biologics can make a difference and assess the opportunities for making a commercial and practical impact on global health.

Plant-derived vaccines and smart drugs

Selene Baschieri
Laboratory of Biotechnology, ENEA C.R. Casaccia, Roma, Italy

The use of plants as natural sources of medicinal compounds deepens its roots in the past, probably since the dawn of civilization, and is in a perennial evolution by virtue of the advances in chemistry methods. Conversely, the use of plants as biopharmaceuticals' factories is a modern discipline started in the early '90s of the last century and in active expansion, hand in hand with the progress in plant biotechnology.

In these years, efforts of the lab have been directed to the improvement of plants as technological platforms for the production of biologics, mainly antibodies and antigens. Beside, through different approaches (e.g. using plant pathogens) we also focused on the delivery step of these molecules. Delivery is indeed crucial in influencing efficacy/bioavailability of a vaccine/drug and remains among the major challenges in biomedicine.

The acquired knowledge provided us with a set of novel tools we are currently testing in different contexts. Our goal is to have the opportunity to boost their application-driven development trying to contribute to the present challenges in human and animal health, possibly through the cross-pollination among diverse disciplines.

Plant-made nanomaterials for diagnosis and therapy

Linda Avesani[1], R. Zampieri[1], M. Merlin[1], E. Gecchele[1], C. Lico[2], E. Bartoloni Bocci[3], E. Tinazzi[4], A. Brozzetti[3], M. Pezzotti[1], M. Santoni[1], A. Falorni[3]
[1]Dept Biotechnology.University of Verona; [2]ENEA, Rome; [3]Department of Medicine. University of Perugia; [4]Department of Medicine.University of Verona

Viral structures can be considered as proteinaceous structures designed by nature and shaped by evolution in great varieties with an appropriate size for nanoscale applications and several intriguing hallmarks: - the ability to self-assemble in structurally simple scaffolds homogeneous in size and shape; - their wide range of dimensions and symmetries; - their plasticity in terms of resistance to harsh environments and conditions and - their rapid and scalable manufacturing in several recombinant systems.

Here we describe an approach that combines plant molecular farming and plant viruses for the production of nanomaterials and their use for the therapy and diagnosis of human autoimmune diseases.

Regulatory Network Dynamics: from Interaction to Function

Comprehensive systems analysis of the transcriptome and its regulation of a genome reduced bacterium

Luis Serrano Pubul
Centre for Genomic Regulation (CRG), The Barcelona; Institute for Science and Technology (BIST)

Determining the gene regulatory network of an organism is fundamental to understand its cellular behavior. Studies of transcription regulation are limited to annotated transcription factors (TFs) and neglect non-canonical regulators. Here we describe the first bacterial DNA-interactome. We identified all potential DNA-binding proteins in Mycoplasma pneumoniae, mapped their binding sites and identified their effector genes. This enable us to discover new TFs, structural proteins – some moonlighting, and novel regulators, like modulators of the RNA polymerase and metabolic and signaling enzymes. Integration of this data revealed that the majority of transcription regulation is not mediated by canonical TFs, but occurs at the level of non-TF regulators, metabolic control, riboswitches, RNA degradation, supercoiling, terminator readthrough and chromosome topology. We quantified the non-redundant contribution in a set of 53 environmental perturbations, showing that the contribution to gene expression variance of non-canonical regulators is 5 times larger than the one of TF-based regulation. This comprehensive analysis highlights the importance of non-TF-mediated regulation in bacteria.

Deciphering yeast physiology by a multi-scale framework integrating cell cycle and metabolism

Matteo Barberis[1,2]
[1]Synthetic Systems Biology and Nuclear Organization, University of Amsterdam, Amsterdam, The Netherlands; [2]Molecular Cell Physiology, VU University Amsterdam, Amsterdam, The Netherlands

Cell division cycle and metabolism are coupled networks. Cell growth and division require synthesis of macromolecules which is dependent on metabolic cues. Conversely, metabolites involved in storage metabolism fluctuate periodically as a function of cell cycle progression. High-throughput and mechanistic interactions are reported between these two networks, and computer models of cell cycle and metabolism are being developed. However, to date no effort has been made to explore how cell's physiology is regulated by the integration of these networks in any organism.

Here I will present a multi-scale framework that integrates a Boolean cell cycle model with a constraint-based model of metabolism in budding yeast, including mechanistic and high-throughput interactions. Directionality and effect of interactions are incorporated iteratively through an evolutionary optimization algorithm. Remarkably, a number of interaction networks is able to reproduce changes in metabolic pathway activity and enzyme concentration dynamics. The multi-scale framework may be employed to capture the mechanistic basis of robustness of cell cycle networks by predicting metabolic causes of cell cycle arrest.

Understanding transcription factors biology through genomics

Davide Cacchiarelli
Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA)

Cellular reprogramming through manipulation of defined factors holds great promise for large-scale production of cell types needed for use in therapy, as well as for expanding our

understanding of the general principles of gene regulation. However, the extent by which some transcription factors regulatory network can convert some cell types, but not others, remain poorly understood. Here I will provide new approaches to understand how single cells, during cell conversion processes navigate towards a final cell identity, highlighting cell fate branches, and dynamic networks. I will also introduce the concept of cell fate alignment with the goal to understand, with respect to a given cell fate identity, the extent and efficiency of a given reprogramming approach.

Altogether, branch analysis and trajectory alignment are a broad tool to yield insights into the molecular network mechanisms of reprogramming, cell differentiation, and a wide array of other biological processes.

The employment of systems biology in treatment of liver diseases

Adil Mardinoglu
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To develop novel strategies for prevention and treatment as well as to gain detailed insights about the underlying molecular mechanisms of liver diseases, it is vital to study the biological functions of liver and how liver interacts with other human tissues as well as with the gut microbiota. Biological networks including metabolic, transcriptional regulatory, protein-protein interaction and co-expression networks can provide a scaffold for studying biological pathways operating in the liver in connection with disease development in a systematic manner. In my presentation, I will present our recent work where biological networks have been employed to identify the reprogramming in liver physiology in response to complex liver diseases. I will further discuss how this mechanistic modelling approach can contribute to the discovery of biomarkers and identification of drug targets which may lead to design of targeted and effective treatment strategies. Finally, I will present a roadmap for the successful integration of models of the liver and other human tissues with the gut microbiota to simulate whole-body metabolic functions in liver diseases.

PARALLEL SYMPOSIA

Emergence and Spread of Archaic and Modern Humans: News from Bones and Genomes

The evolution of the genus *Homo* was driven by a series of geographical dispersals

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Our understanding of the evolution of the genus *Homo* greatly changed in the last decades and is now viewed as a bushy tree (according to the metaphor introduced by S.J. Gould), supported by a fossil record that is now far richer than before. It is characterised by frequent dispersals across large part of Africa and Eurasia, combined with isolation of small groups in eco-geographical refuges across a range of diverse biomes. Both phenomena (dispersal and isolation) played crucial roles in term of adaptive pressures, genetic drift, and admixture among human populations of the same or different species. This scenario is not in contrast at all with the available archaeological signals and/or with the growing molecular evidence.

Evolution of *Homo sapiens* in Africa: integrating genes, fossils and behaviour

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Leverhulme Centre for Human Evolutionary Studies, University of Cambridge, UK

Research into human evolution has become strongly multi-disciplinary, with major contributions to understanding deriving from archaeology, fossils and genomics, all supplemented by the contextual evidence of climate and environment. This has been the case since the first mitochondrial DNA results were published in 1987 and the Out of Africa Model of modern human origins became established. However, while the Out of Africa Model has remained the most compatible with the data, the accumulation of evidence in all fields has radically changed it from its original form as a rapid, simple 'origin-dispersal-replacement' process. This paper will assess the evidence for the evolution of the modern human phenotype, behaviour and population genetics, and explore how a longer chronology, multiple dispersals, gradual changes in behaviour, convergence and admixture, now provide rich insights into the evolutionary processes involved in the emergence of our species.

Genomics of the bell beaker phenomenon and the transformation of Western Europe

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The Bell Beaker (BB) archaeological complex, characterized by distinctive bell-shaped vessels and also associated to war and hunting objects, emerged in southwestern Europe around 2,750 years cal BCE. It quickly spread to north and central Europe, reaching the North European Plain and the British Isles. Its geographic scale is unprecedented in the history of the European continent, but its significance and dynamics has been the subject of much debate. A large group of paleogenomic researchers and archaeologists have recently analyzed 400 ancient samples from the Calcolithic to the Bronze Age, including 226 individuals associated to BB artefacts. The initial spread of BB complex out of Iberia seems to be a movement of ideas, while a subsequent reflux to Great Britain, Iberia and Northern Italy is clearly associated to migrations that brought the steppe ancestry from central Europe to the rest of the continent. In Great Britain, the BB arrival replaced about 90% of the previous Neolithic substratum. Additional analysis of about

300 ancient samples from Iberia shows the impact of these reflux movement during the Bronze Age, which is especially dramatic in the Y-chromosome.

Recent, ancient and archaic genomic signatures in the Italian populations

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The genome variability of Europeans has been heavily influenced by pre- and historical events that involved populations characterised by a limited number of ancestries. Here we investigated the genetic structure and ancestry signatures of the Italian population using a genome-wide dataset representative of modern-day samples from all across Italy and several European and world-wide populations. Italian genomes captured signatures from the Steppe and additional components from the East, and revealed variation in Neanderthal contribution. Differences in ancestry composition as the result of migration and admixture in the last few millennia generated in Italy the largest degree of population structure observed so far in Europe.

Genetic and Epigenetic Mechanisms Regulating Transgenerational Inheritance

Molecular mechanisms underlying X-Y chromosomes segregation during meiosis

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Klinefelter Syndrome (KS) is a chromosomal disorder (47, XXY) that cannot be pass from one generation to the next due to the sterility of the carriers. Changes that cause KS are thought to occur as random events during meiosis, as no predisposing genetic alterations have been associated to it, yet. Crossing-overs (COs) are physical exchanges between homologous chromosomes that ensure their faithful segregation during meiosis. COs formation is initiated by double strand breaks in the genome generated by SPO11. In mammals, *Spo11* generates two main splice variants: beta and alpha. We will show that knock-in mice expressing the single SPO11-beta isoform (*Spo11*-beta-only) suffer of a XY chromosome non-disjunction defect, differentiate XY diploid sperms and despite their reduced testis size, are fertile. This implicates that KS-predisposing mutation can be inherited. Intriguingly, testicular atrophy and XY aneuploidy occurs only when the *Spo11*-beta-only genotype is express in the context of a specific genetic background. This implies that yet unknown modifier genes interact with *Spo11*, shaping the risk of generating KS individuals.

Epigenetic inheritance through the germline in Drosophila

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Fertilization occurs when the two gametes, after epigenetic reprogramming, fuse to produce a totipotent zygote. Although any defects in gametes' reprogramming can cause infertility, the mechanisms underlying this process, remain poorly understood.

Our lab now focuses on understanding the epigenetic events contributing to the formation of functional gametes, to the establishment of totipotency and to the conversion of the totipotent zygote's quiescent genome into a transcriptionally competent one.

We recently showed that H3K27me3 repressive histone mark is retained on post-meiotic oocytes and it is intergenerationally transmitted from the germline to the early embryo (Zenk F., et al. 2007, Science) The maternally inherited H3K27me3 regulates the activation of enhancers and lineage-specific genes during development. Thus, we suggest that H3K27me3 serve as a template of epigenetic memory that can be transferred through the maternal germline and instruct the developmental program of the next generation.

Genetic & epigenetic regulation of the mammalian germline

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Mammalian ontogeny is associated with distinct phases of global epigenetic reprogramming – which occur during preimplantation development and in primordial germ cells (PGC) – and comprehensively remodel the epigenome. These events are thought to reset cellular potential for development, and also act as a barrier against the inheritance of epigenetic information between generations. Our studies have investigated the dynamics and mechanisms of epigenome reprogramming in mouse and human PGCs. Whilst erasure of global DNA methylation is comprehensive and likely entails multiple systems, we unexpectedly identified genomic regions

that escape reprogramming and are consequently epigenetically transmitted through the germline. We have further used unbiased CRISPR screening to understand the regulatory factors that drive PGC development and the associated epigenetic programme. Using this approach, we have traced the genetic determinates that underpin the cell-fate transitions from naïve pluripotency to germ cell fate. Taken together we have gained insight into both the genetic and epigenetic regulation that underpins mammalian germline development.

Chromatin regulation of heat stress memory in plants

Isabel Bäurle
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In nature, plants often encounter chronic or recurring stressful conditions. An increasing number of observations suggest that plants can remember a past exposure to stress in order to be better prepared for a future stress incident. My lab studies heat stress memory in Arabidopsis thaliana as a model case for environmental stress memory. Seedlings acquire thermotolerance through a heat treatment at sublethal temperatures (priming heat stress (HS)) that enables them to survive an otherwise lethal HS. This thermotolerance is actively maintained for several days as indicated by the existence of mutants which are able to establish thermotolerance, but fail to maintain it. HS induces sustained histone methylation at HS memory-related loci that outlasts the transcriptional activity of these loci and hence marks them as recently transcriptionally active. This is correlated with hyper-induction of gene expression upon a recurring HS, - a signature readout of transcriptional memory. Thus, the physiologically defined phenomenon of HS memory has a molecular equivalent in the transcriptional memory and associated changes in chromatin structure.

Proteins as Drug Target and Drugs, and Protein Degradation as a Therapeutic Strategy

Structural determination of proteins of *Helicobacter pylori* relevant for bacterial survival and pathogenicity

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The bacterium *Helicobacter pylori*colonizes the stomach of more than half of the world's population, with the highest rates in developing countries, making it one of the most successful bacterial pathogens. Although the infection is mostly asymptomatic, *H. pylori* is responsible of severe gastroduodenal pathologies, including gastritis, ulcer and eventually gastric adenocarcinoma and MALT lymphoma. *H. pylori* has become an important target for research in the last thirty years, both from the medical and from the biological point of view. Despite the identification of the bacterium dating back to 1984, its pathogenesis remains poorly understood at the molecular level. Indeed, it is estimated that the function of approximately 30%-40% among the about 1550 genes encoded within its genome is only hypothetical or completely unknown. In our laboratory we are working on the structural characterization, either by X-ray single crystal diffraction or cryo-EM, of proteins of the bacterium relevant for pathogenesis or host colonization. In this communication we will focus on some secreted proteins, whose function was unknown or undefined.

Kinases as drug targets: state of the art and future perspectives

Antonella Isacchi
Biotechnology Department Nerviano Medical Sciences Nerviano (Milano) Italy

Protein kinases constitute one of the largest families of enzymes and are key regulators of multiple physiologic as well as pathologic processes. Kinase gene alterations lead to their anomalous expression and activation in specific cancer subsets. Kinases are an important class of pharmacological targets because of their biological relevance as well as their druggability by small molecule inhibitors binding the conserved ATP-pocket. There are currently >40 drugs approved in Oncology, still targeting a very limited portion of the human kinome, leaving much of the kinase therapeutic potential unexplored. Nerviano Medical Sciences has developed a Kinase Platform approach to accelerate development of kinase inhibitor drugs. Among these entrectinib, a Trk, Ros and Alk inhibitor, will be presented as an example of personalized medicine development in Oncology The clinical success of kinase inhibitors in different tumor types, together with the emergence of drug resistance mechanisms, provide a rationale for the future development of kinase drugs addressing mechanisms of resistance as well as novel biological mechanisms in cancer.

The CMC and biological development path of an enzyme replacement therapeutic drug - from idea to EMA approval

Cecilia Weigelt
Biotech Research & Product Development, Chiesi Group, Lidingo (Sweden)

A high level presentation of the long and crooked road that ultimately leads to the registration of a new biological drug. From the first attempts of (at that time) a small Biotech to rise funding and to engage in a consortium with several University groups to generate the first proof of principle data, to the full scale CMC development and transfer of processes to contract manufacturing organizations for full scale GMP production. The presentation will also touch

upon the analytical development and extensive characterization of the target molecule required by the authorities to pass the needle eye for final approval.

Drug design for targeted protein degradation

Alessio Ciulli
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University of Dundee

Bivalent degrader molecules (also termed PROTACs), which target proteins for degradation through recruitment to E3 ligases, represent a revolutionary new class of compounds with therapeutic potential. Formation of a ternary complex between the degrader, the ligase and the target leads to the tagging by ubiquitination and proteasomal degradation of the target protein.

In 2015, we disclosed our discovery of MZ1, a potent degrader made of a ligand for the E3 ligase von Hippel-Lindau (VHL), and JQ1, a pan-selective ligand for the BET proteins Brd2, Brd3 and Brd4. We made the unexpected but fascinating observation that MZ1 induces preferential degradation of Brd4 over Brd2 and Brd3 - despite engaging BET proteins with the same binary affinity. This demonstrated a now well-established feature of PROTACs: they can achieve a more narrow degradation profile in spite of broad target engagement. Our first co-crystal structure of a PROTAC ternary complex (VHL:MZ1:Brd4) revealed the structural basis of degradation selectivity, and illuminated the role of cooperative molecular recognition and inducing de novo protein-protein interactions in the ternary complex for PROTAC mode of action.

Crossing Biological Barriers, in Health and Disease

Modeling the blood-brain barrier (BBB) in healthy and diseased states

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The endothelial cells lining the brain capillaries separate the blood from the brain parenchyma and play a key role in maintaining the brain homeostasis. This "blood-brain barrier (BBB)" strictly limits the passage of blood-borne substances, drugs included. Therefore, the development of new strategies to cross the BBB for the treatment of brain diseases is a challenge, but it is of fundamental importance to consider that in several brain pathologies, the BBB is altered and this can pose serious problems to deliver drugs into the brain. We recently showed that molecular alterations occurring at the level of brain vasculature in a disease state or in aging dramatically affects the ability of BBB targeting-particles to reach the brain (*Dal Magro et al. Nanomedicine 2018*). Since, most of the in vitro and in vivo tests evaluating the ability of drugs to cross the BBB are performed using 'healthy' models, without considering the BBB status, we are developing a new and original in vitro BBB model that reflects the most important alterations of the BBB in Alzheimer disease (Project No: JPCOFUND_FP-829-031). This will represent a novel and highly valuable tool for drug design and testing.

Innovative strategies to overcome ocular barriers

Sara Nicoli
University of Parma, Food and Drug Department

Blindness and visual impairment affect millions of people worldwide and have a very important impact on patients' quality of life. Despite the recent availability of new and effective drugs, targeting the different ocular structures is difficult due to the presence of numerous barriers protecting the inner tissues.

We have evaluated different approaches to overcome ocular barriers, using freshly explanted porcine tissues as model. In particular, the potential of cell penetrating peptides (CPPs) and the use of polymeric nanomicelles as drug carriers will be presented. The results highlighted that CPPs could be useful to enhance drug transport in the cornea and depending on the drug target (intra or extra cellular) different CPPs should be selected.

Polymeric micelles prepared using a water-soluble derivative of vitamin E (TPGS: d-alpha tocopheryl polyethylene glycol 1000 succinate) and poloxamer 407 as excipients, were used as nanocarrier for cyclosporine, a hydrophobic therapeutic peptide effective for the treatment of anterior and posterior segment eye diseases. The data demonstrated the capability of the micelles to effectively solubilize the drug and enhance drug delivery to the cornea and across the scleral tissue.

Overall the results obtained contribute to the study of non-invasive administration modalities for ophthalmic drugs, a significant topic that still represent an unmet medical need.

Cationic amino acid transport: physiology and pathology

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Four distinct systems, named y^+, y^+L, $b^{0,+}$, and $B^{0,+}$, account for Cationic Amino Acids (CAA: arginine, lysine and ornithine) transport in mammalian cells. In non polarized cells CAA uptake

is mainly mediated by the ubiquitous Na^+-independent, membrane potential-dependent system y^+ (CAT family of transporters). In epithelial cells, CAA accumulation is performed by the operation of the Na^+/Cl^--dependent system $B^{0,+}$ ($ATB^{0,+}$ transporter), located at the apical side. At the same side, system $b^{0,+}$, formed by the subunits rBAT and $b^{0,+}AT$, accepts, beside CAA, cystine; it is essential for (re)absorption of this amino acid. CAA efflux at the basolateral side is performed by system y^+L, formed by the heavy chain 4F2hc and the alternative light chains y+LAT1 and y+LAT2; it exchanges CAA with a neutral amino acid plus sodium.

Mutations of rBAT or $b^{0,+}AT$ cause cystinuria, a disease characterized by excess of cystine in the urine leading to the formation of stones in the kidney. Mutations of y+LAT1 cause LPI (Lysinuric Protein Intolerance), a multisystem disorder presenting urea cycle defects consequent to low CAA plasma levels, ascribable to defective intestinal/renal absorption/reabsorption.

Challenges and Opportunities to Develop Drugs Traversing Biological Barriers

Krisztina Herédi-Szabó
SOLVO Biotechnology, Szeged, Hungary

The drug development landscape is shifting towards developing more drugs with low passive permeability. For these compounds it is crucial to have an active path through the cell membrane, therefore their interaction with transporter proteins will shape their ADME properties. Transporter proteins play a role in either taking up compounds to the cells (SLC or uptake transporters) of efflux compounds from the cell (ABC or efflux transporters).

There are several examples where an efflux transporter, such as P-gp or BCRP limits the intestinal absorption of drugs or lead to adverse effects through drug-drug interactions (DDI). Some compounds, (e.g. metformin or several statins), rely on an active uptake process for their pharmacological effect. In the liver canalicular membrane or the kidney proximal tubule cells, transporter interactions do not always lead to changes in plasma concentration but influence the pharmacodynamics. Transporters can be a target for drug delivery - chemical modifications of the drug can increase its bioavailability by being a transporter substrate.

The challenges and opportunities of transporter-mediated interactions will be presented through case studies.

RNA Biology

Concepts and controversies in epitranscriptomics

Dan Dominissini[1,2,3]
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The identity and location of modified nucleotides in the transcriptome make up the epitranscriptome. Recent conceptual and technological breakthroughs have introduced the notion that these internal modifications of mRNA and non-coding RNA are abundant, dynamic and reversible events which possess key regulatory roles in a growing number of RNA processing steps. Paramount among those is N6-methyladenosine (m6A). Parallel to development of important insights into the biology and regulation of the epitranscriptome, controversies began to arise, some pertaining to the function of m6A and its regulators while others challenging the very existence of several modifications. The major controversies revolve around: (1) The dynamic and reversible nature of m6A; (2) the identity of an m6A demethylase or otherwise – FTO substrate specificity; (3) the role of m6A in regulation of splicing; (4) the redundancy of m6A readers; and (5) the complement of mRNA modifications, methods for their detection and mapping, particularly m1A, m5C and Nm. In my talk, I will try to reconcile some of the apparently discrepant findings and bring the audience up to date with current concepts in epitranscriptomics.

Dissecting the RNA structurome to decipher *in vivo* RNA folding dynamics

Danny Incarnato
IIGM, Turin

Deciphering RNA folding pathways and predicting the structure of complex three-dimensional ribonuclear complexes is central to elucidating their biological function. RNA is a single-stranded molecule, able to fold back on itself to form intricate secondary and tertiary structures. A n nucleotides-long RNA can virtually assume up to 1.8n different conformations, but only a tiny subset of such structures is actually sampled in vivo. These structures are crucial to the ability of RNA to perform complex biological functions such as catalysis, regulation of gene expression, and macromolecular scaffolding, making the understanding of how it folds a key need. In this short lecture I will take you through the current knowledge on RNA structure formation dynamics and on how RNA structure thereby tunes cellular processes, as well as future perspectives and still open questions in the field.

Aggregation, Neurodegeneration and RNA networks

Gian Gaetano Tartaglia
Centre for Genomic Regulation and ICREA, Barcelona, Spain

Nucleic acids and proteins are the most important biomolecules in any organism, with the former carrying genetic information and the latter executing and regulating life processes. Characterizing protein interactions s key to unravel the functionality of genomes and can open up therapeutic avenues for the treatment of a broad range of diseases. In this talk, I will focus on the work done in my laboratory to study the role played by proteins and RNAs in transcriptional and translational regulation as well as neurodegenerative disorders (examples include Parkinson's a-synuclein, Alzheimer's disease amyloid protein APP, TDP-43 and FUS). Using both computational and experimental approaches, we aim to discover the involvement

of RNA molecules in regulatory networks controlling protein production. More specifically, I am interested in understanding mechanisms whose alteration lead to aberrant accumulation of proteins and RNAs. We have previously observed that interactions between proteins and their messenger RNAs induce feedback loops that are crucial in protein homeostasis. Recently, we started to unveal the interactions of mystery trascripts, the long non coding RNAs

Lnc-049, a cytoplasmic long non-coding RNA involved in skeletal muscle differentiation

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Lnc-049 is a muscle specific long intergenic noncoding RNA expressed in early phases of *in vitro* myoblast differentiation with a role in mature muscle fiber formation. Endogenous RNA-pull down experiments were performed to identify protein and RNA partners. The most interesting interactor was the ATP-dependent RNA helicase DHX36 that is able to unwind G4-quadruplexes participating in the regulation of mRNAs stability and translation. Among the 61 mRNAs identified as interactors of lnc-049, we focused on Mlx, an important myogenic transcriptional factor.

We demonstrated that lnc049 controls the translation of Mlx-γ isoform through the interaction of a short region (part of a LINE element) with the first exon of its target mRNA. DHX36 helicase is required to resolve a G-quadruplex sequence present in the first exon of Mlx-γ favoring its translation. In the presence of lnc-049, the quadruplex RNA is mantained unwound by the interaction between Mlx-γ and the lncRNA, inducing translational repression. Notably, Mlx-γ repression affects the nuclear translocation of the other two Mlx isoforms (α and β) thus preventing the transcriptional activation of their pro-myogenic target genes.

Inflammation and Disease

IL-1R8, a negative regulator of Inflammation and checkpoint molecule

Cecilia Garlanda
Department of Biomedical Sciences, Humanitas University

IL-1 family members are central mediators of inflammation and homeostatic differentiation and activation of immune cells. IL-1 receptor (ILR) and Toll Like Receptor (TLR) pathway activation is crucial for immune surveillance against infectious agents and sterile damages, but given its broad inflammatory potential it is tightly regulated through ligands with antagonistic or anti-inflammatory activity, or decoy and negative regulatory receptors. IL-1R8 (also known as SIGIRR) is a members of the ILR family acting as a negative regulator of the IL-1 system. IL-1R8 dampens ILR- and TLR-mediated cell activation and is a component of the receptor complex recognizing the anti-inflammatory cytokine IL-37. IL-1R8-deficiency is associated with different pathologic conditions, ranging from infectious and sterile inflammation, to cancer-related inflammation. In addition, IL-1R8 acts as a novel checkpoint molecule of NK cells, tuning NK cell anti-tumor and antiviral activity. Thus, IL-1R8 contributes to the delicate equilibrium between host defense and detrimental inflammation, cancer-related inflammation and immunesurveillance of cancer.

ATP: the quintessential DAMP

Francesco Di Virgilio
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Release of intracellular components is a key trigger of inflammation. Extracellular ATP is the most common, ubiquitous and efficient damage-associated molecular patterns (DAMPs) (1). ATP is endowed with a remarkable plasticity thanks to a panoply of plasma membrane receptors (P2 receptors, P2Rs) (2). It is now well established that extracellular ATP at inflammatory sites, the tumor microenvironment included, is several fold higher than in the healthy microenvironment (3). At inflammatory sites, ATP activates both P2YR and P2XR subtypes. Among P2Rs, the P2X7R is more closely associated to inflammation being coupled to NLRP3 inflammasome stimulation, release of IL-1β and IL-18, generation of reactive oxygen species (ROS), dendritic cell activation and T lymphocyte proliferation (4). Blockers of the P2X7R are in clinical test for the treatment of inflammation (5)

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PAMPs: linking bacterial recognition and inflammatory response

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The innate immune system uses germline-encoded receptors called Pattern Recognition Receptors, PRRs, to recognize and respond to a wide range of microbial stimuli. PRRs recognize common, conserved, and indispensable microbial molecular profiles called pathogen-associated molecular patterns (PAMPs). PAMPs are molecules shared by and exclusive to microorganisms and include bacterial lipopolysaccharide (LPS), peptidoglycan (PGN) and lipoproteins (BLP). Toll-like Receptors (TLRs) are the best characterized PRRs and each recognizes unique

microbial PAMPs. Upon binding to a PAMP, TLRs initiate a signaling cascade that converge onto molecular effectors up-regulating genes that are involved in the inflammatory response against the pathogenic intruder. TLR4 recognizes lipid A, the anchor-membrane domain of LPS. However, in some situations, Lipid A can trigger systemic inflammation that causes tissue damage and occasionally death. To evade this immune response, some bacteria have diversified their Lipid A structures to result in attenuated inflammation. On this basis, targeting the TLR pathway is currently considered a novel therapeutic strategy in order to treat some inflammatory disorders.

Glucocorticoid and B cell functions: Role of Glucocorticoid-induced leucine zipper (GILZ)

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Glucocorticoids (GC) are commonly used for treatment of autoimmune and inflammatory diseases. Although GC are potent life-saving drugs, the clinical effects are transitory and chronic use of GC is accompanied by serious side effects. Therefore, new drugs substituting GC are needed. We have cloned a gene, glucocorticoid-induced leucine zipper (GILZ) that is induced by GC and mediates many GC anti-inflammatory effects. Using a GILZ knock-out (KO) mouse model we have showed that, similar to GC, GILZ regulates B cell survival and differentiation. GILZ deficient B cells are partially resistant to GC-induced cell death, indicating that GILZ is a mediator of pro-apoptotic effects of GC on B cell survival. We have also generated a B cell-specific gilz KO mice, where we found that lack of GILZ in B cells leads to an increased production of IFN-gin B cells and it is associated with an enhanced susceptibility to experimental colitis in mice. Altogether, these findings indicate GILZ as a regulator of B cell maintenance and its deregulation could be implicated to disease predisposition. GILZ may represent a new potential therapeutic target in the treatment of autoimmune/inflammatory diseases.

Carbon Cycle and Climate Change: the Future

Soil, the second active planet scale interface: effects and perspectives of climate change

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Stakeholders, media and general pubblic are well informed of the damage caused by the uncontrolled use of fossile fuels, which results in unprecedented rates of emissions of carbon dioxide into the atmosphere. Yet, even the most attentive among them are still unaware that soils take an active and substantial part in determing the balance between production and sequestration of the main green house gases. About 30 % of the increase in the concentration of carbon dioxide, observed from the second half of the 19th till the end of 20th century, resulted from emissions caused by cultivation of unfarmed lands and intensification of agricultural activities. In this context, a controlled use of fossil fuels is not the only way to reduce the emissions of greenhouse gases. Soils are indeed, after the ocean, the second active planetary interface with the atmosphere and the way they are managed contributes greatly to aggravate or mitigate this problem. The global pool of soil organic carbon constitutes the largest C pool, after that of the ocean, yet to consider the role of soil in the mitigation of the greenhouse effect as solely limited to its C sequestration potential is restrictive.

Climate change and soil biotic carbon cycling

Nicholas John Ostle
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The combined challenges of continuing climate change and increasing demand for natural resources and food are putting considerable pressure on global soils. The interactions between climate and plant-soil systems are critical to the functioning and resilience of soil systems. In this presentation I will show specific examples of current research into plant-soil ecological systems that are being changed by landuse and climate. Case studies from graassland systems examine the role of biological diversity, above and below-ground, as a determinant of soil carbon cycling and biogeochemical functions. The role of improved soil management as a tool to mitigate greenhouse gas feedbacks to climate change will be discussed with new data from research into soil security and sustainable production.

Evolution of stomatal and mesophyll conductance: implications for carbon and water exchange in drylands

Mauro Centritto, M. Haworth
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Stomatal (G_s) and mesophyll (G_m) conductance are the two major stages involved in the regulation of the uptake of CO_2 for photosynthesis and water loss via transpiration. Selective pressures induced by declining [CO_2] over the past 100 Myr have favoured greater allocation of the epidermis to stomata, increased amphistomaty (the presence of stomata on the abaxial and adaxial surfaces) and faster control of G_s in the more recently derived angiosperm groups. Modification of photosynthesis to enhance the water use efficiency (WUE) of C3 crops requires concurrent increases in stomatal density and the capacity of stomata to react quickly to environmental pressures. On-going work will assess the possibility of similar patterns in G_m in different plant groups. Optimisation of G_s and G_m is especially important in drylands to maximise

photosynthetic gain during the brief periods conducive to photosynthesis and prevent excessive water loss. Although the development of crops with improved G_s to G_m ratios would enhance WUE, ensuring higher productivity in drylands, its impact on the teleconnections with the global climate system remains unclear.

Flux of carbon from leaves to roots in plants under environmental stress.

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Soils are a major sink for carbon sequestration and are the subject of renewed interest in a context of climate change. A significant part of carbon in soils is captured by plants through photosynthesis in leaves, transported to the roots and exuded in the soil. Our research focus is the translocation of carbon, as sucrose, from leaves to roots, a major step in the distribution of carbon from the atmosphere to the soil. We investigated the translocation of sucrose and its delivery in the roots by developing tools adapted to the model plant *Arabidopsis thaliana*. We identified the molecular players involved. The effect of water shortage on sucrose delivery to the roots was also studied and the role of a small number of sucrose membrane transporters was found. Moreover, despite severe reduction of plant growth in case of water shortage, the length of the primary length, the surface of soil explored by the roots were only marginally affected in water-stressed plants. In parallel, the transport of sucrose to the roots was enhanced, on a relative basis (Durand et al., 2016, Plant Physiol, 170 : 1460). We will discuss these results in connection with the sequestration of carbon in soil.

SHORT TALKS BY SPONSORS

IncuCyte® S3: Live-Cell Analysis System

T. Claudio Bencivenga
Sartorius Corporation, Italy

The IncuCyte® Live-Cell Analysis System, developed by Essen BioScience, Inc., is the first system to quantify cell behavior in real time (from hours to weeks) while cells remain undisturbed inside a standard incubator. The IncuCyte® System automatically collects and analyzes images around the clock, providing continuous insight into active biological processes that is difficult to achieve with endpoint assays. The IncuCyte suite of assays are designed specifically for live cell assays to not perturb cell health.

Whether evaluating general cell health metrics such as proliferation, cytotoxicity, and apoptosis or functionally-specific processes such as immune cell killing, chemotaxis/migration, antibody-dependent cell-mediated cytotoxicity (ADCC), or stem cell differentiation, the IncuCyte® System is a purpose-built solution, that will increase lab productivity and deliver new insights into the biology of your cells.

When computer science meets life science: novel bioinformatics approaches to improve NGS data interpretation

Walter Sanseverino
Sequentia Biotech SL, Campus UAB, Edifici Eureka, Barcelona, Spain

Next Generation Sequencing is experiencing a boom. From human genome research for personalized medicine, to investigation into plant and animal life, many scientists are joining the NGS wave in order to develop solutions for many of the world's pressing issues. The problem is that, whilst existing sequencing technologies are ever-more mature and accessible, the methodologies for researchers to move, store and analyse data are still very immature. Moreover, there is a limited number of bioinformatics experts who can process the data, and the data is so vast that they do not always generate the best results. Sequentia is developing a solution that replaces today's overly complex data analysis tools. With its democratic approach, Sequentia bridges the gap between NGS data production and interpretation. On one hand, its highly personalized consulting service ensures unique and niche projects reach the deep interpretation and integration of biological data to its maximal potential; on the other hand, its powerful yet truly user-friendly SaaS (Software as a Service) solutions allow research groups to perform their own data analysis intuitively, quickly, efficiently and affordably, accelerating the transformation of data into knowledge. Sequentia is creating a new, evolved bioinformatics paradigm, created by scientists for scientists, to enable them to spend the bulk of their time analysing and interpreting their results and driving groundbreaking, new ideas, rather than in setting up and processing their analysis. Sequentia intends to make data interpretation as accessible as data generation in all fields from biomedicine to agrifood, to help empower researchers across all the biotech spectrum, and in this way, democratize sequencing.

POSTER AND SELECTED SHORT TALKS

1 - Environmental Microbiology and Biotechnology

O1.1 - Environmental microbial signatures revealed by metagenomic analysis of the airways of cystic fibrosis patients

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In the present work, the temporal dynamics of the airway microbiome in cystic fibrosis (CF) is investigated by using shotgun metagenomic sequencing, paying special attention to the episodes of acute pulmonary exacerbation. Twenty-two CF subjects, with a moderate pulmonary disease, were followed over a 15-month period during which one or more exacerbation events might have occurred. Results showed patient-specific bacterial colonization even at the strain level. An extraordinary resilience of the main CF pathogens and a heterogeneous taxonomic microbiome distribution across patients and within time points were found. Metabolic pathways and functional genes were mainly correlated with the environment (i.e. patient conditions), highlighting the role of the functional part of the microbiome in modulating the interaction with the host. Overall, such results suggest the need to develop models of microbiome x host genotype x environment interaction to be used for future personalized therapeutic approaches.

This work was supported by Grants from Italian Cystic Fibrosis Foundation (FFC#14/2015; FFC#19/2017).

O1.2 - Frog-skin esculentin-1a derived peptides against *Pseudomonas aeruginosa* keratitis: anti-biofilm activity and immobilization to soft contact lenses

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Pseudomonas aeruginosa is a Gram-negative bacterium capable of colonizing biotic/abiotic surfaces and causing severe infections such as keratitis which mainly affects contact lens (CL) wearers. This is because *P. aeruginosa* can easily colonize the CL surface forming biofilms which are less susceptible to conventional antibiotics. For these reasons, novel antimicrobial strategies and compounds are needed. Antimicrobial peptides (AMPs) hold promise in this respect. Recently, two frog-skin AMPs, named Esc(1-21) and Esc(1-21)-1c have been characterized for their strong anti-*Pseudomonas* activity. Here we evaluated their activity against *Pseudomonas* biofilm formed on soft CLs resulting in Esc(1-21)-1c showing the highest percentage of killing

(up to 85% *vs* no killing of Esc(1-21) at 4 μM for some bacterial strains). Importantly, when the peptides were covalently immobilized on soft CLs surface, they were found to retain antimicrobial activity without any harmful effect on mammalian cells or lens properties. Overall, the usage of these peptides tethered to medical devices represents a promising approach for prevention/treatment of CL-associated *P. aeruginosa* keratitis.

O1.3 - Exploitation of biological As(III)-oxidation in water treatment systems: potentialities and microbiome profiling in bioreactors

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Arsenic(As) is one of the most toxic element worldwide. Despite As toxicity, many microorganisms evolved different mechanisms to metabolize it and withstand high concentrations, thus revealing the microbial potentiality in bioremediation applications. Microbiological As(III)-oxidation is one of the most promising application as a precursor step in As removal from contaminated groundwater, since conventional iron-based treatments are more effective in removing As(V) rather than As(III). This work is aimed to evaluate the potentialities of biological As(III)-oxidation in lab-scale biofilters treating As-contaminated groundwater through the selection and the establishment of biofilms composed by native water microbial communities. The oxidative performance of the engineered systems was evaluated on different filling materials (sintered glass rings, coarse sand) by using a variety of experimental conditions (various filling materials, flow rates, As(III) concentrations, As(III):As(V) ratio, bed volumes). The As(III)-oxidizing microbial communities colonizing biofilm in bioreactor were exhaustively described by applying flow cytometry, qPCR and high-throughput 16S rRNA gene sequencing

O1.4 - Flying to Mars: the human microbiome under isolated conditions

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The salivary microbiome is composed of all commensal bacteria living in the human salivary glands and it represents the first defence line against external infections. It is able to interact with microbes from other districts of the human body, such as the gut, playing a role in the adaptation of such communities to environmental stresses. Although the interest toward this environment is increasing, little is known about how its composition changes during time and how this can be affected by other people sharing the same environment. Here, salivary samples coming from the Mars500 mission, the longest ground-based space simulation ever (520 days long), were sequenced using a targeted metagenomic approach based on the 16S rRNA gene. According to our results, the diversity of the salivary microbiome across different individuals is decreasing during the period of isolation and, surprisingly, this behavior continues even after the end of the mission. Isolated conditions produced an inter-individual selection that was mainly driven by a set of OTUs representing more than 40% of the total biodiversity.

O1.5 - Biotechnological exploitation of bacterial communication processes: from quorum sensing inhibition to the generation of synthetic cells interfacing with natural cells

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Most bacterial species coordinate group activities *via* an intercellular communication system known as quorum sensing (QS). In the last decades many studies aimed at untangling QS networks governing bacterial social behaviours, others at exploiting QS circuits as tools in synthetic biology applications or as targets for new therapeutic approaches. In the human pathogen *Pseudomonas aeruginosa* a multilayered QS circuit controls the expression of key virulence factors, thus representing both a suitable system to study complex networks leading to the emergence of social traits in unicellular organisms, and a potential target for the development of anti-virulence drugs.

In this context, this work aims at *i)* understanding new regulatory properties emerging from the peculiar topological architecture of the *las* QS circuit, *ii)* exploiting the *rhl* QS system for the generation of synthetic cells interfacing with natural cells, *iii)* identifying new anti-virulence drugs targeting the *pqs* QS system. Data produced in this work increase our knowledge of QS circuits and pave the way for future therapeutic applications based on synthetic cells and on repurposed drugs with anti-virulence activity.

P1.1 - High similarities in the gut microbiota of the two cetacean species *Tursips truncatus* and *Stenella coerulealba*

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The evaluation of symbiotic microbial communities occurring in the intestinal tract of animals has 11 received great interest in the recent years. However, still few is known on cetaceans, despite their 12 relevance in the ecology of marine communities. Here, we investigate the gut microbiota of the two 13 cetacean species Stenella coeruleoalba and Tursiops truncatus by sampling intestinal mucosa from 14 specimens retrieved stranded along the Tyrrhenian coast of Tuscany (Italy). We revealed an abundance 15 of members from Clostridiaceae and Fusobacteriaceae, which in total accounted for more than 50%. 16 Sex, preservation status and also species, did not correlate with differences in the microbiota. Indeed, 17 a high similarity of the taxonomic composition between the gut microbiota of the two species was 18 found. In conclusion our results confirmed that the gut microbiota is more similar to that of 19 phylogenetically unrelated carnivorous mammals and may suggest an influence of the diet on the 20 taxonomic shaping of the gut microbiota.

P1.2 - Drinking water microbiota: where biodiversity rules

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Drinking water and biodiversity are strictly related, a relation of fundamental importance, but unfortunately still underestimated and not fully understood.

We focused our research on the analysis of the bacterial communities residing in drinking water treatment plants (DWTP), starting from groundwater, across carbon filters, to post-chlorination, using high-throughput DNA sequencing techniques.

Our results revealed that there is a striking neglected biodiversity, that depicts DWTP as a complex ecosystem, with a peculiar microbiota, despite the low abundance of microorganisms.

The composition of bacterial communities varies across the DWTP: Parcubacteria dominates treated water, while groundwater has the highest heterogeneity. Carbon filters probably act as substrate for microorganism growth and contribute to seeding water downstream, since chlorination does not modify the incoming bacterial community.

Multi-level approaches, based on the integration of recent innovations in technology and a research responsible and innovative will allow us to appreciate the real biodiversity of water ecosystem and to better manage this resource.

P1.3 - Bioremediation in marine environment, opportunities and challenges approaching the Blue Growth

Renata Denaro
Institute for Coastal Marine Environment, National Research Council (IAMC-CNR)

The fossil fuel-based economy is expected to decline completely by 2050. Notwithstanding, the global demand for energy is expected to rise by 25% in the period 2014-2040 and 1/3 of this will be satisfied by oil. This is not consequence-free, in fact oil pollution impacts human and animal health but it has also deleterious effects on biodiversity, marine industries as fishery, aquaculture, desalination plants, salt production, coastal engineering and shipbuilding, tourism, recreational activities and cultural/natural goods. Finally, the affected regions suffer a significant socio-economic decline also in terms of jobs. Bioremediation is a treatment which employs

living resources (as bacteria), to break down hazardous pollutants into less toxic or non-toxic substances. In spite its eco-friend character, bioremediation in marine environment is still underexplored because of legal, environmental, technological, political and educational barriers. Correct management and communication strategies can help to exploit this opportunity taking advantages in terms of new available products and services and also for new jobs according to the Blue Growth european strategy.

P1.4 - Long-term occurrence of antibiotic resistance genes and class 1 integrons in plankton-associated bacteria

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Massive use of antibiotics in human care and animal farming led to the release of high amounts of antibiotics in aquatic environments, causing the selection of antibiotic resistant bacteria (ARB). While the role of water and sediment in hosting and spreading ARB is well known, no information are available about the involvement of phyto- and zoo-plankton in this respect. This study aims to investigate if marine plankton community could be a reservoir of antibiotic resistance genes and class 1 integrons. Twenty-nine formaln-fixed CPR (high-speed plankton sampler designed to be towed from ships over long distances) samples, collected from different locations (Southern North Sea, Irish Sea, Newfoundland and North Atlantic) from 1970 to 2011 were analysed for the abundance of sulphonamide resistance gene (*sul*2) and class 1 integron-integrase gene (*intI*1) by qPCR and Droplet Digital PCR (ddPCR). The two genes were present in a large fraction of the samples (48% for *sul*2 and 76% for *intI*1). This study reveals long-term occurrence and spread of *sul*2 gene and class 1 integrons in the plankton-associated bacterial communities in the ocean.

P1.5 - Biofilms in cooling towers: biodiversity, structure and seasonality

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Biofilms widely colonize cooling towers promoting biofouling with equipment damages and economic impact. Although the role of biodiversity in biofouling has been recognized, few data are available on the microbial composition and structure of these biofilms. Using NGS, CARD-FISH/CLSM, FLBA and SEM, we provided insights into the biodiversity and structure of biofilms collected from different cooling tower types across a set of European industrial systems. The bacterial diversity showed the existence of a biofilm core microbiome constituted by members of *Sphingomonadaceae*, *Comamonadaceae* and *Hyphomicrobiaceae*, whose occurrence also in the water entering in the cooling systems suggested their possible role in the initial colonization. In addition, diversity and structure of biofilms collected from a cooling tower opened to the atmosphere showed seasonal patterns mainly driven by phototrophic microorganisms. Decreasing trend in bacterial biodiversity from winter to autumn, concomitant with the increasing in taxon richness of the phototrophic populations was observed. We may suggest a strategy accounting for seasonal biofilm variability and focusing on potential pioneer

species role.

P1.6 - Innovation towards sustainable future: new perspectives for bacterial-enhanced phytodepuration

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Bacterial communities of different *Phragmites australis* compartments and soil from the phytodepuration plant of GIDA S.p.A. will be periodically analyzed to: i) determine the composition of bacterial communities and evidence their variation due to seasonal changings and/or wastewater characteristics; ii) select strains able to degrade pollutants; iii) evaluate the influence of microbial consortia on phytodepuration.

The composition of cultivable bacterial communities associated to different tissues of the plant and soil were identified before the activation of phytodepuration plant (with a different distribution of bacterial genera among tissues) and after 4, 8 and 12 months from it. The total bacterial communities were also analyzed through 16S targeted metagenomic sequencing. Cultivable strains collected from root tissue have been tested for their ability to degrade a synthetic waste water containing boron, iron, selenium, and chlorides, allowing to select strains able of enhancing phytodepuration efficiency. Bacterial consortia will be tested in vitro with the purpose to innovate sustainable methods for bioremediation.

P1.7 - Clothes from a dead body: the complexity of bacterial populations

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Decomposition of living beings is a complex ecological process involving prokariota and eukariota. The development of next generation sequencing (NGS) technologies and the availability of bioinformatic tools allowed fast analysis of bacteria populations from environmental matrices. This work shows the analysis of bacteria present on the clothes of a human cadaver found floating in an external pond. As far as we know this is the first NGS analysis of thanatomicrobiome for a body found in water. The NGS analysis clearly shows the presence of the genus *Clostridium*, considered an important element of thanatomicrobiome, together with environmental bacteria. The data are in agreement with other published studies describing rapid postmortem overgrowth of *Clostridium* spp. At the same time the presence of environmental species (e.g. *Pseudomonas* spp) underline the interaction with the environmental bacterial populations.

P1.8 - Bioactive protein-based films containing an antimicrobial apolipoprotein B fragment

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Host defense peptides (HDPs) are short amphipathic peptides playing central roles in innate defense systems. Two novel HDPs have been recently identified in apolipoprotein B (residues 887-922) and partially characterized. Since we demonstrated that peptide $ApoB_L$, 38-residue long in the recombinant form, is able to effectively act *in vitro* as acyl acceptor substrate for microbial transglutaminase (mTG), we tested both enzyme and $ApoB_L$ as additives of a protein-based film forming solution to prepare bioactive films for food coating. The results of our

experiments demonstrate that (i) mTG modulates $ApoB_L$ biological effect because, only when the peptide was incorporated into the film matrix in the absence of enzyme, film antimicrobial activity was fully retained, (ii) Lys cationic residue(s), responsible for $ApoB_L$ ability to interact with microorganism membranes, is(are) neutralized by the mTG-mediated crosslinking of the peptide to the protein network. These findings open new interesting perspectives in the field of active biopackaging devoted to improve the shelf life of different food products. (*Supp. by Ital. Min. For. Aff. & Inter. Coop.; IV Progr. Quadro Coop. Italia/Messico*).

P1.9 - Production and characterization of keratinase from a newly thermophilic actinvomycete *Actinomadura keratinilytica* strain Cpt29 isolated from Algeria

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An extracellular thermostable keratinase (called KERAK-29) was purified and biochemically characterized from a thermophilic actinomycetes *Actinomadura keratinilytica* strain Cpt29 newly isolated from Algerian poultry compost. The isolate exhibited high keratinase production when grown in chicken-feather meal media (24,000 U/ml). Based on matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS) analysis, the purified enzyme is a monomer with a molecular mass of 29233.10-Da. The data revealed that the 25 b , N-terminal residue sequence displayed by KERAK-29 was TQADPPSWGLNNIDRQTAFTKATSI, which showed high homology with those of Streptomyces proteases. This keratinase was completely inhibited by phenylmethanesulfonyl fluoride (PMSF) and diiodopropyl fluorophosphates (DIFP), which suggests that it belongs to the serine protease family. Using keratin azure as a substrate, the optimum pH and temperature values for keratinase activity were pH 10 and 70 °C, respectively. KERAK-29 was stable between 20 and 60 °C and pH 3 and 10 for 5 and 120 h, respectively, and its thermoactivity and thermostability were enhanced in the presence of 5 mM Mn^{2+}.

P1.10 - Magnetic nanoconjugated teicoplanin: a novel tool for bacterial infection site targeting

Giorgia Letizia Marcone[1], I. Armenia[1], F. Berini[1], V. T. Orlandi[1], C. Pirrone[1], E. Martegani[1], R. Gornati[1], G. Bernardini[1] , F. Marinelli[1]

Nanoconjugated antibiotics can be regarded as the next generation drugs due to their potential to overcome multidrug resistance in pathogens. Iron oxide nanoparticles (IONPs) have been used in biomedical sector due to their biocompatibility and magnetic properties. IONPs have been also investigated as potential nanocarriers for antibiotics to be magnetically directed to/ recovered from infection sites. Herein, we conjugated the 'last resort' antibiotic teicoplanin to IONPs. Classical microbiological methods, fluorescence and electron microscopy analysis were used to compare antimicrobial activity and surface interactions of naked IONPs, amino-functionalized NPs and nanoconjugated teicoplanin with teicoplanin. As bacterial models *S. aureus*, *B. subtilis* and *E. coli* were used. The results indicated that teicoplanin conjugation conferred high and prolonged antimicrobial activity to IONPs toward Gram-positives, while no antimicrobial activity was detectable toward *E. coli*. Sensitivity of bacteria to NPs varied according to the bacterial surface and it was species-specific. Finally, NP-TEICO inhibited also *S. aureus* biofilm formation improving the activity towards adherent cells.

P1.11 - Antibacterial and wound healing effects of colloidal silver on human keratinocytes

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Wound repair is one of the most complex biological processes consisting in the activation of numerous intracellular and intercellular pathways able to restore tissue integrity and homeostasis. Open wounds have to be treated to prevent further injury and bacterial invasion, thus facilitating healing. Ionic silver has found large application due to its antibacterial and antifungal activity, especially for the treatment of skin infections. The interest in colloidal silver, a suspension of submicroscopic metallic silver particles, increased during the last years, given the resurgence of antibiotic resistance. While retaining antibacterial activity it also displays less or null toxicity respect to ionic silver and antibiotics. We analyzed a suspension of silver nanoparticles for its activity against several bacterial strains and *C. albicans* and observed a cytotoxic effect on most strains analyzed. Further, we explored the effects on human cultured keratinocytes such as HaCaT cells by wound healing assay and immunofluorescence. We interestingly found an increase of wound closure efficiency accompanied by actin cytoskeleton reorganization.

P1.12 - Gaia: integrated metagenomics suite

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Identifying the biological diversity of a microbial population is of fundamental importance due to its implications in industrial processes, environmental studies and clinical applications. Today, there is still a need to develop new, easy-to-use bioinformatics tools to analyze both shotgun and targeted metagenomics with the highest accuracy and the lowest running time. With the aim of overcoming this need, we introduce GAIA, an online Software as a Service (SaaS) solution to perform 16/18S, virome or shotgun analysis. GAIA is able to obtain a comprehensive overview at any taxonomic level of microbiomes of different origins, including environmental (e.g. land, water or organic waste). Recent publications have benchmarked commonly-used 16/18S pipelines (Siegwald, et al. 2017) as well as shotgun metagenomics pipelines (McIntyre, et al. 2017), and we also benchmarked GAIA with the same datasets. GAIA is currently the best pipeline to analyze shotgun metagenomics data as it obtained the highest Fmeasures above all tested pipelines (e.g. CLARK, Kraken, BlastMegan). In addition, GAIA also obtains excellent Fmeasures analyzing 16S data, yielding better F-measures than mothur and QIIME.

P1.13 - Screening and selection of *Tetraselmis suecica* PGP strains

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Microalgae industrial applications have raised global attention to the study of bacteria–microalgae interactions. Associated bacteria communities may affect microalgae growth and metabolism, influencing biomass yield and quality. Nevertheless, only a few studies have addressed a characterization of bacteria associated with microalgae for the selection of plant

growth promoting bacteria (PGPB). In this study, 250 bacterial strains were isolated from three cultures of *Tetraselmis suecica* F&M-M33, identified through 16S rDNA sequencing and phenotypically characterized for PGP traits. Selected PGP strains were employed in co-culture assays to evaluate the effect of single strains on *T. suecica* growth. Then the spent media of axenic *T. suecica* cultures and co-cultures with strain *Vitellibacter* AAD2 and strain *Sphyngopyxis flavimaris* AG5, that mostly enhanced the microalga growth, were analyzed using a non-targeted metabolomic approach. The analysis of results showed that more metabolites were present in the spent medium of the axenic *T. suecica* cultures than those of co-cultures, suggesting a scavenging action of bacteria.

P1.14 - Dry-fermented salami: evaluating natural products as an alternative to sodium nitrite

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Nitrite is used in meat products as a multifunctional additive to improve their quality and safety. Anyway, the meat industry is seeking for alternatives as nitrite reaction products may have carcinogenic effects.

This study aimed at exploring the use of two natural extracts to replace nitrite in natural dry-fermented salami. Salami were manufactured with three different additives: NIT, containing sodium nitrite; GSE, using grape-seed extract and hydroxytyrosol; and CHE, using chestnut extract and hydroxytyrosol.

Significative differences among treatments were observed for color, with GSE and CHE showing the lowest score, and for pH, higher in NIT. CHE showed the greatest amount of carbohydrate fermentation compounds.Salami microbial communities in the three treatments were similar with a predominance of *Staphylococcus xylosus*and *Lactobacillus sakei* (87% of the total community). Differences were observed for five taxa (accounting for less than 0.4%), a higher abundance of *Photobacterium*in NIT was found. Principal food pathogens were not detected using both classic and NGS approaches. In conclusion CHE and GSE may be considered as good alternatives to nitrite for salami curing.

P1.15 - Bioluminescence-based biosensor for the detection of the *Pseudomonas aeruginosa* siderophore pyochelin

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Iron is an essential nutrient for bacteria. To face iron limitation, the pathogenic bacterium *Pseudomonas aeruginosa* (Pa) produces two siderophores, pyoverdine (PVD) and pyochelin (PCH), which are regarded as key factors for infection. Quantification of these siderophores is crucial both in Pa cultures and clinical samples. While PVD can easily be detected by fluorescence spectroscopy, PCH must be extracted from either cultures or clinical samples, making quantification more difficult and less reliable. We generated a bioluminescence-based biosensor which allows direct (i.e. extraction-independent) PCH quantification in both cultures and clinical samples. The biosensor consists in the promoter of the *pchE* PCH-biosynthetic gene fused to the *luxCDABE* operon, inserted into the chromosome of the Pa siderophore null mutant Δ*pvdA*Δ*pchD*Δ*fpvA*. Different parameters were considered to calibrate the biosensor performances, in order to achieve rapid, sensitive and specific response to PCH. The biosensor was successfully employed to quantify PCH in Pa culture supernatants, and used to set up a screening method to detect PCH-producing Pa strains on solid media.

P1.16 - Natural tools in the battle against cariogenic bacteria: *in vitro* antibacterial effects of polyphenolic extracts of honey and myrtle, also in combination with pomegranate peel extracts, *vs S. mutans* and *R. dentocariosa*

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Standard antibiotics, used to treat oral infections, are increasingly ineffective and often cause side-effects that limit their long-term use. The need for new anticariogenic substances has shifted interest to natural sources, like medicinal plants and derivatives able to inhibit oral pathogens growth. In this regard, the aim of the work has been to evaluate the *in vitro* antibacterial activities of extracts from *Myrtus communis* leaves and *Robinia pseudoacacia* honey against two important cariogenic bacteria, *Streprococcus mutans* and *Rothia dentocariosa*. Combination with peel pomegranate extracts was also investigated. The values of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) have been used to quantize the antibacterial activity of extracts. The Fractional Inhibitory Concentration (FIC) index as a predictor of synergy has been investigated using the extracts combined together. Time-kill curve method was used for evaluate the best combination efficacy against single and mixed bacterial cultures. Our preliminary results suggest that extracts of honey, myrtle and pomegranate may be valid alternatives in the prevention and treatment of caries.

P1.17 - The foodborne strain *Lactobacillus fermentum* MBC2 triggers *pept-1*-dependent prolongevity effects in *Caenorhabditis elegans*

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Lactic acid bacteria (LAB) are involved in several food fermentations. The many properties of LAB, including the ability to provide health benefits, are strain-specific. In this work, the probiotic potential of *Lactobacillus fermentum* MBC2 previously isolated from Mozzarella di Bufala Campana was investigated, through *in vitro* and *in vivo* approaches. *Caenorhabditis elegans* was used as an *in vivo* model to analyze prolongevity and anti-aging effects. *L. fermentum* MBC2 exerted beneficial effects on nematode lifespan, influencing energy metabolism, oxidative stress resistance and aging. Studies on PEPT-1 mutants demonstrated that *pept-1* gene was involved in the anti-aging processes through DAF-16. Indeed, *L. fermentum* MBC2-fed mutants showed a high accumulation of lipofuscin and lipid droplets, whereas the oxidative stress protection exerted by *L. fermentum* MBC2 resulted PEPT-1 independent. Moreover, analysis of acid tolerance, bile tolerance and antibiotic susceptibility were evaluated. Overall, these findings provide new insight for the application of this strain in the food industry as newly isolated functional probiotic.

P1.18 - Anaerobic digestion of food waste: the effect of waste activated sludge addition on microbial population composition

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Owing to the industrial development and population growth worldwide, the amount of food waste (FW) is expected to increase dramatically in the near future. FW, being rich of organic content, is a strategical feedstock for the anaerobic biological conversion and production of methane. However, the availability of prompt biodegradable carbohydrates and the high loading conditions associated to a long-term digestion was found to cause the inhibition of the methanogenic population mainly caused by the intermediate accumulation. Anaerobic co-digestion of FW with a typically recalcitrant substrate like the waste activated sludge (WAS) is a feasible option to improve the process efficiency and stability. The aim of this research was to investigate the microbial population dynamics in long-term semi-continuous co-digestion systems in relation to different feedstock and organic load by using *in situ* cell detection techniques and high-throughput 16S rRNA gene sequencing. The WAS was found to provide specialised beneficial microorganisms for the AD process and, being it a slowly biodegradable substrate, allowed to avoid VFA accumulation by improving the process stability and methane yield.

P1.19 - Phytoremediation of whey by *Chlorella minutissima* induces high lipid accumulation during mixotrophic growth

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C. minutissima is one of the major microalgae used for intensive production of algal biomass, for its high growth rate and the ability to accumulate critical amount of mono and poly unsaturated fatty acids. Annually, the Italy dairy chain produces over 6 M tons of whey, a special classified waste by-product due to its high BOD (biological oxygen demand). In this study the growth of *C. minutissima*, between different concentrations of whey, in *batch* culture condition, was evaluated. As well as the consumption of organic and nitrogen compounds. In addition, qualitative and quantitative analysis was conducted on microalgae fatty acids accumulation, through gas chromatography techniques with GC coupled with MS and FID detector. Results show an important reduction of the serum protein content (80%) after 13 days at both concentrations of serum tested. Moreover, it was observed a 4-fold increase in concentration of the main fatty acids (C16:0, C18:0 and C18:1 ω-9) than the microalgae control cultures. The data collected allowed to assume the hypothetical use of *C. minutissima* in a phytoremediation process of dairy wastewater, using whey as valid alternative for mixotrophic growth.

2 - Genomics, Proteomics and Systems Biology

O2.1 - Meta-analysis of RNA-seq data to gain insight into crop responses to environmental stresses

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Transcriptomic studies are usually conducted in a singular time, they do not provide any repetition across different seasons and frequently they are performed in field conditions where environmental variability is high and disturbing factors are frequently present. For these reasons, more meta-analysis are needed in order to validate singular transcriptomic works with other similar studies performed with same research purposes. Meta-analysis of transcriptomic data will identify commonalities and differences between differentially regulated gene lists and will allow screen which genes are key players in gene-gene and protein-protein interaction networks. The objective of this work is to identify specific and common molecular features (genes, proteins, gene sets, pathways), linked to both abiotic and biotic stress resistances among key crops. The identification of common genes between different biotic stress will allow to gain insight into these general responses and help the diagnosis of an early "stress state" of the plants. These analyses will help in monitoring stressed plants to start early specific management procedures for each disease or disorder.

O2.2 - Analyses of genomic R-loop maps induced by G-quadruplex ligands in human cancer cells

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R-loops and G-quadruplexes (G4) are non-B DNA structures that can be involved in transcription regulation and genome instability. G4s are DNA structures composed of stacked guanine tetrads, notably located at telomeres and oncogene promoters. R-loop consists of a DNA-RNA hybrid and a displaced ssDNA. We determined R-loop profiles and their modifications in U2OS cells treated with G4-binders. Our data show that R-loop profiles are similar between control and treated cells, but the number of peaks and genome coverage suggest an overall increase of R-loops in treated cells. Increased R-loops at transcription start sites depends on gene expression and presence of a CpG-island, suggesting that transcription and G-rich sequences may favour the binding of compounds to their targets. R-loop peaks are often extended in treated cells, and analysis of co-occurance of R-loop extension and experimentally-observed G4 in the ssDNA supports a model in which G4 structures in the displaced strand of an R-loop can be stabilized by a G4 binder therefore stabilizing the overall structure. Thus, our data indicate that G4-binder-induced DNA damage is mediated by unscheduled R loops in human cancer cells.

O2.3 - VDAC (voltage dependent anion selective channel) promoters elements and their involvement in metabolic stress

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VDACs are pore-forming proteins located in mitochondrial outer membrane where allow exchanges of ions and metabolites. We are interested in the transcriptional profile of VDAC isoforms in conditions of metabolic stress induced by hypoxia and nutrient deprivation. These

conditions cause mitochondrial dysfunction, a common feature in many diseases.

We exposed HeLa cells at nutrient and O_2 depletion and performed Real-time PCR on VDAC genes. Interestingly, VDAC1 and VDAC2 mRNAs increased with time, while VDAC3 remained unchanged or slightly down-regulated.

In parallel, we performed a bioinformatic prediction of the TFs binding sites on VDAC promoters (-1000/+200 in gene). TFs sites associated with mitochondrial biogenesis, in particular NRF1, were found: some of them are active. VDAC1 and VDAC2 genes promoters have similar structure and elements. VDAC3 gene promoter has instead a different organization. The differentiated promoter structures fit with the expression patterns of the three isoforms, studied by RT-PCR in metabolic stress conditions.

In conclusion, we propose here the first study on regulatory mechanisms controlling VDAC isoforms expression in metabolic stress conditions.

O2.4 - Nanostructured surfaces promote differentiation and maturation events in different cell types

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Cells are competent to perceive biophysical signals of their microenvironment and to convert them into biochemical responses. These signals comprise the microenvironmental nanotopography. In accordance, cells can sense surface differences that can have a strong impact on cell's behavior. We investigate how nanoroughness of zirconia surfaces can guide cellular activities in the context of neuronal differentiation and β-cell replication and maturation.

We performed a proteomic analysis to assess the molecular mechanisms responsible for the different behavior observed in cells grown on gelatin, flat zirconia or zirconia nanostructure. We found that nanostructured cluster-assembled substrates promote differentiative behavior (neuritogenesis and synaptogenesis) of neuronal cells and β-cell (survival and function) by mechanotransductive events via remodelling of the actin cytoskeleton and nuclear architecture. These findings suggest that tailored nanostructured substrates coupled to proteomic approaches may provide a unique strategy to identify novel hints for cell replacement strategies and tissue engineering to treat neurodegenerative diseases and diabetes mellitus.

O2.5 - Integrating Serum Proteomics, Metabolomics and Lipidomics to Study the Effect of Sport Activity

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It is well known that physical activities improve overall health and counteract metabolic pathologies. In the last years many studies have investigated the effects of stressful physical activities but the integrated study of serum proteomic, metabolomic and lipidomic profiles is still lacking.

Untargeted shotgun proteomics of serum proteins was performed after the immunodepletion of the most abundant serum proteins. The samples were analysed using a liquid chromatography

and gas chromatography coupled to mass spectrometer.

The purpose of this study was to compare pre and post exercise blood samples in order to provide both qualitative and quantitative information, to understand the effect of sport activity on human health and to integrate multi-omics results. The multi-omics approach allowed the identification of several modulated proteins, metabolites and lipids, but also the enriched pathways after the sport activity. In particular, detoxification pathway as well as immune response, lipid transport, and coagulation were affected by physical activity.

P2.1 - From diseases and phenotypes to processes and pathways

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Advanced sequencing technologies allow studying the genetic components of phenotypic traits. We present eDGAR[1] and PhenPath, two resources designed to assist researchers in unveiling the processes involved in the manifestation of normal and pathological traits.

eDGAR collects data on gene-disease associations: given a polygenic disease (or a set of genes), it reports the features shared among the associated genes/proteins (transcription regulation, GO terms, KEGG and REACTOME pathways, direct and indirect interactions from BIOGRID and STRING).

PhenPath collects data on gene-phenotype associations: it compares different phenotypes retrieving the shared biological process and molecular mechanisms at the basis of their appearance. Moreover, our NET-GE[2] algorithm enriches each gene set related to diseases/phenotypes for more GO terms, KEGG and REACTOME pathways.

With our resources, researchers can associate diseases/phenotypes with the genes involved in the characterizing biological processes, for an immediate understanding of molecular mechanisms and possible cures.

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P2.2 - Transcriptome analysis of zinc homeostasis genes reveals a selective altered expression of zinc transporters in colorectal cancer

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ZnT transporters are responsible of zinc efflux and are encoded by ten genes belonging to Solute Carrier family 30A (SLC30A1-10), while ZIP transporters are responsible for the influx of zinc into the cytoplasm and are encoded by fourteen genes belonging to Solute Carrier family 39A (SLC39A1-14). Transcriptome analysis has been performed to assess the mRNA levels of ZnT- and ZIP-encoding genes in colorectal cancer (CRC) samples matched to normal colon tissues and in CRC cell lines. Results revealed an up-regulation of specific ZnT and ZIP transcripts in CRC. Up-regulation of SLC30A5, -6, -7 transcripts, encoding zinc efflux transporters ZnT5, -6, -7, localized on endoplasmic reticulum (ER) membranes, might be part of a coordinated transcriptional program associated to the increased activity of the early secretory pathway, while transcriptional upregulation of several specific ZIP transporters (SLC39A6, -7, -9, -10, -11) could contribute in meeting the increased demand of zinc in cancer cells. Moreover, exon-level analysis of SLC30A9 revealed the differential expression of alternative transcripts in colorectal cancer and normal colonic mucosa.

P2.3 - Glutamate as a multi-purpose nutrient for budding yeast

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The yeast *Saccharomyces cerevisiae* is able to grow on a vast number of nitrogen sources by mean of the activation/repression of different pathways.

Glutamate is a good nitrogen source for yeast, and it could in principle constitute an additional carbon source funneling TCA. We applied cell biology, transcriptomics metabolomics and mathematical modeling to investigate the influence of glutamate on cellular physiology and metabolism. We found a significant alteration in the global transcription profile, cell size, metabolism and stress resistance in cells growing on glutamate, relative to ammonium, used as a reference nitrogen source.

Although glutamate can theoretically act both as a nitrogen and carbon source, wild-type *S. cerevisiae* strains are unable to grow on synthetic media supplemented with glutamate in the absence of a carbon source, such as glucose. Mutants capable of growing on glutamate-supplemented medium in the absence of a carbon source were obtained by direct evolution. Results from preliminary physiological, molecular and computational analysis of the mutants - whose mutated genes encode components of nutrient signal transduction pathways – will be presented.

P2.4 - Isolation and characterization of a primary human corneal epithelial cell line

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Primary human corneal epithelial cells, HCE-F, were isolated from a donor cornea and characterized by morphological and molecular means. The morphological characteristics of HCE-F were studied by optic and scanning-electronic microscopy. HCE-F cells formed a polygonal mosaic and showed a flattened shape morphology: the presence of microvilli and microplicae between adjacent cells were consistent with their epithelial nature. Molecular analyses showed that HCE-F expressed characteristic markers of corneal epithelial cells. The cytokeratin (CK) profile on SDSD-PAGE showed the typical epithelial pattern with CK3, CK5, CK8, CK12, CK17, CK18 and CK19. A western immunoblot showed that HCE-F expressed – as expected by corneal epithelial cells – integrin β-1, CK3/12 and PAX-6. Furthermore, immunofluorescence staining of HCE-F showed positivity for the epithelial barrier markers ZO-1, PAX-6 and occludin. Barrier properties were confirmed by the measure of transepithelial electrical resistance (TEER). Taken together, our results demonstrate the epithelial nature of HCE-F cells, which may serve as an in vitro model of corneal epithelial barrier.

P2.5 - Nitrobindin: an ubiquitous family of all β-barrel heme-proteins

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Nitrobindins (Nbs) form a new class of heme-binding proteins, which has been uncovered in the genome of several species, including *Homo sapiens sapiens*. Structural characterization of Nb from *Arabidopsis thaliana* and from the *C*-terminal heme-binding domain of THAP from *H. sapiens sapiens* indicated that these proteins are composed almost exclusively of *β*-strands and are possibly involved in NO chemistry. To highlight the structural bases of Nbs reactivity, *Mycobacterium tuberculosis* Nb (*Mt*-Nb) and *Homo sapiens* Nb (*Hs*-Nb) have been expressed, purified, and characterized spectroscopically. Ongoing experiments of laser flash photolysis measurements of CO and NO binding kinetics together with the resolution of the three-dimensional structure of *Mt*-Nb will allow us to understand the structure-function relationships of Nbs. Furthermore, ectopic expression of *Hs*-Nb in HEK293 cells will allow the identification of the interarctors of this protein still lacking a biochemical and physiological function

P2.6 - The molecular landscape of the amyloid-driven microglia transformation

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Inflammatory processes are considered the hallmark of chronic diseases. In Central Nervous System, microglia represent the macrophage-like sentinel cells that react to several microenvironmental cues and are responsible of inflammatory processes.

In the Alzheimer disease (AD) the presence of supramolecular structures made up by beta amyloid peptides leads to the activation of microglia with production of inflammatory agents.

Proteomics has been a suitable methodological approach to study changes in protein composition, and were successfully carried out to pick out molecular and functional signatures of a distinctively amyloid activated microglia phenotype.

Results of our recent proteomics studies on cytoplasm and plasma membrane revealed a limited down and up-regulation of the genome expression and few changes in plasma membrane colonization. These changes mainly affect protein involved in the inflammatory and the cytoskeleton re-modelling pathways. The new phenotype arising from these modifications is associated with the toxic behaviour of microglia in AD that sustains the chronic inflammation leading to the development of the neurodegenerative processes typical of this disease.

P2.7 - A mouse model of creatine transporter deficiency syndrome: proteomic approach focused on mitochondrial proteins

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Creatine (Cr) transporter deficiency syndrome-1 (CCDS1) is an X-linked metabolic disorder

causing intellectual disability. Evidence of mitochondrial dysfunction in animal models of Cr deficiency suggests that mitochondrial function may also be abnormal. In this study we investigate the mitochondrial proteome in a CCDS1 mouse model to identify potential protein alterations. Mitochondria were obtained by differential centrifugation from brain of wild type (CrT $^{+/y}$) and knock-out (CrT $^{-/y}$) mice. Mitochondrial proteins extracts were separated by 2-DE and proteomic profiles compared by SameSpot. Fifty-nine spots resulted significantly differentially expressed in CrT $^{-/y}$ with respect to CrT $^{+/y}$. Spots of interest were cut and analyzed by mass spectrometry. Identified proteins essentially appertained to mitochondrial respiratory chain and oxidative stress defense system. Ingenuity Pathways analysis was performed and the network generated involves free radical scavenging and neurological disease. Moreover, sirtuin and Nrf2 pathways resulted inhibited and activated, respectively. Overall our results suggest that CCDS1 activates the processes of ATP production and oxidative stress control.

P2.8 - Post translational modifications of sea urchin toposome induced by environmental factors

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Ocean acidification and exposure to generic pollutants, as metals and toxin, are considered one of the most pervasive human impacts on global marine life. The sea urchin (*P. lividu)s* is recognized as a good model to study the response of marine organisms to environmental stress.

P. lividus protein toposome, and its post-translational regulation, plays important roles in the gametogenesis and embryo development. To detect possible nitration events induced by environmental factors on toposome, the protein was purified from gonads.

Animals were collected prior to the bloom of *Ostreopsis cf. ovata* (control), at bloom phase (toxic), and after detoxification in controlled conditions for different periods.

Identification of peptides with nitrated Tyr or Trp was carried out using LTQ-Orbitrap Velos MS. The nitration level in the toposome protein was compared with that of larval stages from sea urchin eggs treated with metals, used as a proxy of environmental pollutants.

This study highlights for the first time that post-translational modifications induced by environmental factors can affect the function of the toposome in sea urchin.

P2.9 - No Evidence of mtDNA methylation by real-time direct Nanopore sequencing

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The occurrence of DNA methylation in the human mitochondrial genome (mtDNA) and its functional role is since long largely debated and diverging results have been obtained so far. In any case, when detected, the level of methylation is very low.

The Oxford Nanopore Technologies (ONT) sequencing devices may allow direct real-time DNA methylation analysis, based on the observation of peculiar current signals at level of methylated/non-methylated cytosines when ssDNA is crossing the pore.

We compared the methylation profile of ONT sequence data from native and amplified mtDNA from OWTC cell lines, using these latter as a negative control. The observed comparable average methylation level for native and amplified mtDNA, 2.9% and 3.8%, respectively, does

not provide evidence of mtDNA cytosine methylation above the expected signal noise. On the contrary, a remarkable signal was observed by analyzing the methylation pattern in nuclear DNA from NA12878 benchmark data (coverage > 20) with observed average levels of 60% and 7.7% for native and amplified DNA, respectively.

P2.10 - Characterization of the protein corona adsorbed onto novel nanomaterials in biological media

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Nanomaterial are used in many fields and their application continue to expand. Upon introduction into physiological environments, they readily associate proteins forming a protein corona (PC) on their surface whose presence influences the surface characteristics and may impact their interaction with cells. It is therefore necessary to understand PC formation.

A series of experiments has been conducted to determine the connection between nanoparticle geometry and biomolecular corona formation. To this aim, gold nanoparticles (NPs) with two different shapes, urchin-like (GNP1) and spheres (GNP2), were considered. Two main strategies were applied that, combined, may provide a thorough understanding of protein dynamics at the nanoparticle interface. The nanoparticle size distribution was assessed by Dynamic Light Scattering and Single Particle Extinction Scattering (SPES). To qualitatively and quantitatively characterize the protein composition of the corona a proteomic analysis has been performed by NanoLC-ESI tandem mass spectrometry. The analysis has been conducted on the urchin-like (GNP1) and spheres (GNP2) gold NPs incubated with 98% serum.

P2.11 - AIR: Artificial Intelligence RNAseq

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In the field of genomics, sequencing technologies have drastically changed in the last few years and the output of complex data generated has outpaced the solutions available for analysis, integration and interpretation. RNA Sequencing has emerged as the number one technique in transcriptomics and thus the solution we propose is based on this. A.I.R.: Artificial Intelligence RNASeq is the first easy to use SaaS (Software as a Service) built with solid scientific methods. AIR is able to perform a robust DEG and GEOA analysis with different statistics to solve three important obstacles in the genomics field simultaneously: the informatics problem (specifically data storage, automatization of results and duration of analysis); the scientific problem (data interpretation and data integration, as well as providing new bioinformatics and statistical functions); the social problem (the lack of availability of skilled bioinformaticians). The overall objective of this project is to introduce a disruptive innovation that will allow researchers to perform transcriptomics data analysis easily, quickly and affordably. AIR is accessible at http://transcriptomics.cloud

3 - Chromosome Biology, Cell Division and Cell Cycle

O3.1 - Morgana/CHP-1 is required for oogenesis and early embryonic divisions in *Drosophila*.

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Morgana/CHP-1 is a ubiquitously expressed, highly conserved CHORD (Cysteine and Histidine Rich Domain) containing protein. Null mutations in the *Drosophila morgana (mora)* gene cause lethality and elicit a complex mitotic phenotype that is fully rescued by the human orthologue of *mora*. *morgana -/-* mice die at the preimplantation stage, and *morgana +/-* primary cells and mice display supernumerary centrosomes and increased susceptibility to neoplastic transformation. Here we investigate role of Mora in *Drosophila* embryonic development. Females expressing a *mora* shRNAi construct in their germline lay eggs that are not fertilized or give rise to highly aberrant embryos. Time-lapse analysis of embryos injected with an anti-Mora antibody revealed that acute disruption of Mora function causes chromatin and spindle defects, but does not affect centrosome number, suggesting tissue-specific functions of Mora. Proteomic analysis conducted in embryos expressing Mora-GFP revealed that Mora interacts with several components of the Hsp90-R2TP-TTT supercomplex, suggesting that Mora plays a regulatory role within this large complex.

O3.2 - RANBP2: a nucleoporin with key roles in protein SUMOylation

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Protein conjugation with SUMO (small ubiquitin-like modifier) peptides is a post-translational modification that modulates protein interactions and localization in rapid dynamic processes, e.g. the DNA damage response and the mitotic division. The nucleoporin RANBP2 has SUMO-ligase and SUMO-stabilizing activity and it is often deregulated in cancer. Using in situ PLA, live imaging and functional assays, we identify RANBP2-guided SUMOylation processes required for at least three steps during mitosis:

a) the localization of Topoisomerase II-alpha at centromeres and, hence, sister centromere decatenation prior to chromosome segregation;

b) the accumulation of the kinase Aurora-B at kinetochores, underlying Aurora-B's ability to correct erroneous kinetochore-microtubule interactions;

c) the localization and function of NuSAP (Nucleolar and Spindle Associated Protein) in stabilizing microtubule/kinetochore interactions.

These results indicate that local protein SUMOylation is essential to key mitotic steps and highlight a regulatory role of RANBP2, suggesting that RANBP2 - when deregulated or dysfunctional - may contribute to originate mitotic errors and aneuploidy.

O3.3 - Citron kinase inactivation inhibits medulloblastoma progression by inducing apoptosis and cell senescence.

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Medulloblastoma (MB) is the most common malignant brain tumor in children. Current treatment for MB, consisting of surgery followed by irradiation of the whole neuraxis and high-dose multi-agent chemotherapy, is only partially effective and is associated with highly invalidating side effects. Therefore, the identification and validation of novel target molecules, capable of contrasting MB growth without disturbing brain development, is needed. The Citron kinase protein (CITK), encoded by primary microcephaly gene MCPH17, is required for normal proliferation and survival of neural progenitors. Constitutive loss of CITK leads to cytokinesis failure, chromosome instability and apoptosis in developing brain, but has limited effects on other tissues. We here show that CITK deletion leads to cytokinesis failure and DNA damage also in medulloblastoma cells, impairing proliferation and inducing cell senescence and apoptosis via TP53 or TP73. Similar effects were obtained in a primary mouse MB model. Most importantly, CITK deletion decreases tumor growth and increases survival in these mice. These results suggest that CITK can be a useful therapeutic molecular target for MB treatment.

O3.4 - Cohesin over-expression promotes colorectal cancer development

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Cancer cells are characterized by chromosomal instability (CIN) and it is thought that errors in pathways involved in faithful chromosome segregation play a pivotal role in the genesis of CIN. Cohesin forms a large protein ring that binds DNA strands by encircling them. In addition to this central role in chromosome segregation, cohesin is also needed for DNA repair, gene transcription regulation and chromatin architecture. Though mutations in both cohesin and cohesin-regulator genes have been identified in many human cancers, the contribution of cohesin to cancer development is still under debate. Here we showed that *SMC1A* cohesin core gene was present as extra-copies, mutated, and over-expressed in human colorectal carcinomas. We then demonstrated that cohesin over-expression led to the development of aggressive cancers in immunocompromised mice through gene expression dysregulation. Collectively, these results suggest that defective cohesin plays a major role in the development of human colorectal cancer.

O3.5 - A genotype/phenotype correlation study to dissect the clinical features of the PRUNE-1 syndrome (#617481)

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Neurodevelopmental disorder with variable brain anomalies, including progressive cerebral and cerebellar athrophy with brain stem lesions (NMIHBA; OMIM #617481) is an autosomal recessive condition caused by mutations or deletions in PRUNE1 locus (Zollo et al, 2017). To date, twelve variants affecting PRUNE1 functional domains have been reported in 23 unrelated families (some with a degree of consanguinity), with p.D106N being the most frequent. Here, we dissect the genotype/phenotype correlation using fibroblasts derived from affected patients. We describe the mechanism of action of mutated Prune1 proteins (mostly mediated by its interaction with NME1) affecting microtubules (MT) mitotic dynamics through alterations in GTP pool at MT-plus end, by using fibroblasts carrying p.D106N, p.D106N/L270P (from three new Italian families), and p.H292Qfs*3 mutations (Karakaya et al, 2017). We also show the ability of a not-toxic small molecule to "*rescue*" the proliferation and mitotic defects *in vitro*. Further studies will be aimed to bring these molecules soon in therapy in human.

P3.1 - Satellite DNA is responsible for centromere clustering in mammals

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In the 3D architecture of mammalian nuclei, centromeres cluster at the nuclear and nucleoli periphery. It is a matter of debate whether centromere clustering depends on the presence of satellite repeats or on the centromeric function. We discovered that, in equid species (horse and donkey), several centromeres are satellite-free, whereas many satellite DNA loci are not centromeric (Wade et al Science 2009; Piras et al PLoS Genet 2010; Nergadze et al Genome Res 2018). Thus, Equids represent a unique model for investigating the basis of centromere clustering.

By studying their 3D distribution, we showed that centromeric and non-centromeric satellite DNA loci form clusters, indicating a tendency of satellite sequences to coalesce irrespectively of the centromeric function. On the other hand, satellite-less centromeres, although localizing mainly at nucleoli and nuclear periphery, do not associate to the satellite-based centromeres.

These observations are in agreement with the notion that in the mammalian nucleus, chromosomal loci tend to associate with each other according to their repeat enrichment as a result of mutual repeat recognition (Solovei et al Curr Opin Cell Biol 2016).

P3.2 - Two immortalized rat astrocyte cell lines as *in vitro* model for specific cell proliferation studies: cytogenetic and epigenomic characterization and diversification

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Here we report differences between: 1) a heterogeneous population of primary rat brain astrocytes (Primary), in culture since several years ago, and 2) a cloned cell line (Clone), obtained from the Primary cells. Both populations maintain astrocyte morphology but, according to cytogenetic and epigenomic characterization, differ for the chromosomal asset from rat normal cells (42 chromosomes): Primary cells show mostly a bimodal karyotype with 41 or 43 chromosomes, and Clone has a unique-modal karyotype of 43 chromosomes. Interestingly, we also found that both cell lines show genome-wide DNA hypomethylation, with Clone showing even more pronounced demethylation respect to Primary cells. These features, together with a faster doubling time, confer to Clone an altered proliferation control phenotype. Conversely, the Primary cell population is more similar to normal cells. Used together the two cell populations are a promising model to investigate in vitro modifications of genome, epigenome and others 'omics', mimicking tumor clonal evolution-derived heterogeneity, particularly useful in studies on CNS cancers, which derive mostly from glial cells.

P3.3 - The tumor suppressor p14^{ARF} hampers proliferation of aneuploid cells induced by CENP-E partial depletion

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The Spindle Assembly Checkpoint (SAC) is a cellular surveillance mechanism that ensures

faithfully segregation of chromosomes. Reduced expression of some of its components weakens the SAC and induces chromosome instability and aneuploidy, both hallmarks of tumor cells. Centromere Protein-E (CENP-E) is a crucial component of the SAC and facilitates kinetochore microtubule attachment required to achieve and maintain chromosome alignment. To investigate the possible role of $p14^{ARF}$ on aneuploid cells proliferation we induced aneuploidy in primary human fibroblasts (IMR90) and in near diploid tumor cells (HCT116) by partial depletion of CENP-E obtained by RNA interference. Our results show that in contrast to IMR90 aneuploid cell number that was drastically reduced tending toward wild type condition, HCT116 aneuploid cells were slightly decreased at late time points. This euploidy restoration was accompanied by increased $p14^{ARF}$ expression in IMR90 cells and followed ectopic $p14^{ARF}$ re-expression in $p14^{ARF}$-null HCT116 cells. Collectively, our results strongly suggest that hampering proliferation of aneuploid cells is an additional role of the $p14^{ARF}$ tumor suppressor.

P3.4 - Identifying new genes linked to cell division and regulation of brain size

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During evolution, the human brain increased in size and complexity when compared to other Hominidae. In particular, changes in brain globularization and skull shape took place during the transition from *H. neanderthalensis* to *H. sapiens.* These changes are thought to be due to fixed mutations in genes involved in brain development. In order to identify regulators of human brain size, we selected candidate genes that carry fixed sequence changes in *H. sapiens* compared to *H. neanderthalensis*, and performed siRNA based phenotypic screening in human hTERT-RPE-1 cells. The screening identified several genes with roles in mitosis, in particular in spindle assembly and chromosome segregation. siRNA-induced mitotic defects, resulted in p53 activation and cell cycle arrest or apoptosis, depending on the target. Moreover, we observed defects in serum starvation-induced ciliogenesis following gene silencing. To better understand the consequences of the defects that we observed in cultured cells on brain development, we are performing experiments *in vivo* in chicken embryos with selected candidates. The results of the screening and current characterization of candidate genes will be presented.

P3.5 - Alternative Lengthening of Telomere (ALT) activated by telomere damage in human primary fibroblasts

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The large majority of cancers (80–85%) maintain telomere length by expressing telomerase whereas the other ones (15-20%) utilize the Alternative Lengthening of Telomeres (ALT) pathway. Although the telomerase mechanism is well known, the molecular details of ALT remain poorly described. Previous studies provided evidence that X-rays modulate telomere length at 15 days after exposure in human primary fibroblasts (HFFF2). In order to understand the mechanism responsible for such modulation, we treated HFFF2 with 4Gy of X-rays and analyzed telomere length/dysfunction, telomerase activity, ALT markers and epigenetic changes at different times after irradiation. Results demonstrated that irradiation modulates telomere length with ALT mechanism instead of telomerase activity. Furthermore the analysis of Telomere Induced Foci (TIFs), indicated a telomere dysfunction and ChIP results support also a epigenetic modification after telomere damage. These data confirm our previous hypothesis that ALT is a mechanism

activated by normal cells as a response to physiological telomere dysfunction.

P3.6 - Yeast Gcn5 histone acetyltransferase is present in the mitoplasts independently from the SAGA complex

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In yeast, SAGA is the main acetylation complex and it is composed by two multiprotein modules having different catalytic activity: HAT for acetylation and DUB for deubiquitylation.

We have previously shown that the KAT2-Gcn5 protein of the HAT module is required for respiratory metabolism and oxygen consumption, indicating a role of acetylation in mitochondrial functions. Moreover in respiratory condition, the expression level of *GCN5* is upregulated at protein as well as at mRNA level. We will report an important difference on the effect of the *GCN5* deletion in two strains having different mitochondrial (mt) DNA organization. We also report the mitochondrial localization of the Gcn5 protein obtained by Western Blot and supported by physiological, genetic and microscopic analysis.

The presence of Gcn5 in mitochondria is investigated for the first time and sheds new light in functioning of this versatile protein as nuclear gene transcription regulator as well as mitochondrial factor. The data do not allow to asses a mitochondrial function for Gcn5 and its role remains to be established. On the basis of the DAPI results we may envisage a new function in the mt DNA maintenance.

P3.7 - Role of the Aurora-A kinase and its regulator TPX2 in control of spindle orientation in human cells

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Mitotic spindle organization and function are essential for proper execution of mitosis, preventing the generation of aneuploid cells that may be prone to transformation. The Aurora-A kinase (Aurk-A) is a key regulator of spindle assembly; it is overexpressed in tumours together with its major regulator TPX2 and their involvement in tumorigenesis is under active investigation. We previously characterised a role of Aurk-A in spindle orientation in human cells, through phosphorylation of the NuMA protein.

We now show that formation of the Aurk-A/TPX2 complex is required for correct spindle orientation. In addition, we observe that chromosome mis-segregation induced by Aurk-A inhibition is exacerbated in cells undergoing misoriented cell division. Independently interfering with spindle orientation, through inactivation of the orientation factor LGN, yields telophase chromosome bridges and micronuclei, and a subsequent G2 arrest in p53-proficient cells. These results indicate that activity of the Aurk-A/TPX2 complex is crucial for spindle orientation, and support the hypothesis of a link between spindle mis-orientation and chromosomal instability, of potential relevance in cancer.

P3.8 - Role of M2 muscarinic receptor in epithelial ovarian carcinoma (EOC)

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Epithelial ovarian cancer (EOC) is one of the most common female cancer. EOC arises from the ovarian surface epithelium (OSE) which undergoes a process of injury-repair at each ovulation. In the ovary, a non-neuronal cholinergic system is present, and acetylcholine (ACh) is produced by granulosa cells and luteal cells. We have characterized the expression of muscarinic receptors in two human ovarian carcinoma cell lines (SKOV-3, TOV21-G) and in one immortalized ovarian surface epithelium cell line (i-120). We observed a lower expression of M2 muscarinic receptor subtype in cancer cells, compared to i-120. We investigated its role in the control of cell growth and survival. Treatment with arecaidine propargyl ester hydrobromide (APE), a selective M2 agonist, cell number decreases in a dose and time dependent manner. In the i-120 we observed high levels of cell death, which may be related to the high expression of M2 receptor. Conversely, in cancer cells, there is significantly lower cell death but the APE delays the cell cycle progression. These results suggest that M2 receptor has a negative role on cell growth/vitality, therefore, its downregulation may favor tumor progression.

P3.9 - Degree of X-Y aneuploidy in sperms of mice with altered expression of Spo11 splice variants varies with genetic background

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SPO11 protein is able to catalyze DSBs formation during meiosis. In mammals, Spo11 produces Spo11-beta and Spo11-alpha isoforms. Transgenic expressing single Spo11-beta isoform in C57-genetic-background (GB) shows defects in X-Y pairing and sterility. We generated C57/129 knock-in mice expressing SPO11-beta under Spo11-promoter (Spo11-beta-ki-only) and analysed their phenotype. Contrary to transgenic model mice, they were fertile. Their phenotype was highly variable: some males displayed a reduced testicular weight [ST], others exhibited a size similar to controls. To understand these differences, we generated "mix"-congenic mice and asked what is the effect of a specific GB (C57). We observed phenotype of "mix"-congenic mice was stable, indicating phenotype variability depends on GB variability. Shift into C57 rescued ST-phenotype. Analyses of Spo11-beta expression and activity did not reveal significant differences among Spo11-beta-ki-only models. 1% of spermatozoa from ST males were diploid for sex chromosomes. This indicates alterations of Spo11 might predispose to conception of progeny with an unbalanced sex chromosomes number. Efforts are underway to identify modifier genes.

P3.10 - The centrosomal localization of p53 is critical for spindle pole integrity in human nontransformed cells but not in cancer cells

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During mitosis, two newly formed centrosomes assemble a bipolar spindle and ensure faithful segregation of chromosomes. We recently showed that, in untransformed human cells, integrity of centrosomes in M phase and proper spindle formation require p53 mitotic centrosomal localization. On the contrary, human tumor cells can survive in the absence of p53. To investigate how cancer cells evade centrosome-associated p53 control, we will generate a

normal human cell line in which it will be possible to remove p53 in a fast, efficient, reversible and inducible way using the auxin-inducible degron system (AID) and the CRISPR-Cas9. We verified that degron tag both at N- and C-terminal does not affect p53 transcriptional activity and centrosomal localization. We also verified the AID system activity and observed that the treatment with the auxin, in the presence of its receptor, is able to degrade the p53 protein only when it is fused with the degron tag. Based on these results, we will tag endogenous p53 with degron sequence in nontrasformed human cells. This will provide a cell model to study how human tumor cells evade loss of spindle pole integrity and prevent mitotic catastrophe.

4 - Transcriptional Mechanisms and Epigenetic Modifications

O4.1 - ATM unconventional splicing modulation by glucocorticoids

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Ataxia Telangiectasia (A-T) is a rare incurable genetic disease, caused by biallelic mutations in the Ataxia Telangiectasia-Mutated (ATM) gene. Treatment with glucocorticoid analogues has been shown to improve the neurological symptoms of patients but the molecular mechanism underlying the glucocorticoid action in A-T is not yet understood. Here we report a new protein variant, named miniATM, obtained by an unconventional SDR splicing induced by dexamethasone. The functionality of this miniATM variant was ascertained and it was shown able to cooperate with the cellular machinery to rescue some cellular signaling failed in A-T cells. Interestedly the same SDR-mediated transcript, so far demonstrated *in vitro*, is present in the RNA expression pattern of patients subjected to long-term treatment with dexa delivered through autologous erythrocytes (IEDAT, EudraCT Number 2010-022315-19). Surprisingly, quantitative PCR showed higher level of miniATM transcript in responder patients. Currently, the transcript is tested as molecular marker in a phase III clinical trial (ATTeST).

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O4.2 - Strategies to inhibit PRC2 methyltransferase activity in *Arabidopsis* seedlings

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Polycomb repressive complex 2 (PRC2) represents a transcriptional epigenetic repression system, responsible of trimethylating histone H3 at lysine 27 (H3K27me3) through its catalytic subunit (EZH2). In plants PRC2 controls diverse developmental processes, from fertilization to flowering.

In *Arabidopsis*, PRC2 is involved in endosperm formation, and lack of PRC2 lead to embryonic lethality and seed abortion. This has severely hampered studies on the function of PRC2 during seed development.

Since in animals EZH2 overexpression and the consequent high level of H3K27me3 are hallmarks of several cancers, several PRC2 inhibitors have been designed. Curiously, a pharmacological approach has never been tested on plants, although it may provide a useful tool for the study of PRC2 in plants.

Taking advantage of the homology of EZH2 between animals and plants, we have assessed the efficacy of the UNC1999 inhibitor, previously reported to be highly effective in vitro, and of a new selective EZH2 inhibitor on Arabidopsis seeds.

Our results showed a reduction of H3K27me3 and a consistent increase in the expression level of PRC2 targets, thus giving new opportunities to deepen the studies on PRC2.

O4.3 - Epigenetic Marks at Satellite-Free and Satellite-Based Centromeres

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Vertebrate centromeres contain long stretches of highly repeated DNA sequences (satellite DNA). Nonetheless, exceptional centromeres, devoid of satellite DNA, exist. We previously demonstrated that the karyotypes of Equids are characterized by the presence of satellite-free and satellite-based centromeres and represent a unique biological model for the study of centromere organization and behaviour (Wade et al, Science 2009 326:865-7; Piras et al, PLoS Genet 2010 6:e1000845; Purgato et al, Chromosoma 2015 124:277-87; Nergadze et al, Genome Res 2018 28:789-99). The main epigenetic mark of centromere function is the centromeric variant of histone H3, CENP-A. Moreover, a centromere specific balance between heterochromatic and euchromatic histone modifications characterises the centromere of all eukaryotes.

Here we employed high resolution multi-colour immunofluorescence on chromatin fibres to analyse six post-translational histone modifications at satellite-free and satellite-based horse and donkey centromeres. The results were compared with ChIP-seq data. This study has important implications in understanding the molecular bases of mammalian centromere function.

O4.4 - How oxidative stress affects telomere structure and telomeric epigenetic modifications?

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Due to their high content in guanine residues and their low repair efficiency, telomere represents the preferential target of oxidative damage. We previously demonstrated that acute hydrogen peroxide treatment induced persistent 8-oxoG at telomere. In order to understand the mechanisms by which oxidative stress compromised telomere length and integrity in human primary fibroblasts, we performed different analysis to study telomeric protein, telomere damage and replication. Results obtained by ChIP showed a significant reduction of telomeric binding protein, TRF1 and TRF2, at telomere 48hrs after treatment; furthermore, a higher frequency of γH2AX-TIFs respect to 53BP1-TIFs allowed us to suppose telomeric replication fork arrest rather than a DSB. This hypothesis was also confirmed by CO-FISH analysis, that reported a significant increase of unreplicating telomeres in treated cells compared to the control. All these findings lead us to state that 8-oxoG has a central role in telomere shortening, the binding of telomeric proteins and replication fork arrest that in turn induce telomere dysfunction, responsible of oxidative stress-induced chromosome instability, previously reported.

P4.1 - Che-1 is targeted by c-Myc to sustain proliferation in pre-B-cell acute lymphoblastic leukemia

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Despite progress in treating B-cell precursor acute lymphoblastic leukemia (BCP-ALL), disease recurrence remains the main cause of treatment failure. New strategies to improve therapeutic outcomes are needed, particularly in high-risk relapsed patients. Che-1/AATF (Che-1) is an RNA polymerase II-binding protein involved in proliferation and tumor survival, but its role in hematological malignancies has not been clarified. Here, we show that Che-1 is overexpressed in pediatric BCP-ALL during disease onset and at relapse, and that its depletion inhibits the proliferation of BCP-ALL cells. Furthermore, we report that c-Myc regulates Che-1 expression by direct binding to its promoter and describe a strict correlation between Che-1 expression and c-Myc expression. RNA-seq analyses upon Che-1 or c-Myc depletion reveal a strong overlap of the respective controlled pathways. Genomewide ChIP-seq experiments suggest that Che-1 acts as a downstream effector of c-Myc. These results identify the pivotal role of Che-1 in the control of BCP-ALL proliferation and present the protein as a possible therapeutic target in children with relapsed BCP-ALL.

P4.2 - Top1p mediates Fob1p/Sir2p interaction and contributes to rDNA silencing

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S.cerevisiae rDNA locus consists of a 9.1 Kb unit repeated in tandem about 150 times. These sequences are interrupted by non transcribed spacers (NTS1 and NTS2) containing two cryptic promoters, E-PRO and C-PRO, whose transcription of non coding RNA is normally repressed, but not in absence of Sir2 deacetylase. Hyperacetylation and loss of silencing at rDNA are also shown in DNA topoisomerase I (Top1p) mutants and it has been demonstrated that Top1p is able to recruit Sir2p at this locus.

Top1p nucleolar localization is partially due to Nsr1p, whose absence determines a Top1p amount reduction of about 30% at rDNA.

Moreover it has been reported that Top1p loses its capability to cleave rDNA at replication fork block sequences (RFB) in absence of Fob1p, a protein that, upon RFB binding, blocks the replication fork which proceeds in the opposite direction compared to 35S transcription.

In order to understand if Fob1p plays a role in Top1p recruitment at RFB or in its cleavage activity, we have analyzed fob1Δ cells and we have concluded that Fob1p, by recruiting Top1p, also contributes to Sir2p recruitment at rDNA, thus determining rDNA silencing and preventing unequal recombination.

P4.3 - 3D-FISH and Hi-C to study the organization in the cell nucleus of the 7q36.3 chromosomal region, frequently rearranged in leukemic cells

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In addition to 3D-FISH, the recently developed biochemical procedure Hi-C has been used to look at long-range chromatin interaction and three dimensional organisation of the genome in the cell nucleus, providing higher-resolution than 3D-FISH. We analysed the organization of genes localized in the telomeric band 7q36.3 by high resolution Hi-C method in several human and mouse cell lines, including the leukemia derived cell line K562, that was also studied using 3D-FISH. The latter method enabled us to highlight the nuclear repositioning of the telomeric region 7q36.3 in the K562 cells, possibly related to the ectopic expression of the *HLXB9*/*MNX1* gene. However, the change in nuclear location was not clearly identifiable by Hi-C data in this cell line. Thus, 3D-FISH and Hi-C are approaches that complement each other and can inform on genome organisation at different levels. The information derived from the application of these methods will allow a better investigation of the organization in the nucleus of specific chromosomal regions in different situations, including cellular differentiation and genetic diseases.

P4.4 - Ignoring the fats: *Tm7sf2* gene disruption impairs *in vitro* adipocyte differentiation of mouse embryonic fibroblasts

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Tm7sf2 gene, encoding for NET47 protein, has been primarily associated to cholesterol biosynthesis due to its sterol C14-reductase activity. In HT1080 fibroblasts, the overexpression of NET47 modulates the expression of the master regulator of adipogenesis PPARγ, suggesting additional unknown functions for this protein. We subjected fibroblasts isolated from wild type and *Tm7sf2-KO* mouse embryos to *in vitro* differentiation into adipocytes. *Tm7sf2* mRNA expression was upregulated in wild type cells during adipogenesis, whereas *Tm7sf2*-KO cells showed impaired differentiation in terms of lipid droplets formation and adipogenic genes expression. Moreover, the lack of *Tm7sf2* gene blocked the initial mitotic clonal expansion and reduced the expression of the early stage marker C/ebpβ and of the terminal differentiation regulators C/ebpα and Pparγ. Interestingly, Pparγ was retained at the nuclear rim during differentiation of *Tm7sf2*-KO cells, representing an additional layer in the regulation of its activity. All these data underline a yet undiscovered role for *Tm7sf2* gene in adipose tissue development and could expand the understanding of the molecular basis of adipocyte differentiation.

P4.5 - Che-1/AATF-induced transcriptionally active chromatin promotes cell growth in Multiple Myeloma

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In recent years, several exciting discoveries have shown that aberrant epigenetic modifications play a major role in the genesis and progression of cancer. Multiple myeloma (MM) is a cancerous pathology resulting from a clonal expansion of plasma cells, characterized by abnormal production and secretion of monoclonal antibody proteins. Here we demonstrate that the RNA Polymerase II (Pol II) binding protein, Che-1 is required for MM cell growth by sustaining genome wide transcription and recruitment of Pol II to the DNA. Notably, we found that Che-1 localizes on active chromatin and that its depletion leads to accumulation of heterochromatin by a global decrease of histone acetylation. Strikingly, transgenic mice expressing human Che-1 in plasma cells develop MM with clinical features resembling those observed in the human disease. Moreover, Che-1 downregulation decreases BRD4 chromatin accumulation to further sensitize MM cells to bromodomain and extra-terminal (BET) inhibitors. In summary, our findings identify Che-1 as a key player for maintaining open chromatin required for sustaining MM growth.

P4.6 - Ubiquitin protease Ubp8 is necessary for *S. cerevisiae* respiration

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Mitochondrion is the energy centre of the cell and it is necessary for respiration. In normal condition, mitochondria form a dynamic network regulated by opposing fusion and fission events. Quality control of mitochondrial network is due to Ubiquitin–Proteasome system that regulates both mitophagy and single protein degradation. SAGA complex is a transcriptional co-activator with two catalytic domains: the deubiquitination module with DUB Ubp8 and the acetylation domain with HAT Gcn5. Previously, we demonstrated that SAGA complex and Gcn5 are required for budding yeast respiration and that HDAC Hda1 counteracts Gcn5 activity. In this work, we showed that DUB Ubp8 is necessary for respiration and its expression levels are upregulated in respiratory condition. We demonstrated that the E3 Ub-ligase Psh1 counteracts Ubp8 and its deletion is able to rescue defective respiratory phenotype of strain deleted in Ubp8 gene. Interestingly, we found for the first time that Ubp8 localizes in both nucleus and mitochondria highlighting a double function linked to cell respiration. Moreover, our results suggest a direct interplay between acetylation and ubiquitination in metabolic regulation.

P4.7 - *Drosophila melanogaster* as a model to study *in vivo* the functional role of Transposable Elements in Huntington's disease pathogenesis

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Huntington's disease (HD) is a late-onset disorder characterized by progressive motor dysfunction, cognitive decline and psychiatric disturbances. The disease is caused by a CAG repeat expansion in the IT15 gene, which elongates a stretch of polyglutamine at the

aminoterminus of the huntingtin protein. Despite the impressive data that have been accumulated on the molecular basis of neurodegeneration, no cure is still available. It is therefore important to keep investigating potential previously unnoticed pathways that may be altered in HD and target of therapeutic treatments.

Transposable elements (TE) are mobile genetic elements that constitute a large fraction of eukaryotic genomes. Retrotransposons replicate through an RNA intermediate and represent approximately 40% and 30% of the human and *Drosophila* genomes. Mounting evidences suggest mammalian L1 elements are normally active during neurogenesis. Interestingly, recent reports show that unregulated activation of TE is associated with neuropathology.

Our experimental results obtained in *Drosophila* HD model, suggest that TE activation may represent an important piece in the complicated puzzle of polyQ-induced neurotoxicity.

P4.8 - Genome-wide RNA editing analysis in human neurological disorders

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RNA editing is a widespread co/post-transcriptional mechanism that modifies RNA sequences by insertions, deletions or base conversions. The most common RNA editing event in humans include the deamination of Adenosine (A) in Inosine (I) catalysed by ADAR enzymes. Inosine is recognized as Guanosine by cellular machineries and is abundant in the brain, mostly in untranslated mRNA regions. RNA editing deregulation has been linked to several psychiatric, neurological and neurodegenerative diseases. To better understand the correlation between RNA editing and neurological pathologies, we present here the analysis of more than 830 RNA-Seq experiments from different SRA BioProjects related to Alzheimer, Parkinson, Amyotrophic lateral sclerosis, bipolar disorder, autism and major depression.

Preliminary results based on AEI and REI metrics suggests that RNA editing is dysregulated in neurological disorders and especially in neurodegenerative diseases. Further analyses to identify common dysregulated RNA editing signatures in human neurological pathologies are ongoing, hoping to provide novel insights into the challenging search of biomarker candidates for the design of innovative drugs.

P4.9 - Constitutional methylation of *BRCA1* gene as breast cancer risk factor

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Methylation of CpG islands in promoter region of genes is an epigenetic modification that

causes silencing of genes and might be associated with cancer risk if it is present in peripheral blood. It has been shown that constitutional methylation of *BRCA1* promoter correlates with breast cancer risk, especially with triple-negative tumors.

In this study we evaluated breast cancer risk depending on *BRCA1* methylation in peripheral blood and assessed correlation with clinical features of tumors. We examined three groups of women: 519 unselected breast cancer cases, 500 triple-negative breast cancer cases and 500 healthy controls. All women were negative for 13 common Polish *BRCA1* germline mutations. Moreover, 274 FFPE tumor tissues from our cases were tested to estimate association between constitutional and somatic *BRCA1* promoter methylation. Methylation status was assessed using methylation-sensitive high-resolution melting (MS-HRM).

The results show that *BRCA1* methylation detected in peripheral blood is strongly associated with the risk of TNBC (OR 5.26, $p<0.001$), correlates with methylation in paired tumors, and is significantly associated with tumor type and size.

P4.10 - Dexamethasone effects on HDAC4 in A-T cell lines

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Ataxia Telangiectasia (A-T) is a rare and incurable hereditary syndrome, caused by biallelic mutations in the Ataxia Telangiectasia-Mutated (ATM) gene, which codifies for a protein kinase mainly involved in DNA damage response. Treatment with glucocorticoid analogues has been shown to improve the neurological symptoms of patients but the involved molecular mechanism remains unknown.

Since ATM deficiency causes nuclear accumulation of dephosphorylated histone deacetylase 4 (HDAC4) in neurons of A-T patients and promotes neurodegeneration, we investigated the effects of dexamethasone (dexa) on HDAC4 in A-T cell lines. Preliminary results indicate that dexa treatment is able to increase HDAC4 nuclear accumulation, in spite of its phosphorylation status by disulfide bridges reduction. Probably the observed accumulation leads to the increased interaction between HDAC4 and HIF1-α modulating some downstream genes specifically in A-T cell lines.

These results indicate a direct activity of HDAC4, apart its epigenetic role, in A-T cell lines triggered by dexa treatment.

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5 - Oncogenes and Tumor Suppressors

O5.1 - HMGA1/E2F1 axis and NFkB pathways regulate LPS progression and trabectedin resistance

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Although the evolvement of sarcoma treatment, many patients develop recurrence suggesting the need to identify novel therapeutic targets. To this end, we found that HMGA1 is involved in the progression of dedifferentiated and myxoid liposarcoma. The immunohistochemical and RT-PCR analyses of 68 liposarcomas revealed high expression of HMGA1 in myxoid and dedifferentiated liposarcomas compared to differentiated subtypes. HMGA1 depletion and overexpression experiments showed the contribution of HMGA1 to cell proliferation, motility, invasion and drug resistance in dedifferentiated and myxoid liposarcoma cells. The *in vitro* treatment of myxoid liposarcoma with trabectedin, a potent anti-tumoral drug, caused the down-regulation of HMGA1/E2F1axis-regulated mesenchymal markers vimentin and ZEB1. These data were confirmed in patients' biopsies. Furthermore, trabectedin inhibits *in vitro* NFkB pathway in mixoyd liposarcoma sensitive but not resistant cells, and the inhibition of NFkB pathway re-sensitizes the resistant cells to trabectedin. These data support the rational for combining NFkB inhibitors and trabectedin in trabectedin-resistant liposarcoma patients.

O5.2 - Impact of the inhibition of the mitochondrial Serine hydroxymethyltransferase enzyme in mitochondrial respiration and cancer cell growth

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Cancer cells adapt their metabolism in order to support enhanced proliferation and survival. In these cells, mitochondrial folate enzymes are strongly upregulated e.g. serine hydroxymethyltransferase (SHMT; EC:2.1.2.1). In the human, two isoforms of SHMT are found: the cytoplasmic SHMT1 and the mitochondrial isozyme (SHMT2). Its physiological function is to catalyze the conversion of serine and tetrahydrofolate (THF) to glycine and methylene-THF.

When serine cleavage is catalyzed, this reaction generates single carbon units that are required for biosynthesis of purine, thymidine, antioxidant and tRNA.

Our group has been interested in the effect of SHMT inhibitors such as a pyrazolopyran compound, namely 2.12 [1].

Since growing evidences support the leading role of the mitochondrial SHMT2 to catabolize serine, we are currently studying the effect of such inhibitors of SHMT2 by following biochemical properties, cancer cell proliferation and mitochondria respiration, also using selectively

modified cells (knockout for SHMT2). Our results show that our compounds are more effective against serine catabolism, and can be useful tools to monitor selectively the SHMT activity in mitochondria.

O5.3 - Secreted YB-1 regulates NFkB signaling pathway in receiving cells

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The prototype cold-shock Y-box binding protein 1 (YB-1) has recently been identified as a key orchestrator of kidney inflammation and fibrosis. YB-1 regulates cell proliferation and is considered a *bonafide* oncogene. The plethora of functions assigned to YB-1 is strictly dependent on its subcellular localization. In resting cells, YB-1 is mainly cytoplasmic and regulates translation. Under cellular stress, YB-1 contributes to the formation of cytoplasmic stress granules (SGs). Upon DNA damage, YB-1 translocates to the nucleus and participates in DNA repair mechanisms. It has recently been discovered that YB-1 can be secreted and functions as an extracellular mitogen. Here, we have analyzed YB-1 subcellular localization in HEK293T cells, under normal and stress conditions. We found that in response to oxidative insults, the assembly of YB-1 in SGs was accompanied by a significant increase of YB-1 protein secretion. Treatment with extracellular YB-1 induces expression of pro-inflammatory genes and activates NF-kB signaling in receiving cells. Collectively, our data suggest a role for secreted YB-1 as a paracrine signal orchestrating oxidative stress-induced inflammatory response.

O5.4 - Regulation of ovarian cancer progression by IQGAP1 through endothelin-1 receptor signalling

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The invasive phenotype of serous ovarian cancer (SOC) cells is linked to the formation of actin-based protrusions, invadopodia, operating extracellular matrix (ECM) degradation and cell invasion. Growth factor receptors cause engagement of integrin-related proteins, like IQGAP1, to a F-actin core forming an "adhesion ring" needed for invadopodia function. Here, we investigated whether IQGAP1 and endothelin-1 (ET-1)/β-arrestin1 (β-arr1) axis could act as signal-integrating node for adhesion components, cytoskeletal remodelling and ECM degradation. In SOC cells, ET-1 receptor (ET-1R) activation affects IQGAP1 expression and localization and IQGAP1/β-arr1 binding promoting the regulation of invadopodia-related pathways, such as Rho GTPase. Disrupting IQGAP1/β-arr1 interaction prevents the number of invadopodia and aggressive features. In vivo, targeting ET-1R/β-arr1 signalling controls SOC metastatic dissemination, associated with reduced expression of IQGAP1. These data establish the ET-1R-driven β-arr1/IQGAP1 interaction as a prerequisite for the dynamic functions and integration of adhesive and proteolytic pathways in fostering invadopodia and metastatic process in SOC.

O5.5 - The p53 Mitotic Centrosomal Localization Contributes to Mitotic Surveillance Pathway

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Centrosome aberrations contribute to chromosomal instability (CIN). The p53 tumor suppressor localizes at the centrosomes at each mitosis–p53 mitotic centrosomal localization (p53-MCL)– in ATM-dependent manner. Here, we show that in non-transformed human cells, acute inhibition of p53-MCL causes centrosome fragmentation and mitotic catastrophe. In contrast, tumor cells tolerate low levels of p53-MCL and its inactivation has no effect on centrosome integrity and cell survival. Mechanistically, p53-MCL contributes to the mitotic surveillance pathway that sense centrosome-loss and signals to cell cycle arrest through non-canonical p53 activation. We define that centrosome-loss, leaving human p53 orphan of its mitotic centrosome localization, promotes the formation of discrete foci of Ser15-phosphorylated p53 that, by recruiting 53BP1 triggers its own further activation and subsequent cell cycle arrest.

P5.1 - The natural sesquiterpene β-caryophyllene acts as an enviromental decontaminant through the interference with the STAT3 signalling pathway

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Enviromental pollution represents a very important issue for human health and poses multiple challenges for the management of toxicity pollutants. Thus, characterizing pollutants molecular mechanisms is an urgent need for an effective decontamination and for reducing their impact on human health. We investigated the involvement of STAT3 pathway in the pro-cancerogenic effects of cigarette smoke and β-hexachlorocyclohexane (β-HCH). Furthermore, the possible role of the sesquiterpene β- caryophyllene *(CRY)* as an environmental decontaminant has been assessed. The activation of STAT3 signalling pathway triggered by β-HCH (10 μM) and CSC (75 μg/ml) and the ability of CRY to interfere with this process were investigated in MDA-MB-468 breast cancer cells, performing western blot, proliferation and migration assay and qRT-PCR.

Preliminary results allow us to hypothesize that CRY produces antiproliferative effects by inhibiting the CSC and β-HCH-induced STAT3-phosphorylation.

These results support the possible role of CRY as chemopreventive agent and suggest further studies to validate the idea of using this natural compound as an environmental decontaminant.

P5.2 - Gene expression profiles in genome instabllity-based classes of colorectal cancer

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Broad copy number aberrations (BCNAs) represent a common form of genome instability in colorectal cancer (CRC). CRCs show large variations in their level of aneuploidy: microsatellite-instable (MSI) tumors are known to have a near-diploid karyotype while microsatellite-stable (MSS) tumors show high level of chromosomal instability. However, MSS tumors have great heterogeneity in the number of BCNAs, with a minor percentage of samples showing an almost normal karyotype. In the present work we subdivided MSS CRCs according to the number of BCNAs in low-BCNA (LB) or high-BCNA (HB) and characterized their transcriptome profiles. LB tumors were enriched for mucinous CRCs and their gene-expression profile resembled that of MSI samples for what concerns a subset of genes involved in secretory processes, mucosal protection, and extracellular matrix remodeling. HB tumors were predominantly non-mucinous adenocarcinomas and showed overexpression of a subset of genes typical of surface colonocytes and EGF signaling. A classification of colorectal tumors based on the number of BCNAs identifies two groups of MSS tumors which differ for histopathology and gene expression profile.

P5.3 - Can the mitotic centrosomal localization of p53 define ATM variants of uncertain significance (VUS)?

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The discovery of genetic variants is deeply accelerating and contributing to the definition of several rare genetic and multifactorial diseases, such as neoplasms. High throughput sequencing, as Next Generation Sequencing, although specific, is enormously increasing the number of genetic data coming from patients. Many mutations often remain orphan of definition and classification, leading to a misinterpretation of their association within specific pathologies (variants of uncertain significance-VUS). Therefore it is imperative to find easily applicable tests able to functionally and clinically categorize VUS.

Our group recently developed a diagnostic test able to determine the absence or presence of pathogenic *ATM* gene mutations in PBMC or LCL, by analyzing the localization of p53 at centrosomes during mitosis (p53-MCL). Preliminary data showed that ATM VUS-carriers can behave differently, meaning these variants can have different pathological significance. ATM, indeed, is considered an intermediate cancer risk factor for breast cancer and an important marker of patients' sensitivity to radiotherapy, highlighting the importance of this functional test within clinical practice.

P5.4 - A positive feed-forward regulatory loop between METTL3 and WTAP sustains the oncogenic role of the m6A methylation complex in myeloid leukemia

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The Wilms tumor 1 (WT1)-associated protein (WTAP) is upregulated in many tumours, including, acute myeloid leukemia (AML), where it plays an oncogenic role by interacting with different proteins involved in RNA processing and cell proliferation. In addition, WTAP is also a regulator of the nuclear complex required for the deposition of N6-Methyladenosine (m6A) into mRNAs, containing the METTL3 methyltransferase. However, it is not clear if WTAP may have m6A-independent regulatory functions that might contribute to its oncogenic role. Here, we show that both knockdown and overexpression of METTL3 protein results in WTAP protein upregulation, indicating that METTL3 levels are critical for WTAP protein homeostasis. However, we show that WTAP upregulation is not sufficient to promote cell proliferation in the absence of a functional METTL3. Our results indicate the existence of a positive feed-forward regulatory loop, where METTL3 upregulates WTAP, which is relevant to increase WTAP expression concomitantly to the METTL3/METTL14 core m6A methylation complex and sustain the oncogenic role reported for the m6A modification complex in leukemia.

P5.5 - PI3K role in the c-Met-mediated migrating and invading behavior of NT2D1 non-seminoma cells

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The HGF/c-MET system is de-regulated in several human cancers, even if really few data are available on its expression and role in Testicular Germ Cell Tumors (TGCTs). Recently, we demonstrated that c-MET is expressed in three different cell lines representative of seminoma and non-seminoma TGCTs (Oncotarget, under review). However, NT2D1, a non-seminoma cell line, revealed the greatest response to HGF treatment in terms of cell proliferation, migration and invasion. In this study we have evaluated the role of PI3K in the HGF-dependent migration and invasion responses of NT2D1. To this aim, we stimulated with HGF the NT2D1 cells cultured with or without the PI3K inhibitor LY294002. Our results demonstrate that PI3K is involved in HGF-dependent NT2D1 migration and invasion, since PI3K inhibition abrogates the response to HGF. However, it is worth mentioning that the invasive capability of NT2D1 significantly increases administering the LY294002 alone, suggesting that PI3K adaptor protein, in this cell line, has also an anti-invasive role when recruited in c-Met-independent pathways. Further investigation is needed to understand the complex role of PI3K in NT2D1 invasive behavior.

P5.6 - In lung cancer, oncogenic YAP/TAZ lead to the inhibition of oncosuppressive TGFBR2 through transcriptional and post-transcriptional mechanisms

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Lung cancer is the leading cause of cancer death worldwide and YAP/TAZ are pro-oncogenic in this context. Numerous studies characterized YAP/TAZ target genes, while scarce evidence exists on YAP/TAZ regulated miRNAs. Among a list of miRNAs regulated by YAP/TAZ in NSCLC, we focused on the oncogenic miR-106b-25 cluster hosted in the *MCM7* gene. We found that YAP/TAZ positively regulate MCM7 and hosted miRs, thereby promoting cell proliferation through the post-transcriptional inhibition of several oncosuppressive targets, among which p21 and TGFBR2. In NSCLC, TGFBR2 is oncosuppressive and maintained at low levels by YAP/TAZ. Interestingly, this occurs through a post-transcriptional mechanism mediated by miR-106b-25, and through a transcriptional mechanism mediated by the EZH2 repressor, that we identified as a novel YAP/TAZ target. In lung TCGA, YAP and EZH2 are not prognostic alone, while resulting strongly prognostic together, suggesting a possible cooperation. The present work provides novel evidence supporting the pro-tumorigenic functions of YAP/TAZ. Moreover, we shed novel light on the possible YAP/TAZ-TGFβ-EZH2 crosstalk and their possible therapeutic targeting in lung cancer.

P5.7 - hMENA regulates the cross-talk between tumor cells and cancer associated fibroblasts *via* Gas6/Axl axis

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Cancer-associated fibroblasts (CAFs) play a critical role in the complexity of tumor-stroma interaction, affecting tumorigenesis and therapeutic responses. Understanding CAF functional heterogeneity and how it influences tumor development and drug resistance is crucial for the development of new therapies. We demonstrated that hMENA, an actin-regulatory protein, and its tissue-specific isoforms influence signaling pathways involved in cancer cell invasion and epithelial mesenchymal transition. Here, we define a novel function of hMENA in CAF activation and in the regulation of tumour/stroma cross-talk, *via* the modulation of Gas6-Axl signaling. We demonstrated that hMENA/hMENADv6 identify a subset of pro-tumoral CAFs. CAFs over-expressing hMENADv6 secrete the Axl ligand Gas6, favoring the invasiveness of Axl-expressing NSCLC and PDAC cells. Notably, we demonstrated that hMENA/hMENADv6 regulate Axl expression in tumor cells, sustaining the Gas6-Axl axis. Furthermore, a high hMENA/Gas6/Axl gene expression signature is associated with poor prognosis in PDAC patients indicating that the network-based on hMENA/Gas6/Axl expression may represent novel prognostic and therapeutic targets.

P5.8 - Role of Aurora-A kinase/TPX2 complex in control of chromosome stability and cell trasformation in human cells

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The Aurora-A (AurkA) kinase and its major activator TPX2 are key regulators of mitosis. AurkA and TPX2 co-overexpression occurs in tumors and we proposed the AurkA/TPX2 complex as an oncogenic unit. Known functions of AurkA and TPX2 suggest that their role in oncogenic transformation involves chromosomal instability and/or deregulation of the p53 response. To investigate these possibilities we generated non-transformed cell lines overexpressing them, either alone or in combination.

We show that overexpression of the whole AurkA/TPX2 complex yields strong defects in chromosome segregation and cell division. On the other hand, excess TPX2 interferes with correct nuclear reconstitution at mitotic exit, in an AurkA-independent manner. We are currently assessing the p53 response in the aberrant daughter cells -and hence their fate - under these conditions. Finally, we unexpectedly observed that excess TPX2 yields accumulation of overexpressed AurkA in interphase nuclei, a condition that has been reported as highly oncogenic. Results are therefore contributing to clarify the oncogenic potential of the AurkA/TPX2 complex.

P5.9 - Ribosome-free L3 regulates cell proliferation independently of p53 by targeting E2F and inhibiting its function

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Our previous data demonstrated that nucleolar stress induced by anticancer drugs in colon cancer cells devoid of p53 leads to the activation of ribosomal protein L3 as inhibitor of cell

cycle. In particular, nucleolar stress induces L3 expression and promote its nucleolar exit. To understand the molecular mechanism underlying this effect, HCT 116p53-/- cells were treated with Actinomycin D to induce nucleolar stress and with cycloheximimide to block NMD pathway. Results showed that the delocalization of L3 during nucleolar stress was associated with an increase alternative L3 pre-mRNA, substrate of the NMD.

Next, we quantified known cell cycle-associated E2F target genes as CycD1, CycE1 and CDK1 by quantitative RT-PCR in HCT 116p53-/- and L3ΔHCT 116p53-/- cells, in which L3 is stably silenced. We detected a significant up-regulation of E2F target genes associated with L3 knockdown. Results from reporter gene assays in presence and in absence of L3 upon drug-induced nucleolar stress demonstrated that L3 acts as positive regulator of E2F transcriptional activity.

In conclusion, L3 regulates cell proliferation independently of p53 by targeting E2F and inhibiting its function.

P5.10 - Nucleolar variant of ErbB3 regulates RNA polymerase I activity

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The nucleolus is the primary site of the transcription of the ribosomal genes. Rapid growth and division of cells, including tumor cells, is correlated with intensive protein biosynthesis. We have identified a short variant of ErbB3 that localizes in the nucleolus of several cell lines, and, in this compartment it interacts directly with UBF a major transcriptional factor that modulates RNA Pol I activity. Interestingly the presence of this short variant in the nucleolus is inversed correlated with the level of pre-rRNA.

The localization of ErbB3 in the cell is under neuregulin control. By performing experiments of immunoprecipitation with anti ErbB3 antibodies, following by immunoblotting we have demonstrated that the interaction between the ErbB3 variant and UBF depends by neuregulin stimulation.

ErbB3 receptor represent an emerging cancer target because it plays an important role in drug resistance therapies: interfere with the function of this ErbB3 nucleolar variant could represent an innovative approach to counteract cancer progression.

P5.11 - FAK pharmacological inhibition disrupts hepatocellular carcinoma growth and interferes with liver cancer stem cells markers

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Introduction: Heterogeneous nature of hepatocellular carcinoma (HCC) may depend on different hepatic cells, among which liver cancer stem cell (LCSCs). Sorafenib, a multikinase inhibitor, that is the only approved systemic therapy, may cause resistance. We recently unveil a novel unprecedented role of focal adhesion kinase (FAK) in HCC growth.

Aim: We pointed to explore the effect of treatment of HCC cell lines with different pharmacological inhibitors of FAK on tumour growth and on the stemness markers of LCSCs.

Materials and Methods: FAK depletion was obtained with stable silencing or pharmacological inhibition in HCC cells.

Results: Inhibition of FAK with drugs reduce the viability and growth rate of HCC cells in a dose dependent manner. Moreover, our preliminary data demonstrated that different HCC cell lines (HepG2, Huh7 and Hep3B) displayed a differential expression pattern of ESC markers compared to primary human hepatocytes (PHH). We found that FAK silencing or pharmacological

inhibition reduced the expression of NANOG, OCT-4 and PSMD10.

Conclusions: Our results demonstrate that FAK depletion could alter the HCC progression may affecting the expression of several LCSC markers.

P5.12 - Role of rpL3 in the translational reprogramming of colon cancer cells resistant to 5-FU

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In the last years, a lot of evidences have been accumulated suggesting that translational reprogramming plays a key role in tumor initiation, invasion and multidrug resistance (MDR). Translational reprogramming is associated to alteration in eukaryotic translation initiation factors (eIFs) activity, in particular the overexpression of the eIF4F complex, is associated with chemoresistance in melanoma, colon and thyroid cancer cell lines. In our previously study, we have analyzed the role of human ribosomal protein rpL3 in MDR. Our studies revealed that rpL3 is a key determinant in cellular stress responce and in MDR in HCT $116^{p53-/-}$ cells. Here we report results from GST pull-down and mass spectrometry demonstrating that eiF4A, a component of eIF4F complex, is able to interact with rpL3 *in vitro*. Immunoprecipitation experiments confirm the presence of a complex rpL3/eiF4A/eiF4G in HCT $116^{p53-/-}$ cells treated with 5-FU. The translation status of known mRNAs regulated by eIF4A (i.e. BCL2-family, Cyclins and c-Myc mRNAs) has been analyzed by qPCR in 5-FU resistant HCT $116^{p53-/-}$ cells and in L3DHCT $116^{p53-/-}$ cells. Results from these experiments will be presented.

P5.13 - The pleiotropic roles of the human RNASET2 tumor suppressor gene

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The *RNASET2* gene encodes for a highly conserved extracellular ribonuclease which has recently been shown to carry out a marked oncosuppressive role by means of modification of the tumor microenvironment. In particular, focusing on two independent ovarian cancer cell models, we recently reported that RNASET2-mediated tumor suppression *in vivo* involves the recruitment toward the tumor mass of cancer-suppressive innate immune cells belonging to the monocyte/macrophage lineage.

Given these premises, we extended our experiments to analyze the effect of RNASET2 on the polarization of human macrophages *in vitro*. Furthermore, since all previous *in vivo* experiments have been conducted using nude mice, we started developing a syngeneic mouse model in order to study the role of this gene in an immunocompetent model.

Finally, we recently demonstrated that RNASET2 also behaves as a stress response gene and strongly affects the actin cytoskeleton. This prompted us to postulate a role for RNASET2 as a "moonlighting" protein. To shed more lights on its pleiotropic functions, we also started to investigate the role of this gene in the context of mammary tumorigenesis and morphogenesis.

P5.14 - Positive caricature transcriptomic effects associated with broad copy number aberrations in colorectal cancer

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We re-examined the correlation between Broad Copy-Number Abnormal regions (BCNAs), distinguished in gain- and loss-type, and transcriptomic profiles in colorectal cancer (CRC). Transcripts are classified as "OverT" or "UnderT" if overexpressed or underexpressed comparing CRCs bearing a specific BCNA to CRCs not bearing it and as "UpT" or "DownT" if upregulated or downregulated in cancer compared to normal tissue. BCNA-gene dosage effects were evaluated by changes in the "Chromosomal Distribution Index" (CDI) of different transcript classes. Data show that UpT are more sensitive than DownT to BCNA-dosage effects. Over-UpT genes are upregulated in cancer and further overexpressed by gene dosage, defining the so called "*positive caricature transcriptomic effect*". When Over-UpT genes are ranked according to overexpression, top positions are occupied by genes implicated at the functional and therapeutic level in CRC. We show that cancer-upregulated transcripts are sensitive markers of BCNA-dosage effects and propose that analysis of positive caricature transcriptomic effects can provide clues toward the identification of BCNA-associated cancer driver genes.

6 - Photosynthesis, Metabolism and Environmental Stress

O6.1 - Strigolactones and abiotic stress in plants: modulation of abscisic acid transport

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Given their role in regulating leaf transpiration and stomata conductance, strigolactones (SL) have emerged as new class of hormones acting in concert with abscisic acid (ABA) to modulate plant tolerance in response to water deprivation.

In order to dissect the molecular mechanisms underlying the cross talk between these phytohormones, we evaluated the effect of SL treatment on the localization and abundance of ATP BINDING CASETTE G25 (ABCG25), a well-studied ABA exporter in *Arabidopsis thaliana* (At). At seedlings expressing a sGFP:ABCG25 construct were subjected to several hormonal and drug treatments, and the localization and the accumulation of GFP was then monitored by confocal microscopy in different subcellular compartments in root tips. The same construct was also inserted in SL-insensitive and SL-deficient At mutants to investigate the dependency of ABCG25 spatial regulation by SL and abiotic stress treatments from known genetic components of the SL pathway. We show that fine tuning the localization and recycling of this transporter by SL might provide a possible new mechanism of ABA homeostasis regulation during the SL-ABA cross-talk, which is crucial to drought tolerance.

O6.2 - Abscisic acid dynamics in leaves, shoot and roots of *Populus nigra* exposed to drought: relationships with water relations and carbohydrate status

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Abscisic acid (ABA) is the main endogenous messenger of drought. ABA synthesis is suggested to take place in the roots, from where it can be transported to the shoot. However, recent evidences show that ABA is also formed in leaves and stems. We performed a time-course analysis of *Populus nigra* responses to drought at physiological, biochemical and molecular levels to investigate 1) the daily pattern of generation of hydraulic and ABA-based chemical signals and 2) the role of ABA in carbohydrate metabolism. Our results showed close coordination of stomatal conductance, mesophyll conductance and leaf hydraulic conductance under water deficit, which likely maximized water use efficiency. The analyses of gene-expression patterns of 9-cis-epoxycarotenoid-dioxygenase and of [ABA] in all tissues confirmed a general up-regulation of ABA biosynthesis under drought. Soluble carbohydrates and ABA were significantly correlated, suggesting a putative function for ABA in carbohydrate mobilisation under water deficit. This study indicates ABA as a multifunctional signaler of drought stress eliciting tissue specific responses to coordinate whole plant acclimation to drought

O6.3 - Rearrangements in carbon metabolism under osmotic stress: from central metabolism to stress response

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Water shortage is a common and growing problem among crops. Plants typically overcome water stress accumulating compatible osmolytes. Recently, a positive correlation between starch degradation and proline biosynthesis has been highlighted in Arabidopsis plants under osmotic stress (OS). However, the connecting route between the two pathways is still putative and as such uncharacterised.

In an attempt to address the question, we identified ten T-DNA lines of *Arabidopsis thaliana* and quantified oxidative stress as marker for sensitivity to drought. Sucrose-phosphate Synthase A2 (*spsa2*), Sucrose Synthase 1 (*sus1*) and Glucan, Water Dikinase (*gwd2*) emerged as interesting and further characterized through the measuring of water loss, leaf starch, soluble sugars, cell wall, amino acids and proline in response to OS. Compared to wild-type plants, all T-DNA lines showed a strong reduction in sugar and proline concentrations, confirming an incomplete stress response that correlates well with the higher oxidative stress shown by the mutants.

Despite qPCR analyses revealed a 5-fold induction of *SPSA2* only, our results strongly support the involvement of the three enzymes in the OS response.

O6.4 - COP1 mediates light-controlled stamen growth in *Arabidopsis thaliana*

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In seedlings, COP1 (CONSTITUTIVE PHOTOMORPHOGENIC 1), a well characterized repressor of light-mediated responses, promotes hypocotyl elongation through the targeted degradation of transcription factors such as HY5, HYH, PIL1 and HFR1, which in turn repress hypocotyl growth also by downregulating the expression of AUX/IAA19, a negative regulator of auxin signaling.

Here we show that COP1 regulates stamen length. Indeed, our results indicate that a conserved regulatory module, similar to hypocotyl, could control stamen elongation. We show that *cop1* mutant stamens have a reduction in their overall growth and display higher expression of light-induced genes. Furthermore, HY5 and HYH, but not HFR1 and PIL1, regulate stamen elongation, as their respective mutants have increased stamen length. Transcriptomic and quantitative PCR analyses indicate that, similarly to seedlings, *AUX/IAA19* is downregulated in *cop1-4* stamens and upregulated in *hy5 hyh* stamens.

Taken together, our results highlight a novel role for COP1 in promoting stamen elongation and suggest that light could control stamen and hypocotyl growth through a shared set of signaling components.

O6.5 - Archeology of stress-resistance genes: a novel strategy to improve photosynthesis and productivity in crops

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Non Photochemical Quenching (NPQ) dissipates excess energy into heat to protect the photosynthetic apparatus from excess light and prevent formation of reactive oxygen species. NPQ is activated by PSBS and LHCSR proteins, in plants and algae respectively. In the

evolutionary intermediate *Physcomitrella patens*, a moss, both gene products are active.

We overexpressed *A. thaliana npq4* mutants, which lack PSBS and are therefore completely devoid of NPQ, with LHCSR from *P. patens (npq4+PpLHCSR)*. LHCSR-dependent NPQ was detected with low amplitude and increased upon extended illumination at highly stressing conditions. Under fluctuating light, the growth rate of *npq4* was strongly impaired; the growth of *npq4+PpLHCSR* was increased respect to *npq4* but slightly lower than WT implying that *PpLHCSR* was as photoprotective as PSBS in WT.

When we overexpressed *PpLHCSR* in WT its growth rate was higher respect to WT implying enhanced photoprotection.

Sustainable increase in productivity for food and fuel crops can be provided by rescuing ancient genes involved in the photoprotection during the land colonization by lower plants.

P6.1 - Salt stress mitigation in lettuce (*Lactuca sativa* L.) by the application of a new glutamic acid-based biostimulant

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Salt stress is one of the most common abiotic stress in agriculture worldwide, hence the need to develop products able to mitigate its adverse effects.

GHI_18_VHLGlu is a biostimulant based on plant-derived amino acids, 20% glutamic acid and 4% glycine betaine, developed by the Green Has Italia S.p.A.

The aim of this work was to evaluate the effects of GHI_18_VHLGlu on lettuce grown under salt stress (NaCl 100mM) in controlled conditions. GHI_18_VHLGlu was applied by fertigation 4 times (1.5mL/L) at 7-day intervals. Untreated and unstressed plants were used as controls. In general, salt stress negatively affected leaf fresh biomass and root growth. Moreover, stressed plants showed a higher content of H_2O_2 and catalase (CAT) and superoxide dismutase (SOD) activities as consequence of the oxidative damage. On the other hand, H_2O_2 content was lower in treated stressed plants, compared to untreated stressed plants, due to an enhanced ROS scavenging activity. Without stress, the application of GHI_18_VHLGlu led to a significant higher leaf fresh biomass. In conclusion, the treatment with GHI_18_VHLGlu showed a mitigating effect against salt stress in lettuce.

P6.2 - A single cystein-enriched phaseolin expressed in transplastomic tobacco plants accumulates as a biopolymer

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Recently, transformation of chloroplast genome has been used for the production of heterologous proteins. We transformed tobacco chloroplasts with two different versions of the storage protein of *Phaseolus vulgaris,* phaseolin (with or without signal peptide), in which a cysteine residue has been added to its C-terminal region. This modification allows for the formation of inter-chain disulfide bonds, as previously demonstrated in our lab. Our purpose here is to demonstrate the different ability of chloroplast compartments (stroma and thylakoids) in the formation of phaseolin polypeptides held together by disulfide bonds. We observed that the presence of the signal peptide directs phaseolin into the thylakoid compartment, where the protein is able to form disulfide bridges and high molecular weight polymers, which represent about 0.05 % of the total soluble proteins. The formation of phaseolin polymers, not detected in *P. vulgaris*, could be very interesting for industrial purposes. The chloroplast could be utilized as a platform for the production of a biopolymer that derives from an edible protein. A possible application is the production of biodegradable films.

P6.3 - Characterization of calcium uptake in pea stem and root mitochondria

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Ca^{2+} uptake and storage is pivotal for mitochondrial-cell signaling. Recent researches evidence that modulation of Ca^{2+} uptake in plant is driven by a uniporter (MCU), similar to the

animal counterpart (Wagner et al., 2016). In the light of this evidence, we performed a direct characterization of plant Ca^{2+} uptake, driven by substrate-dependent electrical potential, in plant mitochondria. Ca^{2+} uptake was evaluated by fluorimetric assays by Calcium Green 5N probe with mitochondria isolated from stems and roots of etiolated pea seedlings grown in either: i) calcareous sand or, ii) calcium-free substrate (vermiculite). In the first case, mitochondria from both root and stem showed no Ca^{2+} uptake; in the second, the uptake was observed only in the presence of electrical potential and limited in amplitude, evidencing a very low threshold level of saturation, depending on which organ mitochondria were obtained from (root > stem). The phenomenon was negatively modulated by oxidants and stimulated by Pi and reductants. These results suggest that in pea mitochondria the uptake of Ca^{2+} could be modulated by the matrix calcium level.

Wagner S et al (2016) J. Exp. Bot., 67: 3809-3829.

P6.4 - Leaf responses to water stress of four grapevine varieties grown in the Northern Italy

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Leaf physiological and morpho-anatomical responses to summer stresses were investigated in four grapevine varieties widely cultivated in the Veneto Region (North East of Italy): Glera, Garganega, Moscato giallo and Merlot. The study was conducted over two seasons (2016-2017) on potted grapevines grown in an open air environment at the CREA-VE (Conegliano, TV). In the pre-veraison period, the vines were subjected to progressive water limitation and compared to well watered plants. A clear difference in the regulation of leaf water relations and in foliar Abscisic acid (ABA) content was observed between varieties. Under water stress conditions Moscato giallo showed a water-saving behaviour and displayed the highest water use efficiency (WUEi). Significant changes in leaf inclination, stomatal density and size were observed under water stress for some varieties, suggesting that leaf morpho-antomical adaptations to water deficit could participate alongside pyhisiological ones in drought resistance in grapevine cultivars.

P6.5 - Cullin neddylation is regulated by water deprivation in *Arabidopsis thaliana*

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Cullin-RING ubiquitin ligases (CRLs) regulate different aspects of plant development as they control the degradation of many proteins through their ubiquitylation. CRLs are activated by modification of their cullin subunit with the ubiquitin-like protein NEDD8 (neddylation) and deactivated by NEDD8 removal (deneddylation). The COP9 signalosome (CSN) acts as a molecular switch of CRLs activity by reverting their neddylation status.

We have shown that the majority of cullin proteins are progressively neddylated during seed maturation and deneddylated upon seed germination. This developmentally regulated neddylation shift is absent in *csn* mutants, suggesting that CSN is required to sustain seedling development in Arabidopsis. Here we show that CSN composition is altered in dry seeds, suggesting that a defective CSN might cause the increase in CRL neddylation. Furthermore, *csn*

germinating seeds are sensitive to desiccation, and conditions mimicking water loss or inducing osmotic stress also promote cullin neddylation. This indicates that water deprivation regulates CSN activity and the CRL neddylation status which in turn regulates protein ubiquitylation and degradation.

P6.6 - Redox homeostasis in rice varieties having different sensitivity to salt stress

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Salt tolerance is a complex trait varying between and within species. Alteration in the regulation of redox homeostasis has been investigated in rice cell cultures obtained from Italian and Pakistan rice varieties having different salt-tolerance: the salt tolerant varieties Baldo and KS-282 and the salt-sensitive varieties Vialone Nano and SuBas.

The cell cultures arising from the tolerant varieties showed the ability of better tuning the accumulation of internal H_2O_2 and to put in place an effective response mediated by genes involved in ROS and ion homeostasis. Our data demonstrate that the tolerant cell lines have a higher endogenous ROS scavenging systems and a better capability to optimize metabolic pathways involved in redox homeostasis in response to salt stress than sensitive ones. This ability might be involved in the shaping of the H_2O_2 signalling which triggers the tolerance mechanism. In fact, only cells capable to control H_2O_2 levels are tolerant to salt.

P6.7 - Nitrogen metabolism at the tillering stage affects grain yield and grain protein content in durum wheat

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Nitrogen is one of the most limiting factor for plant growth and development and its abundance in the soil affects crop yield and quality. In wheat, yield and grain protein content (GPC) are negatively correlated and this correlation is closely dependent on genotype. The objective of this work was to understand how two durum wheat cultivars, Aureo and Vespucci, use nitrogen supplied by fertilization. The results indicate that after fertilization, nitrogen content increases in both cultivars; the increment in yield is higher in Vespucci than in Aureo, whereas a simultaneously increase in both parameters occurs in Aureo. Biochemical analyses conducted during tillering stage confirm genotypic specificity in nitrogen use. In leaves of both cultivars amino acids, proteins and chlorophylls, which give an estimation of total nitrogen, increase significantly after nitrogen fertilisation. However, the increase in aminoacid content is higher in Aureo than Vespucci and this is positively correlated with a higher nitrate reductase (NR) activity. Our results suggest that an enhancement of NR activity in leaves during the tillering stage could be used as a precocious marker of GPC increase.

P6.8 - Mediterranean barley (*Hordeum vulgare*) genotypes as a tool to identify specific roles for HSP70s in response to abiotic stress

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Crop landraces play a crucial role in agronomic genetic improvement. This mostly unexplored resource acquires an increasing value in the Mediterranean area, periodically (and increasingly) subjected to abiotic stresses, namely drought and/or salinity.

To face these adverse conditions, plants exhibit multiple and variegated responses; among them, the critical role played by Heat Shock Proteins 70 (HSP70) - the most important group of chaperons in plants - can be highlighted.

In order to evaluate the role(s) of these proteins against salinity and drought, we performed a bioinformatic, molecular and biochemical investigation on barley (*Hordeum vulgare*) HSP70s in 3 Italian varieties and 4 Tunisian landraces.

The expression and protein occurrence of different HSP70s varied under stress: our results showed the involvement of specific isoforms in different barley genotypes. Particularly the expression of MLOC_67581, MLOC_50972, encoding for cytosolic and mitochondrial HSP70 isoforms, differently changed.

Therefore, we suggest that a constitutive expression of specific chaperons could be a strategy adopted by landraces to enhance drought and salt tolerance in the Mediterranean environment.

P6.9 - The nematode *Caenorhabditis elegans* to evaluate the effects of some atmospheric particulate matters

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Particulate Matter (PM) is a complex mixture of suspended solid e liquid particles that includes many chemical species with a toxicological activity. Meanwhile, the toxicity of particulate matter is extensively studied *in vitro*, very few investigations have been addressed *in vivo*.

Caenorhabditis elegans is a soil nematode that lately has been frequently used as a simple pluricellular model organism to quantify the toxic effects of environmental xenobiotics, as trace metals and organic pollutants.

Thus our goal was to investigate the toxicological pathways involved in the PM interaction with living organisms using this nematode, which combines the *in vivo* and *in vitro* approaches to provide information on single cell and whole-animal responses.

The effects of PM samples originated from two sources (brake dust and pellet ash) were analysed. In particular, we evaluated the effects of two fractions of these atmospheric particulate matters with respect to the total: water-soluble and insoluble fractions. The toxicity of these fractions was analyzed by evaluating the ability to affect the *C. elegans* survival as well as their capacity to induce oxidative stress in worm populations.

P6.10 - Metabolite distribution during ripening of fruits of sweet cherry landraces of Campania (Italy)

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Sweet cherries are fruits rich in nutritrional and nutraceutical metabolites determining many

of their organoleptic properties on one hand and their health-promoting effects preventing several degenerative diseases on the other. The metabolite distribution is strongly dependent on genetic variability, more than environmental and agronomic factors. To characterise the sweet cherry germoplasm of Campania region for its conservation and valorization, a comparative study has been made on the changes in sugars, organic acids, polyphenols, anthocyanins, ascorbic acid, antioxidant activities during fruit ripening in autochthonous landraces cultivated in the regional farm "Improsta". Fruits were collected at different ripening stages and used for the analyses. The results supported that metabolite concentrations were dependent on the landraces and on their fruit ripening stages. Among the sugars, glucose and fructose increased during ripening while sucrose was always at low level. Sorbitol reached values over 20% of the overall sugars in some landraces and highly increased in the late ripening stages. Polyphenols and of the other compounds also showed specific patterns.

P6.11 - Antioxidant defense systems in cyanobacteria: the extremophile *chroococcidiopsis* SP. CCME 029 versus the normophyle *synechocystis* SP. PCC 6803

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The higher resistance to radioactive and hydrogen peroxide exposures of the extremophile *Chroococcidiopsis* sp. CCMEE 029 in comparison with the normophyle *Synechocystis* sp. PCC 6803, has been recently reported.

The aim of the present work is to understand whether this different resistance is due to potentiate systems of reactive oxygen species (ROS) scavenging. On this basis, the ascorbate – glutathione cycle enzymes and metabolites as well as the gene expression of putative ROS scavenging enzymes system previously identified by analysis of genomic databases, have been analyzed in the two strains grown in optimal condition and under oxidative stress conditions.

Our results indicate that the extremophile bacteria have potentiate ROS scavenging capability and is able to better modulate its ROS defence when exposed to oxidative stress.

P6.12 - Development of a synthetic molecular oxygen sensor in plants

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Due to the involvement of oxygen in many essential reactions, all organisms possess molecular strategies that tune physiology and metabolism according to its availability. In mammals, the expression of hypoxic genes is tightly regulated by heterodimeric Hypoxia Inducible Factors (HIF). HIF activity is mainly regulated by an O_2-dependent proteolytic pathway that starts with HIF-α hydroxylation by PHD enzymes and follows with its ubiquitination by the E3 ligase pVHL. As the HIF pathway is not conserved in plants, we devised to exploit engineered versions of it to design and optimize an orthogonal O_2-responsive sensor there. We could efficiently combine modular components, consisting of sorted mammalian, yeast and plant protein domains, to generate novel O_2-responsive circuits in Arabidopsis. Such synthetic devices are meant to work in vivo as (i) specific switches for targeted responses to hypoxia and (ii) reporters of cellular O_2 dynamics. Accordingly, we focused on deploying our strategy to obtain genetically enhanced plants with better tolerance to low-oxygen stresses (e.g. submergence), as proof-of-concept of the possibility of synthetic biology to assist agricultural practices.

P6.13 - Ecotype variability in secondary metabolism of *Moringa oleifera* exposed to severe water deficit and re-watering under high temperature condition

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Moringa oleifera is a fast-growing hygrophilic species cultivated in many dry and hot regions of the Southern Hemisphere. The widespread utilization of this plant has led to the creation of different ecotypes, distinguished by morphological and physiological characteristics. In this work, two ecotypes of *M. oleifera*, one from South Africa (SA) and one from Paraguay (PA), were subjected to drought and re-watering under high temperature conditions (day/night, 38/19°C).

Both SA and PA plants significantly reduced growth, relative water content, water potential and photosynthesis under water deficit, while completely recovered the photosynthesis after re-watering. However, during drought, PA accumulated a higher content of carbohydrates and phenylpropanoids than SA. Conversely, in SA, a stronger increase of the de-epoxidation state of xanthophylls was observed. We show that different classes of secondary metabolites, phenylpropanoids in PA and isoprenoids in SA, offered protection under abiotic stresses in the two ecotypes and allowed the recovery after drought. This provides evidence for a high metabolic plasticity of *M. oleifera*, which seem to be dependent on its native provenance.

P6.14 - Effect of geomagnetic field on plant fatty acid content

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The geomagnetic (GMF) field is an inescapable environmental factor for plants on Earth that affects plant growth and development. However, its impact on plant growth and the mechanism of magnetoreception in plants is not clearly understood. To evaluate the effect of GMF on plant fatty acid composition and content, *Arabidopsis thaliana* Col-0 developing plants were grown under near-null magnetic field (NNMF, ≤ 40 nT) and GMF (~43μT). Fatty acids were analysed by GC-MS for their identification and GC-FID for the quantitative analysis. NNMF was found to significantly affect the Arabidopsis lipid composition, particularly during bolting, flowering and seed set.

P6.15 - Direct measurement of native plant sirtuin activity using a Homogeneous Time-Resolved Fluorescence (HTRF®)-based assay: first application to durum wheat mitochondria

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Sirtuins play a critical role in post-translational modification of proteins by catalyzing the NAD^+-dependent deacetylation of ε-*N*-acetyl lysine residues. Compared to mammals, much less is known about plant sirtuins. Only recently, a sirtuin-mediated fine-tuning of mitochondrial energy metabolism has been suggested in *Arabidopsis*. Nevertheless, to date, a direct assessment of sirtuin activity has never been reported in plant cell extracts and/or subcellular organelles. Here, a HTRF®-based assay was properly adapted and applied for the first time to measure sirtuin activity in highly purified mitochondria from durum wheat (DWM). A NAD^+-dependent

deacetylase activity equal to 268±10 mU·mg^{-1} of protein was measured, resulting *i*) linearly dependent on DWM protein, *ii*) abolished by boiling DWM and *iii*) completely inhibited by nicotinamide, a specific sirtuin inhibitor. Moreover, DWM-sirtuin activity was not significantly affected by resveratrol and quercetin, both reported as activators of the well-studied human sirtuin 1 isoform. Overall, results obtained in this study demonstrate that the adapted HTRF® assay may represent a useful tool to study native plant sirtuin activity.

P6.16 - Salt stress responses in rice: focus on the redox control of root growth

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Crop growth and productivity drop under unfavourable environmental conditions. To cope with stressful situations, plants evolved defensive strategies involving a complex redox signalling network. Oxidative and antioxidative signals are also involved in the regulation of normal growth and developmental patterns. Soil salt concentration is increasing in many areas of our Country reducing plant vegetative growth. In order to unveil the role of redox controls on plant growth inhibition, two rice varieties showing different sensitivity to salt have been investigated. Baldo (B), the resistant line, showed reduced growth inhibition when subjected to 100 mM NaCl compared with Vialone Nano (VN), the sensitive one. Since roots are the first organs perceiving soil salinity, redox shifts occurring in this organ have been monitored. In particular, changes in ascorbate and glutathione level and redox state were observed over treatment time. It is known that glutathione portioning between nucleus and cytosol is involved in plant cell proliferation. On this basis, confocal studies have been performed to follow sub-cellular glutathione compartmentalization in salt conditions limiting root growth.

P6.17 - Design and set up of a plant growth chamber for stable isotope labeling to investigate carbon attraction toward fruit sinks and plant reserves upon and after drought stress

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Root orchestrates the defense adaptations to drought, acting as a sink of the carbon in competition with other plant organs during growth slowdowns.

We aim to study C allocation kinetics in grapevine organs in a controlled drought system basing on pulse-chasing isotopic strategy. The isotope acts as a tracer of the floematic flows that are oriented towards different sinks during drought/rehydration cycles.

Photosynthetic assimilation, stomatal regulation and respiration are checked in the various phases to size $^{13}CO_2$ enrichment flows in the chamber. Maximum assimilation ranges from 9 to 12 mmol m^{-2} s^{-1}, reduced by stomatal control 3 o 4 times at the end of the drought period. Light responses are evaluated to optimizing chamber illumination. Saturation of net photosynthesis occurs around 1000 mmol m^{-2} s^{-1} PPFD: thereafter photoinhibition impairment starts in old leaves, whereas young leaves experience half of maximum assimilation without drastic photoinhibition. Temperature effects on photorespiration are checked and considered. A model optimizing light and temperature is proposed for plants in the various stages of the experiment.

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P6.18 - Apoplastic pH influences grapevine recovery responses to short and prolonged drought

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Alteration of sap pH is one of the first chemical changes that occurs within the xylem vessels of plants exposed to drought. Xylem sap acidification has been recently documented in a number of species; under drought, a drop in pH has been observed to be accompanied by the accumulation of soluble sugars. Here, two different *Vitis vinifera* cultivars (Barbera and Grenache) were exposed to either short (completely stopping irrigation) or to prolonged drought (continual reduction of 10% water). When comparable severe stress and high embolism levels were reached, the potted grapes were re-watered. Changes in pH, sugar and ABA concentrations in the xylem sap along with physiological parameters (leaf gas exchange and xylem pressure) were monitored during the experiment. We hypothesize that a severe short drought induces a fast drop in xylem pH leading to an accumulation of sugars, the main osmoticum necessary for recovery, in the apoplast. On the other hand, prolonged stress causes no significant changes in pH and rather promotes an increase of starch content and a decrease in sugar content thus delaying the recovery process.

P6.19 - Transcriptional response of different *Vitis vinifera* 'Nebbiolo' clones involves molecular signals regulating berry development in coordination with stress defence mechanisms

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Many research works have unveiled how grapevine cultivars respond to environmental conditions; however, the molecular mechanisms underlying the interplay between clones (vegetatively propagated lines of selected mother plants) and environment need to be elucidated.

This study was aimed to explore the complexity of the clone x environment interaction by investigating changes controlled by clone, vineyard or developmental stage.

The transcriptome of berries collected over ripening in different vineyards from three 'Nebbiolo' clones was analyzed by integrating RNA-seq data with analysis of candidate genes, quantification of secondary metabolites and agronomical parameters.

Transcripts associated to sugar transport and anthocyanin biosynthesis were differently modulated among clones, while genes involved in secondary metabolism and defence, such as stilbene synthase genes, were affected by vineyard, consistently with stilbenoid accumulation.

This attests that clone-specific responses exert a role in shaping agronomic performances of a grape variety in different environments, thus providing indications for orienting viticultural practices in light of both cultivation area and clone choice.

P6.20 - 3D visualization of air-filled tissues in two flood-prone halophytes reveals contrasting bottlenecks for long-distance O_2 transport

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Limonium narbonense Mill. and *Sarcocornia fruticosa* (L.) A. J. Scott are two key species in saltmarsh ecosystems of the Northern Adriatic lagoons, occurring along the whole flooding gradient. Both species show high tissue porosity but different O_2 dynamics when flooded (Pellegrini et al., 2018). We analysed the connection of air-filled tissues (aerenchyma) in order to identify possible bottlenecks for long-distance O_2 transport resulting in the observed contrasting responses to submergence. We investigated aerenchyma structure and connectivity in different plant organs of both species, using X-ray phase contrast micro-tomography at the SYRMEP beamline of ELETTRA synchrotron (Trieste, Italy). The micro-tomography scans revealed vast differences between the two species and the presence of an extensive aerenchyma with long but discontinuous air channels in *L. narbonense*, which supports the hypothesis of limited oxygenation of rhizome and roots during submergence. This architecture can be a possible trade-off mechanism reducing gaseous toxins (H_2S) mobility from entry points in the roots to the aboveground tissues.

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P6.21 - A leaf selfie reveals drought resistance: visualizing embolism formation in the venation network using a smartphone

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Under intense drought, the drop of xylem water potential can lead to the loss of plant water transport efficiency by formation of air emboli in xylem conduits. Leaves, as the terminal organs of the water transport pathway, are highly exposed to the risk of dysfunction caused by drought. Several techniques were proposed to measure "vulnerability curves" (VC curves) of leaves to quantify the vulnerability to emboli formation, but most of these are destructive and possibly prone to artefacts. Micro-CT and optical (OV) method have been proposed as "non-destructive" techniques, as they allow measuring leaves from intact plants. The OV method permits continue observation of embolism formation and spread in several plant organs. In this study, we propose a new and cheap set up of this technique, based on a smartphone and a led light source. We were able to accurately measure VC curves in leaves of *Ostrya carpinifolia*, *Oryza sativa* and *Laurus nobilis*. The results highlight the potential for broad application of OV method to a diversity of physiological, ecological and agronomical questions associated with quantification of xylem embolism formation in plants and related drought resistance.

P6.22 - Physiological evaluations of tomato cultivars under high temperature stress conditions

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Previous studies have demonstrated that the yield reduction of tomato under sub-high

temperatures is also related to a decline in the photosynthetic rates. In this work nine tomato landraces were cultivated in two different areas in Campania under three different conditions: open field with normal (April) and late (May) sowing and under plastic tunnel. Photosynthetic parameters, maximum quantum efficiency of PSII, chlorophylls, antioxidants contents and yield were determined. All the landraces showed variable responses for almost all the traits investigated in the different conditions. In the genotypes cultivated under tunnel, where temperatures reached 38-42 °C, there was a clear correlation between some photosynthetic parameters (i.e ETR) and final yield. This correlation was not found in normal and late sown plants. High temperatures affected chlorophylls and antioxidant contents. In particular there was a reduction in the content of chlorophylls and carotenoids and an increase in the level of ascorbic acid when plants were subjected to high temperatures. Genotypes potentially tolerant to high temperatures have been identified through the various parameters investigated.

P6.23 - Micro-CT assisted analysis of xylem recovery from water stress in poplars subjected to chemical inhibition of cellular activity

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Perennial plants maintain xylem hydraulic functionality to sustain photosynthetic capacity upon periods of severe drought. Under water stress, plants adjust their xylem pH and gene expression priming stem by accumulation of sugars and ions in the apoplast for xylem functional recovery. Here, we studied if application of ortho-vanadate solution, aimed to inhibit the metabolic activity of wood parenchyma cells and block proton pumps, can affect the xylem sap properties and hinder or delay the recovery process after soil rehydration.

Poplar trees were used for *in vivo* analysis of embolism using X-ray microCT. Despite restoration of water potential, the ortho-vanadate treated rehydrated poplars revealed a significant impediment of in removal of drought-induced embolisms, while control plants showed almost full recovery from embolism. These findings indicate that embolism removal is an energy dependent process that requires accumulation of sugars in the apoplast, while visual observations indicate that process is spatially coordinated, with embolism formation accruing from inside out and recovery from outside in; thus underlining the importance of xylem proximity to phloem (sugar source)

P6.24 - First evidences of the existence of Glyoxalase I activity in durum wheat (*Triticum durum* Desf.) mitochondria and its activation under hyperosmotic stress conditions

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Glyoxalase I (GloI) is a ubiquitous enzyme responsible for the primary defence against dicarbonyl glycation induced by methylglyoxal (MG), a toxic by-product of glycolysis and Calvin cycle. In plants, MG levels rise under various abiotic stresses, so GloI may play a crucial role in providing stress tolerance. GloI was demonstrated to localize in chloroplast, cytosol and nucleus. Moreover, a proteomic study suggested GloI existence also in mitochondria from

potato tubers; nevertheless, so far, a mitochondrial GloI activity has never been measured. Here, for the first time GloI activity was assessed in highly purified mitochondria obtained from durum wheat (DWM). In particular, a high GloI activity was measured showing hyperbolic kinetics with Km and Vmax values equal to 225±15 μM and 0.134±0.002 $U \cdot mg^{-1}$ of protein, respectively. Interestingly, an increase of GloI activity up to about 20% and 50% was obtained in DWM purified from salt- and osmotic-stressed seedlings, respectively, thus suggesting a key role of this enzyme in counteracting dicarbonyl glycation damage to DWM proteome. Identification of proteins target of dicarbonyl glycation in DWM merits further investigations.

P6.25 - The assembling process of photosynthetic glyceraldehyde-3-phosphate dehydrogenase: the regulation, the structure and something more

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Photosynthetic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is the third enzyme of the C3 cycle. Through its reaction the NADPH produced by the light phase of photosynthesis is consumed, leading to the production of the first sugar of the whole photosynthetic process. In higher plants two isoforms of GAPDH co-exist in the chloroplast stroma. The homo-isoform, formed exclusively by subunits A, is inactivated in the dark by the CP12-mediated interaction with phosphoribulokinase. The hetero-isoform, containing A and B subunits, turns off its activity through an autoassembly process that leads to the formation of inactive oligomers.

To study the dynamism of the assembling process, the AB-GAPDH system was analysed by Size Exclusion Chromatography coupled with Small Angle X-ray Scattering and cryo electron microscopy (cryo-EM) followed by single particle analysis. Both techniques showed the coexistence of different oligomerization states, allowing to speculate on a further *in vivo* physiological role (e.g. proteostasis) in addition to the regulatory one. Focusing on the most populated oligomerization cluster, cryo-EM structure of the inactive A_8B_8-GAPDH was solved at 6.6Å resolution.

P6.26 - Seasonal changes in *Chaetomorpha linum* bioremediation capability of acquaculture wastewater

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Marine pollution due to aquaculture wastewater is a widespread and increasing ecological problem. The use of algae in bioremediation of nutrient surplus in wastewater could be useful to achieve a more sustainable aquaculture. Since growth and metabolism of generalist seaweeds are strongly affected by changes in environmental conditions, in this study the seasonal uptake and assimilation capability of nitrogen (N) and phosphorous (P) excess of *Chaetomorpha linum* was tested.

C. linum, taken in different seasons (spring and winter) and subjected to acclimation in controlled lab conditions for 2 months, were grown in marine water with excess of ammonium, nitrate and phosphate. The results indicate that the best N and P removal performances were achieved by algae harvested in spring; however, acclimation of winter algae significantly enhances nitrogen uptake.

The seasonal capability of *C. linum* in N removal was more in depth investigated, studying in the algal tissues the changes in pigment content, intracellular inorganic and organic nitrogen pools and activities of the enzymes involved in N metabolism.

P6.27 - Tissue-specific hormone profiles from woody poplar roots under bending stress

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Mechanical forces induced by bending are able to trigger an asymmetrical response in *Populus nigra* L. woody taproots: the recruitment of new lateral roots on the convex side and the deposition of reaction wood (RW) on the opposite concave side. Since these responses seem to be induced by asymmetric activity and differentiation of cambium cells, in the present work, we investigated how mechanical forces could influence the activation of specific phytohormone signaling pathways of the root vascular cambium.

Thus, cambial zone and its surrounding areas were isolated from convex and concave sides of bent poplar root and analyzed by UHPLC-MS/MS to profile IAA metabolites, ABA and CKs.

In the concave side, IAA gradient plays a pivotal role in the control of cambial growth rate and xylem differentiation, and it could be at the basis of the strictly unidirectional RW production toward this side. Furthermore, the higher levels of ABA and all CKs metabolites in the concave side support their involvement in RW production, whereby ABA could mediate the adaptation to the deforming conditions generated by bending, while CKs could act in synergy with IAA to control cell differentiation.

7 - Genetics of Microorganisms

O7.1 - Hijacking bacterial iron metabolism using the transition metal gallium

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Iron (Fe) is a key nutrient for pathogenic bacteria. During infection, mammals use Fe-binding proteins to withhold Fe to invading microbes. To counteract Fe shortage *in vivo*, pathogens adopt various strategies, the most widespread being the production of siderophores and heme-uptake systems. With the aim of targeting Fe metabolism as part of antibacterial strategies, Fe-acquisition pathways have been hijacked to selectively deliver drugs. Recently, the Fe-mimetic metal gallium [Ga(III)] has successfully been repurposed as an antimicrobial drug. Ga(III) disrupts Fe-dependent metabolism, inhibiting microbial growth. Using the human pathogen *Pseudomonas aeruginosa* (Pa) we showed that the antimicrobial properties of Ga(III) are potentiated by complexation with the pyochelin siderophore, and that protoporphyrin IX-bound Ga (GaPPIX) enters Pa cells through heme uptake-routes and inhibits bacterial respiration. We have broadened our investigations on the effect of Ga compounds on multidrug-resistant bacteria of the ESKAPE group, and on bacterial pathogens causing lung infection in cystic fibrosis patients. Our results raise hope for the future development of Ga(III)-based antibacterials.

O7.2 - Playing with the rhizobial Mega-Apps: creation and multi-omics characterization of a genomically hybrid strain in the nitrogen-fixing symbiotic bacterium *Sinorhizobium meliloti*

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Many rhizobia harbour a multipartite genome and symbiotic phenotypes are mainly encoded by genes residing on secondary replicons.Approaches for modifying such phenotypes may involve large-scale genome manipulation and mobilization of secondary replicons among different hosts to obtain genomically-hybrid strains with improved abilities.Here we report the creation of a cis-hybrid strain in a model multipartite genome species,the plant-symbiotic bacterium *Sinorhizobium meliloti*.We moved the secondary replicon pSymA from a donor *s*train to an acceptor strain. The hybrid strain was screened for a panel of complex phenotypes(carbon/nitrogen utilization phenotypes,intra- and extra-cellular metabolomes,growth abilities,symbiosis).Then,metabolism reconstruction and constraint-based modelling were used for *in silico* prediction of metabolic flux reorganization.Interestingly,the symbiotic phenotype showed a marked cultivar-specific improvement with the hybrid strains compared to both parental strains.These results provide a proof-of-principle for the feasibility of genome-wide replicon-based remodelling of rhizobial strains for improved biotechnological applications in precision agriculture.

O7.3 - The role of Type VI Secretion System on the competitiveness of *P. aeruginosa* clinical isolates

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The evolution of chronic *Pseudomonas aeruginosa* infections in Cystic Fibrosis (CF) is characterised by the accumulation of pathoadaptive, loss-of-function mutations.

In this study an early (23) and a late clonal (175) isolate from the same patient and VrPa97, a closely related acute strain, were analyzed. The killing of *C. elegans* by 175 was remarkably reduced compared to 23 and VrPa97. Growth rate of 175, when co-cultured with VrPa97 or 23, radically decreased, suggesting the occurrence of a cell-to-cell inhibition mechanism. Genomic analyses revealed that 175 carried a stop mutation in *tssK3*, part of the H3 cluster encoding a Type VI Secretion System (T6SS). Accordingly 175 showed an impaired T6SS in an *E. coli* killing assay.

ΔtssK3 mutants were constructed in PAO1 and PA14. Both mutants showed a reduced capacity to kill *E. coli*, suggesting that *tssK3* plays an important role in the correct functioning of H3-T6SS, allowing *P. aeruginosa* to attack neighbour cells.

Our findings suggest that while early CF isolates still have a functional T6SS that provides an advantage in outcompeting other microorganisms, once persistence has been established, a strain may no longer needs the T6SS.

O7.4 - Characterization of an *E. coli* suppressor mutant that can survive and assemble a functional LPS transport machinery in the absence of the essential inner membrane-tethered LptC

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Lipopolysaccharides (LPS) an essential component of the outer membrane (OM) of Gram-negative bacteria is exported to the to the cell surface by the trans-envelope Lpt multiprotein machinery constituted, in *E. coli*, by seven essential proteins (LptA-G). LptBFG constitute an IM ABC transporter, LptDE form the OM translocon for final LPS assembly, whereas LptC, an IM-anchored protein with a periplasmic domain, interacts with the IM ABC transporter and the periplasmic protein LptA. Although essential, LptC can tolerate several mutations and its role in LPS transport is unclear.

We are currently characterizing a class of viable mutants lacking LptC, and bearing amino acid substitutions at residue R212 in LptF. Since a six-component Lpt machinery appears to be functional in LPS transport, LptC could function either as an adaptor protein for complex assembly or as a modulator of ATPase activity or both. We found that in suppressor mutants the six-component Lpt machine is assembled and that the mutated $LptF_{R212}$ protein is able to recruit LptA thus bypassing LptC in Lpt complex assembly. The effect of LptF mutations on the ATPase activity of LptBFG transporter will be discussed.

O7.5 - DksA-dependent regulation of quorum sensing in *Pseudomonas aeruginosa*

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In some bacterial pathogens the stringent response regulator DksA affects the expression of

virulence traits.

The animal and plant pathogen *Pseudomonas aeruginosa* has two DksA paralogs: DksA1 is constitutively expressed and contains a Zn-finger motif; DksA2 does not contain Zn and it is expressed only under Zn starvation condition. The current model predicts that DksA2 might complement DksA1 function in the latter condition.

Here *P. aeruginosa* single and double deletion mutants in *dksA1* and *dksA2* genes have been characterized under standard growth conditions (*i.e.* in rich medium). Results showed that DksA1 positively regulates the production of quorum sensing (QS) signal molecules and of QS-dependent virulence factors. Accordingly, *dksA1* deletion reduces *P. aeruginosa* virulence in a lettuce leafs infection model. *In trans* expression of *dksA1* or *dksA2* restores the wild type phenotypes in the double mutant, supporting the hypothesis that the two proteins are interchangeable. Experiments aimed at assessing the impact of *dksA1* and *dksA2* on *P. aeruginosa* transcriptome, and killing assays in a simple animal model (*Galleria mellonella* larvae) are in course.

P7.1 - CodY-mediated regulation of gene expression in Group B *Streptococcus*

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Group B *Streptococcus* (GBS) is a harmless commensal of the adult gastrointestinal and genitourinary tracts able to switch to a pathogenic lifestyle, causing invasive infections in newborns, elderly and immunocompromised patients. CodY is a GTP and branched chain amino acids (BCAA, isoleucine leucine and valine [ILV]) responsive global transcriptional regulator controlling major metabolic pathways and virulence genes in response to nutrient availability in many Gram+ bacteria. Here, we investigated the ability of CodY to control gene expression in GBS.

A *codY*-null derivative of GBS strain NEM316 exhibited a growth phenotype similar to the wild type (WT) strain in both complex and chemically defined medium. The expression of four genes carrying a putative CodY binding motif in their regulatory region was significantly higher in the *codY* mutant than in the WT strain. Moreover, while in NEM316 the expression of these genes increased at decreasing BCAA concentrations, no changes at varying ILV levels were observed in the *codY* mutant, suggesting that CodY activity in GBS is modulated by BCAA. These and additional results concerning the role of CodY in GBS will be presented.

P7.2 - Connection between hypoxia and glucose regulation in the expression of the glucose transporter gene *RAG1* in *Kluyveromyces lactis*

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Hypoxia induces a transcriptional response involving genes from several metabolic pathways in *K. lactis*. We demonstrated that the glucose sensor Rag4 mediated the hypoxic transcription of the *KlPDC1* gene. By reverse genetics, we also showed that the hypoxic transcription of lipid biosynthetic genes, mediated by the factor *Kl*Mga2, was dependent on glucose signaling. The expression of the glucose transporter gene *RAG1* is regulated by glucose through cascades involving Rag4 and other proteins, including the casein kinase Rag8 and the repressors Sms1 and *Kl*Rgt1. A second pathway involves the chromatin remodeler *Kl*Snf2 and Sck1. We have found that the transcription of *RAG1* is strongly induced by hypoxia in the presence of glucose. Promoter analysis allowed the identification of sequences necessary for such induction. We also identified Sck1 as the main element of the glucose signaling pathway(s) involved in the hypoxic signaling. We demonstrated that a differential binding of Sck1 to specific sequenceson *RAG1* promoter depends on hypoxia. Moreover, our results showed a dependence of Sck1 expression on hypoxia. Our study revealed a novel role of Sck1 as a hypoxic modulator in *K. lactis*.

P7.3 - Azurin: phylogenetic distribution, possible ecological role and quantification in colorectal cancer (CRC) patients

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Azurin, a Cupredoxin family's protein, firstly isolated from *Pseudomonas aeruginosa,* has a cytotoxic activity against tumor cells in vitro and in vivo. Phylogenetic analysis revealed

the presence of Azurin coding genes mainly in γ, β-*Proteobacteria* and CFB group. A possible ecological role of Azurin has been investigated by observing bacterial phenotypic changes at massive Azurin production conditions (selective media).

Real-time PCR for the detection and quantification of Azurin in 10 healthy and 10 CRC patients was performed. Samples of stool, saliva, and biopsy have been analysed and compared for the presence of Azurin coding genes from different bacterial groups.

The results highlighted an enrichment of Azurin coding genes of stool samples; at the same time, High-throughput sequencing analysis revealed an enrichment in *Pseudomonas* genus in biopsy samples. Overall, an evident correlation with CRC and Azurin presence has not been evidenced in comparison with healthy subjects.

Project "Tumore del colon-retto: caratterizzazione funzionale/metabolica del microbiota e ruolo dei probiotici nella modulazione della risposta immune specifica", funded by FAS (now FSC) Regione Toscana.

P7.4 - Characterization of an efflux pump in *Mycobacterium smegmatis*

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Tuberculosis (TB) remains one of the most important infectious disease worldwide. It is still endemic in many low and middle-income countries and the emergence of *Mycobacterium tuberculosis* multi-drug resistant strains continues to plague TB control. *M. tuberculosis* has the remarkable capacity to survive within the hostile environment of the macrophage; the molecular mechanisms behind the success of this pathogen are still poorly understood. We recently described the role of a TetR-like protein of *M. smegmatis* and *M. tuberculosis* in regulation of the *MSMEG_3762/63/65* and *Rv1687c/86c/85c* operons, respectively, coding for efflux pumps, using a combination of mutagenesis, local and global gene expression analyses and DNA binding studies. In *M. smegmatis*, *MSMEG_3762* and *MSMEG_3763* are annotated as ABC transporter ATP-binding protein and ABC transporter, respectively, as well as their orthologues in *M. tuberculosis*. In this contest, we have isolated a strain carrying a deletion in the *MSMEG_3763* gene, and phenotypes related to efflux-mediated resistance to several anti-TB drugs and to acid-nitrosative stress, mimicking the macrophage environment, are under investigations.

P7.5 - Towards microbe-assisted therapy of colon cancer: exploring oral and intestinal human microbiota in patients with CRC

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In this study, NGS analysis was used to investigate and compare human microbiota from three different compartments (saliva, feces, and cancer tissue) of patients with colorectal cancer (CRC) and control patients. Furthermore, *Fusobacterium nucleatum* abundance was evaluated through qPCR.

Data obtained revealed both a different bacterial communities composition and a differentiation in *F. nucleatum* abundance between healthy and CRC subjects. Differences were observed among the three different analysed compartments too.

Firmicutes, *Bacteroidetes*, and *Proteobacteria* were the more abundant bacterial phyla

detected in all samples. An enrichment of *Bacteroidetes* within fecal samples of CRC patients was observed, while *Firmicutes* were over-represented in the fecal samples of healthy controls.

Further investigation will highlight the possible correlation between CRC and bacterial community, as well as the correlation between the presence of *F. nucleatum* and the clinical course of CRC patients.

Project "Tumore del colon-retto: caratterizzazione funzionale/metabolica del microbiota e ruolo dei probiotici nella modulazione della risposta immune specifica", funded by FAS (now FSC), Regione Toscana.

P7.6 - *Streptomyces coelicolor* extracellular vesicles: isolation, purification and characterization

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Extracellular vesicles (EVs) are membranous particles with nano-scale diameter that contain macromolecules and metabolites. EVs have been characterized in Gram-negative bacteria and more recently in several Gram-positive bacteria.

Streptomyces coelicolor is a filamentous Gram-positive bacterium, having a complex life cycle characterized by the production of an enormous repertoire of bioactive metabolites and extracellular enzymes.

In order to purify EVs from *S. coelicolor* liquid cultures, a density gradient ultracentrifugation-based protocol was applied and the presence of particles compatible with EVs was revealed by TEM, SEM and AFM. Moreover, DLS analysis confirmed that particle sizes range between 100 and 200 nm and Raman microscopy showed that actinorhodin antibiotic is associated with EVs.

Electrophoretic analyses showed that vesicular cargo includes proteins, DNA and RNA.

Finally, LC-MS/MS analysis revealed that EV's proteome is mainly constituted by proteins involved in the differentiation of *S. coelicolor.*

This study, revealing the presence of EVs in liquid cultivations for the first time, may shed light on the role of EVs in the physiology and development of *S. coelicolor*.

8 - DNA Replication, Repair and Recombination

O8.1 - Identification of the biological target of new platinum, copper and nickel complexes

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The biological activity of some thiosemicarbazones is linked to the modulation of ribonucleotide reductase (RNR), an essential enzyme in DNA synthesis and repair.

In previous studies, we showed the antiproliferative properties of $[Ni(tcitr)_2]$ (Buschini et al., 2009) and $[Cu(tcitr)_2]$ (Bisceglie et al., 2012) complexes. In particular, $[Ni(tcitr)_2]$ induces G_2M cell cycle arrest and p53 independent-intrinsic-apoptosis in U937 cells (Buschini et al., 2009). $[Ni(tcitr)_2]$ also causes DNA damage and alters DNA conformation creating knot-like structures and hairpins (Buschini et al., 2014).

In this study, we compared the biological activity of citronellal thiosemicarbazone complexes with platinum, copper and nickel ions. Starting from these derivatives, we also detected the antiproliferative and genotoxic activity of dimethylated compounds toward a selection of cancer cell lines. Furthermore, we analysed the modulation of the expression of both subunits of RNR after treatment with metal complexes.

Our results suggest that probably RNR is not the main target of these metal complexes but could be involved in cellular response.

O8.2 - MUS81 endonuclease activity is regulated by CHK2 for replication forks rescue upon replication stress and BRCA2 depletion

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MUS81 is a structure-specific endonuclease involved in producing Double Strand Breaks (DSBs) to resolve late recombination intermediates at G2/M transition and perturbed replication forks under persisting replication arrest or checkpoint deficiency. MUS81 activation needs to be tightly regulated to mediate the cellular response to replicative stress in order to maintain genome stability. In yeast, MUS81 activity is negatively regulated by *Cds1* kinase, but how it is accomplished in human cells is little known. We identified a new mechanism elucidating a replication stress-induced activation of MUS81 by CHK2 kinase. Our data demonstrate that CHK2 is able to bind MUS81 *via* the ForkHead-Associated (FHA) domain and phosphorylates it on Serine 97 *in vitro*. Interestingly, CHK2 activity also supports the formation of early MUS81-dependent DSBs in BRCA2-depleted cells. Moreover, CHK2-MUS81 interaction is reduced by the I157T mutation of CHK2 that is associated with Li-Fraumeni syndrome and elevated breast cancer risk. Thus, we propose CHK2-MUS81 interaction as a new target in BRCA2-deficient cancer cells to modulate the response to chemotherapeutics that cause replication fork degradation.

O8.3 - Low doses of gamma irradiation render *Drosophila melanogaster* resistant to the DNA damage

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A major issue of radiation biology research is evaluating whether low doses of ionizing radiation (LDR) potentially affect human health. Despite the large number of studies addressing this issue, the analysis of low dose radiation human risk continue to be a focus of significant controversy. We have found that wild-type Drosophila melanogaster flies, chronically exposed to a priming g-radiation dose of 40cGy, delivered at the LIBIS facility with a dose rate of 2.5 mGy/h during embryo-to-third instar larvae development, exhibited a strong reduction (~50%) of chromosome break frequency after a challenge dose of 10Gy with respect to untreated flies directly exposed to 10Gy. This indicates that a chronic exposure of 40cGy renders flies more resistant to genotoxic threats suggesting that LDR can induce an adaptive response to DNA damage in Drosophila somatic tissues. This effect is associated with changes in the dynamics of g-H2AV recruitment at DNA damage sites, as revealed by cytological and WB analyses. In addition our genetic and RNA-seq data indicate that this response largely depends on the genetic background. Our data will shed light on the in vivo effect of LDR also in humans.

O8.4 - Identification of molecular biomarkers useful for predicting the likelihood of radiation-induced side effects in oncological patients undergoing radiotherapy

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During radiotherapy (RT) non-tumor tissues are unavoidably exposed to radiation. Negative side-effects that impact on life quality depend on an individual response, which in turn is influenced by genetic factors. By combining clinical parameters, gene expression analysis of the DNA-Damage Response (DDR) pathway and an in vitro individual radiosensitivity (IRS) index, we aimed to define an individual radiophenotype. Cutaneous toxicity, pain, itch, and asthenia were recorded according to CTCAE V4 criteria as a measure of radiation toxicity. The expression of 15 genes of the DDR pathway was analyzed by qRT-PCR before and 24 h later the first fractionated dose of RT. To determine IRS, a G2-chromosomal assay was applied on blood samples collected before RT and cultured in presence/absence of caffeine, an inhibitor of the G2 checkpoint. Finally, clinical parameters, gene expression and IRS were matched to define a genetic signature apt to reflect an individual radiophenotype. The expression of some DDR genes correlates with clinical parameters; this result may contribute to the ability of predicting RT side-effects, with the future perspective of applying personalized RT treatments.

O8.5 - Live cell imaging analysis of the influence of p21CDKN1A on PCNA-partners turnover at UV-induced DNA damage sites

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The $p21^{CDKN1A}$ protein is involved in various cellular processes including the Nucleotide Excision Repair (NER) through the interaction with Proliferative Cell Nuclear Antigen (PCNA). In this study we have investigated whether p21 may influence the turnover of PCNA-interacting partners, such as DNA Polymerase (Pol) δ and DNA Ligase (Lig) 1, at the DNA damage sites. To this end, we used three different p21 mutants: T148D (p21DD), $KRR^{154\text{-}156}AAA$ (p21AAA), and K161Q, K163Q (p21-2KQ), all characterized by resistance to degradation. Live cell imaging analyses of GFP-tagged p21DD and p21AAA mutants showed that only the first interacted with PCNA and delayed both the recruitment and the dissociation kinetics of PCNA partners (Pol δ and Lig 1) from DNA damage sites. The analysis of HA-tagged p21-2KQ mutant showed an increased persistence of DNA Pol δ at DNA damage sites. Interestingly, the presence of p21 also influenced PCNA levels at DNA damage sites. These results suggest that the exchange of PCNA partners, at least during NER, is influenced by p21, whose degradation can be then interpreted as the regulatory mechanism temporally defining the activity of PCNA-dependent NER steps.

P8.1 - The Werner exonuclease is involved in fork processing and checkpoint activation in response to low-dose of camptothecin

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The WRN protein is a RecQ helicase that acts in promoting repair or recovery of perturbed forks. It possesses an exonuclease activity whose biological relevance is poorly defined. Here, we investigated the role of WRN exonuclease in mediating replication fork processing and checkpoint activation in response to low-dose of Topoisomerase I inhibitor camptothecin. Using cells expressing the exonuclease-dead WRN and performing multiple assays to evaluate the response to perturbed replication, we demonstrated that loss of exonuclease activity increases nascent ssDNA formation, which is partially MRE11-mediated but independent from DNA2. Also, we found that this alternative fork processing prevents MUS81-dependent DSBs formation and affects reinforcement of CHK1 phosphorylation. Mechanistically, these results might correlate with our observation that loss of WRN exonuclease activity impairs chromatin recruitment of proteins implicated in fork reversal. Our findings identify two roles for WRN exonuclease: upstream of the fork reversal reaction cooperating with proteins involved in this process, and downstream formation of reversed forks. More studies are ongoing to clarify this mechanism.

P8.2 - DNA damage response kinases as a target for the differentiation treatment of acute myeloid leukemia

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Acute myeloid leukemia (AML) is a genetically heterogeneous malignancy characterized by the expansion of hematopoietic stem/progenitor cells (HPCs) blocked at different stages of maturation/differentiation. AML causing-mutations modify chromatin organization at genes sites regulating HPCs proliferation, terminal differentiation, and DNA repair, contributing to the development and progression of the disease. Recent findings underline increased DNA damage and abnormalities in the DNA damage response (DDR) as a critical feature of AML blasts. Therefore, we tested the effect of specific DNA damage kinases (ATM, ATR, CHK2 and CHK1) inhibitors on several AML cell lines. Results demonstrated that AML blasts are sensitive to ATM, ATR, and CHK1 inhibitors that induce the expression of the CD11b myeloid differentiation marker and promote the transition of AML blasts to granulocyte, as demonstrated by morphological and biochemical markers. The capability of DDR kinase inhibitors to promote the differentiation of AML cells represent a novel attractive strategy to treat leukemia by inducing the terminal differentiation of the leukemic blasts without exerting cytotoxic effects.

P8.3 - A DDB2 mutated protein, unable to interact with PCNA, affects DNA repair and confers proliferation advantage

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Nucleotide Excision Repair (NER) mechanism removes UV-induced DNA damage. In global genome repair (GG-NER), a subpathway of NER, DNA binding protein 2 (DDB2) forms with DDB1 the UV-DDB complex able to recognize DNA photolesions. DDB2 directly interacts with PCNA through a conserved sequence and the loss of this association determines impairment

in NER process. Our results, obtained using stable transfected clones producing $DDB2^{Wt}$ or $DDB2^{PCNA-}$ proteins, showed that $DDB2^{PCNA-}$ cells acquired an evident motility and disorganized multilayer of grown. These data suggested an increased resistance to UV-C radiation. Preliminary results, obtained by soft agar assay, showed that $DDB2^{PCNA-}$ clone is able to grow in anchorage-independent manner. Moreover, this clone expresses lower levels of E-cadherin protein, comparing to $DDB2^{Wt}$ and control cell lines, but higher levels of metalloproteinases 2 and 9 yielding the microenvironment prone to digest extracellular matrix. All these findings leave to suppose that cells expressing $DDB2^{PCNA-}$ might undergo malignant cell transformation.

P8.4 - G-quadruplex ligand RHPS4 radiosensitizes glioma xenograft *in vivo* through a differential targeting of bulky differentiated- and stem-cancer cells

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In previous studies we showed that the G-quadruplex (G4) ligand RHPS4 display *in vitro* radiosensitizing effect in U251MG GBM radioresistant cells through the dysfunctionalization of telomeres. In the present study we show that the combination of RHPS4 administration and ionizing radiation (IR) exposure is very effective in blocking tumor growth in mice as evaluated up to 65 days in a heterotopic mice xenograft model. The long-term tumor control observed in combo-treated mice suggested the targeting of the stem cell compartment. Thus, *in vitro* experiments in stem-like cells derived from U251MG and in 4 patient-derived glioma stem cell lines (GSC) were performed. Interestingly, RHPS4 alone was able to strongly reduce stem cell proliferation but combined treatment did not determine any increased effect due to high GSC telomeric-resistance to RHPS4 as shown by the absence of telomere fusions and telomere doublets. The mechanism by which RHPS4 target the stem compartment remains to be elucidated but, interestingly, RHPS4 treatment determined a strong replicative stress in GCSC, further exacerbated by the concurrent reduction of Rad51 and Chk1 protein level and gene expression.

P8.5 - Investigating PCNA acetylation to identify novel biomarkers of DNA replication stress in normal and tumor cells

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Proliferating Cell Nuclear Antigen (PCNA) plays a critical role in replication and repair pathways allowing the binding of enzymes and regulatory factors to DNA. PCNA functions are regulated by post translational modifications, among which acetylation is important for regulating protein stability after DNA damage. In fact, failure to acetylate PCNA results in its retention on chromatin after DNA damage. To understand the role of PCNA acetylation during DNA replication, we performed treatments with TSA (inhibitor of histone deacetylases) and C646 (inhibitor of acetyl transferases). The results showed that after TSA treatment chromatin bound PCNA level is increased in human primary fibroblasts, whereas inhibition of acetylation does not affect this level. We performed experiments on HeLa cells expressing PCNA mutated on its acetylation sites to detect an interaction with E3 ubiquitin ligases MDM2 and Cul4A. Preliminary data showed that the interaction with MDM2 is influenced by the mutation. Other studies will be needed to understand how ubiquitylation and degradation of PCNA are linked to PCNA stability during DNA replication.

P8.6 - TRF1 PARylation by PARP1 is required for the accomplishment of telomere replication

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PARP1 is the most abundant chromatin-associated protein after histones. It is activated upon DNA damage and in turn regulate DNA repair. PARP1 is also activated by replication fork stall upon a replication dependent damage where it promotes HR dependent and fork restart. At telomeres, the topological stress generated by G-quadruplex stabilization was reported to increase PARP1 recruitment at telomeric sites. So, we asked wether PARP1 could play a role in telomeric replication in absence of genotoxic stress. Here, we show that telomeric endogenous TRF1 and TRF2 associate with PARP1 in a cell-cycle dependent manner. In particular, we asked wether TRF1 could be parylated by PARP1 and if this modification could impact on TRF1 binding to chromatin during replication. We showed that TRF1 is a direct PARP1 target and, in addition, PARP1 interference impinge on TRF1 dynamics at replicating telomeres and induces an increase of telomere fragility. Interestingly, PARP1 inhibition affect telomere stability in a higher extent compared to TRF1.This suggests that PARP1 activity could modulate more than one component of telomere replication machinery.

P8.7 - XPD mutations differentially affect the cellular composition of TFIIH in XP and TTD patient cells

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TFIIH is a transcription/DNA repair factor composed of the core-TFIIH and the CAK sub-complexes, bridged together by the XPD subunit. XPD plays a direct role in regulating TFIIH structure and functionality. Indeed, free-CAK regulates cell cycle progression by phosphorylating cdk proteins, whereas TFIIH-bound CAK (holo-TFIIH) phosphorylates the RNApol II and activates transcription elongation. Mutations in different sites of XPD give rise to distinct clinical entities, including the cancer proneness xeroderma pigmentosum (XP) and the multisystemic cancer-free trichothiodystrophy (TTD). To understand whether XPD mutations affect the composition of TFIIH complex and, in turn, the CAK-mediated signalling pathways, we investigated the association/dissociation of the core-TFIIH with CAK in fibroblasts from XP or TTD patients mutated in XPD. By chromatin immunoprecipitation and immunofluorescence, we found an altered cellular distribution and chromatin association of the two sub-complexes specific for XP or TTD cells, thus suggesting the activation/repression of specific signaling pathways in patient fibroblasts.

P8.8 - A new functional *in vitro* "cell-free" assay as a method to study the DNA repair synthesis

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DNA Damaged binding protein 2 (DDB2) is a key factor involved in the recognition of ultraviolet chromatin photolesions in Nucleotide Excision Repair mechanism (NER) thanks to its ability of creating a complex together with DDB1 (UV-DDB complex). A mutated form ($DDB2^{PCNA-}$) was recently studied to demonstrate a loss of direct interaction with Proliferating

Cell Nuclear Antigen (PCNA), a protein that plays an important role, not only in cell proliferation but also in repair machinery. Comparing cells transfected with pcDNA3.1-DDB2^{Wt} vector to the pcDNA3.1-DDB2^{PCNA-} one, after UV-C damage, the DDB2^{PCNA-} cells shown a delay in UV damage recognition and NER factors recruitment on the chromatin lesion sites. To confirm the reduction on the repair efficiency, we have set up a new functional *in vitro* "cell-free" assay to evaluate the DNA repair synthesis. To this end, we used isolated nuclei from cells synchronized in late G_1-phase by Mimosine and then irradiated. Using this new method, we observed a different behaviour of DDB2^{Wt} and DDB2^{PCNA-} recombinant protein to bind and promote a DNA repair synthesis after UV-C damage.

P8.9 - Investigating the role of WRNIP1 in response to CPT-induced DNA double strand breaks

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DNA double-strand breaks (DSBs) are cytotoxic lesions that threaten genomic integrity. Failure or misrepair of DSBs has deleterious consequences for cells, including genomic instability and cell death. Werner helicase interacting protein 1 (WRNIP1) is a member of the AAA+ ATPase family, which we have recently involved in preventing MRE11-mediated degradation of hydroxyurea-stalled replication forks. Beside an ATPase activity, WRNIP1 contains a ubiquitin-binding zinc finger (UBZ) domain whose function is still poorly characterised. This study aims to explore the role of WRNIP1 and its enzymatic activities in response to treatment with the anticancer campthotecin (CPT), a DNA topoisomerase inhibitor that induces DSBs. Our results show that WRNIP1 promotes cell survival upon CPT-induced DNA damage. Consistently, loss of WRNIP1 exhibits enhanced accumulation of DNA damage and chromosomal aberrations after CPT treatment. Interestingly, our findings demonstrate a crucial role of WRNIP1 in DSB repair and a different requirement of its enzymatic activities in this context. Altogether, our data implicate WRNIP1 and its activities in response to CPT-induced DSB repair.

P8.10 - RAD52 protects replication forks from nascent strand degradation

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Homologous recombination (HR) is a key mechanism devised to maintain genome integrity in the cells. Defects in HR may lead to genome instability and, in most cases, to cancer onset. In yeast, Rad52 is an essential HR protein, while in humans its function is redundant with that of BRCA2. However, the precise role of RAD52 at stalled replication forks is still unknown. Here, we investigated whether and how RAD52 promotes stalled fork restoration.

We found that, early after hydroxyurea-induced replication arrest, inhibition or depletion of RAD52 causes accumulation of ssDNA at nascent strand that does not derive from DSBs processing, but it is dependent on MRE11 nuclease activity. Notably, the effect of RAD52 inhibition was reduced upon concomitant inhibition of RAD51. We analyzed also the recruitment of some proteins involved in fork reversal and we found that RAD52 inhibition affects SMARCAL1 and RAD51 recruitment at stalled replication forks. Moreover, we demonstrated that RAD52 is important to prevent DNA damage during S-phase. Altogether, our findings contribute to elucidate the role of RAD52 in fork stability and relevance for genome integrity.

P8.11 - Genotoxic/mutagenic potential of mobile-phone radiofrequency (915 MHz) in human lymphocytes exposed *in vitro* to continuous or fractional treatments

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Genotoxic and mutagenic effects induced by exposure to mobile-phone radiofrequency (915 MHz RFR, SAR<2W/Kg) were studied in human lymphocytes from 12 healthy donors, equally divided by sex and smoking habits. A total of 10 hours exposure was administered to blood sample cultures placed in a Transverse Electromagnetic (TEM) cell, following either a continuous (C-RF) or a fractional (F-RF) exposure mode consisting of four 2.5-hour RF exposure cycles with a 10-minute interval. Alkaline Comet and CBMN assays were used, for measures of DNA primary-damage and mutagenic effects, respectively. Statistically significant increases of DNA primary damage were found exclusively after F-RF exposure. The CBMN-test showed a significant MN increase after C-RF. Detailed analyses between groups evidenced differences between the sexes and the cigarette smoking habits. Data indicate that mobile-phone 915 MHz RF exhibit genotoxic and mutagenic effects. The higher level of DNA-damage induced by F-RF mode compared to C-RF one invokes a role of adaptation mechanisms taking place in the last one. The unexpected differences between sexes and smoking habits are interesting results to be further investigated.

P8.12 - RHPS4 telomeric replication stress in BLM CRISPR/Cas9 knockout glioma cells

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Telomeres pose a challenge to DNA replication machinery, due to their repetitive nature which tend to form secondary structures called G-quadruplex (G4s). Cells rely on different helicases to resolve G4s during telomere replication. Indeed, telomeres act like fragile sites in replication stress conditions. RecQ helicase BLM plays a specific role in S-phase in telomeric replication: cells lacking BLM show telomere fragility, even in absence of replication stress. Recently, we showed that in U251 glioma cells, RHPS4 molecule can bind telomeric G4s, modulates protein level of cell cycle regulator CDK2, the replisome component PCNA, and ATR-CHK1 S-phase checkpoint pathway. Cells also show an increased telomeric fragility, suggesting that RHPS4 induce replication stress in S-phase. To understand which players are involved in the response of the stabilized G4s by RHPS4, we generate BLM knockout clones of U251 cells by CRISPR/Cas9 targeting the helicase domain of the protein. We confirmed the knockout checking for BLM protein levels. In addition, the replication defects in absence of BLM after drug treatment were evaluated, uncovering the action of the helicase in telomere replication.

P8.13 - ATM and CDK1 phosphorylate WRN for a correct and timely execution of DSBs processing

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DNA double-strand breaks (DSBs) are critical lesions that are mainly repaired by homologous recombination (HR). HR is initiated by DNA end-resection to produce single-stranded tails

for RAD51-mediated strand invasion. These processes are extensively investigated but the regulatory mechanism(s) ensuring their timely execution is incompletely understood. The WRN protein is an important molecular player in resecting DSBs and is regulated by CDK1. However, WRN is also phosphorylated by ATM and ATR, which act upstream or downstream end-resection, respectively.

This study describes that WRN, through accurate and ordered multiple phosphorylation events orchestrated by CDK1, ATM and ATR, is able to control initiation of long-range resection and ensuing HR of DSBs.

We show that initiation of long-range end-resection requires ATM-dependent phosphorylation of WRN. Surprisingly, dephosphorylation of the CDK1 site S1133 and phosphorylation of the ATM/ATR site S1141 are involved at the end of long-range resection to support HR.

Collectively, our data support a model whereby a sophisticated regulation of WRN provides a molecular switch to bridge a correct execution of end-resection with HR.

P8.14 - Antioxidant treatment preserves genome stability in SMC1A-mutated Cornelia de Lange syndrome cells

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Cornelia de Lange syndrome (CdLS) is a rare disease characterized by cognitive impairment, multisystemic alterations, and premature aging. Furthermore, CdLS cells display gene expression dysregulation and genomic instability. Here, we demonstrated that treatments with the antioxidant drugs ascorbic acid and riboceine reduced the level of genomic instability and extended the in vitro lifespan of CdLS cell lines. Gene expression profiling showed that antioxidant drugs caused dysregulation of gene transcription; notably, a number of genes coding for the zinc finger (ZNF) containing Krueppel-associated box (KRAB) protein domain (KRAB-ZNF) were found to be down-regulated. Taken together, these data suggest that antioxidant drugs have the potential to ameliorate the developmental phenotype of CdLS.

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P8.15 - DNA damage repair in Friedreich Ataxia: a novel insight for the comprehension of the pathological phenotype

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Friedreich ataxia (FRDA) is an autosomal recessive disorder caused by a homozygous GAA repeat expansion in the first intron of the *FXN* gene (9q21.11), leading to insufficient levels of protein. Because FRDA cells incur increased oxidative stress, it is reasonable that an impaired DNA damage response may contribute to the pathological phenotype. However, whether DNA damage could be involved in FRDA is a still overshadowed field. PARP1 and its partners are emerging as central mediators for the immediate detection of DNA damage in human cells, thus the aim of this work is to investigate PARP1 pathway in the frame of FRDA disease. A deeper knowledge of the involvement of this pathway in FRDA may shed light in fact on the relationship between DNA repair, the altered DNA replication dynamics and heterochromatinization affecting the mutated gene. Using lymphoblastoid cells derived from FRDA patients and their relatives (carriers and healthy subjects), we have analyzed: 1) the expression of PARP1 and other related genes by qPCR and western blot; 2) the response of normal, mutated and heterozygous cells to oxidative stress induced by H_2O_2 or menadione. Data analysis is still in progress.

P8.16 - Post-translational modifications of Drp1 link mitochondrial dysfunction to neurodegenerative features in Cockayne Syndrome A cells

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A growing body of evidence indicate that the neurological defects in Cockayne Syndrome (CS) may be due to loss of mitochondrial function, whereas the impaired transcription-coupled repair could account for the skin photosensitivity. Evidence has been provided that human CS cells present an altered redox balance and excessive mitochondrial fission due to hyper-phosphorylation of the mitochondrial fission protein (Drp1)(Pascucci et al.; 2012, 2016).

We report that MDIVI, a mitochondrial fission inhibitor, is able to rescue the dysfunctional mitochondrial phenotype of CS-A cells, and plays also an anti-apoptotic role by reducing the translocation of Bax at mitochondria. Preliminary data show that CS cells are characterized by the formation of higher levels of nitric oxide (NO) and superoxide anion compared to normal fibroblasts. These reactive species could induce post-transcriptional modification of target molecules, which could contribute to the development of several neurodegenerative disorders.

Therefore, the study of post-translational modifications of mitochondrial proteins could further contribute to the molecular characterization of the pathological features of CS.

P8.17 - *In vitro* evaluation of DNA and chromosomal damage induction by Silver nanoparticles

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In the framework of RinnovaReNano project potential cytotoxicy and genotoxicy effects of silver nanoparticles (AgNPs) have been evaluated on two different cell lines: BEAS-2B (normal human bronchial epithelium) and RAW264.7 (murine macrophage). AgNPs 20nm (HIQ-Nano s.r.l.-Lecce, Italy) were characterized by DLS both in batch dispersion and in different cell culture media.

Cytotoxicity investigations were performed by the MTS viability assay only on BEAS-2B treated for 24 hrs with 1, 10, 25, 50 and 100 ug/ml of AgNPs.

Genotoxicity effects of AgNP were evaluated by comet and CBMN assays on both cell lines. In particular, BEAS-2B and RAW264.7 were treated with AgNPs in a range of doses of 1-50 ug/ml for comet assay (4 and 24hrs of treatment) and 1-25ug/ml for CBMN test (48hrs of treatment).

Results showed a DNA damage increase in BEAS-2B after 4 and 24hrs of treatment that seems to evolve in chromosomal damage after 48hrs of treatment. On the contrary, in RAW264.7 no increase of DNA damage at any time and dose of treatment was observed, even though an increase of micronucleus frequency at the highest dose tested was highlighted.

P8.18 - Nucleotide Excision Repair (NER) XPG endonuclease is induced by loss of p300/CBP acetyl transferase activity and transcription stress

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The endonuclease XPG participates in Nucleotide Excision Repair (NER), but it is also

involved in basal transcription to promote chromatin looping and DNA demethylation. In addition, XPG is required for processing of RNA/DNA hybrid (R-loops), whose malfunction may cause double strand breaks formation. Here we have investigated whether p300 and CREB-binding protein (CBP) lysine acetyl transferases (KAT) may play a role in XPG chromatin association during basal transcription. We show that chromatin-bound XPG is acetylated in the absence of DNA damage and interacts with both p300 and CBP. Depletion of KAT activity by RNA interference or chemical inhibition with C646 resulted in the redistribution of XPG in nuclear focal areas, possibly concomitant with protein modification. XPG relocation was also observed after inhibition of RNA polymerase II with 5,6-dichloro-1-β-D-ribofuranosy benzimidazole (DRB). Both DRB and C646 triggered a DNA damage response associated with R-loop formation, as detected with S9.6 and histone γ-H2AX immunostaining. These results suggest that basal chromatin association of XPG is altered by impairment of p300/CBP KAT activity and transcription stress.

9 - Non-coding RNA

O9.1 - LncRNAs in motor neuron differentiation and physiopathology

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Long noncoding RNAs (lncRNAs) emerged as essential players in nervous system physiopathology. In this study we highlight their relevance in motoneurons (MNs), the cell type affected in severe neuromuscular disorders such as FUS dependent Amyotrophic Lateral Sclerosis.

MNs were *in vitro* differentiated from HB9::GFP mouse embryonic stem cells (mESCs), FACS-purified and analyzed through RNA-Seq. Data were compared to available mESC dataset. We identified 305 noncoding transcripts turned-on in MNs (q-value<0,05) and, using ENCODE annotations, we assessed their tissue specificity, finally focusing on 12 lncRNAs. Based on coding potential, expression during differentiation, localization and conservation in human, we selected four candidates. We evaluated their levels in MNs derived from $mFUS^{-/-}$ or knock-in $mFUS^{P517L/P517L}$ mESC, unveiling lncRNA differential expression in the different FUS backgrounds. For functional analysis we established lncRNA K/O mESC lines using the CRISPR-Cas9 technology.

Our work contributes to provide a global view of MN transcriptome. We identified and characterized interesting lncRNAs, which are currently undergoing phenotypical and functional screening.

O9.2 - HULC/miR-186/Twist: a novel molecular axis in the resistance of Ewing Sarcoma to YK-4-279

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Ewing Sarcoma (ES) is characterized by the specific chromosomal translocation between *EWSR1* and *FLI1* genes that results in the production of the aberrant transcription factor EWS-FLI1. YK-4-279 (YK) compound is emerging as a promising agent to address ES pathology because of its ability to block the interaction between EWS-FLI1 and DHX9, a DNA/RNA helicase crucial for EWS-FLI1 oncogenic activity.

Here, we identified HULC (*highly upregulated in liver cancer*) as one of the most downregulated lncRNAs in YK-treated ES cells. Mechanistically, HULC acts as competing endogenous RNA by sequestering miR-186 and repressing its tumor suppressor activity. The negative modulation of HULC after YK treatment correlates to the downregulation of Twist1 protein, which is a direct target of miR-186 in ES cells. Interestingly, high levels of Twist1 are associated to poor prognosis in ES patients and its ectopic overexpression increases the resistance of ES cells to YK treatment. Conversely, inhibition of Twist1 by miR-186 mimic or Twist1 siRNA sensitizes ES cells to YK.

O9.3 - An innovative technology for the direct detection of microRNAs in biofluids

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Circulating microRNAs have been widely proposed as new promising non-invasive biomarkers for several diseases, including cancers. However, their clinical use is limited by available technologies. Most assays for microRNAs quantitation still lack the sensitivity and specificity required for reliable clinical diagnostics. Here, we propose a new assay, that has single-base specificity, and no need for either RNA extraction or target pre-amplification and labeling. This novel compact and miniaturized platform is based on a Silicon Photomultiplier (SiPM)-based device manufactured by Optoi, integrated with DestiNA's abasic PNA probes and SMART chemistry, and a chemiluminescence-based assay. The device showed a Limit-of-Detection (LoD) of 1.6 pM and was validated for the direct detection of hsa-miR-21 in plasma samples of lung cancer patients. We evaluated the concentration of hsa-miR-21 in lung cancer plasma samples via calibration curves with gold-standard TaqMan RT-qPCR. Subsequently, we detected hsa-miR-21 from eight lung cancer plasma samples directly adding magnetic beads coated with DestiNA probes in the plasma samples, obtaining a proof of concept for this innovative technology.

O9.4 - SINE-encoded ncRNA profiling unveils a new layer of epigenetic dysregulation in cancer

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SINE (Alu and MIR) retrotransposons accounts for 13% of the human genome. They are subject to tight epigenetic silencing, but Alu expression was reported to globally increase upon viral infection and in cancer. We recently developed a computational approach exploiting RNA-Seq data to profile SINE expression at single-locus resolution. By Alu transcriptome profiling of tumor RNA-seq datasets in the comprehensive Cancer Genome Atlas (TCGA) and TARGET programs, we are revealing Alu dysregulation signatures of acute myeloid leukemia and other cancer types. We also found that Adenovirus 5 small E1A protein activates Pol III-dependent transcription of hundreds of Alu elements in immortalized fibroblasts, likely through reorganization of the host epigenomic landscape. Alu RNA overexpression in non-infected fibroblasts was found to upregulate the expression of pro-mitotic genes at the mRNA level, but also to produce widespread mRNA-independent alterations in the proteome, in agreement with multiple roles of Alu RNA in gene regulation. Our data point to Alu epigenetic dysregulation as a previously underappreciated molecular signature and mechanistic avenue of cancer.

O9.5 - MiR-663 sustains NSCLC by inhibiting mitochondrial outer membrane permeabilization (MOMP) through PUMA/BBC3 and BTG2

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Treatment of lung cancer is an unmet need as it accounts for the majority of cancer deaths worldwide. The development of new therapies urges the identification of potential targets.

MicroRNAs' expression is often deregulated in cancer and their modulation has been proposed as a successful strategy to interfere with tumor cell growth and spread. We recently reported on an unbiased high-content approach to identify miRNAs regulating cell proliferation and tumorigenesis in non-small cell lung cancer (NSCLC). Here we studied the oncogenic role of miR-663 in NSCLC biology and analyzed the therapeutic potential of miR-663 targeting. We found that miR-663 regulates apoptosis by controlling mitochondrial outer membrane permeabilization (MOMP) through the expression of two novel direct targets PUMA/BBC3 and BTG2. Specifically, upon miR-663 knockdown the BH3-only protein PUMA/BBC3 directly activates mitochondrial depolarization and cell death, while BTG2 accumulation further enhances this effect by triggering p53 mitochondrial localization. Moreover, we show that miR-663 depletion is sufficient to elicit cell death in NSCLC cells and to impair tumor growth in vivo.

P9.1 - Neurogenin 2 expression is regulated by the long noncoding RNA HOTAIRM1 during *in vitro* neuronal differentiation

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Neurons derive from neural progenitors whose differentiation occurs in two distinct steps, each one involving specific regulatory cascades. The initial production of neuronal precursors is followed by the formation of differentiated cell types that acquire and maintain their identity.

The molecular mechanisms underlying these timely-regulated steps are object of intense studies.A prime role in this process is played by proneural genes. They function by activating neuronal-differentiation gene cascades, inhibiting glial cell fates and regulating cell cycle. The proneural transcription factor Neurogenin 2 (Ngn2) is pivotal in the decision between stem cell self-renewal and differentiation. Its expression is tightly regulated during differentiation and even subtle alterations of Ngn2 levels could affect cell fate.

Ngn2 is activated in neuronal precursors to be repressed in post-mitotic cells through a still unknown mechanism. We identified a role in this process for the long noncoding RNA HOTAIRM1, so far described as a regulator of the HOXA genes. We characterized the neuronal isoform of this RNA and found that the nuclear counterpart controls *Ngn2* expression at the epigenetic level.

P9.2 - Noncoding RNA circuitries in motor neuron physiopathology

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Motor neurons (MNs) convey motor impulses from nervous system (NS) to effector organs. They represent the cellular targets of devastating neuromuscular disorders, such as amyotrophic lateral sclerosis (ALS). The central role of MNs in NS physiopathology calls for detailed studies of the molecular networks underlying their differentiation and (dys)function. Taking advantage of an in vitro system to direct mouse stem cells to MN fate, the whole MN transcriptome was profiled. We are analysing how coding and noncoding RNAs (both short and long) integrate into genetic circuitries underpinning MN specification or degeneration.

As for microRNAs, we highlighted a novel, miRNA-mediated post-transcriptional interplay between the ALS-associated RNA binding protein FUS and Gria2, a subunit of the glutamate receptors involved in excitotoxicity, an ALS pathological pathway.

We are also focusing on long noncoding RNAs (lncRNAs), critical regulators of NS development and activity, still poorly characterized in MNs. Molecular and functional analyses of selected transcripts allow us to clarify the mode of action of lncRNAs in MN differentiation and diseases.

P9.3 - Study of circular RNAs function in murine motor neurons

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The RNA binding protein FUS is known to be involved in Different steps of RNA merabolism and is altered in Amyotrophic Lateral Sclerosis (ALS) . Recently, we demonstrated that FUS is

also involved in circRNA biogenesis. We performed a RNA sequencing using murine motor neurons (MNs) derived from embryonic stem cells (mESC). We analysed circRNA expression in wild type MNs as well as in MNs Knockout for FUS and we found several circRNAs affected in FUS KO condition. Among these we selected two circRNAs in order to study their function in MNs, circ31 and circ16. We decided to generating mESC KO by using CRISPR/Cas9 technology in order to identify altered pathways by performing RNA sequencing (RNAseq); we have set up and performed circ31 and circ16 pull down in MNs in order to identify protein interactors by mass-spectrometry analysis and we are studying the subcellular localization of circ-31 and circ-16 by using Fluorescent in situ hybridization both in normal and in stress condition.

P9.4 - microRNA-375 distinguishes neuroendocrine from non-neuroendocrine lung tumors in FFPE samples

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Lung cancer is one of the leading causes of death worldwide. The main challenges in treating this disease are the early diagnosis and the correct classification of the tumour. Clinical variability of lung cancer is high and frequently disentangling this complexity is demanding. In this context, correct discrimination of pulmonary neuroendocrine (NE) from non-NE tumours (squamous cell carcinomas and adenocarcinomas) may be problematic and of critical relevance. The spectrum of NE tumours is various and each type has molecular and phenotypical differences. We explored the possibility of using microRNAs in Formalin-Fixed, Paraffin Embedded (FFPE) samples in order to classify lung tumours. In this study, we analyzed a series of 82 FFPE lung tumour tissues by RT-qPCR and we found that microRNA-375 expression is able to classify low-grade NE lung tumors from non-NE lung tumors, but not large cell NE from small cell lung cancer tumours. Albeit miR-375 deregulation has been observed in several neuroendocrine tumours (intestine, thyroid etc.) this is to our knowledge the first time its upregulation is described as a potential biomarker for lung NE tumours.

P9.5 - *Charme* long noncoding RNA links up with chromatin shaping the 3D nuclear organization

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The mammalian genome contains thousands of long noncoding RNAs (lncRNA), which have been proposed to be fundamental in the regulation of many biological processes. Among them, nuclear lncRNAs are generally associated to chromatin and they can act as genome architects contributing to the formation or disassembly of 3D structures. We identified a subset of new polyadenylated and multi-exonic lncRNAs differentially expressed during murine *in vitro* myogenesis. In particular *Charme* is an abundant and highly conserved noncoding transcript specifically required for *in vitro* muscle differentation. Interestingly, its ablation *in vivo* resulted in a peculiar cardiac phenotype in which the morphology of the heart is remodelled. Mechanistically, *Charme a*cts in the nucleus as a structural RNA, contributing to the formation of chromosome territories where coordinated expression of pro-myogenic genes occurs. Our recent finding that *Charme i*nteracts with Matrin3, a major component of the nuclear matrix, also

creates an intriguing connection between *Charme* expression and chromatin shaping. New data on the need of such interplay for the acquisition of muscle identities will be presented.

P9.6 - The lncRNA HOTAIR governs epigenetic modifications causal to EMT and is controlled by HNF4a

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We recently demonstrated that the long noncoding RNA HOTAIR, largely deregulated in epithelial cancers and positively correlated with their progression, is causal for the repressive activity of the master factor Snail on epithelial genes (Battistelli et al., Oncogene 2017). Based on this evidence, murine HOTAIR sequences has been mutagenized to obtain deletion mutants able to modulate/interfere Snail function in EMT. In particular, the overexpression of the HOTAIR module correspondent to the sole Snail binding domain in Snail-positive cells was shown able to compete with the Snail/HOTAIR/EZH2 tripartite complex formation, resulting in a partial EMT reversal and loss of invasive properties.

Moreover, concerning HOTAIR expression, we also provided evidence that HNF4α, inducer of epithelial differentiation, directly represses HOTAIR transcription in the EMT reverse process called Mesenchymal-to Epithelial Transition. Mechanistically, HNF4α causes the release of a chromatin loop on HOTAIR regulatory elements thus exerting an enhancer-blocking activity.

P9.7 - Neuronal circular RNAs in a mouse model of autism spectrum disorders

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Circular RNAs (circRNAs) represent a class of non coding RNAs characterized by a covalently closed structure, resulting from backsplicing reaction. circRNAs are highly expressed in the brain and potentially involved in plasticity mechanisms. The Autism Spectrum Disorders (ASDs) are developmental disorders characterized by impairments in social interactions and communication, and by repetitive and stereotyped behaviors and interests. It has been hypothesized that the deregulation of the activity-dependent signaling network at the synapses could represent the key molecular component of this pathology (Ebert and Greenberg, 2013). The aim of this study is to elucidate a possible role of circRNAs in ASDs, in particular in neuronal signaling pathways implicated in the pathology.

P9.8 - Dysregulation of microRNA biogenesis in cancer: the impact of mutant p53

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The global miRNA deregulation observed in human cancers is often the result of defects in the miRNA biogenesis pathway. Still, the mechanisms through which miRNAs are regulated in cancer and the connection between oncogenes and miRNA biogenesis remain poorly understood. The TP53 tumor suppressor gene is mutated in half of human tumors resulting in an oncogene

with Gain-Of-Function (GOF) activities. Only a handful of miRNAs have been identified as direct targets of mutant p53 (mutp53) at transcriptional level, and very few data about the role of mutp53 on deregulation of miRNA biogenesis in cancer are available yet.

In the process of studying the mutp53 involvement on the deregulation of miRNA biogenesis, we have obtained some results that strongly suggest a new transcriptionally independent function of mutp53 in miRNA maturation, through a mechanism by which this oncogene is able to interfering with Drosha and Dicer activity, inhibiting miRNA biogenesis. The relevance of the proposed mechanism reside on the oncosuppressor functions of mutp53 dependent miRNAs such as induced cell death in colon cancer cells and tumor growth inhibition *in vivo*.

Collectively, our results depict a novel molecular axis (HULC/miR-186/Twist1) taking part to the antitumorigenic effect of YK in ES.

P9.9 - miR-214 overexpressing mice develop more aggressive mammary gland tumors

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MicroRNAs are small non-coding RNAs acting as negative regulators of gene expression and controlling tumor progression. miR-214 is upregulated in malignant melanomas and breast tumors where it governs dissemination via a pathway involving TFAP2C, ALCAM and the anti-metastatic miR-148b. Inhibition of miR-214 together with overexpression of miR-148b lead to strong reduction of metastasis. To study miR-214 role in endogenous tumors, we generated and crossed a miR-214 overexpressing mouse model with a mouse model of breast cancer progression (MMTV-PyMT). In the double transgenic animals, mammary gland tumor onset was delayed, but miR-214 overexpressing tumors were more aggressive than controls leading to enhanced metastatization. These tumors show increased mesenchymal traits, angiogenesis and inflammation, thus suggesting a contribution of both tumor and stromal miR-214 in tumor progression. miR-214 is highly expressed in stroma cells and its overexpression in the stroma context is sufficient *per se* to promote tumor dissemination. Our results suggest that miR-214 overexpression in an established tumor onset increases metastasis formation acting both on tumor and stromal cells.

P9.10 - The pncCCND1_B non-coding RNA assembles a RNA-binding protein complex to regulate cyclin D1 transcription in Ewing sarcoma cells

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Ewing sarcoma (ES) is a pediatric tumors of bones and soft tissues. The most common mutation is the chromosomal translocation t(11;22), which fuses the *EWSR1* and *FLI1* genes, leading to the *EWSR1/FLI1* chimeric oncogene. The resulting EWS-FLI1 oncoprotein affects transcription and processing of genes involved in proliferation and transformation, among which the *CCND1* gene. *CCND1* is significantly upregulated in ES, and its dysregulation likely

contributes to defective regulation of cell cycle progression. We found that EWS-FLI1 regulates both transcription and splicing of *CCND1,* and its interaction with the RNA helicase DHX9 differently affects these activities. Impairment of DHX9-EWS-FLI1 interaction, by YK-4-279 molecule, reduces EWS-FLI1 activity but enhances SAM68 recruitment to the *CCND1* promoter. Interestingly, SAM68 associates with a *CCND1* promoter associated noncoding RNA (*pncCCND1_B*), negatively affecting *CCND1* transcription. Conversely, IGF-1 mitogenic stimulation of ES cells inhibits *pncCCND1_B*-Sam68 interaction and up-regulates *CCND1* expression. Thus, these studies uncover the *pncCCND1_B* and Sam68 as novel mediators of the oncogenic activity of EWS-FLI1 in ES cells.

10 - Plant Nutrition and Biofortification

O10.1 - Strigolactones effects on root acidification ability

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Phosphorus (P) is an essential element that plays crucial roles in root growth; the phytohormones strigolactones (SL) are also involved in nutrient signalling pathways as their synthesis and exudation are enhanced as part of the acclimatization process to low P[1]. We investigated the effect of exogenous SL and SL-like application (or insensitivity) on the acidification of the growth medium by the root system. *Arabidopsis thaliana* (WT Col-0 and insensitive to SL and SL-like molecules; *d14/kai2* double mutant) and WT tomato seedlings cv M82 were grown for 2 weeks in agarized medium with different P levels and containing synthetic SL or not; then transferred on bromocresol purple-containing plates[2] to visualize acidification halos. Qualitative results obtained so far suggest that in Arabidopsis, exogenous SL could alter root acidification ability in a *d14/kai2*-dependent fashion; and that in tomato, this effect was dependent also on P availability. The possibility that such effect is mediated by specific microRNAs, and its relevance for the plant mineral nutrition are being explored.

[1]Kohlen, W. *et al*. (2011) Plant Physiol 155: 974–987
[2]Planes, M.D. *et al*. (2015) J Exp Bot 66: 813-825

O10.2 - Lead exposure differentially affect growth, antioxidative network and nutrient uptake in metallicolous and non-metallicolous *Zygophyllum fabago* populations

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Zygophyllum fabago is a pioner species that thrives in mine soils characterized by different levels of heavy metals and nutrient deficiencies. In order to obtain information on the adaptative mechanisms allowing *Z. fabago* to combat heavy metal toxicity, seeds collected from three populations, both non-metallicolous (NM) and metallicolous (M), grown in natural environments, were germinated in presence of sub-lethal doses of Pb. Ionomic profiles of seeds were coherent with the different edaphic conditions but they also indicated that these populations pursued specific strategies in order to guarantee the appropriate supply of nutrients in seed storing tissues. Their fenotyping characterization and germinability as well as seedlings fitness clearly showed that M populations were well adapted to high Pb concentrations. Moreover, M seedlings had constitutive higher levels of metabolites involved in antioxidative pathways and, after Pb exposure, showed a more efficient ROS-scavenging capability. The presence of high concentrations of heavy metals in their natural environmant acted as a sort of *"metallopriming"* in this pioneer species that enabled them to counteract the Pb toxic effects.

O10.3 - Scouting cross-kingdom transfer: effect of plant microRNA on the expression of human genes involved in cell proliferation

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An interesting relationship has been observed between diet and human pathologies, including

cancer. Epidemiological studies suggest a role of fruits and vegetables in protection against several diseases and nutrients have been demonstrated to alter gene expression by DNA methylation and histone modifications. Diet has also been found to modulate miRNA expression, leading to a subsequent regulation of the effector genes. On these basis, we have carried out a pilot study, using a combined "*in-silico and wet*" approach, to investigate the potential effects of edible plant miRNA on the expression of human genes involved in cancer onset and progression. Transfecting two human colon cancer cell lines with a mix of plant miRNA selected by *in silico* analysis, we observed a reduction of cell proliferation. Interestingly the expression profile analysis in the same cells showed that 433 genes are differentially expressed (DE), among which 121 are non-coding RNA. Moreover the 69,3% of DE genes, mainly involved in cancer progression and metastasis, are down regulated, suggesting a direct or indirect repression effect of plant miRNAs on a broad range of human genes involved in cell proliferation.

O10.4 - Tvv1 retrotransposon family is activated by seaweed extracts in Micro-Tom genome

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Solanum lycopersicon L. species is a worldwide cultivated crop. Its genome is composed of 62% of retrotransposons LTR and their mobilization is stress related. Recently seaweed extracts (SWE) have been used to improve tomato plant growth and salt stress tolerance. So we have study the effect of SWE (Bioatlantis and Agriges) on Tvv1 retrotransposon family activation in Micro-Tom genome by SSAP profiling. SWE produced new polymorphic bands indicative of different *Tvv1* subfamilies. Research by MiBase database showed that the 70% of new transposition events were preferentially inserted in the retroelements belonging to LINEs and Ty1-*copia* families. Three new insertions were found upstream to the start codon within lipase gene, in *Rcd1* gene and in the transit-polypeptide of Ycf15 chloroplastic gene. In-*silico* analysis of U3 *Tvv1* region promoter showed the presence of three putative *cis*-acting elements, involved in the transcriptional activation of plant defense genes. Our results represent the first direct demonstration that SWE are able to generate new polymorphisms. Their mobilization could play a supplemental role in response to environmental stresses.

O10.5 - In vitro antioxidant and anti-inflammatory activities of some v*iola* L. species in Turkey

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Compounds isolated from *Viola* include flavonoids, phenylpropanoids, terpenoids and essential oils. Polyphenols and flavonoid-rich medicinal plants are known to have protective roles against various diseases. *Viola* L. has exhibited a number of activities such as antioxidant, antibacterial, anti-inflammatory, immunomodulatory. The aim of this study is to investigate the antioxidant and anti-inflammatory activity of methanolic extracts of 9 different *Viola* species *distrubed to Noth Anatolia.* The results showed that all the extracts possessed ABTS free radical scavenging effect as an indicator of antioxidant activity and human red blood cell membrane stabilizing effect as an indicator of anti-inflammatory activity. *Viola alba* subsp. *alba* exhibited the highest red blood cell stabilizing effect (IC50=0,12 mg/ml) compared to other extracts and acetylsalicylic acid which was served as reference (IC50=0,27 mg/ml). *Viola alba dehnhardtii*

was the most active extract on ABTS free radical scavenging, followed by *Viola alba* subsp. *alba* (IC50=0,030 and 0,035 mg/ml, respectively). Further studies are needed to elucidate possible mechanisms and active ingredients.

P10.1 - Determination of magnesium (^{26}Mg) uptake fluxes in grapevine rootstocks through ICP-MS analysis

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Magnesium (Mg) is a macronutrient involved in important biochemical and physiological processes in plants, influencing their growth and development. Mg deficiency is a disorder common in vineyards causing important losses of production.

Grapevine rootstocks are known to display differential tolerance to Mg deficiency. However, the molecular and biochemical bases of these differences are far to be understood. Mg transport across root plasma membrane can be a key step explaining the different tolerance to Mg deficiency. Therefore, a method based on the use of ^{26}Mg as a stable tracer and its quantification by ICP-MS was optimized. The method was first validated on Fercal cutting roots , evaluating the transport rates and the influence of temperature on ^{26}Mg transport. Then, ^{26}Mg uptake was studied in 1103P and SO4, two rootstocks showing different tolerance to Mg deficiency, grown hydroponically in the presence or absence of the nutrient. The experiments were performed with roots from both cuttings and microcuttings, evaluating also the competition of K on ^{26}Mg uptake. Further analyses for the determination of V_{max} and K_m of the high-affinity component of ^{26}Mg uptake are in progress.

P10.2 - Implication of copper and iron availability and sources in plant growth and development

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Copper (Cu) is an essential mineral nutrient for plant growth and development. Copper is present in the active site of numerous enzymes involved in metabolic processes. At the same time, Cu is highly reactive and toxic via the Fenton reaction. Iron (Fe) and Cu homeostasis are deeply linked in the cell metabolism. Iron-Cu crosstalk may influence mineral acquisition and transport. *Non-graminaceous* plants have a reduction-based mechanism to acquire Fe, while grasses rely on the biosynthesis and release of phytosiderophores (PS) and uptake of Fe–PS complex (*Strategy II*). Copper, on the other hand, could be taken up by either type of mechanism: reduction-based and Cu-PS complex transport.

Understanding crosstalk between Fe and Cu nutrition has become a topic of great interest, in terms of reduced crop yield due to the accumulation of Cu in some agricultural soils, thus the need to develop novel strategies to improve growth on those soils.

Based on these considerations, this work investigates the interaction between Cu and Fe acquisition in maize (Cu-tolerant species) and tomato (Cu-sensitive species) with the purpose to better understand how they reciprocally affect Fe and Cu uptake.

P10.3 - Water-extractable humic substances speed up transcriptional response of maize roots to nitrate

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Humic substances are known to positively influence plant growth and nutrition, however the molecular bases of this response are not fully clarified so far.

In the present work, the physiological effects of WEHS (Water-Extractable fraction of Humic Substances) on nitrate acquisition in maize roots were correlated with changes in the root

transcriptomic profile. A faster induction of a higher capacity to take up nitrate in maize roots was caused by WEHS. Comparing the root transcriptomic profile of *Nitrate*- and *Nitrate*+*WEHS*-treated plants *versus Control* (-N) ones, more 2000 transcripts were modulated in presence of WEHS. Among these, genes involved in nitrate transport and assimilation (NRT1s, NRT2s, NAR2.1, NR, GS, GOGAT, CNX, UPM) were strongly modulated by WEHS. Furthermore, also genes known to be related to the nitrogen limitation responses were affected by WEHS, such as transcripts coding for transcription factors (as LBD37, NIN-like protein, NFYA, GRF5) and for enzymes of hormones' metabolism. The modulation of these transcripts might play a crucial role in coordinating the early response to nitrate, favouring its uptake and assimilation in WEHS-treated plants.

11 - Cellular Stress, Apoptosis and Autophagy

O11.1 - A new telomeric and ESCRT complex associated factor

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Nuclear envelope ruptures are doubly linked to cancer: they create chromatin herniations and genome instability and favor cancer cell diffusion in confined spaces. The ESCRT machinery, which has been associated with membrane sealing at trafficking vesicles and at the midbody in cytokinesis, also controls nuclear envelope integrity. AKTIP/Ft1 is a new factor enriched at the nuclear envelope, controlling telomere function, genome stability and nuclear envelope integrity. We identified multiple striking similarities of AKTIP with ESCRTs. AKTIP interacts with vesicle factors, it is enriched at the nuclear envelope and at the midbody, and is structurally similar to the ESCRT I TSG101, which recruits ESCRT complex proteins. Along with this, Ft1 is a concausal element in the diffusion of lymphomas in mice and AKTIP reduction in human lymphoma has been reported. Our hypothesis is that the mechanistic explanation for AKTIP/Ft1 in telomere function, genome stability and cancer diffusion is linked to activity at the nuclear envelope. AKTIP/Ft1 depletion would impinge on nuclear envelope and chromatin organization. Fragile nuclei and DNA damage would contribute to diffusion of lymphomas.

O11.2 - YB-1 recruitment to stress granules in Zebrafish reveals a differential adaptive response

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Survival of mammalian cells exposed to adverse environmental conditions, requires a radical reprogramming of protein translation. Mammalian cells respond to different stressors, organizing structures called Stress Granules (SGs), cytoplasmic foci where untranslated mRNAs are sorted or processed for reinitiation, degradation, or packaging into mRNPs. The Y-box binding protein 1 (YB-1) is an evolutionarily conserved protein that is recruited to SGs and influence their assembly. Here we provide the first analysis of SG formation in response to different stressors in zebrafish (*D. rerio*). We show that, unlike in mammalian cells, YB-1-positive SGs were not assembled following arsenite, copper or hydrogen peroxide treatment in zebrafish PAC2 cells. However, in zebrafish, thermal shock induced YB-1 aggregates containing G3BP1 thereby implying that they were *bona fide* SGs. Moreover, zfYB-1 knockdown prevented formations of SGs under thermal stress and compromised cell viability highlighting the essential role of YB-1 in SG assembly and cell survival. Together our findings reveal that mammalian and zebrafish have evolved a differential adaptive response to environmental stress.

O11.3 - An RBP complex containing DHX30 and PCBP2 operates translation control acting as gatekeeper for p53-dependent apoptosis

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To decipher the molecular mechanisms linking p53 activation in cancer cell to the induction of specific cell outcomes, we recently uncovered how the invariably multifunctional p53 transcriptional output can be directed towards the activation of apoptosis at the level of translation control. This regulation appears to be mediated by a protein complex binding to a 3'-UTR *cis*-element (named CG-motif) present in several apoptotic mRNAs, such as BAK1. PCBP2 and the RNA helicase DHX30 were identified by pull-down, mass spectrometry, co-immunoprecipitation, proximity ligation, alpha assays and polysome profiling as key members of this RNA binding complex. DHX30 in particular was shown to act as a rate limiting inhibitor of apoptosis of p53 wild type cancer cells treated with the MDM2 inhibitor Nutlin-3. For example, the apoptotic prone SJSA1 cells express low levels of DHX30 and a derivate clone with inducible expression of DHX30 shows reduced BAK1 protein levels and a reduction in apoptotic markers, when treated with Nutlin. Conversely, cell cycle arresting HCT116 or U2OS cells express higher levels of DHX30 whose depletion results in increased BAK1 expression and induction of apoptosis.

O11.4 - Nucleolipidic-based Ru(III) nanosystems induce multiple cell death pathways activation in preclinical models of human breast cancer

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According to WHO, breast cancer incidence is increasing and treatment options are limited by toxicity or acquired resistance, so that novel anticancer drugs are required to effective kill specific cancer type. Impaired apoptosis plays a central role in cancer development, limiting the efficacy of conventional therapies. As well, a growing body of evidence is implicating autophagy in both cancer development and therapy. Current research efforts are focused on a deeper understanding of responses to treatments, including cell death pathways activation by novel ruthenium-based drugs, proposed as effective alternative to cisplatin. Aiming at improving their suitability for biomedical applications, we have developed a suite of nucleolipidic Ru(III) complexes preparing stable liposomal nanoformulations endowed with significant antiproliferative activity. Behind an in-depth microstructural characterization, we have focused on the ability of these nanosystems to inhibit proliferation in human breast cancer models. Preclinical investigations revealed cellular responses consistent with both autophagy and apoptosis, suggestive of a possible interplay between different cell death pathways.

O11.5 - Involvement of mitochondria and endoplasmic reticulum in stress response to the environmental pollutant 4-Nonylphenol in a human liver cell line

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4-Nonylphenol (4-NP) is a widely diffused environmental contaminant, employed in several industrial applications. Humans are constantly exposed to 4-NP by ingestion of contaminated food and water. Consequently, the liver, involved in the detoxification of xenobiotics, may

be heavily damaged by chronic exposure to this pollutant. In this study, we investigated the cytotoxic effects of 4-NP on a human cell line from liver (HepG2) by performing MTT and caspase assays. Furthermore, we investigated mitochondria and endoplasmic reticulum (ER) involvement in stress response in the presence of 4-NP. We performed Western blot and PCR analyses to determine the expression of early ER-stress markers (GRP78 and spliced XBP1) and of mitochondrial dynamic behaviour markers, Mfn2 and DRP1. We found that 4-NP was cytotoxic and induced apoptosis in HePG2. Moreover, NP-4 triggered an ER-stress response and alterations of mitochondrial dynamics. These findings support the hypothesis that prolonged exposure to 4-NP through the diet may lead to local damage, involving ER-stress and mitochondria dynamics, at the level of liver tissues, with potentially negative consequences for liver functionality.

P11.1 - 17b-estradiol regulates mitochondrial metabolism in glioblastoma cells

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Glioblastoma multiforme (GBM) is the most malignant type of primary brain tumor in humans and it is often associated with a poor prognosis. High levels of estrogens exhibit oncogenic potential in various organs such as breast, prostate, endometrium and lung through their classic receptors ERa and ERb. To this regard, ERb has been described as a possible tumor suppressor, whereas ERa is involved in cancer progression. Several studies showed that glioblastoma cells express higher ERa receptor levels than the ERb subtype. The aim of the present study was to evaluate in vitro the role of 17b-estradiol (E2) in GBM cell proliferation and metabolism. We observed that E2 was able to regulate positively mitochondrial dynamics, increased OXPHOS genes and mitochondrial biogenesis. In conclusion our data support the hypothesys that locally produced estrogens in glioblastoma cells may act as autocrine factors. In particular, E2 may affect glioblastoma cells by different possible mechanisms, including the regulation of estrogen receptor-mediated transcription of genes involved in cell survival, proliferation, tissue invasion and rewiring mitochondrial fitness in GBM cells.

P11.2 - Alpha Lipoic Acid shows antioxidant and chelating properties against the toxic effects induced by iron overload treatment

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Secondary iron overload syndromes are due to hematological diseases such as thalassemias, transfusion-dependent anemias and myelodysplastic disorders. The aim of the present study was to investigate whether alpha- Lipoic Acid reduces cellular damage induced by iron overload focusing on its antioxidant and chelating properties in vitro and in vivo (Zebrafish).The treatment with Ferric Citrate Ammonium plus alpha- Lipoic Acid was able to reduce the oxidative stress, measured by different methods, induced by FAC alone. Interestingly, ALA co-treatment improves both mitochondrial integrity, increasing EF-Tu protein levels, and cellular homeostasis, decreasing the autophagolysosomes formation compared to iron alone treatment. In addition, co-treatment was able to induce glutathione synthesis and to restore the mitochondrial membrane potential after iron accumulation. in vivo results showed that ALA protects zebrafish intestine, liver, heart and gills from iron overload showing its ability to prevent histological alterations and to reduce oxidative stress. Our findings back up the novel idea that ALA supplementation could be of help in countering secondary iron overload related diseases.

P11.3 - Connections among lipid biosynthesis, ROS metabolism and longevity revealed by the deletion of the hypoxic regulator *KlMGA2* in *Kluyveromyces lactis*

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Fatty acids (FAs) and unsaturated FAs (UFAs) are essential components of functional

membranes. Our studies on the yeast *K. lactis* suggest that glucose, oxygen and temperature regulate FAs biosynthesis through the hypoxic regulator *Kl*Mga2. This transcription factor is homologous to Mga2 and Spt23 of *S. cerevisiae*, two ER proteins activated in hypoxia. Their major target is the FA desaturase gene *OLE1*. The deletion of *KlMGA2* in *K. lactis* generated a viable strain, exhibiting several deficiencies, like reduced cellular fitness and growth, Rag- phenotype, reduced FA content and consequently altered membrane composition, reduced respiration rate and collapsed mitochondria. All these defects were restored by addition of UFAs. Interestingly, absence of *KlMGA2* gene caused increased resistance to oxidative stress and extended longevity. These phenotypes were probably due to an increased expression of catalase and superoxide dismutase genes. These results indicate the existence of a correlation between hypoxia, fatty acid biosynthesis and ROS metabolism in the yeast *K.lactis*, with *Kl*Mga2 playing a role as a direct mediator of both hypoxic response and oxidative stress response/adaptation.

P11.4 - Development of a combination strategy based on ER and oxidative stress in Acute Myeloid Leukemia

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We have previously shown that Acute Promyelocytic Leukemia (APL) cell lines and primary blasts are highly sensitive to a combination of Retinoic Acid (RA) and ER and oxidative stress inducers (Tunicamycin, Tm, and Arsenic Trioxide, ATO), at doses not detrimental for healthy hematopoietic progenitors. This treatment caused aggregation of the APL oncoprotein PML-RARα. Thus, mutant or fusion proteins, easily prone to aggregation or mis-folding, could render the cells sensitive to levels of ER and oxidative stress that can be recovered in their absence.

We found that Acute Myeloid Leukemia (AML) cells, bearing different fusion proteins and the FLT-3-ITD mutation, are highly sensitive to the combination of sub-lethal amounts of RA, Tm and ATO. We observed prolonged activation of the antioxidant response and of the unfolded protein response (UPR), activated by ER stress. The antioxidant agent N-acetyl-cystenine and a UPR inhibitor determine resistance to the treatments. Importantly, the combination of ER and oxidative stress significantly reduces the colony forming capacity of primary FLT3-ITD positive leukemic blasts, but not of healthy progenitors.

P11.5 - Grape seed extracts shows anticancer activity in mesothelioma cell lines

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Grapevine (*Vitis vinifera* L.) is a plant rich of bioactive compounds that have beneficial cardiovascular, chemopreventive, and cytoprotective effects. These molecules include polyphenols that are known for anti-inflammatory, antimicrobial and antioxidant activities and are widely used in nutraceutical and cosmetic fields. Here we describe the biological activity of grape seed and skin extracts of two Italian grape varieties, Aglianico (red) and Falanghina (white) in human mesothelioma cell lines. Grapes seed extracts were able to induce apoptosis in a dose and time-dependent manner in different mesothelioma cell lines. In particular, both extracts induced

apoptosis in cells sensitive (MSTO-221H, NCI-H2452) or insensitive (Ist-Mes2) to standard chemotherapy. Apoptosis was triggered by intrinsic pathway with mitochondrial cytochrome *c* release. We believe these results are relevant for wine and food waste transformation companies allowing the recovery of a waste product of grapes to be reused as a source of nutraceuticals or new drugs.

P11.6 - Novel insights on Sorcin (SOluble Resistance-related Calcium binding proteIN)-dependent drug-resistance in H1299 cancer cell line and its molecular binding partners

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Sorcin regulates calcium channels/pumps in the ER, prevents ER stress and increases escape from apoptosis.

It is overexpressed in several human tumors, is a marker of Multi-Drug Resistance (MDR), is highly expressed in chemoresistant tumor cells, confers MDR when overexpressed, and it is often coexpressed with the xenobiotics efflux pump *Mdr1*.

Although Sorcin is an interesting potential cancer target, its interactors upon calcium binding are poorly known. To gain information about its network of interaction we performed Proteomic Peptide Phage Display, a novel high-throughput method used to characterize protein-protein interactions. To this end we used a peptide library derived from intrinsically disordered regions of human proteome, enriched in short linear motifs.

Our results show that:

Sorcin binds directly to doxorubicin acting as a buffer in the cytoplasm enhancing its accumulation outside the nucleus and then its extrusion through MDR1 pump;

Sorcin silencing, in H1299 cell line, increases the sensitivity towards the chemotherapeutic drug and subsequently cell death upon treatment;

additionally we unraveled the preferred binding motifs towards Sorcin and novel binding partners.

P11.7 - Protective effects of melatonin in inflamed intestinal epithelium are associated with reduced NF-κB activation and changes in DNA methylation status

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Melatonin is the main product of the pineal gland but is also released in the gastrointestinal tract (GIT). Production of melatonin at GIT is independent of the photoperiod and contributes almost completely to plasma melatonin concentration during daylight hours. The physiological role of melatonin at GIT is poorly characterized but recently anti-inflammatory effects have been reported. In this study, we evaluated the effect of Melatonin in intestinal epithelial cells (IEC) stimulated by Interleukin-1β. Our results clearly show that melatonin at micromolar concentrations inhibits the inflammatory response in IEC. The protective effect is expressed through a marked decrease in release and expression of inflammatory mediators, inhibition of DNA damage, and reduced

activation of the NF-κB. Moreover, our results provide evidence that local inhibitor effect of Melatonin can involve an epigenetic mechanism also.

In conclusion, our findings suggest that the intake of small amounts of melatonin, comparable with those found in pharmaceutical preparations used for sleep disorders, can also exert beneficial effects to the gastrointestinal physiology.

P11.8 - Systemic blood pressure alterations contribute to death of retinal ganglion cells in glaucoma

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Glaucoma is chronic optic neuropathy characterized by progressive loss of retinal ganglion cells(RGCs). Normal tension glaucoma (NTG) is reported to be related with systemic hemodynamic factors, such as fluctuating blood pressure and systemic hypotension. In this study, we investigated the syetemic blood pressure alterations contribute to death of RGCs in glaucoma. We used a rat ocular hypertension glaucoma model induced by episcleral vein cauterization and the rats were treated with a hydrochlorothiazide. Apoptosis of RGCs were examined by TUNEL. Expression of various markers related to RGC apoptosis and glial cell activation were analyzed by western blot analysis and immunohistochemical staining of the retina. IOP remained elevated in the cauterized eyes for the 12 weeks experiment. The average number of RGCs decreased significantly, and TUNEL-positive cells were detected in the ganglion cell layer (GCL). Expression of GFAP was increased throughout the retinal layer. These findings suggest that systemic hemodynamic factors may contribute to the changes of glial cells in retina cell death without elevated IOP. The role of this phenomenon needs further investigation.

P11.9 - Role of heme oxygenase-1 in uveal melanoma progression

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Heme Oxygenase-1 (HO-1) catalyzes the enzymatic degradation of heme with the simultaneous release of CO, $Fe^{2+}$and biliverdin. Uveal melanoma (UM) is the most common primary intraocular tumor in adults, with about 1200-1500 new cases occurring per year in the U.S. The aim of the present study was to characterize the HO-1 system in UM cell line and how pharmacological regulation regulates cancer progression. Such results were further confirmed in UM biopsies from our database. Cell proliferation and colony formation capacity weres evaluated by cytofluorimetric assay, Xcelligence technology, clonogenic and wound healing assay. Such analyses were also performed following Hemin (5 and 10 μM) treatment, a pharmacological inducer of HO-1 and CO releasing molecule (CORM-3 and CORM-A1). Our results showed that hemin significantly increased HO-1 gene and protein expression resulting in a significant increase in cancer progression. These results were further confirmed by CO releasing molecules and in patients' biopsies. Our results suggest that HO-1 plays a major role in UM growth and may represent a good candidate to be exploited as a target for new chemotherapic agents.

P11.10 - A comprehensive multisciplinary study on the pathophysiology of the prototype inborn error of metabolism alkaptonuria

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Alkaptonuria (AKU) is a disease associated to low activity of homogentisate 1,2-dioxygenase (HGD that catabolizes homogentisic acid - HGA) due to *HGD* mutations, but with no genotype-phenotype correlation. In AKU, HGA is not metabolized and accumulates as a pigment, following oxidation+polymerization in connective tissues. There is no licensed therapy for AKU.

Thanks to a multisciplinary approach combining genetic analysis, in silico studies, molecular modeling, set up of serum, cell and tissue models, cell biology, biochemistry and post-genomics, histopathology, bioinformatic and analytical chemistry complemented by clinical data, we provided novel relevant insights into AKU molecular mechanisms, connecting cell oxidative stress, apoptosis, autophagy, amyloidosis.

An overview of our more recent findings is presented showing how AKU is a very complex disease. Particularly, we focused on the AKU-associated comorbidities system and on a dedicated digital integrated ecosystem collecting multidisciplinary data, thus providing tools for physicians and researchers, allowing biomarkers discovery for prognosis (so far absent) and diagnosis, therapeutic targets and clinical monitoring.

P11.11 - Elucidation of alpha-synuclein aggregation process by FRET-based biosensors in living cells

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Alpha-synuclein (αS) is the main component of Lewy Bodies, typical hallmarks of Parkinson's Disease. Under pathologic conditions, αS can undergo conformational changes and turn onto β-sheet pathological structures. In order to track αS aggregation *in vivo*, Fluorescent Resonance Energy Transfer (FRET)-based biosensors have been developed. Cyan and Yellow Fluorescent Proteins were cloned separately, at the C-term of WT αS in two different vectors for cytosolic or endoplasmic reticulum (ER)-associated expression. Biochemical and functional validation of the αS biosensors, done in SH-SY5Y cell line and subsequently in an inducible cell model stably expressing αS, under normal and stress conditions, indicated a correct size and localization of the biosensors in both cell lines. Although both basal conditions or the treatments investigated until now did not provide a clear indication of FRET signal, expression of the biosensors in the ER under stress give rise to striking fluorescent dot-like structures resembling protein aggregates. More studies will be necessary to understand the origin of these species and to see if their aggregation can generate a FRET signal *in vivo.*

P11.12 - Polyphenols from *Arabidopsis thaliana* exert protection against Amyloid beta toxicity

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and primary form of dementia. In AD, increased amyloid-beta (Aβ) production leads to oxidative stress, neuroinflammation and neurodegeneration. Polyphenols are well known for their antioxidant, anti-inflammatory and neuroprotective effects and have been proposed as possible therapeutic agents. We investigated the effects of a poyphenolic extract (PE) of *Arabidopsis thaliana* on inflammatory response induced by Aβ on microglia and on the neurotoxicity in a *Drosophila* model of AD.

BV2 cells treated with both Aβ and PE showed a lower proinflammatory (*IL-6*, *IL-1β* and *TNF-α*) and a higher anti-inflammatory (*IL-4*, *IL-10*, *IL-13*) cytokine production compared to cells treated with Aβ only. PE treatment also led to activation of Nrf2-antioxidant response element signaling pathway resulting in protection from the toxic effects of Aβ. To establish whether PE is effective against Aβ-induced neurotoxicity in *Drosophila*,we evaluated the climbing ability of transgenic AD flies expressing human Aβ42 fed with PE. PE significantly restored the impaired locomotor activity further confirming their neuroprotective effects also *in vivo*

P11.13 - The aberrant expression of the mesenchymal variant of FGFR2 in the epithelial context inhibits autophagy

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Signaling of the epithelial splice variant of fibroblast growth factor receptor 2 (FGFR2b) triggers both autophagy and differentiation, while the aberrant expression of the mesenchymal FGFR2c isoform in epithelial cells induces impaired differentiation, EMT and tumorigenic features. Here we analyzed the possible impact of FGFR2c forced expression on the autophagic process in human keratinocytes stably expressing the FGFR2b or FGFR2c isoform. Biochemical and fluorescence approaches, coupled to the use of selective inhibitors of the autophagic flux, showed that ectopic expression of FGFR2c and its signaling inhibit the autophagosome formation. In addition, the decrease of BCL-2 phosphorylation appeared to indicate that FGFR2c signaling could interfere with the early step of the phagophore nucleation. These results provide the first evidence of a negative impact of the out-of-context expression of FGFR2c on autophagy, suggesting a possible role of this receptor in the modulation of the recently proposed negative loop between autophagy and EMT during carcinogenesis.

P11.14 - Role of heme oxygenase 1 in glioblastoma cell proliferation and progression

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Glioblastoma (GB) is the most common and malignant subtype among all brain tumors. Due to its high proliferation rate and the infiltrative and invasive phenotype, the prognosis of patients

with GB remains poor. Heme oxygenase-1 (HO-1) is a cytoprotective microsomal enzyme that catalyzing the degradation of heme in carbon monoxide and biliverdin. Our previous data show that HO-1 promote cancer progression and chemoresistance. The aim of the present study was to investigate the role of HO-1 in GB cell proliferation and progression. Pharmacological induction of HO-1 in different GB cell lines resulted in a significant regulation of cell proliferation and GB progression. Furthermore, HO-1 correlated with a more aggressive phenotypes and patient survival. In conclusion, overexpression of HO-1 is associated with cancer cell growth and progression thus suggesting it may represent an important target for new therapeutic agent development.

P11.15 - Protective effects of 2-fucosyllactose in association with hyaluronic acid against oxidative injury induced by hyperosmolarity

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Ocular dryness is a common disorder characterized by tear hyper-osmolarity and oxidative stress. 2-fucosyllactose (2-FL), a sugar found in the colostrum of lactating women, can efficiently treat experimental atropine-induced dry eye model. Aim of this study was to evaluate if 2-FL in association with hyaluronic acid (HA) could protect rabbit corneal SIRC cells *in vitro* from the damage induced by hyper-osmotic conditions. SIRC cells grown at confluence were then incubated for 6 h or 24 h in culture media in which the osmolarity was raised to 514 mOsm, either in the presence, or in the absence of 2-FL (0.5%) and/or HA (0.15%). Oxidative stress was measured by ROS determination with DCFH-DA and by GSH expression with DTNB. Results showed that the mix of 2-FL with HA, more than either compound alone, was protective against the hyper-osmolar damage, inducing a viability recovery of 15%, a ROS reduction of 50% and a recovery of GSH content of 79% at 6h. Protection was still evident at 24h, even though at a lower extent. Therefore, the association of 2-FL and HA could be a useful addition for the protection of the ocular surface in case of hyper-osmolar conditions and/or oxidative stress.

P11.16 - Toll-Like Receptors (TRLs): molecular characterization in endothelial cells

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The endothelium forms a large network that dynamically regulates the function of the vascular barrier, but endothelial cells play also important roles during infection and trauma, due to their widespread distribution throughout the body. Although they are not considered classical immune cells, endothelial cells express innate immune receptors, including Toll-like receptors (TLRs), which activate intracellular inflammatory pathways.

TLRs agonists, including LPS and bacterial lipo-peptides, directly modulate endothelial cell behavior. The activation of TLRs also regulates the permeability of endothelial cells and the expression of intermediaries of the coagulation pathway.

In order to investigate TLRs activation in endothelial system, we used bEND5 cell line, an immortalized mouse cell line from brain capillary endothelial cells. Cells were exposed to various concentrations of LPS and other microbial agonists and the TLRs were evaluated at the level of mRNA and protein expression. Our results justify further investigation on this cell line for its suitability for *in vitro* studies on the role of TLRs in endothelial activation processes.

P11.17 - Is the mitochondrial compartment involved in U118 glioblastoma cell death induced by the antiglycolytic 3-bromopyruvate?

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Glioblastoma (GBM) is the most common brain tumor. The use of antiglycolytics to target its peculiar energy metabolism highly dependent on glycolysis could be an effective complementary therapy. 3-Bromopyruvate (3BP) is an alkylating agent able to inhibit glycolysis and induce cell death in cancer cell lines. We previously demonstrated that 3BP induces cyt c degradation and autophagy in GL15 GBM cells. In this work, we investigated the effect of 3BP treatment in U118 GBM cells. 3BP caused dose-dependent decline in cell vitality and increase in ROS production. In addition, reducing power drop and cyt c release outside mitochondria occurred, suggesting the involvement of the mitochondrial compartment in 3BP effect. However, released cyt c did not trigger the apoptotic pathway. Preliminary data also exclude necrosis. Given the importance of cardiolipin (CL) in anchoring cyt c to the inner mitochondrial membrane and in sustaining the mitochondrial respiratory activity, CL synthase silencing will be performed in U118 cells, in order to assess the relationship between mitochondrial compartmentand 3BP-induced cell death, and the possibility to sensitize cells to low-dose 3BP treatment.

P11.18 - Post-translational modifications of voltage dependent anion selective channel (VDAC) isoforms from rat liver mitochondria

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VDACs are mitochondrial outer membrane proteins whose primary function is to allow the communication and exchange of molecules.

We have recently reported about the peculiar over-oxidation of cysteines to sulfonic acid in rat liver mitochondria VDAC3. In this work we extended the analysis to the other isoforms.

We determined the VDACs post-translational modifications (PTM). We focused on over-oxidation of cysteines and methionines in VDAC1 and 2 that can be important for the ROS regulation. Interestingly, in VDAC2 we found over-oxidation of cysteines exposed to the intermembrane space, despite quantitatively less abundant than in VDAC3. Surprisingly, also in VDAC1 the only two cysteines show over-oxidization, even though at a lower extent.

We have also checked whether other mitochondrial proteins contain this special PTM. In the proteins passed through hydroxyapatite no more over-oxidized cysteine was detected. Thus oxidation of cysteine to sulfonic acid appears to be an exclusive feature of VDACs.

We will discuss the biological meaning of such VDACs PTMs, in the light of a role in ROS signaling and mitochondrial quality control.

P11.19 - The role of AhR signaling pathways triggered by β-HCH in the progression of prostate cancer

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Prostate Cancer (PCa) pathogenesis and progression could be associated with genetic as well as environmental factors. Organochlorine pollutants (OCPs), such β-hexaclorocyclohexane (β-HCH), can induce an imbalance in cellular homeostasis interfering with hormone signaling pathways through endocrine disruption. OCPs can also interact with the Aryl Hydrocarbon

Receptor (AhR). Aside from modulating the transcription of cytoprotective genes encoding for detoxifying enzymes, AhR seems involved in several signaling pathways triggered by different stimuli. In particular, its interaction with the soluble cytoplasmic kinase Src has been largely described. Thus, it is conceivable to hypothesize that β-HCH could affect AhR activity promoting the PCa progression to a more aggressive hormone-refractory phenotype. This study compares Gleason 6 and 9 FFPE prostate tumor tissues and corresponding tumor cell lines, LNCaP (AR^+) and PC3 (AR^-), treated with 10μM β-HCH in the presence or not of a specific Src inhibitor. Both tissues and cells were analyzed for proteins and genes related to AhR/Src pathways and the obtained results confirm the interplay between AhR and Src in response to β-HCH.

P11.20 - Higginsians: two novel molecules having potential anticancer effects

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Fungal secondary metabolites have been an excellent source of new antibiotics,herbicides,and antitumor compounds.Colletotrichum is a fungal genus comprising a large number of endophytic,saprophytic,and plant pathogenic species.It causes anthracnose disease of fruits and leaves in a wide range of hosts,resulting in severe crop reduction and conspicuous post-harvest losses of tropical and subtropical plants.Species belonging to this genus have been the subject of extensive studies involving their pathogenesis,morphology,multigene analysis,and disease life cycle.Bioactivity-guided fractionation of this extract resulted in the isolation of two novel diterpenoid α- pyrones,named Higginsianins A and B that have been chemically characterized.We present data showing that these compounds have selective anti proliferative and cytotoxic activity against a panel of tumor cell lines.In particular,Higginsianin B treatment,caused significant induction of the cell cycle inhibitor p21 while full length PARP protein decrease,suggesting necrotic cell death.Together, these data indicate that Higg B can be used as a treatment against tumor cells in association with general anticancer chemo-therapies .

P11.21 - Effects of hyaluronic acid based formulation containing amino acids, vitamins, and minerals on oxidative stress using an *in vitro* cell model of UV-A induced damage

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Among the causes of skin photoaging, ultraviolet (UV) radiations induced alteration at the molecular and cellular level resulting in dryness and reduction of skin elasticity. We investigated, the anti-aging and antioxidant effects of hyaluronan formulations based hydrogel. An intradermic formulation based on hyaluronic acid (HA), minerals, amino acids and vitamins, was compared with the sole HA of the same size. HaCaT cells were subjected to UV-A and H2O2 exposure and then treated with growth medium (CTR) combined with medium MW HA or HA-formulation to evaluate their protective ability against stressful conditions. Cells reparation was improved by HA-formulations as evaluated using a scratch in vitro model and Time Lapse Video Microscopy. NF-kB, SOD-2 and HO-1, were significantly reduced at the transcriptional and protein level. γ-H2AX and protein damage assay confirmed also the protective effect against to oxidative stress. Cell treatment with this new formulation, shows marked antioxidant action in cells exposed to UV-A and H2O2. A significant protective effect for hyaluronan based formulation was shown respect to M-HA, thus, supporting the formulation use to treat damaged skin.

P11.22 - Sphingolipid alterations in the cross-talk between primed microglia- and dopaminergic neurons

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Chronic inflammation plays a key role in neurodegenerative disease and in particular in Parkinson's disease, where microglial activation emerged as crucial. Despite intensive studies, the relevant changes associated to microglia activation and its negative effects on dopaminergic neurons remain elusive. We investigated the role of sphingolipids as possible mediators in both microglial priming and microglial-induced neuronal damage. We first observed that priming of microglia leads to increased cellular and extracellular sphingosine-1 phosphate (S1P), which acts as a proinflammatory mediator. GlcCer accumulation also occurred, promoting microglial proliferation. In addition, exposure of dopaminergic neurons to primed microglia medium lead to a marked increase of ceramide and S1P reduction, which emerged as critical in inducing caspase-3 activation and neuron apoptosis. Overall, this study reveals multiple sphingolipid metabolic switches in both microglia and dopaminergic neurons. Understanding how these metabolic switches occur and affect essential microglia and neuronal pathways will possibly help devise strategies aimed to protect dopaminergic neurons in degeneration.

P11.23 - Exposure to particles debris generated from passenger tyres induces genotoxicity and inflammatory response on raw 264.7 cell line

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The particulate matter (PM) from tyre wear is 0.8-7% of air PM10. We evaluated the genotoxic-cytotoxic effects and the induction of the inflammatory response by tyre particles, in the murine alveolar macrophages (RAW 264.7). The particles were analyzed by XRD technique (morphological, qualitative and quantitative analysis of Si, C, O, S, Zn, Mg, Al, Ca and Fe). The tyre PM cytotoxicity was evaluated by the MTS assay at 10-25-50-100-200 μg/ml. A viability reduction was shown at the 50-100 μg/ml, 48h treatment. Tyre PM genotoxicity was evaluated by the micronuclei test used as a biomarker for the evaluation of effects from exposure to mutagenic-carcinogenic compounds. The results shows a dose dependent increase of micronuclei at all test concentrations (25-200μg/ml). The induction of the inflammatory response was evaluated by the TNFatest at 4 and 24 h treatment. TNFaassay shows an increase of the release after 4h at 100-200 μg/ml and at 24h at 25-200 μg/ml. For the purposes of environmental and allergies prevention it would be desirable to reduce traffic, develop tyres with less wear and reduce the amount of inorganic elements and heavy metals in the composition of tyres.

12 - Development, Differentiation and Ageing

O12.1 - Studies on cancer-associated *CDKN1B* mutations: unravelling a novel mechanism for tumor suppressor loss of function

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CDKN1B codifies for $p27^{Kip1}$, a protein initially identified as a CDK inhibitor, belonging, with its siblings $p21^{Cip1}$ and $p57^{Kip2}$, to the Cip/Kip family. By its nuclear CDK inhibiting function, $p27^{Kip1}$ plays crucial antiproliferative roles in response to many different antimitogenic stimuli; conversely, when localized outside the nuclei, it participates to different activities, mostly involved in control of cell motility and invasion. Accordingly, low nuclear $p27^{Kip1}$ levels and cytoplasmic relocalization have been associated to aggressive phenotypes and poor outcome of several human tumors, including colon, breast, prostate and ovarian cancers. Therefore, $p27^{Kip1}$ has been considered a non canonical tumor suppressor, since only very rarely found altered in tumors. Recently, due to the next-generation sequencing and genome-wide association analyses, *CDKN1B* has been found mutated in several human cancers, i.e. hairy cell leukemia, breast cancer and MEN4 (1). Here, we present a biochemical and functional study on a missense *CDKN1B* mutation identified in a parathyroid adenoma, strongly affecting $p27^{Kip1}$ tumor suppressive function.

1. Bencivenga et al., Canc Lett 2017, 403:354-356.

O12.2 - Role of sphingosine 1-phosphate signaling axis in the differentiation of epithelial cochlear progenitors

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Age-related hearing loss affects more than 360 million people and the current unique available treatment is cochlear implant. ERM proteins are cross-linkers that connect plasma membrane and the actin cytoskeleton playing a crucial role in mediating signal transduction; they recently emerged as potent targets regulated by sphingolipids. Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid with a strictly regulated metabolism and it's essential for auditory epithelium maintenance. Our aim is to understand the role of S1P on ERM activation and its possible involvement in inner ear progenitor differentiation. We observed that mouse otic cell line US/VOT-E36, progenitors of cochlear epithelial cells, express all the enzymes involved in S1P metabolism, its transporter SPNS2, $S1P_1$ and $S1P_2$ and that S1P induces a rapid and strong ERM activation via its specific ligation to $S1P_2$. Obtained results suggest an involvement of MAPK and PI3K/Akt pathways in S1P-induced ERM activation. We observed that S1P provoked changes in actin organization and both passive and outward K^+ currents in a $S1P_2$- and ERM-dependent way, suggesting that S1P regulates the appearance of a differentiated phenotype.

O12.3 - The mechanism of neuronal toxicity by oligomers and fibrils of a-synuclein

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Alfa-Synuclein (αS) is an intrinsically disordered protein whose aggregation and conversion into amyloid fibrils is associated with Parkinson's disease (PD). The nature of the toxic species and their mechanism of action remains unclear. We compared the cytotoxic effects induced by stable and well-defined aS aggregated species in cultured human neuroblastoma cells and primary rat cortical neurons. We found that at early incubation times αS oligomers, with solvent-exposed hydrophobic clusters, are significantly more toxic than any other aS species, but that after prolonged incubation with cells, aS fibrils became toxic, whereas unstructured monomers and hydrophilic unstructured oligomers are inert. The neurotoxic pathway induced by cross-b oligomers and fibrils appears to be similar, although with different kinetics, efficiencies and ability to embed their structured hydrophobic core into the lipid membrane. We show that fibril-induced toxicity is correlate to their ability to slowly release oligomers, defining the mechanism of neurotoxicity triggered by soluble b-sheet oligomers, that can be either generated during the aggregation process or released from fibrillar species.

O12.4 - Tbx1 interacts genetically with Vegfr3 to regulate lymphangiogenesis in mice

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Tbx1 is the major gene implicated in 22q11.2 deletion syndrome. The complex clinical phenotype includes vascular anomalies.

In mice, loss of *Tbx1* is associated with a strong reduction in lymphatic vessel density in most tissues and downregulation of *Vegfr3*. We are evaluating the genetic interplay between *Tbx1* and *Vegfr3* in lymphatic vessel development in mice.

To test for a genetic interaction between *Tbx1* and *Vegfr3*, we analysed cardiac lymphangiogenesis in conditional double heterozygous ($Tbx1^{Cre/+}$;$Vegfr3^{flox/+}$) embryos at E18.5. We also generated a transgenic mouse that expresses *Vegfr3* upon Cre recombination (TgVegfr3) that would allow us to manipulate *Vegfr3* expression *in vivo* in a tissue- and time-specific way.

Our results reveal a strong interaction in cardiac lymphangiogenesis. In $Tbx1^{Cre/+}$; $Vegfr3^{flox/+}$ embryos, lymphatic vessels were absent on the ventral side of the heart and strongly reduced on the dorsal side. In addition, vessel morphology was altered. Moreover, we show that the Vegfr3 transgene was able to rescue partially the lymphatic defects in the compound heterozygotes.

This is the first evidence that Tbx1 and Vegfr3 interaction genetically in lymphangiogenesis.

O12.5 - Influence of APOε polymorphism and physical activity on the well-being of erythrocytes: antioxidant capability and cellular membrane assessment.

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Neurodegenerative diseases (NDs) are typified by misfolded oligomers, which has been strictly linked to oxidative stress that triggers damaging of DNA and lipids, and contributes to the alteration of cellular membranes. To evaluate the influence of APOε4 polymorphism, strongly associated with NDs, on the well-being of Red Blood Cells (RBCs), a cohort of healthy human subjects was enrolled to measure antioxidant capability (AOC), and the composition/

oxidative status of RBC membrane depending on the level of physical activity and age.

The results showed that the level of physical activity and age influence AOC and lipid peroxidation, instead APOε4 allele polymorphism influence only lipid peroxidation.Experiments are in progress to determine a variation in the RBC membrane's fluidity and composition (phosphatidylcholine and phosphatidylethanolamine).

Preliminarily, our data confirm erythrocytes as a good model to monitor oxidative stress in peripheral cells, and suggest that both genetic (APOε polymorphism) and environmental factors (physical activity) can modulate the membrane alterations observed in NDs.

P12.1 - Progerin expression induces a significant downregulation of transcription from human repetitive sequences in iPSC-derived dopaminergic neurons

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Repetitive DNA sequences (RS) represent about half of the human genome. RS have a central role in human biology, but are notoriously difficult to study. Here it is presented a pipeline that quantifies transcripts containing RS indipendently from their genomic localization, tolerating mutational noise, all with low computational requirements.

The purpose of this study was to quantify the transcription from RS in a progerin-expressing cellular model of aging. Progerin is a form of lamin A protein causative of the Hutchinson–Gilford progeria syndrome that is also incrementally expressed during the aging process.

Progerin expression strongly downregulates the expression of all the classes of RS. The ALU element was overall the most expressed one. It was expressed on average at 192493.5 RPKM (SE=21081.3) in the controls and dropped to 43760.1 RPKM (SE=5315.0) in the progerin-expressing cells, being statistically significant downregulated (p=0.0005).

These results highlight a global perturbation of these transcripts in a cellular model of aging and provide a direct link between progerin expression and alteration of transcription from human repetitive elements.

P12.2 - Gene regulatory network approaches for studying the evolution of gut patterning

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Comparative gene regulatory network (GRN) approaches have been proven to be very useful in studying evolution of specification processes. Using the sea urchin as main model system, we are studying the GRNs that control the formation of feeding related cell types and organs: the circum-esophageal muscles, the pancreatic cell type and the posterior gut, the latter differentiating into stomach, pyloric sphincter and intestine. The comparison of these different GRNs with their putative homologs in other echinoderm (sea star), vertebrate and also protostome animals highlighted striking commonalities: except for the use of some recurrent sub-circuits (such as the *hnf1-ptf1a* sub-circuit controlling exocrine pancreatic-like cell type formation), these developmental GRNs appear to be subject of considerable rewiring even when they contain the same groups of orthologous genes. Even more prominent is the fact that these genes often display extreme conservation of topology of expression within similar cell types or organs. We are currently using an approach integrating multiple NGS applications, including ATAC-Seq, for the prediction and validation of gene interactions.

P12.3 - Nucleolar tau protein colocalizes with UBTF and is related to neuronal *in vitro* differentiation

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Tau protein was originally identified as a cytoplasmic protein associated with microtubules, and high levels of tau phosphorylations clinically define Alzheimer's disease and its stages. Subsequently, tau protein was also observed in the neuronal cell nucleus, where its function

is not yet clearly understood. Here we analysed the nuclear Tau-1/AT8 epitopes, namely the unphosphorylated or phorphorylated tau protein in the Pro189/Gly207 and Ser202/Thr205 residues, respectively, in the SK-N-BE neuroblastoma cells, and after their differentiation in neuronal-like cells or after exposition to Actinomycin-D. Results demonstrated that Tau-1/AT8 nuclear distribution is related to transcriptional activity of the rRNA genes, with AT8 only detected in the nucleolus of the differentiated and Act-D exposed cells. Conversely, Tau-1 was always detected in the nucleolus, and colocalized with the upstream binding transcription factor (UBTF). Our data indicate AT8 as a marker of neuronal cell differentiation, whose presence in the nucleolus appears to be related to rRNA transcriptional inactivation, on the contrary to Tau-1 epitope that seems to be related to the active rRNA genes.

P12.4 - *AHI1* gene mutations alter ciliopathy-associated molecular mechanisms in Joubert syndrome

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Joubert syndrome (JS) is a rare autosomal recessive condition characterized by a peculiar midbrain-hindbrain malformation, known as the molar tooth sign (MTS). To date, most of the identified causative genes of JS encode proteins involved in cilia function or assembly. We focused our attention on mutations in *AHI1*, the first gene to be associated to JS. In order to analyze how these mutations are involved in pathogenesis of JS, primary fibroblasts from two JS patients with different point mutations in *AHI1*gene (G2168A and A2687G) were used as cellular model in comparison with two healthy donors. Our results show that: 1) AHI1 mutations lead to a constitutive hyperactivation of Wnt signaling with an imbalance between canonical and non-canonical pathway, 2) AHI1 mutations affect ciliogenesis both at morphological and number level. At present, the roles of primary cilia during neural differentiation remain largely vague so the next goal will be to reprogram fibroblasts in iPS cells and to differentiate them in neuronal cells in order to investigate how the impairment of cilia could influence the neural development.

P12.5 - *C. elegans* expressing D76N beta-2microglobulin: a useful model for screening drug candidates targeting amyloidosis

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The availability of living organisms to study key molecular events underlying amyloidogenesis is crucial for the exploration of new therapeutic avenues. The natural variant of β_2-microglobulin (D76N β_2-m) is associated with a fatal familial form of amyloidosis. No animal models have been developed for the study of this disease so far. We employed *C. elegans*, a nematode model well-suited to the investigation of age-related diseases, establishing a transgenic *line* expressing the variant in the muscles cells of worms (PavIs1). Using the INVertebrate Automated Phenotyping Platform (INVAPP) and Paragon algorithm, we were able to detect growth and motility impairment in D76N β_2-m expressing worms. We also demonstrated that, by RNA interfering β_2-m gene, we rescued the defective phenotype. Moreover, the INVAPP/Paragon system enabled demonstration of the efficacy of doxycycline, a drug that inhibit β_2-m fibrillogenesis both *in vitro* and *in vivo*. Thus, a useful *C. elegans* model for D76N β_2-m related amyloidosis has been developed and the INVAPP/Paragon system provides a powerful tool to undertake library-scale screening in the search for candidates able to combat amyloid-induced toxicity.

P12.6 - CRISPR/Cas9-induced inactivation of the autism risk gene setd5 leads to social impairments in zebrafish

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SETD5 gene encodes for a putative histone methyltransferase whose loss-of-function (LoF) mutations in humans have been recently associated to intellectual disability (ID) and autistic spectrum disorders (ASD). The aim of this study is to characterize a *setd5* LoF zebrafish model generated by CRISPR-Cas9 gene editing technique. *setd5* LoF causes microcephaly, a significant reduction of body length and locomotor activity in both zebrafish larvae and adults. Moreover, *setd5* LoF zebrafish adult brains display reduced expression of synaptic proteins. In addition, *setd5* LoF adults are characterized by a reduced social interaction when compared to wild type siblings and these autism-like altered behavioral traits triggered by *setd5* LoF are ameliorated by risperidone, an antipsychotic drug commonly used to treat behavioral traits in ASD patients.

The future perspective is to screen for targeted compounds able to rescue the developmental and behavioral defects in setd5 loF mutants, to identify novel promising therapeutic compounds for individuals affected by ASD and ID due to SETD5 haploinsufficiency.

P12.7 - Demography and ancient/recent population dynamics shape the genetic architecture of human longevity

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Longevity is a complex phenotype resulting from tangled gene-environment interactions. Recent GWASs include subjects of different populations to reach higher statistical power and population evolutionary histories and changing trade-offs between genes and environments during aging are neglected. We propose a *"diachronic"* approach that investigates processes occurred at two timescales, *i.e.* evolutionary and lifespan ones. Genome-wide analysis of 333 centenarians (CENT) compared with 773 geographically-matched healthy individuals showed that: (i) centenarians born in Northern Italy unexpectedly clustered with controls from Central and Southern Italy suggesting that Neolithic gene flow did not favour longevity in this population; (ii) centenarians are characterized by a peculiar genetic signature for the modulation of fatty

acids metabolism; (iii) lifelong non-monotonic changes in the frequency of several alleles revealed different pleiotropy and trade-off mechanisms crucial for longevity. We suggest that demographic history and ancient/recent population dynamics have to be considered to identify genes involved in longevity, which can differ in distinct temporal/spatial settings.

P12.8 - Effects of Notch1 knockdown on the proliferation and the differentiation of human articular chondrocytes

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Purpose: Osteoarthritis (OA) is the main degenerative disease of the joint, altering the differentiation pattern of articular chondrocytes. In mouse, Notch signaling is critical regulator of cartilage development and homeostasis. In human, NOTCH1 pathway is overexpressed in OA articular cartilage compared to healthy chondrocytes. Our aim was to investigate if NOTCH1 knockdown could be a potential therapeutic approach in OA.

Methods: Primary human OA articular chondrocytes were NOTCH1 silenced by siRNA and cultured in 3D up to 3 weeks. Proliferation was assessed by cell cycle and DNA quantification. Cell viability, catabolic factors release and cell differentiation were also analysed.

Results: NOTCH1 silencing reduced active proliferation, but had no effects on senescence and cell cycle regulators. In 3D cultures, mimicking OA progressive differentiation, NOTCH1 silenced cells showed higher viability, reduced differentiation and matrix metalloproteases production.

Conclusions : NOTCH1 silenced OA chondrocytes showed a more helthy phenotype, by reducing terminal differentiation and increasing cell viability. NOTCH1 appears a therapeutic target to reduce OA progression.

P12.9 - The keratinocyte differentiation induced by FGFR2b requires sequential involvement of PKCδ and PKCα

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The tumor suppressor epithelial isoform of the fibroblast growth factor receptor 2 (FGFR2b) induces the early differentiation of human keratinocytes. Moreover, protein kinases C (PKCs) are known to regulate the differentiation program in several cellular contexts, including keratinocytes. Therefore, aim of our study was to clarify if FGFR2b could play a role also in the late steps of keratinocyte differentiation and to assess if this receptor-induced process would sequentially involve PKC isoforms. Immunofluorescence, biochemical and molecular approaches, performed on 2D cultures or 3D organotypic rafts of human keratinocytes overexpressing FGFR2b by stable transduction, showed that receptor signaling induced the precocious onset and an accelerated progression through the entire program of keratinocyte differentiation. In addition, the use of specific inhibitors and gene silencing approaches demonstrated that PKCδ controls the onset of FGFR2b-triggered differentiation, while PKCα plays a role restricted to the terminal stages of the process, sequentially acting via the induction of KLF4 and DLX3, two downstream transcription factors linked by negative loops to the hub molecule p63.

13 - Metabolism and its Regulation in Health and Diseases

O13.1 - Beyond intermediary metabolism: characterization of the moonlighting function of human serine hydroxymethyltransferase

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Serine hydroxymethyltransferase (SHMT) is a metabolic enzyme fundamental in the serine/glycine one-carbon (SGOC) metabolism, which fuels *de novo* biosynthesis of purines, pyrimidines and the production of antioxidant molecules. In human cells there are two genes that encode for different SHMT isoforms: SHMT1, which encodes a cytosolic isozyme, and SHMT2, that encodes a mitochondrial one, the latter gene also encodes a second transcript, called SHMT2α, which localizes into the cytosol. Previously published data from our group suggested a cross regulation between SHMT isozymes in lung cancer cell lines. We hypothesize that the cytosolic enzyme is responsible for the observed regulation, which might occur in a post-transcriptional manner, since our recent results demonstrate that SHMT1 binds to specific RNA sequences *in vitro*, suggesting a regulatory role of the enzyme and, strikingly, we demonstrate that, in turn, the protein-RNA interaction contributes to control the enzymatic reaction of SHMT1 inside the cells. We characterized the RNA binding feature of SHMT1, revealing a complex regulatory network between RNA regulation, metabolites levels and enzymatic activity of SHMT.

O13.2 - Blockade of glutamine synthetase enhances inflammatory response in microglial cells

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Aims: Microglial cells are brain-resident macrophages engaged in surveillance and maintained in a constant state of relative inactivity. However, their involvement in autoimmune diseases indicates that in pathological conditions microglia gain an inflammatory phenotype. The mechanisms underlying this change in the microglial phenotype are still unclear. Since metabolism is an important modulator of immune cell function, we focused our attention on glutamine synthetase (GS), a modulator of the response to lipopolysaccharide (LPS) activation in other cell types, which is expressed by microglia. Results: GS inhibition enhances release of inflammatory mediators of LPS-activated microglia in vitro, leading to perturbation of the redox balance and decreased viability of cocultured neurons. GS inhibition also decreases insulin-mediated glucose uptake in microglia. In vivo, microglia-specific GS ablation enhances expression of inflammatory markers upon LPS treatment. In the spinal cords from experimental autoimmune encephalomyelitis (EAE), GS expression levels and glutamine/glutamate ratios are

reduced.

O13.3 - Molecular identification and functional characterization of a novel glutamate transporter in yeast and plant mitochondria

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The genome of *S. cerevisiae* encodes 35 members of the mitochondrial carrier family (MCF) and that of *A. thaliana* 58 MCF members. Here two members of this family, Ymc2p from *S. cerevisiae* and BOU from Arabidopsis, have been characterized. These proteins were overproduced in bacteria and reconstituted into liposomes. Their transport properties and kinetic parameters demonstrate that Ymc2p and BOU transport glutamate. Transport catalyzed by both carriers was saturable, inhibited by mercuric chloride and dependent on the proton gradient across the proteoliposomal membrane. The growth phenotype of *S. cerevisiae* cells lacking the genes *ymc2* and *agc1*, which encodes the only other *S. cerevisiae* carrier capable to transport also glutamate, was fully complemented by expressing Ymc2p, Agc1p or BOU. Furthermore, mitochondria isolated from wild-type, *ymc2*Δ and *agc1*Δ strains, but not from the double mutant *ymc2*Δ*agc1*Δ strain, swell in isosmotic ammonium glutamate showing that glutamate is transported together with a H^+. It is proposed that the primary function of these proteins is to import glutamate into the mitochondrial matrix for energy production, C1 metabolism and protein synthesis.

O13.4 - Synthesis and breakdown of H_2S, the third gasotransmitter

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Along with NO and CO, hydrogen sulfide (H_2S) is a physiological modulator at low concentrations, while being toxic at higher levels. Synthesized by cystathionine β-synthase (CBS) and few other enzymes, H_2S is catabolized in the mitochondria by sulfide quinone reductase (SQR). As many diseases are linked to altered H_2S metabolism, it is important to understand its regulation under (patho)physiological conditions. We reported that both CO and NO inhibit CBS with high affinity (1), especially in the presence of S-adenosyl-L-methionine (2), and that a CBS variant responsible for classical homocystinuria shows unusually high propensity to CO inhibition, suggesting a novel pathogenic mechanism (3). Here, investigating the effect of hypoxia on H_2S metabolism in colon cancer, we report that hypoxia-treated cells have reduced ability to detoxify H_2S and less mitochondria but enriched in SQR, as an adaptive mechanism to jointly ensure higher H_2S levels with pro-survival effects and mitochondria protection against H_2S toxicity.

1) Vicente et al. (2014) J.Biol.Chem. 289, 8579
2) Vicente et al. (2016) J.Biol.Chem. 291, 572
3) Vicente et al. (2017) Oxid.Med.Cell.Longev. 2017, 8940321

O13.5 - Metabolism in Breast Cancer: A Systems Biology Approach

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One of the major challenges of present day biology is the interpretation of data obtained through high throughput experiments. Methods for combining and interpreting the results from several experiments, although desirable, are still not optimized. The purpose of systems biology is to integrate measured data in models of interacting components, in order to describe them and predict their behavior and specific features. Valid human-oriented genome-scale metabolic models (GSMMs) are already available for optimization and refinement, such as RECON. In this study we applied steady-state genome-scale modelling to shed light on Cancer Associated Fibroblasts (CAFs), the main component of stromal cells of a tumor mass. In fact, the presence of CAFs is associated with a poor prognosis and it is known that they adopt unusual metabolic strategies aiming at sustaining the high metabolic demand of proliferating cells. Two human models were combined and allowed to cross-talk, taking advantage of RNA-seq and exo-metabolomics data with the aim of shedding new lights on the metabolic interactions between CAFs and cancer cells and metabolic advantages gained by tumor mass.

P13.1 - The inhibition kinetics of the Ca^{2+}-activated F-ATPase by F1 inhibitors strengthens its role in the mitochondrial permeability transition pore formation

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The mitochondrial F-ATPase is responsible for ATP production, but also, most likely, forms the mitochondrial permeability transition pore (MPTP), in turn involved in mitochondrial dysfunctions and cell death. Ca^{2+} can replace the natural cofactor Mg^{2+} in the F-ATPase catalytic site and Ca^{2+} increase in mitochondria triggers MPTP. Kinetic analyses point out a different response of the Ca^{2+}-activated F-ATPase with respect to the Mg^{2+} F-ATPase to F_1 inhibitors. Quercetine inhibition of the Mg^{2+} F-ATPase depends on ATP concentration, while the Ca^{2+}-F-ATPase inhibition only depends on Ca^{2+} concentration. The Mg^{2+} F-ATPase inhibition by NBD-Cl decreases when ATP decreases, while the Ca^{2+}-activated F-ATPase inhibition by NBD-Cl is lowered by both Ca^{2+} and ATP reduction. However, NBD-Cl exerts on the two differently activated F-ATPases the same uncompetitive inhibition with respect to ATP and mixed-type inhibition with respect to either divalent cations. The uncompetitive inhibition between Ca^{2+} and Mg^{2+} and the higher steric hindrance of Ca^{2+} suggest that, with respect to Mg^{2+}, Ca^{2+} interacts with different aminoacids to activate the enzyme which would dissipate ATP and open the MPTP.

P13.2 - Role of paraoxonase-2 in chemoresistance in T24 human bladder cancer cells

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Paraoxonase-2 (PON2) is an intracellular antioxidant and anti-apoptotic enzyme. An increased PON2 expression was described in several tumors, including bladder cancer (BC). The aim of this study was to investigate the role of PON2 in chemoresistance of T24 cells, a cell line from human urinary bladder carcinoma.T24 cells were transfected with pcDNA3-PON2 plasmid vector. Control cells were transfected with empty vector (pcDNA3) or treated with transfection reagent (mock). Transfected cells were treated with gemcitabine and cisplatin, drugs used in the BC management, and cell proliferation was measured by MTT assay.

Real-Time PCR and Western blot analysisshowed that, compared with mock and pcDNA3-treated cells, T24 transfected with pcDNA3-PON2 displayed significantly increased PON2 expression. PON2 upregulation led to a significant increase in cell growth, despite the treatment with gemcitabine and cisplatin.

Our results are the first evidence suggesting that PON2 overexpression could be involved in the increased chemoresistance of T24 cells and seem to suggest PON2 as a potential target for silencing in BC to improve the susceptibility to chemotherapeutics.

P13.3 - The approach of intrasite differential inhibition of aldose reductase in counteracting the onset of diabetic complications and inflammation

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Aldose reductase (AR), a NADPH-dependent reductase, is involved in the onset of diabetic complications. Nevertheless, the ability of AR to reduce both aldoses and cytotoxic aldehydes, generated from lipids peroxidation, poses the question of whether AR might be classified as a detoxifying enzyme. In addition, AR is able to reduce glutathionylhydroxynonanal, thus leading

to inflammation through NF-kB activation. These considerations, together with a diffuse failure in developing effective drugs from AR inhibitors, raise doubts as to the unequivocal advantages of inhibiting the enzyme. We have recently proposed a new approach to modulate AR activity through the action of intra-site differential inhibitors (ARDIs) that should be able to affect AR activity depending on the substrate the enzyme is working on. Thus, effective ARDIs should inhibit the reduction of aldoses and of glutathionylhydroxynonanal, with a minimum or null impact on the reduction of cytotoxic aldehydes. Synthetic and naturally occurring molecules have been tested for their *in vitro* action as ARDIs on the human recombinant enzyme. In addition, evidences are provided for the presence of ARDIs in edible vegetables.

P13.4 - *Santolina corsica* Jord. & Fourr. extracts exert pro-apoptotic activity and inhibit breast adenocarcinoma cell (MDA-MB-231) migration

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The anticancer properties of *Santolina corsica* Jord. & Fourr. *n*-hexane (EHS) and methanol (EMS) extracts were herein evaluated, in relation to their chemical profile. EHS and EMS were analysed by gas chromatography-mass spectrometry and high performance liquid chromatography-diode array detection, respectively. Quercetin-like molecules were the dominant EMS constituents, while EHS showed high concentrations of sesquiterpenes and fatty acid esters.

The anti-proliferative activity of those extracts was evaluated on a wide panel of cancer cell lines. Interestingly, anti-proliferative activities of both extracts were found to be very specific for cancer cells, since their IC_{50} values measured on a non-tumorigenic breast epithelial cell line (MCF 10A) were remarkably higher than those found for all the tested cancer cell lines. Moreover, our results proved that they were able to activate the extrinsic apoptosis pathway, reducing motility, as well as invasive and migratory potential of MDA-MB-231 cells, so counteracting their metastatic behaviour. Concluding, EHS and EMS might be taken into account for potential therapeutic applications in cancer treatment.

P13.5 - Harnessing immunometabolism to fight cancer: lessons from glutamine

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The tumor stroma consists of different cell types, among which tumor-associated macrophages (TAMs). Signals from cancer cells are known to hijack TAMs to support immune escape, vessel formation, and metastasis. On this basis, a novel therapeutic approach could be to re-educate TAMs to acquire their antitumor, immunostimulatory functions. Emerging evidence suggests that these different functional states (M1, antitumor-like and M2, protumor-like macrophages) are sustained by distinct "immunometabolic" programs driving specific functional properties in macrophages. Our study shows that pharmacological targeting of glutamine synthetase (GS) reprograms M2 macrophages toward a M1-like functional phenotype through a specific metabolic signature. Genetic deletion of macrophagic GS in tumor-bearing mice promotes tumor vessel pruning, vascular normalization, accumulation of cytotoxic T cells, and metastasis inhibition. These data identify GS activity as a mediator of the proangiogenic,

immunosuppressive, and pro-metastatic function of M2-like macrophages and highlight the possibility of targeting this enzyme in the treatment of cancer metastasis.

P13.6 - Hyaluronan oligosaccharides down-regulate thyroglobulin and NIS expression in human thyrocytes

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Hashimoto's thyroiditis is an autoimmune disease characterized by chronic inflammation and altered function of the thyroid, including hyaluronan (HA) accumulation. During inflammation HA can be degraded into small fragments that, by stimulating the TLR-2, TLR-4 and CD44 receptors, modulate inflammation in a NF-κB dependent manner.

Evidences have shown that exists a correlation between inflammatory state and downregulation of thyroglobulin and sodium iodide symporter (NIS), the transporter that mediates the first step in thyroid hormone biosynthesis.

In light of these findings, firstly we evaluated the pro-inflammatory effect of 6-mer HA oligosaccharides on primary human thyrocyte cultures; then we studied the effects of HA induced inflammation on the expression of thyroglobulin and NIS.

Following 6-mer HA treatment IL-1β and IL-6 concentrations were increased, while thyroglobulin and NIS expression was decreased. On the contrary, the blockage of HA receptors reduced inflammatory mediator production, while thyroglobulin and NIS levels were restored.

These data suggest a possible role of HA induced inflammation in the reduction of iodide transport and thyroid hormone biosynthesis.

P13.7 - *Saccharomyces cerevisiae* as a system to discover beneficial molecules for mitochondrial diseases

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Mitochondrial disorders (MD) are a group of rare clinically heterogeneous conditions, due to an impairment in the process of oxidative phosphorylation, responsible for the synthesis of ATP.

MD may manifest at any age and in virtually any organ, although brain, skeletal muscle, liver and heart are most frequently involved because of their high-energy demand (mitochondrial encephalo-cardio-myopathies). Actually for the majority of these diseases no proven effective therapies exist.

The aim of the project is evaluating the effects of thousands of chemical substances in different MD yeast models, previously characterized in our laboratory, to identify beneficial molecules for specific MD or with a general positive influence on mitochondrial functioning. For this aim we adopted an yeast-based screening called Drug Drop Test. Being all molecules tested FDA approved, this approach of drug repurposing accelerates the discovery of new therapeutics for MD.

Some identified molecules have also shown beneficial effects in other model systems, including human cell lines. The confirmation of these positive preliminary data could lead to new pharmacological approaches for MD.

P13.8 - Effects of oligomerization on peroxisomal import and stability of human alanine: glyoxylate aminotransferase

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The impact of the oligomeric state on peroxisomal import is debated. Alanine:glyoxylate aminotransferase (AGT) is a pyridoxal 5'-phosphate (PLP)-enzyme whose deficit causes Primary Hyperoxaluria Type I (PH1). AGT is homodimeric, but we have engineered a mutant stable in solution as monomer. We used monomeric and dimeric AGT as models to investigate the effect of oligomerization on the intracellular behaviour and import of peroxisomal matrix proteins. Monomerization does not prevent peroxisomal import, but strongly reduces AGT intracellular stability, increases its aggregation propensity, and partly holds the protein in the cytosol, where it could be prone to mitochondrial mistargeting. FRAP experiments show that dimeric and monomeric AGT display an identical peroxisomal import rate in-vivo. Thus, the main factor influencing import is not oligomerization *per se*, but rather upstream events related to the kinetics and equilibrium of the folding process. Besides explaining the effects of PH1 mutations that destabilize the AGT dimer, this is the first study addressing the relationship between folding and targeting for a peroxisomal protein.

P13.9 - Expression of nicotinamide N-methyltransferase in oral malignant melanoma

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Nicotinamide N-methyltransferase (NNMT) seems to have a possible role in cancer biology, but despite this, no data have been reported in oral malignant melanoma (OMM).

In this report, 15 OMM and 15 cutaneous melanoma (CM) were examined for NNMT expression by immunohistochemistry. Serial sections from formalin-fixed, paraffin embedded blocks were cut for each case and mounted on poly-L-lysine-coated glass slides. The sections were incubated for 1 h at room temperature with rabbit polyclonal anti-NNMT antibody (1:1500, Sigma-Aldrich). Extension of NNMT expression was evaluated as percentage of positive cells and staining intensity was reported as follow: negative-weak = 0; moderate-intense = 1.

NNMT expression in tumor cells was significantly higher in CM, while NNMT staining intensity was significantly higher in OMM. Moreover, NNMT staining intensity was significantly higher in OMM cases with ulceration. Regarding the prognostic role of NNMT, univariate analysis showed a negative effect of its expression on the disease-free survival.

In conclusion, we showed different NNMT expression patterns between OMM and CM, suggesting the presence of metabolic differences between these tumors.

P13.10 - Elucidating Thioalbamide activity: new insights into TLMs anticancer mechanism

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Thioviridamide is a structurally novel ribosomally synthesized and post translational modified peptide (RiPP) produced by Streptomyces olivoviridis NA005001. It is characterized by an unusual structure including several thioamide groups, and possesses strong anti-proliferative activity in cancer cell lines. These features led us to investigate the diversity of thioviridamide-like pathways across sequenced bacterial genomes. We isolated and structurally characterized three new thioviridamide-like molecules (TLMs), among them, thioalbamide was tested for its anticancer properties.

This new natural product evidenced anti-proliferative activity, at nanomolar concentrations, on MCF7 breast cancer cell line. Our results also provide new insights on the underlying mechanism of such effect, highlighting the ability of thioalbamide to induce mitochondrial dysfunction, oxidative stress and apoptotic cell death. Our findings are confirmed by the evidence that thioalbamide can inhibit the propagation of cancer stem-like cells, which are strongly dependent on mitochondrial function and are responsible for tumor chemotherapy-resistance, metastasis and recurrence.

P13.11 - Histamine catabolism contributes to cardiac injury by sustaining monoamine oxidase-dependent oxidative stress

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Monoamine oxidase is a major source of oxidative stress in cardiac injury. We investigated if the enhanced MAO-dependent hydrogen peroxide production could increase substrate availability using a metabolomic profiling method. We identified N1-methylhistamine as an important substrate fueling MAO in Langendorff mouse hearts. When hearts were pretreated with the MAO inhibitor pargyline we observed N1-methylhistamine accumulation along with reduced oxidative stress. We showed that synaptic terminals are the major source of N1-methylhistamine. In vivo sympathectomy caused a decrease of N1-methylhistamine levels, which was associated with a marked protection in post-ischemic reperfused hearts. We demonstrate that exogenous histamine is transported into isolated cardiomyocytes and triggers a rise in the levels of reactive oxygen species. Pargyline pretreatment induced intracellular accumulation of N1-methylhistamine along with decrease in ROS levels. These findings uncover a receptor-independent mechanism for histamine in cardiomyocytes. Our study reveals a novel pathophysiological causative link between MAO activation and histamine availability during pathophysiological conditions.

P13.12 - Doxorubicin-induced actin cytoskeleton remodeling was regulated by chronophin activation through RhoA/cofilin signaling in Hela cells

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The cytotoxic property of doxorubicin (Dox) has been widely used in anticancer therapy.

However the precise mechanism of Dox-induced cytotoxicity has not been fully explained. Genotoxic stress not only generates free radicals but also affects actin cytoskeleton stability. In this study, we showed that Dox-induced RhoA signaling stimulated actin cytoskeleton alterations resulting in central stress fiber disruption at early time points and later cortical actin formation at the cell periphery in HeLa cells. Interestingly, activation of a cofilin phosphatase, chronophin (CIN), was initially evoked by Dox-induced RhoA signaling, resulting in a rapid phosphorylated cofilin turnover leading to actin cytoskeleton remodeling. In addition, a novel interaction between CIN and 14-3-3ζ was detected in the absence of Dox treatment. We demonstrated that CIN activity is quite contrary to 14-3-3ζ binding, and the interaction leads to enhanced phosphorylated cofilin levels. Collectively, these data indicated that initial CIN activation regulation could be critical in Dox-induced actin cytoskeleton remodeling through RhoA/cofilin signaling.

P13.13 - *A. thaliana* enables low-cost, easy and fast *in vivo* screening of ERQC modulators

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Modulators of the Endoplasmic Reticulum glycoprotein folding quality control (ERQC) machinery have broad-spectrum antiviral activity against a number of enveloped viruses and have the potential to rescue secretion of misfolded and yet active glycoproteins in rare diseases.

Drug *in vivo* screening in mammals is expensive and fraught with ethical complications in the initial stages of drug development programs.

Conservation of the ERQC machinery across eukaryotes enables transgenic plants for *in vivo* drug screening. We have used the plant response to bacterial peptides to test *in vivo* the effects of *N*B-DNJ, a known ER α-glucosidase inhibitor in mammals. *N*B-DNJ influences the *A. thaliana* response to the bacterial peptide elf-18, but not to the flg-22 peptide, likely because of ERQC dependency of the elf18 receptor (EFR) and/or a glycoprotein member of its signaling pathway. The ERQC impaired plant, *ebs1-3* was also used to rescue the secretion of the responsive mutant BRI1-9.

We propose *A. thaliana* as an attactive platform for low-cost, easy and fast proof-of-concept screening of ERQC inhibitors.

P13.14 - Differential oxidative stress in a Guinea pig transfusion model causes by high- and low-affinity PEGylated hemoglobin-based oxygen carriers

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Hemoglobin (Hb)-based oxygen carriers (HBOCs) are designed as blood substitutes for tissue oxygenation. To investigate the correlation between HBOCs oxygen binding properties and observed oxidative damage, we transfused Guinea pigs with two PEGylated

HBOCs endowed with high and low oxygen affinity, but otherwise chemically identical. We analyzed plasma samples for biochemical markers of inflammation, tissue damage and organ dysfunction, and proteins and lipids extracted from heart and kidney for markers of oxidative damage. Tissue 4-hydroxynonenal adducts, malondialdehyde adducts and plasma 8-oxo-2'-deoxyguanosine exhibited significantly higher levels for PEG-HBOCs in comparison with the control autotransfusion group. For malondialdehyde adducts, a higher level in the renal tissue was observed for animals treated with the high affinity PEG-HBOC, hinting at a correlation between oxygen binding properties and the oxidative stress. This conclusion is also supported by the high affinity PEG-HBOC producing greater tissue oxygenation in comparison with the low affinity one,

P13.15 - New insights on the modulation of human D-amino acid oxidase

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Human D-amino acid oxidase (hDAAO, EC 1.4.3.3) is a FAD-dependent enzyme that catalyzes the degradation of D-amino acids [1]. The favorite substrate is D-serine, the main endogenous co-agonist of the N-methyl-D-aspartate receptors. A dysregulation in processes tuning D-Ser concentration is involved in the susceptibility for various pathologies. Because of its key role played in modulating D-Ser, the interest for the elucidation of the regulation of hDAAO has significantly increased. The structural and biochemical properties of hDAAO have been extensively investigated, anyway the modulation of the enzyme functionality remain elusive. hDAAO is known to be regulated by small ligands and cofactor and by the interaction with pLG72 and bassoon [1, 2, 3]. Furthermore, the consequences of these modifications were poorly investigated. Moreover, it has been suggested that this peroxisomal flavoenzyme [4] could be mistargeted or secreted [5, 6, 7]. Here, we investigated hDAAO post-translational modifications (mainly nitrosylation and phosphorylation): the enzyme modifications and their effects on the properties of hDAAO were studied *in vitro* using the recombinant enzyme.

P13.16 - The protein state of matter: defining a key player in health and disease

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Protein folding-unfolding is a key issue in metabolism, also involved in several diseases, such as Alzheimer's and Parkinson's. Thermodynamically the folding event can be considered as a first-order phase transition. Phases can be easily defined in large thermodynamics systems, but caution is needed while speaking about single molecule.

Here we tackle this problem by means of random matrix theory, using as model the molecular dynamics-derived correlation matrices of Trp-cage (a fragment of exendin-4). The eigenvalue spectra of these correlation matrices show that the low rank modes of Trp-cage dynamics are outside of the limit expected for a random system, both in folded and in unfolded conditions. This shows that the unfolded state is much less random than previously thought. We consider also the bulk eigenvalue spectra of correlation matrices, which represent localized vibrations, as probes of the protein local dynamics in different states. These last analyses show that the correlation matrices describing the folded and unfolded dynamics belong to different symmetry classes, proving that protein folding is a new type of phase transition if considered at single molecule level.

P13.17 - *In vitro* model of hepatic steatosis to test the protective effects of essential oils on lipid accumulation

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The childhood obesity poses important health problems, including liver disease(Mathur P et al. 2007). Non Alcoholic Fatty Liver Disease (NAFLD) is characterized by an increase of intracellular fatty acids (Stiuso et al., 2013). Nowadays there is not a specific therapy but only nutritional guide lines (Rahimlou et al., 2015). Gradual weight loss through increased regular exercise and a low-fat diet appears to be effective. The research project aimed at assessing, the effect of different edible plant extracts as potential nutraceutical agents on NAFLD (Stellavato, Pirozzi et al., 2018) using human hepatic cancer cells as in vitro model (HepG2). Specifically, the HepG2 have been treated with a mix of fatty acids to induce NAFLD. After 24h of treatments with carvacrol, thymol and eugenol the reduction of intracellular fatty acids amount is quantified by Oil Red staining and the activation of peroxisome proliferator-activated receptor (PPARs), that closely correlate to lipid metabolism. Taken together these observations may provide preliminary important data for new functional food/ingredients in prevention of the liver pediatric pathology or even in reducing the fat deposit.

P13.18 - Are D-serine and D-aspartate serum levels putative biomarkers in Alzheimer's disease?

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The diagnosis of Alzheimer's disease (AD) may be delayed because early biomarkers are not available. Hyperactivation of N-methyl-D-aspartate receptors (NMDAr) have been associated with synapse dysfunction in AD. In the past years several studies reported that D-serine is a co-agonist at NMDAr in frontal brain area [1] and AD patients show an increased levels of D-serine in the CSF and in specific brain regions [2]. D-aspartate is an alternative NMDAr agonist and elevated levels of this D-amino acid were found in *post-mortem* AD brains [3].

Here, we measured the levels of D-serine and D-aspartate in the serum of AD patients at early stages of illness and in healthy controls, to assess whether their levels are deregulated in AD. This investigation will pave the way to the use of these selected D-amino acids as biomarkers for this invalidating pathology.

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P13.19 - Evaluation of the crosstalk between eIF6-driven translation and mitochondrial metabolism: a multi-approaches definition

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Eukaryotic Initiation Factor 6 (eIF6) is an oncogenic trans-acting factor in ribosome biogenesis and translation. eIF6 binds 60S and impairs recruitment of 40S. Therefore, eIF6 must be released to allow translational start. We already found that eIF6 increases the translation of mRNAs encoding for transcription factors that induce fatty acid synthesis and metabolic reprogramming. eIF6 is a translation factor acting as a master regulator of metabolism, connecting nutrient sensing and growth factor activity to a specific metabolic programme.

Mitochondrial alterations contribute to the development of metabolic syndrome.

However, the mitochondrial impact of eIF6 has not been yet investigated. To this aim, we analysed, from proteomics to metabolomics signatures, the mitochondrial behaviour of AML-12 (non-tumourigenic murine liver hepatocytes) cell line where eiF6 was down regulated by shRNA.

We found that depletion of eIF6 by shRNA induces profound impact on intrinsic properties and functions of mitochondria, triggering ROS production, impairing the energy production, steering the metabolism toward the up-regulation of aerobic glycolysis and the inhibition of oxidative phosphorylation.

P13.20 - Magnetic resonance spectroscopy-based metabolomic approaches revealed a novel molecular target in metformin treated human glioblastoma cells

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Glioblastoma multiforme is the most aggressive malignant brain tumor because it was refractory to conventional treatments. There is an urgent need to develop novel therapeutic strategies. Metabolomic approaches by magnetic resonance spectroscopy could identify new molecular targets for anticancer strategy. Purposes of this study were: 1) to investigate the alterations of metabolome induced by metformin (anti-diabetic drug); 2) to evaluate the possibility to enhance the antiproliferative effects of this drug. Metformin induced cell growth arrest but not cell death and phoshocholine (PCho, a metabolite linked to phosphatidylcholine (PC) metabolism) accumulation in U87MG cells. Exposure to D609, an inhibitor of PC-specific phospholipase C (PC-plc), induced a reduction in PCho content associated to a significant decrease of cell viability and increased cell death. We provided evidences to support: a) activation of PC-plc and PCho accumulation in metformin-treated cells; b) induction of cell death in U87MG cells simultaneously with a combination of metformin and D609. These results provide the grounds for the possible development of a new multi-targeted approach against this malignancy.

P13.21 - Functional analysis of mitochondrial citrate carrier (SLC25A1) variants linked to combined D-2- and L-2-hydroxyglutaric aciduria

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Combined D-2- and L-2-hydroxyglutaric aciduria (D/L-2-HGA) is a neurometabolic disorder (OMIM: 615182), usually lethal in the first years of life. By whole exome sequencing several missense mutations in the *SLC25A1* gene, encoding the mitochondrial citrate carrier (CIC), were identified in patients, whose biochemical hallmark is combined D-2- and L-2-hydroxyglutaric aciduria. The i*n silico* modeling investigations indicated that the pathogenic *SLC25A*1 mutations would have a deleterious effect on protein function, affecting either substrate binding to the transporter or its translocation mechanism. In addition, the transfection of deficient fibroblasts with wild-type *SLC25A1* restored citrate efflux and decreased intracellular 2-hydroxyglutarate levels, confirming that deficient CIC is the cause of D/L-2-HGA.

We implemented the *in silico* scoring system with the functional assay to analyze the importance of residues affected by all 17 missense variants detected in a total of 26 CIC-deficient patients, showing reduced activities of varying degrees.

This allowed not only a clinical and biochemical overview of the D/L-2-HGA patients but also phenotype–genotype correlation studies.

P13.22 - Bitter taste receptor hTAS2R46 activation regulates mitochondrial Ca^{2+}-buffering in airway smooth muscle cells

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Bitter taste receptors (TAS2R) are discovered to be expressed in extraoral tissues such as the respiratory system, in which is involved in relaxation. We have explored the mechanism by which absinthin, the highly specific agonist of hTAS2R46, could counteract the response induced by histamine in airway smooth muscle cell (ASM). We show that absinthin is able to reduce cytosolic histamine-induced Ca^{2+}-rises. To investigate this mechanism, we infected ASM with aequorin-based Ca^{2+} probes targeted to the cytosol, sub-plasma membrane domains and the mitochondrial matrix showing that such reduction is a consequence of an increased Ca^{2+}-influx into mitochondria. Cytosolic Ca^{2+}-decreases and simultaneous mitochondrial Ca^{2+}-increases are sensitive to a mitochondrial uncoupler, an inhibitor of the mitochondrial uniporter calcium, and to the cytoskeletal disrupter latrunculin; it is inhibited by a hTAS2R46 antagonist and is no longer evident in hTAS2R46-silenced cells, demonstrating that it is hTAS2R46-dependent. All these data demonstrated that mitochondrial Ca^{2+}-uptake can be modulated via a G-protein receptor, thereby adding to the complexity of Ca^{2+}-signalling.

P13.23 - High glucose induces phosphorylation and oxidation of mitochondrial proteins in renal tubular cells: a proteomics approach

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Mitochondrial dysfunction has been thought to play roles in pathogenesis of diabetic nephropathy but with unclear mechanisms. We thus performed proteomic analysis of altered mitochondrial proteome in renal tubular cells induced by high glucose. Mitochondria were isolated from MDCK cells after exposure to normal glucose (5.5mM glucose), high glucose (25mM glucose), or osmotic control (5.5mM glucose+19.5mM mannitol) for 96 h, and subjected to comparative proteome analysis. A total of six differentially expressed proteins among groups were identified by nanoLC-ESI-ETD MS/MS and confirmed by Western blotting. Among several types of potential post-translational modifications (PTMs) identified, phosphorylation and oxidation were most abundant in mitochondrial proteins whose levels were exclusively increased by high glucose. The increases in phosphorylation and oxidation of mitochondrial

proteins by high glucose were validated by Pro-Q Diamond phosphoprotein stain and Oxyblot assay, respectively. These data indicate that phosphorylation and oxidation mitochondrial proteins are, at least in part, involved in mitochondrial dysfunction in renal tubular cells induced by high glucose.

P13.24 - Effects' study of hyaluronic acid and its hybrid complexes obtained through NAHYCO™ technology on an *in vitro* model of osteoarthritis based on two articular cell types

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Nowadays, several studies about osteoarthritis (OA) are focused on development of medical devices able to treat the inflammation and loss of joint viscosupplementation. High molecular weight hyaluronan (H-HA) contributes to preserve the functions of joints but its concentration decreases with OA (Stellavato et al. 2016). We explored the properties of hybrid cooperative complexes formed by high and low molecular weight hyaluronan (HCC) compared to H-HA alone, into an *in vitro* model of OA based on human articular chondrocytes and synoviocytes isolated from pathological joints. We showed that HCC significantly reduced specific biomarkers of OA development respect to H-HA, both at gene (interleukins 6, 8) and protein levels (Cartilage Oligomeric Matrix Protein 2, NF-kB and inflammatory cytokines). Rheological analyses revealed HCC suitability for the viscosupplementation of joints. Our data proved that HCC could become a support for the treatment of OA symptomatology.

14 - Human Genetics and Genomic Diversity

O14.1 - Human genetic diversity and the effects of subsistence strategies

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The genetic diversity of humans is influenced by a combination of both biological and cultural factors. Among them, subsistence strategies are seldom considered.

Subsistence strategies describe the ways in which human populations obtain food and other resources. Their associated set of behaviours can deeply influence a great number of biological and social factors such as diet, mobility, marriage patterns, group sizes, social structure, the relationship with the environment, immunity needs. Those, in turn, may have a strong influence on the variation of the genetic diversity through time.

We compare here the genetic diversity through time on the basis of SNP chip and whole-genome data between different lifestyles. We show that, in multiple geographic regions, differences between populations adopting different subsistence strategies are not only significant, but cannot be the result of environmental factors alone.

This evidence highlights that biases associated with subsistence strategies have to be taken into account when analysing populations with different lifestyles.

O14.2 - Inherited variation in the xenobiotic transporter patwhay and survival of multiple myeloma (MM) patients: an IMMEnSE study

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In the last decades, progresses has been made in prognosis of MM, although it remains an incurable disease. Chemotherapy resistance is a major hurdle for treatment efficacy. Drug resistance can be innate and so driven by genes involved in the drug metabolism pathways. We conducted an association study of 71 SNPs within the major genes in those pathways (*ABCB1*, *ABCC2*, *ABCG2*, *NR1I2/PXR* and *NR1I3/CAR*) in the IMMEnSE consortium, consisting of 1365 MM cases with survival information recruited in 5 European countries. Two of the SNPs showed an association with the survival of MM patients, *ABCB1*-rs2235013 (HR=1.52, 95% C.I=1.18-1.95, p=0.00087) and *ABCC2*-rs4148388, (HR=2.15, 95% C.I=1.44-3.22, p=0.0001). *ABCC2* plays a role in transporting outside cells various anticancer drugs, including several used against MM. In silico analyses predict that the variant alleles of 4 SNPs in LD with *ABCC2*-rs4148388 are associated with increased gene expression. Overexpression of *ABCC2* increases

drug clearance and therefore may induce drug resistance mechanisms. In conclusion, we found a promising association between *ABCC2*-rs4148388 and MM outcome that is comforted by a plausible biological explanation.

O14.3 - A Mendelian randomization approach to predict the risk to develop pancreatic ductal adenocarcinoma

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Objective: Using a Mendelian Randomization approach we tested the hypothesis that the cumulative effect of common genetic variants related to pancreatic ductal adenocarcinoma PDAC risk factors could also predict the risk of developing PDAC. Materials and methods: Using the GWAS Catalog web tool, we identified 320 SNPs strongly associated with several PDAC risk factors. The selected variants were analysed within 3727 PDAC patients and 3522 controls. For each risk factor, we generated a genetic risk score (GRS) adding all alleles known to be associated with that factor. Logistic regression analysis was used to estimate the association between each GRS and PDAC risk. To test this approach, we performed preliminary analyses using genetic variants associated with fasting insulin (FI) level. Results: The results show an association between the score obtained from the alleles associated with FI and the increased risk of developing PDAC (OR=1.03, 95% CI=1.01-1.05, p=0.041). Conclusions: The data reported in our study, although preliminary, suggest that using genetic variants in an MR approach could be a valid and innovative tool to increase our knowledge about risk factors for PDAC.

O14.4 - Exploring the genetic history of Panamanians through ancient genomes

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The Isthmus of Panama was an obligatory corridor for Paleo-Indians moving to South America and had a historical relevance as a strategic node of the Spanish Empire in post-Columbian times. Historical and archaeological studies have proposed an unbroken link between present-day Natives (mostly belonging to Chibchan-speaking groups) and their Paleo-Indian ancestors in the area. This continuity was supported by the molecular dissection of modern Panamanian uniparental haplogroups, but was never tested at genomic level, neither modern nor ancient. In fact, due to the peculiar climatic conditions of tropical regions, no ancient genomes from this region have been published so far.

Within this study, we were able to obtain the first low-coverage genomes from human remains of the Isthmo-Colombian area, excavated in Panama City and radiocarbon dated to

pre-colonial times, from 400 to 1300 years ago. The ancient mitogenomes and Y-chromosomes were classified into specific lineages and the novel genomes were compared with a worldwide genome-wide panel, offering particular insights into pre- and post-Columbian history of the isthmus and confirming a long-standing genetic continuity in the area.

O14.5 - The european heritage of american populations

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The genomic diversity of America has been affected by recent admixture and gene flow that started during the Colonial Era and the Atlantic slave trade, followed by waves of immigration in the XIX century.

Several recent studies have identified multiple layers contributing to the genomic jigsaw of modern day Americans. The big expansion of genomic data and methods able to analyze the variability created by recombination also allows to reconstruct very recent contributions to the ancestry profile of human populations.

Here we applied haplotype-based methods on a genome-wide dataset of ~12,000 individuals from twelve American countries and ~6,000 individuals of European descent to investigate the genetic structure and the admixture history of modern American populations and evaluate the European contribution through time. We highlighted the high complexity underlying the genetic contribution of European populations in America both from geographical and temporal perspectives.

These results help to clarify the details of population movements in the entire American continent and provide relevant information for anthropological, medical and epidemiological studies.

P14.1 - Polymorphism rs7214723 in CAMKK1 and myocardial infarction risk

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Myocardial infarction (MI) is the leading cause of death in industrialized countries. Several risk factors, like unhealthy lifestyle, play an important role but it is increasingly suggestive to believe that the genetic factors and the molecular basis of excitation-contraction mechanisms contribute in modifying an individual's risk.

It is assumed that among all the protein involved in calcium signaling in the heart and the excitation-contraction coupling, calmodulin could be an important regulator because bind and modulated Ca^{2+} channels. Since several works show how some polymorphic variants can be considered predisposing factors to complex pathologies, we hypothesize that the identification of some polymorphic variants of proteins involved in the calmodulin pathway, could be important to understand if MI has genetics bases.

We decided first to focus on CAMKK1, Ca^{2+}/calmodulin-dependent protein kinase kinase I,

because recently it has been observed that CAMKKI , as part of the use of MSC cells, improve cardiac function after MI. We analyze the polymorphism rs7214723 and we conducted a case-control study involving 300 patients.

P14.2 - Dynamics of gene conversion in human Y-chromosome palindromes

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The Male Specific region of the human Y chromosome (MSY) is characterized by the presence of eight massive palindromes. Each palindrome is composed of two large arms separated by a non-duplicated spacer. These elements exhibit an arm-to-arm sequence identity >99.9%, due to gene conversion (GC) events between them. The effect of GC on the genetic diversity of palindromes, as well as its rate and extension, remain largely unexplored. To gain new insights into the evolutionary dynamics of the human Y chromosome palindromes, we analysed by high-coverage next-generation sequencing (>50×) a 30 kb palindrome in 157 samples, chosen to represent the most divergent evolutionary lineages of the MSY. In this analysis we overcame the problem of a non-unique mapping of the sequencing reads, and we performed an analysis of the sequencing depth. The availability of a reliable Y chromosome phylogeny (for the 157 samples), based on the analysis of 3.3 Mb of X-degenerate unique regions, allowed us to identify several GC events and a peculiar mutational pattern within arms. Finally, by mapping GC events across the Y phylogeny, we were able to calculate a precise Y-Y GC rate.

P14.3 - Effect of sexual hormones (estradiol and testosterone) on RNASEL and PTGS2 genes expression and miR-146a expression in keratinocyte cells

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Immunity and inflammatory pathways are important in the genesis of melanoma skin cancers. Functional genetic variation in immune and inflammatory modulators has the potential to affect disease predisposition. In two previous works, we investigated associations between a common polymorphism in miR-146a (rs291016) and its interaction with two SNPs in two of its putative targets RNASEL and PTGS2, and their relation to melanoma. The presence of rs2910164 together

with the minor alleles in PTGS2(rs20417) and RNASEL(rs486907) appeared in both cases associated with a higher risk in the male population and a reduction in risk, albeit not statistically significant, in females. (Association of microRNA 146a polymorphism rs2910164 and the risk of melanoma in an Italian population - Exp Dermatol 2015 Oct). To test the possibility that sex hormones may influence this risk association, we analyzed the effects of testosterone and estradiol (Sigma Aldrich) upon expression of miR-146a, RNASEL and PTGS2 in HaCat cells.

P14.4 - Use of three CRISPR/Cas9-based gene editing systems to study specific alleles within *LGALS3* gene

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Galectin-3 is mainly studied in the context of transformation and progression in cancer field. The single nucleotide polymorphism rs4644 (c.191C>A, p.Pro64His) has been associated with the cancer risk. The change from Proline 64 (broadly conserved across phylogenetically distant organisms) to Histidine is predicted to be damaging. The generation of isogenic cell lines that differ only for rs4644 using CRISPR/Cas9 technology was the first aim of this work. Three different human cell lines (TPC-1, HCT-116 +/+ and non tumoral thyroid Nthy-Ori) were used. Different approaches for DNA strand breaks induction (classical and double nickase strategies), for CRISPR/Cas9 delivery (chemical agent and lentivirus system) as well as the methods to perform the repair (single-stranded DNA oligonucleotides and donor vector), were employed and compared to each other to identify the most effective for specificity, time, and costs. Isogenic cell lines were obtained with variable results depending on the strategy. As published data show that galectin-3 co-works with transcription factors, further step will include the evaluation of how global gene expression could change in base on Pro/His variation.

P14.5 - Rapidly mutating Y-STRs in rapidly expanding populations: discrimination power of the Yfiler Plus multiplex in northern Africa

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The male specific northern African genetic pool is characterised by a high frequency of the E-M81 haplogroup, which expanded in recent times (2-3 kiloyears ago). Because of their recent coalescence, E-M81 chromosomes cannot be completely distinguished on the basis of their Y-STR profiles, unless rapidly-mutating Y-STRs (RM Y-STRs) are analysed. In this study, we used the Yfiler® Plus multiplex, which includes 7 RM Y-STRs, to analyse 477 males from 11 northern African populations sampled from four countries. About 46% of subjects were found to belong to the E-M81 haplogroup, after the analysis of 72 stable biallelic polymorphisms. We found low intra-population diversity indexes, in particular in long-term isolated groups. The AMOVA analysis showed significant differences between countries and populations, with a rough differentiation between northwestern and northeastern Africa, where the Egyptians Berbers from Siwa represented an outlier. The number of chromosomes sharing the same haplotype was

drastically reduced (81 vs. 201) and limited to subjects from the same population, confirming the higher power of discrimination of the Yfiler® Plus multiplex compared to the former kit.

P14.6 - Human genetic diversity of drug metabolizing enzymes: implications in doping control tests

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It is well known that phase I and phase II drug metabolizing enzymes (DME) are involved in the metabolism and the elimination of the majority of drugs, therapeutics and xenobiotics. Genetic variability in enzyme activity determines variation in drug response and hence in the toxicity of a drug. In doping control, the most part of the illicit drugs that are analyzed to the aim to catch cheating athletes are metabolized by DME enzymes. So often, as in the case of the exogenous abuse of naturally endogenous substances, the positivity of the anti-doping test is given by the exceeding of threshold limits over the physiological basal levels. Genetic variation of the DME enzymes determines that athletes may respond differentially to the same dose of a banned drug so that some of them may results positive in a doping control test but some other may not. In this study we show examples of genetic polymorphisms of DME enzymes that may have an impact on the results of the doping control analyses and why it is important to take into account the variability exerted by human genetic polymorphisms when interpreting the results of the anti-doping tests.

P14.7 - Refining the genetic history of Panama through modern and ancient mitogenomes

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The Panama isthmus played a major role both during the first peopling of the Americas, connecting the double continent, and as a critical crossroad of people and goods since the European colonization.

Recent genetic analyses of uniparental markers revealed that the gene pool of the currently "mixed" population of Panama is characterized by an opposite trend: a limited presence of paternal Native lineages, but an overwhelming legacy of maternal Native haplogroups, with some dating to the Paleo-Indian period. However, mitochondrial DNA investigations were limited to the control region variation.

In this work, we refined the fascinating genetic history of Panamanians by extending the analysis: 1) to complete mitogenomes; 2) to all ethnic groups currently present in the Isthmus; and 3) by including ancient pre-Colonial samples. The concomitant analyses of 162 modern and 9 ancient complete mitogenomes presented here for the first time and their comparison with available mitochondrial data confirm a genetic continuity since pre-Colonial time as well as a

much higher than previously reported sequence variation as attested by the numerous newly identified Panamanian specific sub-haplogroups.

P14.8 - The genetic impact of ancient and historical migrations in Southern Italy

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Since ancient times Southern Italy and Sicily have been at the centre of the demographic and cultural network criss-crossing the Mediterranean Sea, bringing into contact European, Asian and African populations. As a result, groups from this area are expected to bear genetic signals related to contributions from the Balkans, Continental Europe, North Africa and the Near East.

Here we investigate ancient signatures in populations from Southern Italy by comparing a genome-wide dataset of 2,622 modern samples (mainly from the Mediterranean basin and including 103 from Southern Italy and Sicily) with a panel of ~2,000 aDNAs available in the literature. Preliminary analyses showed that Apulian, Calabrian and Sicilian populations have high affinities with ancient (3,000 BCE - 1,000 BCE) and modern Greeks, and shared signals from North Africa/Levant/Caucasus.

P14.9 - Analysis of LPI-triggering mutations of SLC7A7: evidence for defective trafficking and activity

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Lysinuric protein intolerance (LPI) is a recessively inherited aminoaciduria due to mutations of SLC7A7, the gene that encodes y+LAT1 light chain of transport system y+L for cationic amino acids (arginine, lysine, and ornithine); to date, 51 different LPI-triggering mutations have been identified.

Here we addressed the effects of four mutations on y+LAT1 protein subcellular localization and transport function. To this end, CHO (Chinese Hamster Ovary) cells were transfected with plasmids expressing wild-type or mutated SLC7A7 associated to eGFP tag for protein visualization; system y+L-mediated arginine transport was assessed in both these cells and in blood monocytes isolated from three LPI patients.

The confocal microscopy analysis revealed the proper membrane localization of only one mutated protein and the cytoplasmic retention of all the others; arginine transport was, however, compromised in all mutated cells.

The application of such an approach to other LPI-causing mutations will provide new knowledge on the functional consequences of each genetic defect at cellular level and will lay the bases for the development of new therapeutic strategies.

P14.10 - Role of epigenetics in idiopathic male infertility

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The etiology of male infertility is often multifactorial and many conditions are known responsible for a possible reduction of fertility potential, but among the various causes 31% is represented by idiopathic infertility. Spermatozoa possess germ cell–specific epigenetic patterns.

One important aspect of the germ cell–specific epigenetic pattern is genomic imprinting. We have decided to investigate the methylation status of the MEST gene promoter, as the hypermethylation of this maternally imprinted gene is significantly associated with decreased sperm count, decreased sperm motility and abnormal morphology. We recruited 100 male subjects who underwent in vitro fertilization (IVF). We analyzed the methylation status of the MEST gene promoter, the global methylation status of sperm cell DNA and the fragmentation of sperm DNA. Increasingly significant data, aimed at explaining some cases of still unresolved male infertility, correlate the DNA integrity with infertility. All data collected has been correlated with the outcome of IVF (pregnancy rate).

P14.11 - Epidermal p63 is essential to control systemic levels of TSLP through repression of Klk6-NFAT signaling

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AEC syndrome (OMIM #106260) is a severe genetic disorder caused by mutation in p63 gene, encoding a master regulator of epidermis, and characterized by congenital erythroderma and severe skin erosions. We generated a conditional knock-in mouse expressing an inducible p63 mutation (L514F) in the epidermis, affected by focal skin erosions, reduced resilience to mechanical stress and premature death.

AEC mice develop a progressive and severe skin inflammation associated with strong epidermal induction Tslp, an IL-7 like cytokine that is released into the blood, causing a dramatic expansion of pre B-cells in the bone marrow and giving rise to an autoimmune lymphoproliferative disorder.

In AEC epidermis Tslp induction is accompanied by strong upregulation of the Klk6 protease, whose gene is directly repressed by functional p63. Klk6 treatment or overexpression of its effector NFAT induce Tslp in keratinocytes. Collectively, these data indicate that extensive skin erosions in AEC syndrome can cause systemic inflammation and an autoimmune lymphoproliferative disorder due to excessive Tslp production, and point to novel therapeutic targets for AEC syndrome.

P14.12 - Evaluation of DNA damage in Myotonic dystrophy cells after treatment with different genotoxic agents

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Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adults caused by (CTG)n expansion in 3'UTR of the *Dystrophia Myotonica Protein Kinase* gene. We demonstrated that DM1 cells show a baseline high number of chromosome instability evaluated as Abnormal Nuclear Morphologies (ANMs) and we observed a significant increase of AMNs frequency after H_2O_2 treatment, greater than that observed in treated healthy cells. On the other hand, after X-ray irradiation, we found a similar increase of chromosome breaks and translocation frequencies in both DM1 and healthy cells. Our first attention was focused on oxidative stress (OS) induced DNA damage repair systems (Mismatch repair – MMR and Base Excision Repair – BER), but their pathways analysis by real-time qPCR showed no difference between treated and untreated DM1 and healthy cells. Furthermore, FPG-Comet Assay indicated a similar trend of repair kinetic in both samples. In order to explain the differences between DM1 and healthy cells in the frequency of chromosome instability observed after OS, our attention was therefore focused on OS detectioan in H_2O_2 treated and untreated DM1 cells and healthy cells.

15 - Neurobiology and Neuropharmacology

O15.1 - Modeling Fragile X syndrome with iPSC-derived neurons as a purpose of deciphering the molecular mechanisms and the neurobiological phenotypes associated with the pathology

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Fragile X syndrome (FXS) is the most common inherited form of human mental retardation and it is caused by expansion of CGG repeat in the FMR1 gene. The resulting epigenetic silencing causes the loss of the fragile X mental retardation protein (FMRP) with defects in the regulation of dendritic spine morphology and synaptogenesis.

The aim of our study is to create an *in vitro* model based on human induced pluripotent stem cells (hiPSCs) with the purpose of deciphering the molecular mechanisms and the neurobiological phenotypes associated with FXS.

We developed a culture system for cortical neuron differentiation and we obtained TUJ1-positive cells to study electrophysiological properties and axon growth dynamics of FXS-iPSCs derived neurons.

Moreover, preliminary calcium imaging and patch clamp recordings suggested a good degree of maturation of these neurons, which form a connected and partially synchronized network. Therefore, the creation of a robust *in vitro* model based on hiPSCs can be used to study FXS in a time frame that is relevant to the disease, understand its mechanisms and allow for therapeutic testing, all in cells carrying the genetic background of individual patients.

O15.2 - M2 muscarinic receptors show inhibitory effects on drug resistance in human neuroblastoma cells

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Neuroblastoma is the most common solid extracranial solid tumor occurring in childhood. One of the property of this tumor is the ability of cancer cells to resist to different drugs; this aspect is known as multidrug resistance (MDR). The MDR can be dependent on different mechanisms including the overexpression of multidrug resistance proteins, which can extrude the drugs from the tumor cells.

In the last decades, the involvement of muscarinic receptors in cancer biology has been reported. Here we demonstrate the ability of M2 muscarinic receptor to inhibit cell proliferation in different neuroblastoma cell lines. Comparing the neuroblastoma cell lines SK-N-BE and chemoresistant SK-N.BE (2c), we found that the selective stimulation of M2 receptor by agonist APE, is able to negatively modulate the chemo-resistance, affecting the expression of multidrug resistance pumps and counteracting cell proliferation when co-administrated with low doses of conventional chemotherapy drugs. The co-treatment of the tumor cells with low doses of M2 agonist and doxorubicin or cisplatin may represent a new therapeutic strategy improving the chemotherapy treatment, reducing the related side effects.

O15.3 - Nitro-oxidative stress dependent Nup153 alteration affects neural stem cell function in a mouse model of Alzheimer's disease

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Impairment of adult hippocampal neurogenesis represents an early critical event in Alzheimer's disease (AD), contributing to memory loss and cognitive decline. Recently, the nuclear pore protein Nup153, has been described as a key regulator of adult NSC plasticity through the interaction with the transcription factor Sox2.

Here we investigated the role of Nup153 in NSC dysregulation in a mouse model of AD. We found that hippocampal NSCs deriving from neonatal 3×Tg mice (AD-NSCs) display lower Nup153 level compared to control. Moreover, Co-IP experiments revealed that Nup153 association with Sox2 was significantly reduced in AD-NSCs compared to WT-NSCs.

Overexpression of Nup153 in AD-NSCs increased their proliferation and migration capacity. Inhibition of nitric oxide synthases (LNAME), antioxidant treatment or gamma secretase inhibition increased the level of Nup153 in AD-NSCs, whereas Ab treatment in WT-NSCs led to Nup153 down-regulation. Thus, increased nitro/oxidative stress may induce Nup153 downregulation through the accumulation of Ab42. Our data suggest that Nup153 may represent a potential new target to ameliorate AD-NSC function and to restore their plasticity.

O15.4 - Human serine racemase, a drug target for neuropathologies

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Human serine racemase (hSR) is a dimeric pyridoxal-5'-phosphate (PLP)-dependent enzyme catalysing the racemisation and the dehydration of L- and D-serine. D-serine is a co-agonist of NMDA receptors and its altered levels have been associated to several neuropathologies. hSR activity is regulated by effectors, as Mg^{2+} and ATP, and by nitrosylation. ATP binds hSR cooperatively to two symmetric sites at the dimer interface and activates the enzyme, while nitrosylation was shown to reduce the activity. The crosstalk between PLP active site, ATP allosteric site and nitrosylation site was explored by ^{31}P NMR, limited proteolysis, site-directed mutagenesis and fluorescence spectroscopy, demonstrating that hSR activity is finely tuned via selection of conformations endowed by distinct catalytic efficiency (1, 2). Reduced nicotinamide derivatives binding near the ATP site resulted effective inhibitors (3). These findings pave the way to the design of allosteric inhibitors of the enzyme for therapeutic purposes.

1) Canosa et al., Sci. Rep. (2018) 8:9016.
2) Marchesani et al., Biochim. Biophys. Acta. (2018) 1866, 813-21.
3) Bruno et al., Biochem. J. (2016) 473, 3505-16

O15.5 - Role of LRRK2 in the regulation of dopamine receptor trafficking

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Up to date, the physiological role of LRRK2 remains largely unknown although a large number of experimental evidence highlights a critical role of LRRK2 in the control of vesicle trafficking that in turn may regulate different aspects of neuronal physiology. We have recently analyzed the role of LRRK2 in regulating dopamine receptor D1 (DRD1) and D2 (DRD2) trafficking. They differ in structural, pharmacological and biochemical properties, as well as in localization and internalization mechanisms. Our results indicate that disease-associated mutant G2019S LRRK2 impairs DRD1 internalization, leading to an alteration in signal transduction. Moreover, the mutant forms of LRRK2 affect the DRD2 turnover by decreasing the rate of trafficking from the Golgi complex to the cell membrane. In the attempt to search for a compound able to counteracts the biological effect of LRRK2 pathological mutants we found that an antiepileptic drug, commonly used in human therapy, is able to significantly, although partially, rescue the LRRK2 G2019S biological effect. All the date underlie a possible therapeutic option for PD treatment by modulating the vesicle trafficking.

P15.1 - Study of the role of calcium on glioma T98G cell line

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INTRODUCTION: Cisplatin (CDDP), one of the most effective chemotherapeutic agents, induces in many patients phenomena of resistance and toxicity. For this reason, new platinum drugs have been developed among which platinum (II) complex [Pt(*O,O'*-acac)(γ-acac)(DMS)] (PtAcacDMS), containing acetylacetonate and a dimethylsulphide.

AIM: Determine whether both compounds cause a cytotoxic increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in human T98G cells.

METHODS: Cells were submitted to continued exposure to CDDP 40 μM or PtAcacDMS 10 μM for 48h. Ca^{2+} imaging and immunocytochemistry were used to assess whether the cytotoxic effect of these drugs involves a remodeling of the Ca^{2+} toolkit and an increase in $[Ca^{2+}]_i$.

RESULTS: PtAcacDMS induced an acute increase in $[Ca^{2+}]_i$ respect to CDDP that was likely to be due to extracellular Ca^{2+} entry and enhanced both cytosolic and endoplasmic reticulum Ca^{2+} concentration after 48h.

CONCLUSIONS: Our results suggest that the development of alternative Pt compounds might evolve into new therapies for the treatment of CDDP-resistant tumors.

P15.2 - Megalencephalic leukoencephalopathy with subcortical cysts protein-1 (MLC1) controls astrocyte activation in response to inflammatory signals

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MLC1 is an astrocyte specific protein whose mutations are responsible for MLC, a rare, incurable genetic leukodystrophy characterized by macrocephaly, epilepsy, myelin vacuolation, deterioration of motor and mental functions. Clinical conditions often aggravate during stress/inflammatory conditions. Although MLC1 involvement in cell volume control and ion exchanges has been demonstrated, its proper function is still unknown. We previously revealed that MLC1 inhibits specific signaling pathways controlling volume changes and astrocyte activation in response to stress (EGFR/ERK). Here we studied MLC1 role in response to inflammatory mediators that are highly produced during brain stress (IL-1b). Using U251 cells and astrocytes from WT and *Mlc1* KO mice we found that WT-MLC1 inhibits IL-1β-mediated activation of EGFR, ERK and NF-kB that, on the contrary, are constitutively activated in KO/MLC1 mutant cells. Moreover, Mlc1 KO causes astrocytosis suggesting MLC1 involvement in the re-establishment of astrocyte homeostasis after inflammation-induced activation. These results open the possibility to select targetable pathways to slowdown MLC pathological processes.

P15.3 - CRISPR/Cas9-mediated targeted gene correction in MLC patient iPSCs

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The generation of patient-specific iPSC lines, in association with CRISPR/Cas9 technology, allows making precise changes in the genomic DNA to study the functional role of genes. Genetic variants of interest can be introduced or corrected to generate isogenic pairs of cell lines that can be compared to reveal cellular phenotypic differences attributable to the genetic variant. Mutations in the MLC1 gene cause a rare form of leukodystrophy, ***M***egalencephalic ***L***eucoencephalopathy with subcortical ***C***ysts (MLC). The hallmarks of this disease suggest that brain damage in MLC patients might be generated by abnormalities in astrocyte function. To shed light on MLC1 function in the brain, we are currently establishing iPSC isogenic cell lines edited through CRISPR/Cas9 to restore the *wt* genotype. Through dedicated softwares, we selected three single guide RNAs (sgRNAs) and assayed them in *vitro* with Cas9 protein to verify their efficacy. The best sgRNA has been then tested in HEK293 cells electroporated with a donor ssDNA. We are now using this sgRNA for homology-directed repair in CRISPR/Cas9 experiments on iPSCs from a patient carrying an exon skipping mutation (c.177+1G>T) in the MLC1 gene.

P15.4 - Hippocampal response to glucocorticoids in Duchenne Muscular Dystrophy: a route to post-traumatic stress disorders?

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Duchenne muscular dystrophy (DMD) is a X-linked disease characterized by muscular wasting due to lack of dystrophin (Dp427), a cytoskeletal protein expressed in both muscle and nervous system. Lack of Dp427 may be responsible for several neurological disturbances described in DMD patients. These could be aggravated by chronic stress conditions, related to patients' quality of life, and therapeutic treatments with glucocorticoid (GC). Here we analyze the response of wild type and dystrophic *mdx* mouse hippocampus, a brain area most affected in DMD, to both acute and sub-chronic treatments with GC. We focused on the modulation of GC receptor (GR) expression, activation and localization, and on cell proliferation and apoptosis occurring at the hippocampal subgranular zone. Differently from wild type, *mdx* hippocampal neurons respond to both treatments as they were chronic exposures. This is known to induce impairment of the negative regulatory activity of hippocampus over the hypothalamus-hypophysis-adrenal axis, therefore amplifying GC production. These events may ultimately lead to post traumatic disorders and, consequently, aggravate the already compromised hippocampal physiology.

P15.5 - Evaluation of neurotoxic effects of platinum compounds on developing rat cerebellum: inflammatory and oxidative stress pathways

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Cancer is currently the second leading cause of death, showing uppermost clinical complexity. Chemotherapy, applied systematically, as standard form of therapy using conventional platinum compounds, causes severe side effects. Thus, any efforts to identify and validate new effective drugs, provoking milder side consequences, is timely and essential. With this aim, the neurotoxic

effects of two platinum-based drugs, i.e. conventional CisPt and newly synthetized PtacacDMS, were investigated in rats, exposed to a single subcutaneous injection (5 mg/kg b.w.) at PD10. Attention was devoted to activation of inflammatory and oxidative stress pathways, evaluated at three different timepoints (PD11, PD17, PD30) during cerebellar postnatal development, using immunohistochemistry. Possible changes in cerebellar *gross morphology* were even evaluated. Our findings demonstrated (i) early triggering of both inflammation and oxidative stress, paralleled by reactive gliosis and anatomical alterations of both fissures and lobules, followed by a surprising complete recovery at PD30; (ii) as a promising result, while CisPt showed marked adverse effects, PtacacDMS caused lesser toxicity.

P15.6 - SLM2 splicing targets are modulated by endocannabinoids in hippocampal neurons

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Alternative splicing is regulated in CNS and contributes to neuron function and plasticity. Neurexins are presynaptic molecules encoded by 3 genes (Nrxns 1-3), each of which generates α long and β short isoforms. Their variability is increased by alternative exons (SS1-6), whose assortments generates hundreds of splice variants. Alternative splicing of SS4, regulated by the splicing factor SLM2, confers to Nrxns different specificity for post-synaptic receptors modulating synaptic plasticity. SLM2 also modulates the splicing of other synaptic molecules, like the Calcium/Calmodulin Serine kinase (Cask). Since endocannabinoids (ECs) negatively regulate neurotransmission modulating the synaptic strength, here we investigate the possible effect of ECs on Nrxns and Cask splicing in hippocampal neurons. Treatment of cultured neurons with the EC anandamide (AEA) causes modification in the splicing pattern of Nrxns (±SS4) and Cask, which correlates with change in SLM2 expression. Our results indicate that AEA modulates splicing of synaptic proteins through the regulation of SLM2 expression highlighting a novel link between EC and synaptic plasticity through a splicing-dependent mechanism.

P15.7 - Plant virus nanoparticles for BBB crossing and drug delivery to medulloblastoma

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Medulloblastoma (MB) is the most common paediatric brain tumor. Currently, a multimodal approach that combines surgery, radio- and chemo-therapy is the most effective treatment, but it has high impact on life quality. The main issue in the development of new drugs against MB is that they should cross the blood-brain barrier (BBB) and reach the tumor in therapeutic quantities. To find a solution, bioinspired/bioengineered auto-assembling protein-based nanoparticles (NPs), such as viruses, are currently considered. We are setting up a "smart" imaging/delivery system based on the two differently shaped plant virus NPs (PVNPs) Potato virus X and Tomato Bushy

Stunt virus, we previously tested for biosafety, biocompatibility and biodistribution. In fact, PVNPs produced in plants could virtually solve difficulties in large scale manufacturing, poor stability, costs and limited availability of approved materials for clinical use. PVNPs will be decorated with peptides enabling BBB crossing and MB cells targeting (taking advantages of *in silico* structure-prediction tools) and will be loaded with doxorubicin for imaging/chemotherapy. Preliminary results will be illustrated.

P15.8 - BDNF anomalies in Niemann Pick type C disease

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Niemann-Pick C1 (NPC1) disease is a lysosomal lipid storage disorder due to abnormal function of NPC1, a transmembrane protein involved in intracellular trafficking of cholesterol. Clinically, NPC1 presents massive loss of cerebellar Purkinje cells, ataxia and neurological manifestations including dementia.

We have previously demonstrated that the early postnatal cerebellar development is impaired in Npc1-deficient mice. The reduced cholesterol availability affects SHH signaling at level of the primary cilium that leads to a reduction of granule neuron precursors proliferative potential. The activation of Shh pathway up-regulates the BDNF expression, which in turn regulates synapse formation as well as connectivity within the cerebellar cortex.

By immunohistochemistry/biochemical approaches, we have observed various defects in the cerebellar BDNF signaling in NPC1-hypomorphic mutant mice and alterations in the fine structure and connectivity of mossy fibers which represent one of the major source of BDNF in the cerebellum.

These results pinpoint BDNF dysregulation as a possible candidate into the molecular pathogenesis of Niemann-Pick type C disease.

P15.9 - Expression of acetylcholine nicotinic receptor a7 in rat Schwann cells: implication in nerve regeneration

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Peripheral Nervous System is able to regenerate. During regeneration, Schwann cells (SCs) assume a phenotype known as Repair Schwann Cells, relevant for promoting an anti-inflammatory environment and axonal regeneration. SCs are cholinoceptive and express muscarinic receptors. Recently, we have characterized the expression of α7 nicotinic receptor. Normally this receptor is fainlty expressed in SCs, as observed both in vivo and in vitro. Its expression significantly increased after nerve injury. In fact in presence of Bradykinin (Bk), a neuropeptide known for its pro-inflammatory effects, the expression of α7 significantly increased both in sciatic nerve fibers and in SCs in vitro. The treatment of cultured SCs with α7 selective agonist (R)-ICH3, after BK treatment, counteracts inflammatory environment in terms of cytokines, growth factors and proteases production, causing a decreased expression of TNFα and modulating tPA, uPA, and MMPs involved in the migration processes and growth factor production. These results suggest that α7 nicotinic receptor may be essential in the establishment of a microenvironment improving peripheral nerve regeneration.

P15.10 - Cholinergic effects mediated by M2 muscarinic receptor in human Schwann-like cells induced from adipose mesenchymal stem cells

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Schwann cells (SCs) have an important role in peripheral nerve regeneration but there are several restrictions on their clinical application. Adipose derived stem cells (ASCs) present good properties for cell therapies. When exposed to selective growth factors, they can acquire a SC-like phenotype (dASCs), expressing key SCs markers. Our group has demonstrated in rat model that M2 muscarinic receptor causes *in vitro,* a reversible arrest of cell proliferation, increasing SCs myelinating phenotype. Human dASCs, as rat dASCs, express muscarinic receptors. In the present work we evaluate if M2 muscarinic receptor activation may contribute to human dASCs proliferation and phenotype. M2 selective activation by selective agonist APE, causes a decreased cell proliferation, modulating the expression of gene involved in the proliferative state (i.e. c-jun and egr2) and neurotrophic factors. Although further analyses are needed to best characterise the role of M2 receptor, these data are the first evidence that its selective activation may have effects also on human dASCs proliferation and may favourite a neuroprotective environment relevant for nerve regeneration.

P15.11 - Altered D-serine levels and neutrophils activation as early markers of inflammation: an *in vitro* study

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Inflammation is a hallmark of Alzheimer's disease (AD) and innate immune cells likely contribute to the pathogenesis. In AD affected individuals, neutrophils adhere to and spread inside brain venules and are present in the parenchyma, along with Neutrophil Extracellular Traps (NETs). Hence, the understanding of the molecular machinery involved in inflammatory mediated neuronal damage in these peripheral immune cells is crucial to identify biomarkers for a precocious diagnosis.

The flavoenzyme D-amino acid oxidase (DAAO) was shown to be expressed in the granule fraction of mature human neutrophils. It is responsible for the catabolism of the NMDA receptor co-agonist D-serine, leading to the production of the ROS specie hydrogen peroxide. Worthy of note, increased D-serine levels were detected in AD patients. Preliminary data on healthy donors, showed that D-serine treatment results in neutrophils activation and DAAO upregulation. These findings prompt us to elect activated neutrophils for evaluating the relationship between: altered levels of D-serine, DAAO expression/activity and oxidative stress (i.e. increase ROS concentration), as early biomarkers of inflammation in AD.

P15.12 - Cellular studies on human D-aspartate oxidase

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The flavoenzyme D-aspartate oxidase (DDO) selectively degrades D-aspartate (D-Asp), which in the CNS can act as an agonist of NMDA receptors (NMDAR), modulating their activation state. Studies performed in animals models (*DDO*$^{-/-}$ mice) demonstrated that the persistent deregulation of D-Asp levels causes age-dependent effects: an improvement of cognitive performances related to NMDAR-mediated neurotransmission in young mice, followed by a rapid deterioration of these processes leading to precocious brain aging [1]. These

findings indicated that DDO plays a crucial role in the brain, strictly regulating D-Asp levels and preventing NMDAR hyper stimulation.

The UniProtKB database reports three human DDO (hDDO) isoforms (constituted by 369, 341 and 282 amino acids), but little is known about their expression, distribution at the cellular levels and the mechanisms involved in the regulation of the enzymatic activity. To fill this gap we generated U87 human glioblastoma cell clones stably expressing the hDDO 369 or the hDDO 341 isoforms. Here we reported preliminary data on their subcellular localization and functional properties.

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P15.13 - Transcriptional profile of adult dopaminergic neurons in *Drosophila melanogaster*: a comparison between wild-type and Parkinson's-related *Dj1* mutant flies

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DJ-1 has been associated to a recessive form of Parkinson's disease. DJ-1 likely acts as a neuroprotective protein. *Drosophila* has two *Dj-1* paralogs (*Dj-1α* and *Dj-1β)*, which show a conserved protective function. How exactly DJ-1 exerts its role is still unknown.

A possible mechanism has been linked to a DJ-1 function in transcriptional modulation of protective genes. To explore this hypothesis, we obtained the transcriptional profile of adult dopaminergic (DA) neurons in wild-type and *Dj-1α⁻;β⁻* flies, using a technique able to detect the transcriptional state of specific cell lineages without cell isolation. The approach is based on the use of flies transgenic for the *E. coli* DNA adenine methyltransferase linked to the RNA polymerase II (Dam-Pol II). In wild-type and *Dj-1α⁻;β⁻* DA neurons, we induced the synthesis of Dam-Pol II that bound transcribed genes, methylating Adenines in the GATC sequences. Sequencing of methylated DNAs allowed to profile the RNApol II occupancy in these specific neurons.

A significant variation in the transcriptional profile of DA neurons in the different genetic conditions was detected and a first computational analysis of the data will be presented.

P15.14 - New roles for neuroligins/neurexins families during nervous system development

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Neurexins (NRXNs) and neuroligins (NLGNs) are the best-characterized synaptic cell-adhesion molecules required for synapse maturation and function in the central nervous system. To investigate their possible additional roles during nervous system development, we have evaluated the involvement of NLGNs/NRXNs in neurite outgrowth, using NSC34 cell lines transfected with a construct expressing the fluorescent NLGN3 wt and R541C mutated form. The data obtained suggest that neurons expressing NLGN3 wt, differentiated in presence of 1mM dbcAMP, produce longer neurites respect to neurons expressing NLGN-R541C, that present an higher number of neurites shorter in length. These results suggest that the over-expression of NLGN3 wt may promote the terminal differentiation of cultured neurons. Interestingly the untrasfected NSC34 cells express NLGN2 and NRXN-1 proteins, but their levels are progressively down-regulated when either NLGN-3 wt or NLGN3-451C are over-expressed, suggesting a possible cross-regulation between these proteins. Our data indicate that NLGNs/NRXNs may contribute to neuron differentiation during NS development before acquiring the role of synaptic proteins in the adult.

16 - Immunology and Host-Pathogen Interaction

O16.1 - Strain level analysis of intestinal fungi and immune mediated host-yeast interaction

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Evidences indicated *mycobiota* as a central player in host-microbe interactions. Investigation of gut fungal communities in Inflammatory Bowel Diseases (IBD) revealed a possible role of fungi in the course of inflammation.

We investigated the immunomodulatory features of either *S. cerevisiae* or *C. albicans* isolates from IBD patients, characterizing phenotypically and genotypically for traits related to adaptation to gut environment, in order to understand the strategies related to interplay or evasion of host immune system.

Analysis of cell wall sugar composition allow to discriminate gut-derived strains from isolates from other origins. The comparison of immunomodulatory properties suggests a strain-specific pattern of cytokines in response to *S. cerevisiae* and *Candida* isolates from diverse ecological niches.

Understanding of the impact of fungi on host's immune system depends on strain-specific phenotypic and genomic characteristics, able to modulate their mechanism of interaction with the host immune system. The frontiers of microbiology and immunology studies, thus, should move from species level generalizations to the understanding of strain level networks of interactions.

O16.2 - Beneficial effect of cathelicidin on treatment of hypersensitivity pneumonitis induced by *Pantoea agglomerans – in vivo* studies

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Cathelicidin (CRAMP) is defense peptide with a wide range of biological responses including: antimicrobial, immunomodulatory, wound healing. Due to its original properties the usefulness of CRAMP in prevention/treatment of pulmonary fibrosis was assessed in mice model of hypersensitivity pneumonitis (HP).

The studies were conducted on mice strain C57BL/6J exposed to saline extract of *Pantoea agglomerans* cells (HP inducer). Cathelicidin was administering in a form of aerosol during and after HP development. Changes in composition of immune cells subopulations were monitored in lung tissue by flow cytometry. Extracellular matrix deposition as well as concentration of proinflammatory and profibrotic cytokines were examined in lung homogenates by ELISA method.

Perform studies revealed that cathelicidin silencing immune reaction induced by mice chronic exposure to *P. agglomerans* and significantly inhibited hydroxyproline and collagen deposition in lung tissue. Beneficial effect of CRAMP on HP treatment was associated with restoring the balance in quantity of immune cells and cytokines production.

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O16.3 - How to control *Pseudomonas aeruginosa*-induced pneumonia? A lesson from derivatives of the amphibian skin peptide esculentin-1a

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The bacterium *Pseudomonas aeruginosa* is an alarming human pathogen causing infections e.g. pneumonia, especially in cystic fibrosis patients. Naturally occurring antimicrobial peptides (AMPs) hold promise as novel therapeutics. We discovered that the frog skin-derived AMP Esc(1-21) has rapid killing kinetics against both free-living and biofilm forms of *P. aeruginosa*, with membrane-perturbing activity as a plausible mode of action limiting the emergence of resistance; the ability to protect host from lung Pseudomonas infection after a single intra-tracheal instillation at a very low dosage without provoking an inflammatory response; the ability to stimulate migration of bronchial cells and, presumably, to accelerate the recovery of an injured bronchial epithelium. Furthermore, a diastereomer of Esc(1-21), containing two D-amino acids was found to be less cytotoxic; more stable and with better *in vivo* efficacy. Finally, we discovered that polymeric nanoparticles made of poly(lactic-co-glycolic) acid represent a promising tool for pulmonary delivery of peptides and their sustained release.

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O16.4 - Escaping the oxidative burst: dual role of the *P. aeruginosa* superoxide dismutases

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Macrophages activate different mechanisms to eliminate *Pseudomonas aeruginosa*, an opportunistic pathogen that can cause significant disease. Generation of reactive oxygen species (ROS) represents the first response to incoming bacteria and contributes to their rapid killing. Indeed, we have previously demonstrated that *P. aeruginosa* infection of macrophages leads to assembly and activation of NADPH oxidase (NOX2 isoform), rapidly increasing the production of intracellular ROS (oxidative burst) which in turn contribute to kill the engulfed bacteria.

Here we have investigated the role, if any, of the bacterial scavenger enzymes, such as the superoxide dismutase (SOD), in detoxifying the intracellular environment. SODs dismutate the superoxide (O_2^-) into hydrogen peroxide (H_2O_2), which is in turn degraded into H_2O and O_2 by the catalases. By using *sod*$^-$ mutant strains, we have demonstrated that *P. aeruginosa* SODs contribute to the level of intracellular ROS in macrophages. Additionally, while SOD activity impairs short-term intracellular survival of *P. aeruginosa*, it appears to promote the bacterial long-term persistence.

O16.5 - The contribution of iron uptake to *Acinetobacter baumannii* pathogenicity

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Acinetobacter baumannii (*Ab*) is an emerging bacterial pathogen. The multiplicity of iron uptake systems in *Ab* suggests that iron acquisition could contribute to its success as a human pathogen. In Gram-negative bacteria, receptor-mediated ferric iron uptake depends on proton consumption by the conserved TonB-ExbB-ExbD complex. Active uptake of ferrous iron is mediated by the GTP-dependent Feo system. The *Ab* genomes invariably contain three *tonB* genes (*tonB1*, *tonB2* and *tonB3*) at different genomic locations, whose role in iron uptake is poorly understood. Here, we report that *tonB3* is essential for *Ab* growth under iron limiting conditions, as those imposed by iron-poor media or human serum, whereas *tonB1* and *tonB2* appear unrelated to iron uptake. The *tonB3* promoter contains a functional Fur box and is tightly iron-regulated. The *tonB3* deletion caused over-production of siderophores as the response to severe iron starvation. TonB3 resulted essential for virulence, since *Ab* lethality in animal models was completely abrogated by *tonB3* mutation. Thus, TonB3 is essential for *in vivo* growth and represents a promising target for antibacterial therapies and vaccine development.

P16.1 - Characterization of the effects induced by the HIV-1 recNEF$_{SF2}$ protein on plasmacytoid dendritic cells

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It is an emerging fact that the viral protein Nef of Human Immunodeficiency Virus type 1 (HIV-1) has an important impact on the chemo-cytokine network, possibly contributing to the chronic inflammation observed during HIV disease (Percario et al., 2015). In this context, plasmacytoid dendritic cells (pDCs), specialized to produce type I IFN, play a pivotal role (Aiello et al 2018). We decide to investigate on Nef-pDC interactions using a human pDC cell line treated with recombinant myristoylated HIV-1 Nef$_{SF2}$. We observed that recNef$_{SF2}$ induces the phosphorylation of STAT1 protein, possibly through the release of pro-inflammatory cytokines. Moreover, we analysed the exosome production using a commercially available BODIPY®-C16 fatty acid to label the cells. Surprisingly, we observed that recNef$_{SF2}$ does not increase the production of exosomes in pDC, but it seems to reduce it by about 40%. In addition, recNef$_{SF2}$ is incorporated into the exosome fraction. Further studies are in progress to identify the cytokines released and the response of pDC to exosomes containing Nef.

P16.2 - In silico analysis of Omp19 and M24/M37 family peptidase of *Brucella abortus* to identify the potential T cell epitopes

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Brucellosis is an animal disease caused by gram (-) facultative *Brucella* bacteria and characterized by abortion and reduced fertility in animals. In the present study, various immunoinformatics approaches have been applied to determine antigenic outer membrane proteins of *B. abortus* and to design potential epitope based vaccine against brucellosis. For this purpose Vaxign server was used to identify virulent, membrane associated proteins of bacteria. Results identified 5 membrane proteins; Omp25, Omp31, M24/M37 family peptidase, OmpA-like transmembrane domain and Omp10 for T cell epitope prediction. Prediction of T cell epitope analysis was applied to potent highest antigenic proteins among selected proteins. Omp19 and M24/M37 family peptidase sequences were then subjected to the NetMHCII 2.2 web server and analyzed against 25 MHC II molecules available in this database for T cell epitope. Bioinformatics analysis were identified 13-AGIVLAGCQSSRLGN-27 and 225-GLTSGAIRVGQSLVI-238 epitopes for Omp19 and M24/M37 family peptidase, respectively. These predicted epitopes might be useful for further vaccine design studies against *B. abortus.*
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P16.3 - Role of diacylglycerol kinase α in X-linked lymphoproliferative disease and autoimmunity

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XLP1 is a primary immunodeficiency due to mutations in the SLAM adaptor protein (SAP).

SAP deficiency results in constitutive DGKα activity that decreases TCR signaling and impairs restimulation-induced cell death (RICD). Indeed, pharmacological inhibition of DGKα restores RICD and limits CD8+ accumulation and associated immunopathology in XLP1 models.

In a complementary approach, we promote RICD resistance by treating normal lymphocytes with osteopontin (OPN). Interestingly, DGKα inhibitors restore RICD also in OPN treated cells, indicating a major DGKα role in the OPN signaling and as a general regulator of RICD sensitivity.

Finally, to find new DGKα inhibitors suitable for human use, we used a 2D/3D in silico approach based on chemical homology with the two commercially available DGKα inhibitors. Out of the resulting 127 compounds, ritanserin and CP1 were highly specific for DGKα and showed equal or superior potency currently available one. In cellular models of XLP-1, both ritanserin and Cp1 restored RICD of SAP-deficient CD8+ without significant toxicity, supporting their potential utility for XLP1 therapy.

P16.4 - The frog skin-derived peptides Esc(1-21) and its diastereomer: are they promoters of airway epithelium repair?

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Antimicrobial peptides (AMPs) are products of the innate immune system that control microbial proliferation and ensure epithelial integrity. *Pseudomonas aeruginosa* lung infections, especially in Cystic Fibrosis (CF) patients, often result in the development of lesions. Therefore, we better investigated the mechanism by which two frog-skin derived AMPs, Esc(1-21) and its diastereomer Esc(1-21)-1c, stimulate migration of human bronchial epithelial cells (CFBE) and presumably accelerate repair of damaged bronchial epithelium.

CFBE cell proliferation is not essential for the peptide-induced cell migration and this well correlates with typical morphological changes detected in migrating CFBE cells upon treatment with each peptide. The peptide-induced cell migration involves the epidermal growth factor receptor (EGFR) mediated signalling pathway and metalloproteinases take part in EGFR trans-activation. These findings, along with anti-inflammatory activity of Esc(1-21) and its diastereomer, suggest these peptides as novel candidates for bronchial re-epithelialisation.

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P16.5 - *Saccharomyces cerevisiae* induces trained immunity in social wasp queens

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Invertebrates, lack an immune component based on acquired immunity, as in Vertebrates, and rely only on an innate component, evolved to mount responses against pathogens in the form of immune priming events. Trained immunity is a mechanism that reinforce the activation of the innate component response to a previously encountered pathogens and vicariates in part the acquired immunity of Vertebrates. Here we report the ability of two yeast strains of Saccharomyces cerevisiae, able to induce trained immunity in Mammals, to induce trained immunity in the insect model wasp *Polistes dominula*, and protect against *E.coli* infection, in a Dectin1 dependent manner. Activation by immune training is peculiar to the queens and absent in the workers and changes according to the diapause transition, with different levels, in the

fall and in the spring, following winter hibernation. Metagenome analysis showed that Immune priming also alters the wasp gut microbiota in a strain specific way. The results thus suggest that the different cell wall composition of the yeast, and the different metabolic condition of the insect, determine different level of resistance to the infection by a pathogen.

P16.6 - Circulating biomarkers for tailored treatment strategies in endometrial cancer

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Endometrial cancer (EC) generally has a good prognosis related to the precocity of symptoms; more than 75% of cases are diagnosed at an early stage, with a 10-year overall survival rate of more than 80%. Despite this, about a quarter of patients with EC present an unfavorable prognosis linked both to the advanced stage of disease at the time of diagnosis, and, in the early stages, to the presence of risk factors for recurrence. Therefore, more efforts are needed to improve the stratification and management of EC. We have recently demonstrated the potential of cell free DNA (cfDNA) as a simple and inexpensive tool to better adapt surgical staging and help EC stratification. Cancer mortality in mainly caused by systemic effects, such as dissemination to distant organs, organ failure and thrombotic events, induced by the primary tumor. The mechanism of the release of the chromatin by stimulated neutrophils, called NETosis, may contribute to these systemic effects. In this study, we combined blood levels of citH3, an early marker of NETosis, with cfDNA levels. Our data provide evidences on the role of cfDNA quantitative and qualitative content as prognostic biomarkers in EC.

P16.7 - Cytokine secretion responsiveness of lymphomonocytes following cortisol cell exposure: sex differences

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The stress hormone cortisol is a coordinator of immune response, but its different ability to modulate the inflammatory mediator release in males and females has not been clarified yet. Herein, the release of inflammatory mediators (NF-kB and IDO-1 mRNAs, IL-6, IL-8, IL-4, IL-10, kynurenine) following increasing physiological cortisol concentrations was investigated in a defined *in vitro* model of primary human male and female lymphomonocytes. In basal conditions, male cells presented higher levels of some pro-inflammatory molecules than female cells. Following cortisol exposure, the levels of the pro-inflammatory cytokines were increased in male cells. Conversely, in female cells IL-6 release was unchanged and IL-8 levels were decreased. Anti-inflammatory cytokines did not change in male cells and increased in female cells. Interestingly, kynurenine levels were higher in female cells than in male cells following cortisol stimulus. These results highlighted that cortisol differently affects male and female lymphomonocytes, shifting the cytokine release in favour of a pro-inflammatory pattern in male

cells and an anti-inflammatory secretion profile in female cells.

P16.8 - G6PD deficiency and the redox imbalance: new insights into the susceptibility and the immune response to influenza A virus

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Glucose-6-phosphate dehydrogenase (G6PD) activity, the first and the rate-limiting enzyme of pentose phosphate pathway is responsible for the production of reducing equivalents of NADPH, used for regenerating the reduced form of GSH. It has been reported that G6PD-deficiency could increase the susceptibility to viral infections even if the mechanisms are not well elucidated. The aim of this study was the evaluation of the role of G6PD on influenza virus infection in epithelial and innate immune cell lines. We found that the expression level of G6PD and the activity of the enzyme decreased in infected cells compared to uninfected ones. Epithelial cell line silenced for G6PD and infected with influenza A virus showed an increased expression of influenza A viral proteins relative to control infected cells. TCID50 assay showed a higher viral titer in the supernatants of silenced and infected cells relative to normal infected cells. Thus G6PD may contribute to virus-induced redox imbalance and viral replication. Further studies are in progress to clarify the mechanisms by which G6PD deficiency could influence the spread of viral infections and the host response against virus.

P16.9 - Interleukin (IL)-17/IL-36 axis in the crosstalk between endothelial cells and keratinocytes in inflammatory skin responses

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In inflammatory skin conditions, such as psoriasis, vascular enlargement is associated to endothelial cell proliferation, release of cytokines and adhesion molecule expression. IL-17 is a pro-inflammatory cytokine mainly secreted by T helper 17 cells that is critically involved in psoriasis pathogenesis. IL-36α and IL-36γ are also inflammatory chemokines up-regulated in psoriasis and induced by various stimuli, including IL-17. In this study, we confirmed expression of IL-17 and IL-36 receptors by human dermal microvascular endothelial cells (HDMEC). IL-17 and IL-36 augmented HDMEC proliferation and STAT3 and ERK1/2 phosphorylation. Bio-Plex Pro™ assays showed induction of inflammatory chemokines whereas cytofluorimetric analysis detected ICAM-1 expression when IL-17 or IL-36 synergized with tumor necrosis factor (TNF)-α. VEGF-A and VEGF-C secretion by keratinocytes also augmented by co-treatment with IL-17, IL-36 and TNF-α. Importantly, IL-36 derived from IL-17-treated keratinocytes was responsible for HDMEC proliferation and ICAM-1 expression. Therefore, our data demonstrated that IL-17 and IL-36 are highly involved in endothelial cells/keratinocytes crosstalk in inflamed skin.

P16.10 - CD8 T cell responses to apoptosis-associated antigens in Experimental autoimmune encephalomyelitis (EAE), the mouse model of Multiple Sclerosis

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Autoimmune diseases are characterized by chronic immune activation, possibly sustained by CD8 T cell responses to caspase-cleaved antigens derived from activated apoptotic T cells (apoptotic epitopes, AE). Here, we analysed such response in mice.

Mononuclear cells were extracted from Central nervous system (CNS) or spleen (SPL) of EAE mice and AE-specific CD8 T cells were analyzed by flow cytometry using MHC I dextramers. Immunization with AE peptides in adjuvant was performed in naïve mice and AE-specific CD8 T cell expansion was assessed by flow cytometry.

We observed total CD8 T cell accumulation in CNS of EAE mice compared to naïve, mostly presenting a T effector memory phenotype, and producing high levels of inflammatory cytokines. AE-specific CD8 T cell frequency was higher in the CNS compared to SPL of EAE mice, and their cytokine production correlated with disease severity. By immunizing naive mice with AE peptides, we induced an increase of AE-specific CD8 T cell frequency and cytokine production.

These data indicate that AE-specific CD8 T cells accumulated in the CNS of EAE mice and may be involved in EAE immunopathology through the production of inflammatory cytokines.

P16.11 - NF-κB activation mediated by HTLV-1 transactivator Tax protein is impaired in TRAF3 knock-out cells

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Human T-lymphotropic virus (HTLV-1) is the etiological agent of Adult T-cell Leukemia, a neoplasm of mature activated T cells. HTLV-1 pathogenesis is linked to the expression of the viral regulatory proteins Tax-1 and HBZ. The Tax protein is essential for viral gene transcription and plays a crucial role in T-cell transformation. Among several cellular pathways, Tax-1 constitutively activates the NF-κB signaling interacting with host factors. Recently, we demonstrated that Tax-1 interacts with the TNF-receptor associated factor 3, TRAF3, a regulator of the alternative NF-κB pathway. In order to investigate the role of Tax-1 and the viral antisense protein HBZ in NF-κB deregulation, we produced a $TRAF3^{-/-}$ cell line using CRISPR/Cas9. The TRAF3 knockout HEK293T cell line exhibits a constitutive activation of NF-κB alternative pathway. Interestingly, Tax-induced NF-κB activation was substantially affected by TRAF3 deficiency. In addition, we found that HBZ induced TRAF3 degradation. This study may offer new insights into the molecular mechanism by which viral interactions with TRAF3 impact NF-κB alternative regulation.

P16.12 - HIV-1 NEF_{sf2} protein increases the exosome production in THP-1 human monocytic cells differentiated with PMA

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The protein Nef of Human Immunodeficiency Virus (HIV) is a virulence factor that acts as an adaptor molecule inside the infected cells. It regulates viral production, induces immunoevasion and importantly can be transferred to uninfected cells through exosomes (Percario et al., 2015).

In this study we examined if the recombinant myristoylated HIV-1 Nef_{SF2} was able to induce the production of exosomes in THP-1 cells differentiated with PMA. To this aim, we used

commercially available BODIPY®-C16 fatty acid to label the cells, that once incorporated, produces fluorescent exosomes and microvesicles that we examined and quantified as reported by Sargiacomo and colleagues (Coscia et al., Methods Mol Biol, 2016). We observed that treatment of cells with myrNef$_{SF2}$ increases about twice the production of exosomes but not that of microvesicles. In addition, Nef is incorporated into the exosome fraction. The analysis of the vesicular production induced by cell treatment with Nef mutants suggests that some of its conserved domains are involved in exosomes production. Further studies are in progress to analyze the pro-inflammatory effects induced by the exosomes containing myrNef$_{SF2}$.

P16.13 - The importance of being Proline: biochemical and functional difference between human and mouse CD28 cytoplasmic tail

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CD28 is a crucial co-stimulatory receptor that delivers co-stimulatory signals necessary for optimal T lymphocyte activation. In 2003 Hunig research's group demonstrated that the stimulation of mouse CD28 with superagonistic antibodies (CD28SAb) preferentially activate and expand immunosuppressive regulatory T cells, thus suggesting their use in the therapy of autoimmune diseases. However, the severe systemic inflammatory response syndrome developed by volunteers following the first attempts of administration of CD28Sab in humans, suggested the existence of distinct signaling abilities between human and mouse CD28. Herewith, we provide evidence that a single amino acid variant within the C-terminal proline rich motif of human and mouse CD28 (P^{212} in human *vs* A^{210} in mouse) regulates CD28-induced NF-κB activation and pro-inflammatory cytokine gene expression. Moreover, this $Y^{209}APP^{212}$ sequence in human is crucial for the association with Nck adapter protein involved in the actin cytoskeleton reorganization events necessary for CD28 autonomous signaling

P16.14 - IL-38 has an anti-inflammatory action in psoriasis and its expression correlates with disease severity and therapeutic response to anti-IL-17 treatment

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IL-36 cytokines, a subgroup of IL-1 family, comprise IL-36α, IL-36β and IL-36γ agonists and IL-36RA and IL-38 antagonists. In psoriatic skin, IL-36 agonists are aberrantly expressed and have a pathogenic role, whereas the function of IL-38 antagonist remains to be defined. We demonstrate that skin and serum IL-38 levels are reduced in psoriatic patients and in other skin diseases characterized by neutrophilic infiltrate. Down-regulation of the epidermal expression of IL-38 is related to keratinocyte de-differentiation triggered by psoriasis-related cytokines. We show that in psoriasis the balance of IL-36γ /IL-38 serum levels is in favor of agonist and is associated with disease severity. IL-38 is upregulated by anti-IL17A biological treatment and positively correlates with the therapeutic efficacy of secukinumab in psoriatic patients. Finally, we show that administration of IL-38 counteracts the biological processes induced by IL-36γ in human keratinocytes and endothelial cell cultures and attenuates the severity of the psoriasiform phenotype induced by IMQ in mice, by restoring the physiological programs of keratinocyte differentiation and reducing the immune cell infiltrates.

P16.15 - *Shigella* modulation of polyamines during the invasion of host cells

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Polyamines are small molecules found in all cells and associated with a wide variety of physiological processes. In bacterial pathogens the modulation of polyamine content could represent a strategy to optimize bacterial fitness within the host.

Shigella, the etiological agent of bacillary dysentery, in contrast to its innocuous ancestor *Escherichia coli*, has a polyamine profile characterized by high level of intracellular spermidine.

To understand the role of polyamines in the *Shigella*-host interaction, we analysed the expression of host cell genes involved in the biogenesis and back-conversion of polyamines during *Shigella* infection. Preliminary data indicate that the acetyl polyamine oxidase (APAO) is induced at early stages of *Shigella* infection, while induction of the spermine/spermidine acetyltransferase and of the spermine oxidase is detected later during the infection.

These observations lead us to speculate that the early release of H_2O_2 as secondary product of APAO activity might represent a signal for *Shigella* to activate the expression of *mdtJI* efflux pump operon in order to export bacterial polyamines which have a scavenger function during oxidative stress conditions.

P16.16 - Anti-tumor T cell-mediated immune-surveillance requires the mitochondria pro-fission protein Drp1

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Optimal T cell response against growing tumors requires generation of high amount of effector T cells and their infiltration into the tumor mass. Interestingly, we found that mitochondria pro-fission factor Drp1 regulates three distinct cellular processes, all required to sustain T cell anti-tumor response. Indeed, Drp1 sustains *i)* T cell motility for an efficient extravasation toward

both secondary lymphoid organs and tumors; *ii)* clonal expansion of T cells for the generation of optimal amount of activated T cells; *iii)* the glycolytic switch of effector T cells upon activation by regulating a calcium/AMPK/mTOR/cMyc axis. Thus, in Drp1-deficiency, T cells proliferate less, infiltrate less inside a tumor mass and the lack of glycolytic switch causes the formation of memory-like cells, which preferentially generate exhausted T cells. Consequently, tumors grow faster in a murine mouse model in which Drp1 is specifically removed from T cell lineage, due to a reduced number of tumor-reactive T cells, which also display a more exhausted phenotype. Therefore, our findings highlight the importance of the pro-fission protein Drp1 in sustaining T cell-mediated immune response.

P16.17 - Regulation of *Shigella* virulence genes expression in response to human gut environment

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In *Shigella*, the etiological agents of bacillary dysentery, the virulence genes are located on a large plasmid, pINV. The expression of the virulence genes is regulated by multiple environmental stimuli through a regulatory cascade involving proteins and sRNAs encoded by both pINV and the chromosome. During the invasive process, *Shigella* as other enteropathogens encounter several types of molecules like SCFA, peptides and hormones, produced by the host or by microbiota. It has been reported that, in other related bacterial pathogens, some of these molecules can modify the virulence gene expression.

To investigate the role of different molecules on the *Shigella* pathogenesis, we generated a reporter strain able to modulate the level of luminescence as a function of virulence genes expression. Preliminary results indicate that some molecules tested influence the expression of the genes involved in the invasive process of *Shigella*, thus adding another layer of complexity to the regulation in response to environmental stimuli.

17 - Biotransformations

O17.1 - Optimizing the biotransformation of residual biomasses by ameliorating the effectiveness of cell factories

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Biorefineries can be defined as the combined strategies and techniques aimed to the valorization of renewable biomasses, largely through the exploitation of cell factories or enzymes. The potential of microbial cell factories is huge, thanks to their ability to transform complex and uneven matrixes into useful and valuable compounds. Nevertheless, several aspects of biorefineries still need to be further improved: for example, a viable switch from first generation processes, based on edible crops, to second generation and beyond. Remarkably, the processes that are propaedeutic for the use of residues as substrate for biotransformation often determine the generation or the release of stressing agents. These elements need to be considered while developing bioprocesses and tailoring cell factories. Here we show examples of how stress and stress responses guided our researches on *Saccharomyces cerevisiae* and other non-saccharomyces yeasts, to make them more suitable for industrial application, with a specific attention to the constraints deriving from the use of renewable substrates and from the accumulation of the desired products.

O17.2 - Innovative chitosan-based biodegradable plastics as food coating and wrapping

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Chitin is the second most abundant polysaccharide occurring in nature and its wastes represent a major environmental issue. One possible chitin recycling involves its conversion in chitosan (CH) by alkaline deacetylation. Because of its biodegradability, low toxicity, antibacterial and antifungal properties, as well as possible production in large quantities from seafood industry wastes, CH has been recently promoted as matrix of promising "new economy" bio-based plastics for food coating and protection. The results reported in the present study open new possibilities in the applications of CH-based biomaterials and, as a consequence, in chitin waste recycling. In fact, CH edible films prepared in the presence of spermidine and glycerol were shown to possess newsworthy mechanical and permeability properties compared to the films obtained with CH alone. In addition, these films are also able to be thermo-sealed and retain the antimicrobial and antifungal CH features. Therefore, their use as alternative to the traditional plastics in coatings and wrappings of selected food products is suggested. (*Supp. by Ital. Min. For. Aff. & Inter. Coop.; IV Progr. Quadro Coop. Italia/Messico*).

O17.3 - Identification of two novel hemicellulosolytic enzymes from the hyperthermophilic bacterium *Thermotoga neapolitana* and their exploitation for lignocellulose degradation

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Thermotoga is a genus of (hyper)-thermophilic bacteria belonging to the phylum Thermotogae. Within this genus, *T. maritima* and *T. neapolitana* are of special interest for the production of several hyperthemophilic enzymes suitable for industrial application. In the present study, *T. neapolitana* was selected as a source of hyperthermophilic hemicellulases (HHs) for the pretreatment of lignocellulosic biomass. Two genes were selected upon sequence homology analysis encoding putative hemicellulases and cloned from the gDNA of *T. neapolitana* for the expression in *E. coli*. Enzymatic characterization of the recombinant protein, revealed that such genes encoded two highly processive and thermostable α-arabinofuranosidase and β1,4-endogalactanase with maximal activity at temperature higher than 90°C. Pretreatment of the leaf from barley (*Hordeum vulgare*) with a mixture constituted by the two HHs boosted the degradative activity of commercial cellulolytic product towards such substrate. Thus, the two HHs displayed enzymatic features that may be exploited for improving the degradation of lignocellulose.

O17.4 - Removal and biodegradation of air pollutants by plant-bacteria interactions in urban areas

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Plants and phyllosphere microorganisms have been suggested to effectively contribute to reduce air pollution levels in cities through the adsorption and biodegradation of pollutants onto leaves. We sampled atmospheric particulate matter and leaves of *Magnolia grandiflora* and *Cedrus deodara*, evergreen plant species widespread in the urban area. Polycyclic Aromatic Hydrocarbons (PAHs) on leaves and air particulate matter (PM10) were quantified. Sequencing of 16S rRNA gene, Whole Metagenomics Sequencing and qPCR analyses were applied on leaf samples to gain insight into the interaction between air pollution and phyllosphere microorganisms. The annotation of the predicted genes revealed that the microbial communities on the leaves harbored genes involved in the degradation of hydrocarbons. In particular, 12 catabolic pathways were addressed at degradation of aromatic compounds and they were more abundant on *Magnolia* than *Cedrus*. Notably, the gene coding for naphthalene-1,2-dioxygenase, used as a marker for naphthalene-degrading microorganisms, was found at significantly higher abundance on winter magnolia leaves when naphthalene concentration reachs its peak in air and on leaves.

O17.5 - Purification and characterization of a novel peroxidase from seeds of *Araujia sericifera* Brot.

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Araujia sericifera (family Apocynaceae) is an evergreen climbing plant from South America, introduced in southern Italy at the beginning of last century as ornamental plant. Today, this invasive plant is considered adventitious and a noxious weed. In Italy, *A. sericifera* is known for its fruit that contains hundreds of fluffy white seeds, which are easily dispersed by wind when the fruit splits open.

Furthermore, ripe fruits have also a particular silky fuzz used in artefacts, and for which it is known in Italy as "silk plant". Considering of seeds availability in Caserta territory, our group decided to characterize possible enzymatic or biological activities.

In this framework, we report the purification and partial characterization of a novel basic peroxidase from *A. sericifera* seeds, the first enzyme isolated from this species. Afterwards, the dependence from pH, ionic strength and ions was tested in order to evaluate the biochemical properties of this novel enzyme. Future perspectives of this work will be to investigate the potential use of this novel peroxidase in oxidative reactions with diagnostic, therapeutic and food industries applications.

P17.1 - Development of a microalgal-based powder with thermostable cellulolytic activity

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Chemical or enzymatic treatments are employed to convert lignocellulose to fermentable sugars that, in turn, are used to produce biofuels. Chemical methods are expensive and harmful for the environment, while the use of enzymatic treatments is limited by costs and low efficiency. In order to address these issues, we developed a microalgal-based powder composed of dried *Chlamydomonas reinhardtii* cells accumulating a complete set of hyperthermophilic cellulases in the chloroplast. These strains were also engineered to express the cytosolic Phosphite Dehydrogenase D from *Pseudomonas stutzeri* that allowed the growth of transgenic microalgae in phosphate-depleted/phosphite-repleted media, avoiding the use of expensive sterilization procedures that primarily affect the cultivation costs of microalgae. The microalgal-based powder releases the thermostable cellulases starting from temperatures close to 50°C and showed hydrolyzation efficiencies ranging from 24 to 35% on acid-diluted lignocellulose after 24h of treatment. Moreover, the cellulases released from the algal powder can be reused on fresh substrates up to a total treatment-time of 72h without loss of hydrolyzation efficiency.

P17.2 - Copper homeostasis as a target to improve *Saccharomyces cerevisiae* tolerance to oxidative stress

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Saccharomyces cerevisiae is widely used as cell factory for the biotechnological production of various industrial products. During these processes, yeasts meet different kinds of stressors that often cause oxidative stress. Therefore, the development of robust strains is indispensable to improve production, yield and productivity of fermentative processes. Copper plays a key role in oxidative stress response, as cofactor of the superoxide dismutase 1 and being contained in metallochaperone/metallothioneines with antioxidant properties. We observed a higher naturally copper internalization in a robust *S. cerevisiae* strain engineered to produce l-ascorbic acid (L-AA) compared with the wild type strain. Therefore, we investigated the effect of copper homeostasis alteration on cellular stress tolerance. *CTR1* and *FRE1* genes, codifying for a plasma membrane copper transporter and for a cell-surface ferric/cupric reductase, respectively, were overexpressed in both wild type and L-AA cells. We found that the sole *FRE1* overexpression was sufficient to enhance stress tolerance toward H_2O_2 in both strains. These findings reveal copper homeostasis as a target to develop robust cell factories.

P17.3 - Lignin valorization: a systems biocatalysis approach

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Lignin is an amorphous biopolymer which represents the first renewable source of aromatics on Earth. In nature, biodegradation of lignin is a multi-enzymatic process involving both ligninoytic enzymes and enzymes with ligninolytic-auxiliary activity.

The purpose of this project is to combine different enzymatic activities in a one-pot or a multi-step reaction in order to increase lignin depolymerisation efficiency throughout a biocatalytic approach.

The β-O-4-aryl ether linkages account for approximately 50% of all ether bonds in lignin: five enzymes, namely LigD and LigL (Cα-dehydrogenases), LigE and LigF (β-etherases), and LigG (a glutathione lyase) from the protobacterium *Sphingobium sp.* SYK-6 were used simultaneously allowing to reach the full bioconversion of a racemic mixture of the lignin model compound GGE. The recombinant tetrahydrofolate-dependent *O*-demethylase LigM converts vanillic acid to protocatechuic acid: coupling this reaction with the one of the plant methionine synthase MetE allowed to recycle the cofactor. The combination of these Lig enzymes with known ligninolitic activities (laccases/peroxidases) allowed to isolate promising aromatic compounds.

P17.4 - Milk whey protein-based biomaterials with improved properties

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The dairy industry gives rise to considerable quantities of milk whey (MW) that, because of its high organic content, cannot be discharged directly and, thus, should be treated with additional costs for the manufacturing companies. One possible MW recycle is the use of its protein content (~65% β-lactoglobulin, ~25% α-lactalbumin and ~8% bovine serum albumin) as biopolymer source for production of biodegradable/edible films, coatings or wrappings. In the present study MW proteins (MWPs), following heat denaturation and solubilization at different pHs, were exploited to prepare film forming solutions containing different concentrations of uncharged and/or ionic plasticizers. These new formulations gave rise to stable systems, as demonstrated by ζ-potential measurements and, by casting, to manipulable, resistant and flexible films with features comparable to the ones of various commercial biomaterials. Further experiments, based on enzymatic MWP crosslinking and/or blending with different nanoparticles, are being carried out to further improve the performances of the obtained MWP-based edible films. *(Supp. by Ital. Min. For. Aff. & Int. Coop.; IV Progr. Quadro Coop. Italia/Messico).*

P17.5 - Effect of mesoporous silica nanoparticles on the pectin film mechanical properties

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Nowadays, the increasing attention about the plastic pollution and environmental change open a research field devoted to replace traditional plastics by producing biomaterials with promising properties. Hydrocolloids are the main macromolecules naturally available to produce innovative products, i.e. edible films for the food industry. Citrus peel low-methylated pectin was used to prepare the films in the presence of mesoporous silica nanoparticles (MSNs) to contrast limited mechanical and barrier properties of the pectin film. In fact, due to the low toxicity, high surface area and ease of being obtained, MSNs have been used lately both in drug delivery and food packaging. The results reported in the present study show that MSNs reduced significantly the particles size of pectin aqueous solution while they do not affect zeta potential value. Moreover, MSNs slightly increase the tensile strength and decrease significantly the Young modulus. However, glycerol addition provokes elongation at break and film thickness increase, while reducing Young's modulus and tensile strength. Therefore, these films might be considered good eco-friendly candidates to replace traditional plastics.

18 - Stem Cells, iPS, Cancer Stem Cells

O18.1 - Development of new cranial motor neuron differentiation protocol from human iPS cells carrying ALS mutations

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Human induced pluripotent stem cells(iPSCs) are used for in vitro disease modeling, offering the possibility to generate disease-relevant cell types.Amyotrophic Lateral Sclerosis(ALS) is a neurodegenerative disease of the motor system,caused by progressive degeneration of motoneurons(MNs).Not all MN subtypes are equally vulnerable to ALS disease,although pathogenic proteins are typically expressed in all subpopulations of MNs.ALS prognosis depends on the site of onset of the first symptoms and with bulbar onset ALS representing the form with the worst prognosis.This form affects primarily cranial MNs of the branchiomotor and visceral motor subtype.We present a fast and efficient method to convert human iPSCs into cranial motor neurons. Our method is based on stable integration of an inducible vector that allows controlling the ectopic expression of Ngn2,Isl1 and Phox2a(NIP).NIP induction results into quick and efficient conversion of iPSCs into electrophysiologically mature cranial MNs. Notably,we have extended our method to iPSCs carrying ALS mutations,thus providing a useful tool to analyze the cellular and molecular bases of motor neuron vulnerability in pathological conditions.

O18.2 - New insights on the human bone-marrow and gingival mesenchymal stem cell responses to senescence induction

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Bone-marrow mesenchymal stem cells (BMSCs) has been widely used in regenerative medicine, even if they rapidly undergo to senescence phenomena limiting their use *in vitro*. A promising alternative to BMSCs are the gingival MSCs (GMSCs) for which the susceptibility to senescence induction is still unclear. Herein, we investigated the functional responses of BMSCs and GMCs to two different senescence cellular models that were set up utilizing sub-lethal concentrations of hydroxyurea (HU) and hydrogen peroxide (H_2O_2). Despite these two models effectively induced BMSC and GMSC cells to undergo cellular senescence, the age-related phenotypic changes (SA-β-gal staining) were significantly lower in GMSCs. By evaluating the expression of different senescence-related genes, including p53, p21 and $p16^{INK4\alpha}$, we demonstrated GMSCs maintain a higher proliferation rate and result more resistant to HU and H_2O_2 treatment. BMSC were more prone to apoptotic phenomena and showed higher levels of intracellular reactive oxygen species. In conclusion, GMSCs are more prone to contrast the senescence induction confirming these staminal cell populations as an attractive tool in regenerative medicine.

O18.3 - Generation of an iPSC model to investigate replicative stress as the possible molecular mechanism underlying Schimke immune-osseous dysplasia

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The Schimke immuno-osseous dysplasia (SIOD) is an autosomal recessive genetic osteochondrodysplasia, caused by bi-allelic mutations in SMARCAL1 gene (SML1) that encodes for a protein of the chromatin remodelling SNF2 family. Recent works demonstrated that SML1 is involved in the processing of DNA structures at replication forks and SIOD may originate from accumulation of DNA damage, but the mechanism by which SML1 loss or mutation causes SIOD are completely unknown. We aimed to generate a cell model of SIOD derived from iPSC and characterized by inducible SML1 knock-down. Using this unique model, we looked for a mechanistic view of the relationship between loss of SML1 function, replication stress and SIOD. Our preliminary cell biology and biochemical assays demonstrate that loss of SML1 is sufficient to reduce cell growth, proliferation, induce replication stress and enhance DNA damage response. Further studies are ongoing to determine if pre-differentiation replication stress affects subsequent differentiation ability of iPSC. We expect that our findings might be useful to support further investigation of the molecular mechanism and pathophysiology of the disease.

O18.4 - Dysregulation of RNA metabolism in iPSC-derived FUS mutant human motor neurons, an in vitro model system for Amyotophic Lateral Sclerosis

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We have developed a human iPSC-based in vitro model system for the motor neuron disease Amyotrophic Lateral Sclerosis (ALS). A number of ALS-linked mutations affect nuclear import of the RNA-binding protein (RBP) FUS. Here we report the RNA interactome of wild-type and mutant FUS in human iPSC-derived motor neurons. We show that while the wild-type protein preferentially binds introns, the ALS mutation causes a shift towards 3' untranslated regions (3'UTRs). Among transcripts whose 3'UTR is extensively bound by mutant FUS, we identified genes encoding for the neural specific ELAV-like RBPs. Mutant FUS 3'UTR binding results in altered levels of the encoded proteins. In particular, ELAVL4 (HuD) protein, increased in mutant MNs, interacts with mutant FUS in the cytoplasm and localizes in cytoplasmic speckles. Upon oxidative stress ELAVL4 and mutant FUS co-localize into stress granules. Notably, we identified ELAVL4 within patological aggregates in the spinal cord of ALS patients. We propose a novel pathological mechanism that involves, downstream of FUS mutations, the dysregulation of ELAVL4 and possibly other neural RBPs.

O18.5 - Transcriptome and proteome analysis of FXS patient-derived iPSC lines to investigate FMRP role and its interactors during neural development

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Fragile X Syndrome (FXS), a common inherited intellectual disability, is caused by the silencing of fragile X mental retardation 1 (FMR1) gene. This mutation leads to loss of fragile X mental retardation protein (FMRP), which plays a crucial role in neuronal development controlling mRNAs translation. Patient-specific induced Pluripotent Stem Cells (iPSCs) can be differentiated towards neural fate, offering the chance to study cell population affected by neurological disorder. We have raised a collection of human iPSCs that includes FXS patient-derived iPSCs and mutant FMRP lines derived by CRISPR/Cas9 gene editing and their isogenic controls, aiming to use such model system for better understanding the FMRP network. We have set up proper conditions for iPSC in vitro cortical neuron differentiation, allowing stepwise characterization of neural lineage progression. We will perform RNA-seq and FMRP CLIP-seq analyses on control and mutant iPSC-derived neurons to identify targeted transcripts and to understand how FMRP loss of expression has an impact on targets involved in the disorder. Finally, our iPSC model system will allow in vitro disease modeling and drug screening to treat FXS

P18.1 - Identification of *Prame*-target genes as new markers of 2C-*like* in mouse embryonic stem cells

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ESCs cultures consist of multiple cell populations with different degrees of potency. It was discovered that Retinoic acid (RA) induces ESCs to sporadically enter the high-level of pluripotency 2C-*like* through *Zscan4* and *Gm12794* gene expression. *Gm12794* is a member of the PRAME family (preferentially expressed antigen in melanoma), the ortholog of a human prognostic marker of acute myeloid leukemia. *Prame* overexpression in ESCs causes the block of differentiation with and without ATRA, suggesting a novel molecular mechanisms employed by stem cell to overcome the differentiation pathway. This work is based on the hypothesis that the molecular mechanisms employed by stem cells to overcome cellular differentiation induced by ATRA may be the same mechanisms by which myeloid cancer cell subtypes resist to ATRA differentiating therapy. In this study we have identified *Prame* target genes in ESCs RA-resistant through ChIP-sequencing. Two of target genes have been selected for ChIP validation. Finally, we have observed the influence of *Prame* on their gene expression. Our research provides new insights about the role of RA signaling during ESCs high pluripotency metastate fluctuation.

P18.2 - Long lasting inhibition of MDM2-p53 complex potentiates mesenchymal stem cell differentiation into osteoblasts

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The murine double minute (Mdm2) is a E3 ubiquitin ligase that interacts with several proteins involved in cell cell fate determination. Among several other proteins, Mdm2 binds to the tumour suppressorp53or to the isoform 2 of GRK. Mdm2 contributes to mesenchymal stem cell (MSC) differentiation, nevertheless Mdm2 functions in the osteogenic process derived from MSCs remain unclear.

Herein, different disruptors of Mdm2-p53 complex were used. In our hands, the long-lasting dissociation of Mdm2 from p53, caused by the compound EB148, potentiated MSC differentiation into osteoblasts, and in parallel favoured cAMP accumulation. This effect was probably due to a major availability of Gs coupled receptors that mediated a sustained ERK activation during MSC differentiation. The association between Mdm2 and to GRK2 was evaluated. The data showed an increased association of the two proteins upon challenging with EB148, causing a decrease in the desensitisation of G-protein coupled receptors. Globally, these results suggest that the long-lasting inhibition of Mdm2 activity plays a key role in the mobilization of intracellular proteins that regulate the final outcome of MSCs.

P18.3 - Bergamot natural products eradicate cancer stem cells (CSCs) by targeting mevalonate, RhoGDI signalling and mitochondrial metabolism

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Here, we show that a 2:1 mixture of Brutieridin and Melitidin, termed "BMF", has a statin-like properties, which blocks the action of the ratelimiting enzyme for mevalonate biosynthesis, namely HMGR (3hydroxy3methylglutarylCoAreductase). Moreover, our results indicate that BMF functionally inhibits several key characteristics of CSCs.

More specifically, BMF effectively i) reduced ALDH activity, ii) blocked mammosphere formation and iii) inhibited the activation of CSCassociated signalling pathways (STAT1/3, Notch and Wnt/betacatenin) targeting RhoGDIsignaling. In addition, BMF metabolically inhibited mitochondrial respiration (OXPHOS) and fatty acid oxidation (FAO). Importantly, BMF did not show the same toxic sideeffects in normal fibroblasts that were observed with statins. In summary, our current results directly show that BMF is a natural, nontoxic, inhibitor of HMGR, that can be effectively used to target mitochondrial metabolism (OXPHOS) and fatty acid oxidation (FAO) in breast cancer ER+ cells, preventing the CSCs formation and their propagation via RhoGDIsignalling.

P18.4 - BRafV600E mutation in combination with loss of tumor suppressor Pten in adult NSCs induces astrocytoma formation

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Gliomas are a heterogeneous group of primary tumors of the central nervous system. The landscape of glioma mutations is wide but they mostly concentrate on EGFR, PI3K, CDKN2A, P53 and PTEN genes. BRAF mutations, among which BRAFV600E, are rare but characteristic of ganglioglioma, pilocytic astrocytoma and GBM and are associated with younger patient age. PTEN deletion, instead, coincides with tumor progression toward more malignant grades.

To understand if BrafV600E mutation drives the gliomagenesis we developed a mouse model in which *BrafV600E* and *Pten* mutations are driven by the Tamoxifen-inducible *Sox2CreER,* a deleter specifically active in NSCs of the anterior telencephalon.

Mutant mice developed brain tumors, histologically resembling astrocytomas, in the ventricular cavities and showed neurological symptoms only four weeks following tamoxifen injection.

Our model will allow to understand the molecular changes that occur in NSCs during the malignant transformation. Moreover, given the difficulties in developing valid treatments for these tumors, the therapy with BRAF specific inhibitors will represent a good candidate to improve patient prognosis.

19 - Nutrition Biochemistry

O19.1 - Nutrient deprivation alters lipid phenotype in triple-negative breast cancer

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Tumor cells exhibit an altered metabolism compared with non-transformed cells, consuming glucose, glutamine and lipids as nutrients. *In vitro* and *in vivo* studies have demonstrated that dietary restrictions might induce an increase of life expectancy and a reduction of cell proliferation, ensuring well-being. Breast cancer, frequently associated with obesity, is very intricate disease due to its heterogeneous nature. One subtype is Triple Negative Breast Cancer (TNBC) has an aggressive clinical behavior and is extremely metastatic.

Since there is a complex network that exogenous nutrients and cancer abnormal metabolism, we have evaluated the effects of nutrient deprivation (glucose, glutamine and serum) on cell migration and lipid metabolism in MDA-MB-231 TNBC cell line.

The results obtained indicate that the different medium nutrient restrictions reduce cell viability, tumor cell migration and greatly influence the lipid pattern of MDA-MB-231 cells. The data suggest significant changes in lipid composition, especially in omega-6/omega-3 ratio, triglyceride and sterol content, indicating possible nutritional approaches for the prevention and treatment of breast adenocarcinoma.

O19.2 - Investigating the nutraceutical properties of peptides extracted from Tuscany sourdoughs

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Sourdough is a flour/water mixture fermented by yeasts and lactic acid bacteria (LAB) and it is one of the oldest biotechnological processes in leavening of cereal food production. The use of sourdough in bread making influences many aspects of bread such as taste, shelf life and nutraceutical properties. In fact, during the fermentation process, cereal proteases are activated and strain-specific peptidases are produced thus leading to the formation of peptides mainly from cereal proteins.

A previous study allowed identifying the LABs more effective in producing bioactive peptides starting from 131 strains that were screened for peptidase activities. Among these, 23 strains were selected and further characterized for antioxidant and anti-inflammatory activities. As result, three LAB strains with high biological activity were identified and used to produce cooked breads. Preliminary results show that the antioxidant and anti-inflammatory activity is maintained in the breads. The identification of peptides responsible of the biological activity is also under investigation.

O19.3 - A proteomic approach to study the neuroprotective effect of oleocanthal in SH-SY5Y cells

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Olive virgin olive oil contain many phenolics effective against aging and neurodegeneration, particularly oleocanthal (OC) has been demonstrated to possess neuroprotective and anti-inflammatory activities. The aim of this work is to investigate the neuroprotective effect of OC in neuron-like SH-SY5Y cells before and after oxidative stress induced by H_2O_2. Using 2DE/MS the protein maps for different conditions have been obtained and analyzed. PCR analyses were performed to validate proteomic results. Thirty-three spots resulted differentially expressed after H_2O_2 insult, sixteen after treatment with OC followed by H_2O_2. Spots of interest were excised and identified by LC/MS/MS. OC significantly reverted the down-regulation induced by H_2O_2 of proteasome and ubiquitin proteins moreover it increased the expression of HSP90 and Protein DJ1. Moreover, OC was able to counteract oxidative stress induced by H_2O_2 in SH-SY5Y as measured by MTT viability assay and to increase reduced-GSH level. Our findings suggest that OC may have beneficial health effect in counteracting neurodegeneration.

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O19.4 - Structure-dependent biological activities of food-related stilbene derivatives isomers

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Resveratrol, piceatannol and pterostilbene are stilbene derivatives in which two aromatic rings linked by an olefin bridge. Many stilbene derivatives have proven beneficial to human health, acting on risk factors for cancer, on cardiovascular and neurodegenerative diseases, on diabetes, and on osteoporosis.

All these monomeric polyphenols are particularly prone to oligomerization processes through oxidative coupling, originating complex structures such as dimers or oligomers that may be responsible for their beneficial effects. These natural oligomers present stereogenic centers, that could play a pivotal role in the interaction of this class of molecules with biological targets.

In this study, isomers of these compounds were synthesized, purified, and tested as for their ability to inhibit enzymes relevant to glucose metabolism (such as brush-border glucosidase and pancreatic alpha amylase), and to control inflammatory response in a suitable Caco-2 cell model. Results highlight the requirement for peculiar structural features as for eliciting individual effects, both in terms of the polymerization state of these phenolics and in terms of their three-dimensional structure.

O19.5 - Enhancing stem cell functionality through antioxidant supplementation

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Amniotic fluid stem cells (AFSCs) are characterized *in vivo* by a particular niche allowing their unique role in the body. Maintaining the functionality of stem cells *ex vivo* for clinical applications is essential. Cellular redox status plays an important role in stem cell biology as long the adverse effects by reactive oxygen species (ROS) are excluded. Aim of our study was to investigate the protective effect of two antioxidants, sulforaphane (SF) and epigallocathechin gallate (EGCG), against oxidative stress occuring during *in vitro* AFSC culture. The co-treatment with SF and EGCG was effective in reducing ROS production, increasing GSH levels and enhancing the endogenous antioxidant defences through the up-regulation of glutathione reductase, NAD(P)H:quinone oxidoreductase-1 and thioredoxin reductase. Intriguingly, the co-treatment sustained the stemness state by up-regulating pluripotency markers and influenced senescence associated markers. The co-treatment promoted osteogenic differentiation and up-regulated osteogenic genes. In conclusion, SF and EGCG can be used in combination in AFSC culture as a strategy to preserve stem cell functionality.

P19.1 - Taste sensitivity in parkinsonian patients

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Proper nutrition is necessary to a complete treatment of the Parkinsonian patients, to optimize the pharmacological efficacy and to prevent some complications correlated to the disease. The aim of our work was to focus on the relationship between taste sensitivity and Parkinson's disease (PD). Were enrolled 33 patients (18 men and 15 women) and 32 controls (14 men and 18 women). The taste sensitivity was evaluated using the "Taste strips" test which is performed using solutions at different concentrations of substances for each of the 4 basic tastes (salty, sour, bitter, sweet) and also fat and water tastes. We also evaluated body composition using an impedance analysis. Patients were asked to fullfill a self-assessment lifestyle test and an eating habits questionnaire. The data collected showed a reduction in taste sensitivity in subjects with PD correlated with weight gain. In conclusion, we can confirm that the subjects with PD show a reduction in taste sensitivity. Because taste impairment often results in reduced appetite and possible subsequent malnutrition, careful attention should be paid to reports of changes in taste sense by patients who suffer from PD.

P19.2 - Study of the synergistic effects of sulforaphane, epigallocatechin gallate and plumbagin against H_2O_2 in SH-SY5Y cells

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Nutraceuticals are interesting resources for research in preventive medicine and neurodegenerative diseases that are characterized by an extensive oxidative stress. The aim of this study was to investigate whether a co-treatment (SEP) consisting of sulforaphane (SF), epigallocatechin gallate and plumbagin, all able to activate Nrf2-ARE stress response pathway, is able to counteract H_2O_2 deleterious effects based on a synergistic hypothesis. SEP was more effective, compared to the single compounds, in increasing cell viability (measured by PrestoBlue) after H_2O_2-induced damage and in enhancing GSH levels (by MCB assay). Only SEP and SF significantly reduced ROS levels (by DCFH-DA assay) upon oxidative stress. The synergistic antioxidant activity of SEP was confirmed by the marked up-regulation of different antioxidant enzymes (by RT-PCR). The protection evoked by SEP against H_2O_2 was also confirmed in a 3 dimensional model (3D) of differentiated SH-SY5Y cells we developed. In conclusion, we identified a pleiotropic treatment that can prevent neurodegeneration caused by oxidative stress and we also developed a more precise brain model for the study of nutraceutical compounds.

P19.3 - An *in vitro* retinoblastoma microvascular endothelial cells model: inhibiting effect of Quercetin and its 8-Methylquercetin Pentamethyl Ether derivative

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Angiogenesis is involved in many pathological states such as progression of tumors,

retinopathy of premature and diabetic retinopathy. The latter is a secondary microvascular complication of diabetes mellitus and the leading cause of blindness. The vascular endothelial growth factor (VEGF) is a powerful pro-angiogenic factor that acts through two tyrosine kinase receptors (VEGFR-1 and VEGFR-2). In this work we studied the anti-angiogenic effect of quercetin and its derivates in human microvascular endothelial cells, as a BRB model, after stimulation with VEGFA. The most promising compounds were quercetin (25 μM) and 8-methylquercetin pentamethyl ether (25 μM) that were used both in vitro and in ex-vivo models. Our results showed that these compounds inhibited cell viability and migration and disrupted the formation of micro-vessels in rat aortic ring. Furthermore these flavonoids significantly suppressed the activation of VEGFR2 downstream signaling molecules such as AKT, ERK, and JNK as demonstrated by western blot analysis. Taken together these data suggest that Q and its derivative might be an interesting strategies to contrast angiogenesis-related diseases

P19.4 - The toxic alpha-gliadin peptide 31-43 induces different biological effects in skin-derived cells from celiac subjects and from healthy controls

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Celiac disease (CD) is an intestinal inflammatory disorder triggered by the ingestion of cereals containing gliadins. Type 2 transglutaminase (TG2) expression and activity is strongly implied in CD pathogenesis. Moreover, a subset of toxic gliadin peptides, of which peptide 31-43 (p31-43) is the prototype, modulates intracellular Ca^{2+} homeostasis, inducing TG2 activation in an intestinal cell line. Our aim is to investigate the effect of p31-43 on TG2 expression and activity into a model of skin-derived CD fibroblasts.

We analyzed TG2 levels by PCR and western blot and monitored TG2 activity by a microplate assay using the pentylamin-biotine as substrate. We found a general reduced TG2 activity to the stimulation with p31-43 in CD-derived cells with the respect to control cells. On the contrary, TG2 expression was more markedly induced in celiac cells than in control ones.

The different behavior of p31-43 with the respect to TG2 activation in celiac and control cells is in line with the concept that TG2 participates to define a "CD cellular phenotype", and strengthen the idea that the interplay between p31-43 and TG2 has an important role in CD pathogenesis.

P19.5 - [6]-gingerol effects on the immune system of invertebrates and vertebrates

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Ginger (*Zingiber officinale*) rhizome is commonly used as a spice or as anti-inflammatory and antioxidant. Although the bioactive constituents (gingerols, shogaols) have been identified, to date Ginger mechanisms of action are almost unclear. This study aimed to determine if [6]-gingerol, an abundant component of Ginger, could act as a bioactive molecule and therefore be able to modulate the immune response in *Paracentrotus lividus* sea urchin. In this preliminary study, we analyzed the effects of different concentrations of [6]-gingerol on the immune system of the sea urchin, at different time points (3, 6, 24, 48 hours) after a bacterial stimulation. The mRNA expression of some stress and immune genes (TLR4, PKS, 14-3-3, NfKb, Jun) was analyzed by real-time qPCR. Moreover, preliminary experiments were performed in LPS-stimulated RAW264.7 cells to analyze anti-inflammatory effects of [6]-gingerol. Our results suggested that [6]-gingerol modulates gene expression in both invertebrate and vertebrate

systems, even if further investigations are needed.

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P19.6 - Hydroxytyrosol attenuates the hypomethylation of miR-9 promoters induced by oxidative stress

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Increased oxidative stress and changes in DNA methylation are frequently detected in age-related diseases, including osteoarthritis. We studied the potential chondroprotective role of hydroxytyrosol (HT) and demonstrated opposite variations of miR-9 and *SIRT1* expression following treatment with H_2O_2 or HT in chondrocytes. In the present study we investigate a possible correlation with the methylation status of three miR-9 gene promoters.

MiR-9 was confirmed as a post-transcriptional regulator of *SIRT1*. MiR-9 levels increased after 5-azacytidine treatments in a dose-dependent manner, thus the status of CpG islands surrounding miR-9 promoters is critical in the regulation of gene expression. Moreover, H_2O_2-induced oxidative stress may promote these changes by directly hypo-methylating promoters. Indeed, methylation levels of miR-9 promoters were decreased in cells treated with H_2O_2 and, on the contrary, unaffected in cells pre-treated with HT.

Our findings about HT induction of epigenetic changes complete the dissection of the molecular mechanisms underlying the beneficial effects of this bioactive compound, thus disclosing it as a an interesting therapeutic tool.

P19.7 - Quercetin supplementation decreases erythrocytes oxidative damage at resting and after an acute bout of eccentric exercise in humans

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Quercetin (Q) has a wide range of biological actions however, its effect on redox status has been minimally examined in combination with exercise in humans.

14 males were randomly assigned, in a double-blind crossover design, to a Q (1g/day) or placebo groups. Red blood cells (RBCs) samples were taken under resting conditions, after 2 weeks of supplementation, and after an acute bout of eccentric exercise (EE). Glutathione homeostasis (GSH, GSSG, GSH/GSSG), thiobarbituric acid reactive substances (TBARs), enzyme antioxidant activities (CAT, SOD, GPx) and AAPH–induced oxidative hemolysis were evaluated.

After Q supplementation, the time to reach 50% of hemolysis increased (T50, +13.7 minutes), TBARs levels (-26.2%) and oxidized glutathione (GSSG, -29.7%) decreased in RBCs compared to placebo. After EE, quercetin increased T50, and reduced the increase of TBARs and GSSG exercise-induced. No differences were found in RBCs CAT, SOD and GPx activities.

In conclusion, Q supplementation improves RBCs resistance to oxidative stress therefore making cells more able to cope to oxidative insult. This effect do not involve an up-regulation of endogenous antioxidant processes.

P19.8 - *Meripillus giganteus* extract exerts chemopreventive properties by inducing apoptosis in leukemia cell-lines

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Chemoprevention refers to the possibility to inhibit, slow down or revert the carcinogenic process. In this context the interest towards natural extracts has grown due to their phytochemicals content.

The extract of the edible mushroom *Meripillus giganteus* (MG) showed to exert biological properties such as antioxidant and antimicrobial effects. We evaluated its chemopreventive potential on Jurkat and HL-60 leukemia cell-lines.

Flow cytometry (FCM) analyses of cells treated with MG 0-500 mg/mL for 24-72h allowed to evaluate its ability to induce apoptosis and block the proliferation of cancer cells.

FCM analyses showed that MG induces apoptosis in both cell-lines in a dose- and time-dependent manner. Moreover, data revealed that MG arrests the cell-cycle of HL-60 but has no effect on Jurkat proliferation. To define if MG was selective towards cancer cells, its cytotoxicity was evaluated in human lymphocytes (PBL) from healthy donors. At 24h treatment IC_{50} resulted 385 and 461 mg/mL in Jurkat and HL-60 cells respectively, while it was 761 mg/mL in PBL. These data suggest that MG extract is a partially selective chemopreventive agent.

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P19.9 - Chemical profile, antioxidant properties and molecular fingerprinting of six varieties of *Pistacia vera L.*

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Pistacia vera is the only species of the *Pistacia* genus producing edible nuts, and it have been ranked among the first 50 foods with high AOA. In this work, spectrophotometric assays (Folin-Ciocalteu and DMAC) were combined to HPLC-DAD-MS/MS analysis to investigate the phytochemical diversity within six pistachio cultivars (Bronte, Larnaka, Mateur, Mawardi, Kern and Kerman) both in skin (SHE) and in fruits (FHE) extracts. Chemical assays (ABTS, DPPH and FRAP) along with *in vitro* assays (CAA50 and MTT) were employed in order to evaluate their radical scavenging, antioxidant and anti-proliferative activity. In particular, SHEs showed always the highest antioxidant activity and polyphenols content respect to fees FHEs, but a completely comparable anti-proliferative activity. In agreement with previous reports, the inhibition of cancer cell proliferation cannot be explained just by the total phenolic contents, but is possible that other components may play a specific role. Studies are under way to better characterize these components. Finally, DNA fingerprinting was assessed to understand if differences in chemical composition and bioactivity could be linked to a different genotype.

P19.10 - Evaluation of dysgeusia in cancer patients: development of effective interventions in future

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It is believed that 50-70% of patients with cancer suffer from taste disorders, like dysgeusia. The diagnosis of dysgeusia is important in the prognosis of patients. The present study analyzed the taste alterations in patient population compared to controls. It could open to a new approach for a personalized diet to prevent and/or reduce taste alterations and malnutrition in cancer patients.

Cancer patients (n=45) were compared to healthy controls (n = 32). Taste function test was used to determine taste sensitivity. Different concentrations for each of the 4 basic tastes and also fat and water tastes were evaluated.

A significant difference in taste sensitivity among patients compared to the control group was found. At variance with the control group, taste perception in patients was better in females than in males.

Coping strategies regarding subjective taste impairment should be provided since alterations in taste sensation influence food preferences and appetite. They all have the potential to underpin changes in dietary intake and consequently in nutritional status. Consequently, they can adopt appropriate appetizing strategies and, based on that, change their feeding habits.

P19.11 - Chemical characterization, molecular fingerprinting and antioxidant activity of leaves of *Annona cherimola* Mill

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Annona cherimola Mill (cherimoya) is a fruit crop diffused in tropical and subtropical Countries. Several studies have demonstrated that cherimoya fruit is an interesting source of bioactive compounds, including alkaloids and polyphenols. On the other hand, this fruit, along with other parts of the plant, has been used in traditional medicine against multiple human diseases including cancer. In this study, we explored the nutraceutical value of cherimoya leaves with the aim to evaluate the possibility to use them as an alternative source of cherimoya bioactive compounds. Spectrophotometric assays (Folin-Ciocalteu and DMAC) combined to HPLC-MS analysis were employed for the chemical characterization of leaves from 7 cultivar of cherimoya (*Campas*, *Chaffey*, *Daniela*, *Fino de Jete*, *Torre1*, *Torre2,* and *White*). In addition, ABTS, DPPH and FRAP along with CAA assays were employed to evaluate the radical scavenging and antioxidant activities of the hydrophilic leaf extracts. Finally, DNA fingerprinting was used to assess the influence of genotype on the observed differences in phytochemical profile and functional properties among the studied cultivars.

P19.12 - Mechanism underlying the effect of *Stevia rebaudiana* Bertoni glycosides as insulin-mimetic agents in a Glut-4 -expressing cell model

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Stevia rebaudiana Bertoni is a shrub belonging to the *Asteraceae* family, that has been

used as non caloric, natural sweetener. Its leaves are characterized by a high content of sweet diterpenoid glycosides, mainly stevioside and rebaudioside A, which are responsible for the sweetener power, 200-300 times higher than sucrose.

Although several *in vivo* studies suggest that Stevia glycosides exert also an antihyperglycemic activity, the mechanisms underlying this effect are still unclear.

Therefore, the aim of the study was to deepen the knowledge about both the insulin-mimetic effect exerted by steviol glycosides and their potential antioxidant properties, since oxidative stress is strictly linked to diabetes.

Taken together, the obtained results highlight the insulin-mimetic effect and the antioxidant properties exerted by steviol glycosides, suggesting their beneficial role in health maintenance and in the co-treatment of diabetes.

P19.13 - Primary structure of the myoglobin from Eurasian woodcock (*Scolopax rusticola* L., family Scolopacidae)

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Eurasian woodcock (*Scolopax rusticola* L., family Scolopacidae) is a medium-small and wading plump bird, mostly migratory and well known in Europe as 'game bird', since its meat is consumed in many European countries widely used for culinary purposes.

In this work, the characterization of myoglobin (Mb) from Eurasian woodcock was performed in order to develop molecular biomarkers of meat adulteration against commercial frauds. To this aim, we isolated Mb from *S. rusticola* striated muscles as previously reported (Dosi *et al.* 2006, Comp. Biochem. Physiol. B).

Subsequently, its primary structure was determined by using a strategy based on high-resolution mass spectrometry. The amino acid sequence of Eurasian woodcock Mb was then compared to that of chicken Mb. Proximal (position 93, α-helix F) and distal histidinyl residues (position 64, α-helix E7) are conserved, while only three different amino acid residues are different. Finally, we found that Eurasian woodcock Mb sequence has a calculated average molecular mass of 17324.93 Da ($[M+H]^+$), which is in very good agreement with the experimental value obtained by MALDI-TOF MS on the apo-myoglobin (17324.62 Da; Δ= 0.31).

P19.14 - Baobab (*Adansonia digitata*): a big tree for a "super fruit". A study about the characterization of bioactive molecules in baobab fruit

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Baobab is a fruit-producing tree adapted to growth throughout the hot, drier regions of Africa, with nutritional and therapeutic potential.Either young leaves than whole fruit are sources of macro, micro nutrients and bioactive molecules. The interest for this fruit is growing in western society and in 2009 FDA approved baobab as novel food.Concerning nutritional values, baobab pulp has high vitamin c content, literature data described also total phenolic content but there is a lack in the characterization of this bioactive molecules.The aim of this work was to quantify all total bioactive molecules in the different parts of baobab fruit by using spectrophotometric assays and HPLC coupled with a triple quadrupole mass spectrometer detector for polyphenols identification and a photodiode array detector for their quantification.The analysis of bioactive molecules highlight the quantity of ascorbic acid (135.6 mg/100g) and also of epicatechin

and procyanidin B2 (105, 100 mg/100g).Data suggest that antioxidant capacity is the result of synergic action between ascorbic acid and polyphenols.Further experiments are necessary to evaluate the effects of antioxidant molecules on human health

P19.15 - Transglutaminase as an effective tool to reduce allergenicity of soy proteins in bio-tofu

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Soy and milk are important sources of proteins and essential amino acids. However, together with eggs, peanuts, tree nuts, fish, crustacean and wheat, they are classified as the "big eight" most allergenic foods. Approximately 1%–6% of children and 2%–4% of adults are affected by soybean allergy, while cow milk protein allergy affects 2%–4% of young children and infants. Different technological treatments (such as microwave heating, hydrolysis) have been extensively studied to reduce food allergenicity. Moreover, it is reported that enzymatic crosslinking could also be used to change the immunological properties of food proteins. Transglutaminase is an acyltransferase able to introduce isopeptide bonds in proteinaceous systems. Previous researches have shown that transglutaminase-mediated modification, to a certain extent, can reduce the antigenicity and allergenicity of some protein-based foods. In the present work we demonstrated that the soy protein component of bio-tofu made with soymilk and cow milk in the presence of both transglutaminase and acid lactic bacteria exhibits a lower capacity to bind IgE, suggesting a novel method to obtain a less allergenic food product.

20 - Evolutionary Biology

O20.1 - A biogeographic analysis of loss of planktotrophy in caenogastropod molluscs (Gastropoda, Nassariidae)

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In marine invertebrates the larval development is a key feature for evolution and ecology of species. In planktotrophic development (P), the larvae spend from a few days up to one year in the plankton feeding actively. In non-planktotrophic development (NP), larvae spend very little or no time in the plankton feeding only on yolk supplies. In the Caenogastropoda, NP is mostly considered as a derived condition that arises in response to conditions that counterselect P, allowing independence from trophic environmental availability. It is suggested that NP represents an advantage in phytoplankton-poor regions or in response to major environmental changes occurred in the past.

We tried to detect and analyze, in a phylogenetic framework, the distribution of events of loss of planktotrophy in a group of marine gastropods, aiming at identifying eco-evolutionary patterns. We used a comprehensive robust phylogeny of the family Nassariidae to identify pairs of sibling species, or group of species, that differ in larval development thus representing independent losses of P in the tree. The fossil-calibrated phylogeny allowed dating events of loss of P by using a relaxed molecular clock model.

O20.2 - The genome of *Drosophila subpulchrella* helps polarising the evolution of pest traits in its sister species *Drosophila suzukii*

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Drosophila suzukii, an invasive fruit fly characterised by temperate habitat and unusual egg laying behaviour, is an excellent model to investigate adaptation to new ecological niches. To increase the resolution power of genome analyses, we produced a draft genomes and transcriptome of *D. subuplchrella*, the sister species of *D.suzukii.* Dated phylogenomic trees indicate that they speciated from a common ancestor characterised by reduced generations per year: this is compatible with a long history of adaptation to temperate climates in the subgroup. Uneven sorting of orthologs and of repetitive element is suggestive of some past genetic admixtures between the two species, a hypothesis corroborated by a fertile hybrid obtained in our lab. Comparative genomics indicates that the two species share most of their gustative, but not odorant, gene repertoire confirming a gradual chemosensory specialisation in the subgroup. Overall, our results support a progressive modification scenario toward the peculiar *D. suzukii* biology and ecology. More generally, our study shows that a sister species can highly increase the precision of comparative genomics when studying novel traits and adaptation.

O20.3 - A common regulatory neurogenic toolkit in Bilateria

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The sea urchin apical organ is considered the larval brain and derives from the embryo's apical domain. However, information on the regulatory program used to specify this domain during development and its evolutionary origin is scant. In this work, we analyzed the spatial expression of over 30 regulatory genes, and the regulatory program used to specify the apical domain and proneural cells within it. Our results indicate that the apical domain starts as a single domain at blastula stage and is rapidly patterned into at least seven domains within 21h of development. Furthermore, using morpholino knockdown we show that *foxq2*, *hbn*, and *soxC* are required to specify proneural cells. To clarify the relationship of the apical domain to other nervous structures in bilateria, we identified the orthologues for each of the 33 regulatory genes in other model systems (e.g. fly and mouse) and evaluate the spatial expression in developing brains. We found a large similarity in gene expression between the sea urchin apical domain and other nervous systems in Bilateria. This finding indicates that these regulatory genes might constitute a shared neurogenic regulatory toolkit in bilateria.

O20.4 - On the shoulder of the past. The scapula of the Neanderthal from Altamura, Italy

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The exceptionally well-preserved human fossil skeleton discovered in 1993 in the Lamalunga karstic system near Altamura (Puglia, Italy) has been recently referred to Homo neanderthalensis and dated to a range bracketed between 187.0 and 128.2 ka. Its fragmentary right scapula has been removed from the cave according to a protocol aimed at preventing any biological contamination. The specimen is composed by three large fragments, which were picked up with the aid of telemanipulators in 2009 and 2015 from a small chamber just behind the so-called "abside", where the main assemblage of the human bones lies. The fragments have been digitally acquired via micro-tomography and the 3D volumes were used for composing them according to the original morphology of the scapula, which includes the well-preserved glenoid fossa, the roots of the coracoid and of the acromial processes, the superior third of the axillary border, the scapular spine and part of the supraspinal fossa. The results confirm that the morphology of the scapula from Altamura, despite some peculiar traits, is consistent with the morphology and proportions of both Early and Würmian Neanderthals.

P20.1 - The evolution of p53 gene family in vertebrates

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The origin of the p53 gene family predates multicellular life since members of the this gene family have been found in unicellular eukaryotes. The radiation into TP53, T63, and TP73 is a vertebrate invention. While TP53 is ubiquitous and a stress-response protein regulated at the post-translational level, TP63 and TP73 are expressed in a tissue and differentiation-specific manner and are also regulated at the transcriptional level. TP53 is considered the "guardian of the genome" given its role in protecting cells against the DNA damage and cellular stressors. TP63 and TP73 play a role in epithelial development and neurogenesis, respectively.

The evolution of the p53 gene family has been the subject of considerable analyses. However it is not clear if TP63/73 like or TP53-like gene led to the three genes present in vertebrates.

The aim of this work is to investigate the evolutionary history of the p53 gene family in vertebrates performing phylogenetic analysis and microsyntenic analysis but also analysing the protein domain organization and structure. Our findings here obtained allow us to discuss a possible evolutionary scenario.

P20.2 - Environmental temperature-related relationship of teleost *Rex* transposable elements in the phylogenetic analysis

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The movement and accumulation of transposable elements exerts a very high influence at the level of host genome, e.g. determining genome size and architecture and providing a substrate for homologous recombinations and DNA rearrangements such as inversions, deletions and duplications. In the following work, we focused on the specific RTE family of non-LTR transposable elements, *Rex* elements. Rex1, Rex3 and Rex6 are widely spread among teleosts and deeply active during teleosts genome evolution.Up to now, many studies have evidenced the presence of *Rex* retrotransposon in different species belonging to various teleost orders, in which a correlation between karyotype rearrangement and transposon activity is very well known. Many information were obtained about Rex1 and Rex3 in species belonging to the Antarctic region. To get more information about this extremely attractive RTE family, we extended the analysis to species belonging to the Arctic region. A molecular characterization of the reverse transcriptase sequences was made, together with an extended phylogenetic study in which we evidenced an unexpected environmental temperature-related position of analyzed sequences in the tree.

P20.3 - Virtual anthropology in the study of human evolution and history

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The remains that tipically compose the human fossil and archeological record often bear cracks, damage and deformations. The rapid development of "virtual anthropology" has provided innovative tools to manage, study and preserve cultural and natural heritage. Such tools include Computerized Tomographic Scan (Ct-Scan), Laser-scanning, Photogrammetry, 3D imaging and rapid prototyping. These approaches can contribute to any anthropological context from the discovery of the specimen to research, preservation, and dissemination. 3D imaging thecniques

can substitute physical intervention with a virtual protocol to restore the original shape of a deformed or incomplete specimen. In a similar way, the recovery of digital morphological information using data preserved even on a fragment through the use of 3D comparative samples. Here we present some of the most innovative protocols in virtual anthropology, also aplicable in other fields as natural history and cultural heritage, through the description of recent case studies.

P20.4 - Feeding people in Medieval Rome: isotope evaluation in several communities of the Roman territory

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Along the last decades several necropoleis dated back to the Early Middle Ages have been discovered in Rome, whose molecular evaluation could shed light on the lifestyle and health conditions of the medieval roman population. Remarkable changes occurred at the end of the Roman Empire leading to a crisis of the economic systems that influenced the diet of the roman people.

This study aims to contribute at the characterization of the nutritional habits of the medieval roman population through carbon and nitrogen stable isotope analysis performed on 150 human skeletal remains and several faunal bones.

The isotope evaluation suggests the medieval Romans' diet was mainly based on terrestrial resources consumption, in particular C3 plants: highly protein resources intake seems to be restricted and both marine and freshwater foodstuffs consumption appears to be negligible.

To the best of our knowledge this research provides the first biomolecular data about the medieval roman population, representing an outstanding opportunity to investigate the biologic profile of Romans in this transitional period featured by the downfall of the Roman Empire.

21 - Glycoconjugates

O21.1 - Identification of the antigen recognized by rHIgM22, a remyelination-promoting human monoclonal antibody and his effect on glial cells

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs) and promotes remyelination in mouse models of Multiple Sclerosis. The molecular targets recognized and the molecular mechanism stimulated by this antibody are still unknown. We tested the binding of IgM22 to purified lipids and lipid extracts of mouse brain, CNS myelin, mixed glial cells, and $O4^+$ OLs by using TLC immunostaining. Our preliminary results show that IgM22 binds to sulfatide *in vitro*, while it does not bind to other myelin sphingolipids, suggesting that sulfatide at the OLs surface might be important for the binding of IgM22 to these cells and to myelin. Indeed, in lipid extracts from different sources we found another lipid antigen selectively recognized by IgM22. To attempt the identification of the antigen, samples were purified using column chromatography and the fractions enriched of this second IgM22-immunoreactive antigen were analyzed by ESI Mass Spectrometry.

Furthermore, we assessed the effect of a 24 hours, single dose treatment with IgM22 on OLs, oligodendrocyte progenitor cells (OPCs) and mixed glial cells (MGCs).

O21.2 - The long non coding RNA HAS2-AS1 regulates breast cancer cells aggressiveness acting as a competing endogenous RNA

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The natural antisense transcript for hyaluronan synthase 2 (HAS2-AS1) is a long non coding RNA able to modulate HAS2 expression via epigenetic modifications. Since the activity of HAS2 and the production of hyaluronan are inolved in breast cancer progression, the aim of this work is to understand the role of HAS2-AS1 in tumor progression and hyaluronan metabolism. Functional assays conducted in the breast cancer cell line MDA-MB-231 revealed that the silencing of HAS2-AS1 stimulated cell aggressiveness, without altering HAS2 expression or hyaluronan production. LncRNAs can orchestrate gene expression interacting with other RNA species, i.e. miRNAs. This implies the down-regulation in the interaction of the miRNAs with their mRNA targets, what has been called a sponge effect. Our preliminary results showed that HAS2-AS1 overexpression decreased the abundance of miRNA 186 along with the up regulation of some of its targets. Among them we identified the pro apoptotic receptor P2X7 and other genes involved in cell cycle and apoptosis, explaining the presence of a malignant phenotype after HAS2-AS1 silencing.

O21.3 - Role of TNFα on endothelial glycocalyx

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Endothelial dysfunction is normally considered to be a starting point of atherosclerosis that is identified as a chronic inflammatory disease of the arterial vessel wall. However, the exact mechanisms underlying the impaired vascular structure and activity remain unresolved. Since inflammatory cytokine TNFα appears to be involved in the pathogenesis of atherosclerosis, we treated endothelial cells (HUVEC) with 0.1 μg/ml of TNFα for 24 hours to see its influence on extracellular matrix components as hyaluronan (HA) and syndecans (SDCs).

TNFα in HUVEC leads to an increase of HAS2 expression and HA deposition in pericellular area. SDC4 seems to be important for HUVEC during TNFα stimulation because SCD4 gene is overexpressed, its biosynthesis enzymes are increased and GAGs of its chains are modified showing a heparin-like anticoagulant role. Moreover, SDC4 increasing caused by TNFα seems to prevent the passage of molecules through the endothelial layer, suggesting that SDC4 can be involved in the macromolecules accumulation during the onset of atherosclerosis.

O21.4 - Glycosylation in colorectal cancer: role of B4GALNTII and Sialyl Lewis x antigen

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Glycosylation undergoes profound alterations in cancer.During colorectal oncogenesis a remarkable glycosylation change is associated with B4GALNT2 down regulation,which synthesizes the Sda carbohydrate antigen.On the contrary,the selectin ligand sialyl Lewis x(sLex) is up-regulated.Owing to the common backbone structure of the oligosaccharide(s) carrying either the Sda or sLex antigens, previously we have demonstrated *in vitro* that the biosynthesis of the two structures is mutually exclusive in gastrointestinal tissues.Our survey of "TCGA" database indicates that patients displaying lower B4GALNT2 expression have a worse prognosis than those with higher expression.Thus,we aim at investigating the contribution of Sda and the consequent inhibition of sLex in colon cancer cell biology.*In vitro* assays comparing LS174T cells transfected to overexpress B4GALNT2 and the Sda antigen with mock-transfectants show that the B4GALNT2-expressing cells form less spheroids in 3D conditions and a tendency to a reduced ability to heal a wound.The further study of the biological properties of these transfectants may provide clues on the clinical relationship between B4GALNT2 and prognosis.

22 - Plant Development and Disease

O22.1 - Looking for aminoacid-gated calcium channels in plants: functional, biochemical and structural characterization of one GLR isoform

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External administration of aminoacids (AA) to Arabidopsis seedlings leads to $[Ca^{2+}]_{cyt}$ increase. This response is ascribed to the activity of the Glutamate Receptor-Like (GLR) channels (20 isoforms are present in Arabidopsis). However, a demonstration that GLRs can bind AA, being *bona fide* receptors, is missing.

Arabidopsis seedlings expressing the Ca^{2+} sensor YC3.6 revealed that Gly, L-Glu, L-Cys, L-Ala, L-Ser, L-Asn and L-Met administration trigger root tip $[Ca^{2+}]_{cyt}$ increases. Confocal microscopy analyses of Arabidopsis seedlings show the GLRx expression in root tip meristematic cells. Remarkably, all these 7 AA fail to induce any $[Ca^{2+}]_{cyt}$ transient in two independent *null-alleles* for GLRx. These results support the hypothesis that GLRx works as an AA-receptor.

A recombinant protein, corresponding to the predicted GLRx ligand-binding domain (LBD) was obtained and biochemically characterized for its AA binding. X-ray crystallography allowed to solve the structure of GLRx LBD in its holo form at 2.0Å resolution and to identify the residues required for the AA binding. Site-directed mutagenesis and heterologous expression of mutant GLRx in yeast cells are ongoing.

O22.2 - Fine-tuning the unfolded protein response in plants

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In physiological conditions and in disease,the alteration of ER proteostasis induces accrual of misfolded proteins leading to a potentially lethal ER stress condition that triggers the unfolded protein response (UPR),a conserved signaling pathway able to promote cell growth or death depending on the nature of the ER stress conditions. The regulatory circuitry underlying ER stress resolution is largely unmapped. Here,we illustrate recent advances obtained by studying in *Arabidopsis* the surveillance system that regulates UPR amplitude. We identified the transcriptional cofactor NPR1 as critical redox-regulated UPR modulator,demonstrating convergence of signal coding in ER stress and SA-mediated defense. Moreover,performing GWAS we identified candidate genes associated with ER stress,and we characterized a calcium protein kinase as crucial ER stress regulatory hub. Finally,we present results to demonstrate that plants harness long distance signaling to communicate the occurrence of ER stress in a tissue to systemic tissues,providing a significant step forward in the understanding of the mechanisms underlying systemic signaling transduction of ER stress responses in intact organisms.

O22.3 - Biochemical characterization of Salt tolerance-related protein (STRP): a new protein involved in cold stress in *A. thaliana*

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Salt tolerance-related protein (STRP) is an Arabidopsis thaliana poorly characterized protein

identified in a proteomic screen of temperature stress-responsive proteins.

We observed that STRP rapidly accumulates in the cytosol after 10 min of treatment at 4 °C and that the effect is not dependent on transcriptional activation of the STRP gene. By using a specific proteasome inhibitor, we demonstrated that cold stress stabilizes STRP by inhibition of proteasome mediated degradation.

Transient expression of the STRP-GFP in A. thaliana protoplasts reveals that STRP is localized into the cytosol, the nucleus and associated to the plasma membrane. Cold stress increases STRP levels into the cytosol and nucleus, while the fraction associated to the plasma membrane decreases. We also demonstrated that the STRP is associated to chromatin.

To clarify the physiological function of STRP and its role in response to cold stress, we studied Arabidopsis STRP-overexpressing plants and Strp knockout mutants. Phenotypical analysis of the plants, their response to cold stress and to abscisic acid have been evaluated, demonstrating the crucial role of STRP in cold stress response.

O22.4 - Oxidation of Damage-Associated Molecular Patterns as a homeostatic mechanism in plant immunity and development

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Recognition of endogenous molecules acting as 'Damage-Associated Molecular Patterns' (DAMPs) is a key feature in activating immunity. In plants, a well-known class of DAMPs are the oligogalacturonides (OGs). Hyper-accumulation of OGs severely affects plant growth, suggesting their role in the growth/defense trade-off. We have discovered a homeostatic mechanism that controls the effect of OG hyperaccumulation. Four *A. thaliana* enzymes that oxidize OGs have been identified, named OGOX1-4, which belong to the Berberine Bridge Enzyme-like (BBE-like) family. Oxidized OGs display a reduced elicitor capability and are less hydrolysable by fungal degrading enzymes. Moreover, we identified another BBE-like member (named CELLOX1) which oxidizes cellulose fragments, recently shown as a class of DAMPs, suggesting that BBE-like proteins may control the homeostasis of different cell wall DAMPs. Plants overexpressing OGOX1 or CELLOX1 show altered resistance to different pathogens; moreover, *ogox1* ko mutant and plants over-expressing OGOX1 or CELLOX1 show an altered root growth. We propose that OGOXs and CELLOXs are key elements in the growth/defense trade-off and important players in development.

O22.5 - Pectin methylesterases in plant immunity: activators or susceptibility factors?

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Cell wall (CW) is the foremost interface at which plants and fungi interactions take place. Pectin is methylesterified in the Golgi and secreted in the CW in a high methylesterified form. Pectin Methyl Esterases (PMEs) are critical for the outcome of disease. An high methyl esterification makes pectin less susceptible to cell wall degrading enzymes. PME activity can release active Oligogalacturonides, the best characterized damage-associated molecular patterns (DAMPs) in plants and methanol, a DAMP able to alert adjacent non infected tissues or neighboring plants. Despite this evidence, the role of PME activity during disease remains under debate. Plant PMEs are regulated by subtilisin-like proteases (SBTs) and by PME inhibitors (PMEIs). Our findings

demonstrate the role of specific PMEs, PMEIs and SBTs in plant immunity[1,2].

1.Lionetti V, et al. Plant Physiology 2017;173(3):1844-63.
2.Giancaspro A, Lionetti V, et al. Plant Science 2018 274:121-128

P22.1 - Rolb-transformed tomato plants increase their defence response following Pyrenochaeta lycopersici infection

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Pyrenochaeta lycopersici is among the most relevant soilborne pathogens of tomato. The ascomycete is the causal agent of Corky Root Rot (CRR), a disease characterised by necrotic lesions on the surface of roots. It is well documented for *rolB* a role in enhancing plant resistance to fungal attacks. In the present work, *rolB* expressing tomatoes were tested following infection with *P. lycopersici*. Infected transgenic roots seem to undergo a hypersensitive response at the site of infection, close to root/stem interface, while the wild type roots show more extended necrosis. The exposition to *P. lycopersici* was also conducted *in vitro* on detached leaves, in this case *rolB* plants show fainter symptoms of necrosis compared to wild type plants. Furthermore, we have selected some of the tomato genes known to be involved in the defence response to pathogens and we are currently evaluating their expression in *rolB* infected plants by qRT-PCR analyses. The differences at gene expression level between wild type and *rolB* plants, both infected and not-infected, will be discussed. First results suggest the potential of *rolB* in improving tomato tolerance against *P. lycopersici*.

P22.2 - Plant-microbe interaction in Antarctica

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Endophytes are microorganisms that dwell inside healthy plant tissues without causing any detectable disease symptoms to the host. They are ubiquitously associated with almost all plants and are able to improve plant ecology and fitness, conferring resistance to abiotic and biotic stresses. Their ecological role becomes even more important when plants live in extreme environmental conditions such as those of Antarctica. In recent years, many studies focused on the analysis of soils and roots microbial communities associated to the Antarctic vascular plants *Colobanthus quitensis* and *Deschampsia antarctica*, whereas less it is known about leaf-associated microorganisms.

In this work, we show a preliminary study on the disclosure of bacterial, fungal and viral communities associated to *C. quitensis* leaves. A metatranscriptome analysis revealed the presence of sequences belonging to plant (72%), fungi (23%), bacteria, viruses and algae (5%). The ecological role of viruses was mainly addressed in order to deepen knowledge on the tripartite plant-fungus-virus interaction. Culturable bacteria and fungi have been also isolated and are currently under investigation.

P22.3 - A Berberine-Bridge enzyme-like protein is a specific oxidase that modifies cellulose oligomers and plays a role in Arabidopsis immunity and development

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During infection pathogens secrete enzymes that degrade the plant cell wall. Fragments derived from the cell wall breakdown can be sensed by plant cells as Damage-Associated Molecular Pattern (DAMPs) and activate immune responses. An exaggerated activation of defense responses can lead to hyper-immunity, which causes a reduction of growth. It was recently discovered that the products of the cellulose breakdown, i.e. the cellodextrins (CDs), behave as DAMPs. CDs likely require homeostatic control mechanisms to prevent deleterious responses. We show the biochemical characterization of an *Arabidopsis thaliana* Berberine Bridge Enzyme-like (BBE-like) protein that specifically oxidizes CDs. By ion exchange chromatography, CELLODEXTRIN OXIDASE 1 (CELLOX1), was purified. Plants overexpressing *CELLOX1* display an enhanced resistance to the fungus *Botrytis cinerea* and the bacterium *Pseudomonas syringae,* but an enhanced susceptibility to the bacterium *Pectobacterium carotovorum.* They also display increased root growth. We assume that CELLOX1 is involved in both plant immunity and development by regulating the homeostasis of CDs.

P22.4 - Involvement of *Arabidopsis thaliana* Copper Amine Oxidase β in maturation of root protoxylem precursors induced by leaf wounding

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Root architecture and anatomy are strictly related to crop productivity especially under environmental stress. In particular, metaxylem plasticity enhances water uptake and improves plant performance to protect yield under water stress. Xylem vessels maturation depends on coordinated events of cell wall lignification and developmental PCD, which both are triggered by a developmental- and/or stress-driven oxidative burst. Copper amine oxidases (CuAOs) oxidize polyamines to aminoaldehydes, producing H_2O_2 and ammonia. In this regard, the Arabidopsis AtCuAOβ is expressed in guard cells and root xylem tissues, in which it plays a role in the MeJA-induced early protoxylem differentiation. In this study, the involvement of AtCuAOβ in maturation of root protoxylem precursors induced by leaf wounding was investigated by reverse genetic approaches. An early root protoxylem differentiation was observed in leaf-cut WT plants, while *atcuaoβ* insertional mutants were unresponsive in the same conditions, suggesting that the cell-wall localized AtCuAOβ plays a role in root protoxylem phenotypic plasticity triggered by systemic leaf-to-root signaling pathways after leaf wounding.

P22.5 - SEIPIN proteins affect lipid droplet biogenesis in *Arabidopsis* to promote pollen transmission and reduce seed dormancy

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The physiological role of lipid droplets (LDs) in plants is largely unknown. To gain insight into the function of LDs, we have characterized the *Arabidopsis* homologs of SEIPIN proteins, which are crucial for LD biogenesis in yeast and animals. LDs are the main source of carbon storage, but are also involved in stress responses, development and signaling. SEIPIN1 is expressed in embryo; SEIPIN2 and SEIPIN3 in embryo and pollen. Single, double and triple SEIPIN knockout mutants have been obtained. The double seipin2 seipin3 and triple mutants accumulate enlarged LDs in seeds and pollen, hindering their mobilization during germination. Electron microscopy analysis reveals the presence of nuclear LDs in triple mutant embryos,

supporting that SEIPINs are essential for maintaining the correct polarity of LD budding. In pollen, the perturbations in LD biogenesis and turnover reduced germination *in vitro* with a lower fertilization efficiency *in vivo*. In seeds there was an increase in seed dormancy levels. Our findings reveal the importance of SEIPIN-dependent LD biogenesis in pollen transmission and in adjusting the timing of seed germination, traits of great importance in agriculture.

P22.6 - Post transcriptional control of AtPME17 expressed in *Arabidopsis thaliana* during *Botrytis cinerea* infection

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AtPME17 specifically expressed during *B. cinerea* infection belongs to the Type 1 ProPMEs (1). In addition to the PME domain, AtPME17 contains a Pro-domain located at N-terminus of the *ProPME* gene. The cleavage of the Pro-domain by subtilisin-like Serine Proteases (SBTs) can activate the secreted mature PMEs in the apoplast. In addition to SBT, PME activity is post-transcriptionally regulated by PME inhibitors (PMEIs).PMEIs inhibit endogenous enzymes to protect cell wall integrity for immunity against necrotrophic pathogens (2). Sequence similarities between the Pro-domains with PMEIs indicate a possible role of the Pro-domain in auto-inhibitory activity against the mature PME. proPME17and both the Pro-domains and a pathogen induced PMEI have been independently expressed in *Pichia pastoris,* purified and their activities determined. The possible inhibitory activity of Pro-domain and PMEI against defence-related PMEs is explored.

1-Lionetti V., Cervone F. and Bellincampi D. (2012) Journal of Plant Physiol.169:1623-1630

2-Lionetti V., et al (2017). Plant Physiol.173: 1844–1863 doi:10.1104/pp.16.01185

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P22.7 - Arabidopsis cultured cells without a functional P5C dehydrogenase show reduced oxidative burst following the exposure to fungal elicitor, but only in the presence of a functional Pro dehydrogenase

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Proline metabolism plays a role in many defence responses of plants. These include adaptation to abiotic stress, but more recent findings also suggest a function in the plant immune reaction against pathogens. P5C dehydrogenase, the enzyme that catalyses the final step in proline oxidation, was strongly induced in cereals during compatible, but not during incompatible interactions with rust fungi. Conversely, Arabidopsis *p5cdh* mutants displayed reduced oxidative burst and lower resistance against bacterial pathogens. On this basis, it has been hypothesized that the mitochondrial oxidation of proline may cause the production of reactive oxygen species at levels required to induce a successful response to pathogen challenge. However, conclusive evidence supporting this mechanism is still lacking. To address this issue, we established cell cultures from *A. thaliana* insertional mutants with an array of combinations of functional or

defective enzymes in proline catabolism. Preliminary results showed lower levels of superoxide production by *p5cdh* cells treated with a fungal elicitor. Interestingly, this reduction was only observed in the presence of functional ProDH.

P22.8 - Involvement of Arabidopsis Copper Amine Oxidase β in MeJA/ wounding-induced stomatal closure

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The developmentally-regulated and stress-induced copper amine oxidases (CuAOs) oxidize polyamines to aminoaldehydes producing the plant signal molecule hydrogen peroxide (H_2O_2) and ammonia. The *Arabidopsis thaliana CuAOβ* encodes an apoplastic CuAO expressed in root protoxylem and in guard cells. In this study, its role in MeJA/wounding-induced stomatal closure was explored by pharmacological and genetic approaches. MeJA treatments induced stomatal closure in WT plants after 15'. Leaf/root wounding induced both local and systemic signaling leading to stomatal closure after 5' (local response) as well as 5' and 60' (systemic responses to root or distal leaf wounding), while *atcuaoβ* insertional mutants were unresponsive. No differences in stomatal closure between WT and mutants were observed in physiological conditions. Treatment with the H_2O_2-scavenger *N,N'*-dimethylthiourea reversed the MeJA/ wounding-induced stomatal closure in WT plants, suggesting a key role of the AtCuAOβ-delivered H_2O_2 in these event. Results suggest the *AtCuAOβ* involvement in MeJA/wounding-induced stomatal closure, with a dynamic implying extremely rapid long-distance leaf-to-leaf or root-to-leaf communication.

P22.9 - The Arabidopsis AUXIN RESPONSE FACTOR 8 (ARF8) controls pollen development by regulating exine pattern formation

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In Arabidopsis, pollen maturation is a post meiotic process occuoring inside the anther. It consists of two mitotic divisions leading to tricellular pollen grains and the formation of a resistant cell wall formed by exine and intine layers that protects pollen grains from environmental stresses. Although the role of auxin in pollen mitotic divisions has been established , its role on pollen wall formation remains unclear. We characterized pollen morphology of the Arabidopsis *arf8-7* mutant defective in the AUXIN RESPONSE FACTOR 8, which plays a major role in stamen development. Using electron microscopy analysis we showed that exine formation in *arf8-7* is severely impaired. Accordingly expression of the transcription factors that are essential for pollen exine formation such as *AMS*, *MS188*, and *MS1* is downregulated in *arf8-7*. By inducibly expressing different *ARF8* splice variants in *arf8-7* flowers, we showed that ARF8.1 fully rescues exine defects of *arf8-7* pollen grains and restores the expression of the transcription factors that control exine formation. Our results suggest a key role of auxin via ARF8.1 in controlling pollen wall development by regulation of exine patterning.

P22.10 - Preparing for defense: elicitor-triggered priming of Arabidopsis innate immunity requires *AtLYK2* and *PCaP1*

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Plants have developed defense mechanisms not only to fend off invading pathogens, but also to be prepared to subsequent attack by the same or other microorganisms. In particular, when plants perceive microbe-associated molecular patterns (MAMPs), endogenous damage-associated molecular patterns (DAMPs) or abiotic stresses, they often acquire a primed state of enhanced defense that make them able to mount a faster and stronger response to subsequent infections. To date, the molecular mechanisms underlying this phenomenon are largely unknown. We have recently identified two Arabidopsis thaliana mutants impaired in elicitor-triggered priming phenotype: *AtLYK2*, encoding a LysM-containing receptor-like kinase, and *PCaP1*, also known as MICROTUBULE DESTABILIZING PROTEIN 25 (MDP25), encoding a plasma membrane-associated cation-binding protein. Notably, *atlyk2* and *atpcap1* mutants show unaltered basal resistance to the fungal pathogen *Botrytis cinerea*, but fail to display increased expression of defense-related genes and enhanced resistance after pre-treatment with MAMPs or DAMPs. Moreover, long-term responses to elicitors appear to be altered in these mutants.

P22.11 - Unconventional compounds *versus* endogenous defence mechanisms against powdery mildew: what drives grapevine phyllobiome composition?

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Under a global changing scenario, the reduction of antimicrobial treatments and the application of eco-friendly compounds is an impelling challenge for grapegrowers in the optic of a more sustainable viticulture. We addressed this research to study if non-conventional compounds can counter leaf powdery mildew attack and how they can influence the phyllobiome of Vitis vinifera 'Moscato' and 'Nebbiolo' plants.

The effect of antifungal treatments in shaping the foliar microbial community of powdery mildew-infected vines was evaluated by: i) community-level physiological profiling (CLPP) by using Biolog EcoplatesTM; ii) high throughput sequencing of ITS2 region; iii) depict of virome, viroids and phytoplasma; iiii) analysis of cuticular wax content and secondary metabolites.

Independently of the applied compound, integration of metabolic and fungal profiling analyses revealed a more complex phyllobiome and a lower incidence of the pathogen in 'Moscato', which showed higher concentrations of stilbenoids than 'Nebbiolo'. These results unraveled distinct genotype-mediated defense mechanisms as main factors driving fungal community composition.

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P22.12 - UPR engagement during seed storage protein synthesis

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Seed storage proteins are the major global source of food protein, threatened by the negative effect of climate changes on crop yield and quality. All storage proteins fold and assemble in the endoplasmic reticulum (ER); in most plants, they are then delivered to storage vacuoles. The major proteins of cereals (prolamins) instead accumulate in the ER as insoluble heteropolymers (protein bodies, PBs). During seed development, ER homeostasis is challenged by the massive protein synthesis, but it is not yet known to which extent the different classes of storage proteins induce the unfolded protein response (UPR) and how this is affected by climate changes. We have compared the expression of UPR genes in transgenic *A. thaliana* expressing *Z. mays* PB prolamins or a *P. vulgaris* vacuolar storage protein. Our data indicate that: 1) UPR is activated by prolamins, but not by the vacuolar storage protein; 2) having already a moderate UPR, plants that accumulate prolamins respond more promptly to abiotic stress; 3) two distinct maize prolamins interact at different levels with the UPR machinery. These findings provide molecular tools to sustain grain protein filling under stress.

P22.13 - Genome-wide association study REVEALS Novel PLAYERS IN Plant hormone crosstalk

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Plant hormones salicylic acid (SA) and jasmonic acid (JA) play central roles in biotic stress responses. To deal with specific attackers or to multiple attackers at the same time, SA and JA signaling pathways need to cross-communicate. To identify novel regulators of SA-JA crosstalk, we performed a genome-wide association (GWA) study on natural genetic variation in *Arabidopsis* for the effect of SA on the JA pathway. To this aim, the expression level of the JA marker gene *PDF1.2* was analyzed in 349 wild *Arabidopsis* accessions of HapMap collection, treated with MeJA or SA+MeJA. GWA mapping of the quantitative gene expression data revealed genetic loci potentially associated with SA-JA crosstalk. Among these loci are ARR11 (encoding an *Arabidopsis* response regulator involved in cytokinin signaling) and GLYI4 (encoding a glyoxalase), which were found to affect SA-JA crosstalk by T-DNA insertion mutant screening. Moreover, resistance level against the necrotrophic fungus *B. cinerea* was also found to be influenced by ARR11 and GLYI4. Preliminary functional characterization of the two regulators shed some light on their role in SA-JA crosstalk.

P22.14 - Characterization of *Drechslera gigantea* infection in *Arabidopsis thaliana* and involvement of the unfolded protein response

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The Unfolded Protein Response (UPR) is a set of physiological and pathological responses triggered by the accumulation of unfolded proteins in the lumen of the endoplasmic reticulum (ER) as a consequence of stress conditions perturbing cell homeostasis. UPR has been characterized in respect to abiotic stimuli, like heat and cold stress, whereas information about its involvement in biotic stress is still very limited. *Drechslera gigantea* is a necrotrophic fungus, which produces phytotoxic metabolites named ophiobolins; it causes eye-spot disease in many plants and it is responsible for severe cereal crop losses. Here we describe the infection of *Arabidopsis thaliana*

leaves by the fungus: data about ion leakage, total chlorophyll content, lipid peroxidation, callose deposition and oxidative burst will be presented. Moreover, the molecular characterization of ER stress and UPR during infection will be reported.

P22.15 - Analysis of the *AP1*-, *SEP*- and *AGL6*-like genes expressed in the inflorescence of the Mediterranean orchid *Orchis italica*

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The MADS-box genes involved in the ABCDE model of flower development are classified into five different functional classes (from A to E). We analysed the *AP1/SQUA*, *SEP* and *AGL6* MADS-box genes of *Orchis italica*, a Mediterranean orchid belonging to the Orchidoideae subfamily. Among the 28 MADS-box transcripts expressed in the inflorescence transcriptome of *O. italica*, 4 belong to the class A (*AP1/SQUA*), 2 to the E class (*SEP*) and 3 to the *AGL6* subgroup. The expression profile of these transcripts in the different organs of the flower of *O. italica* shows that they have specific expression in tepals (outer or inner), lip, column (a fusion of male and female reproductive tissues), and ovary. This expression pattern is only in part in agreement with the general ABCDE model and some differences are detectable when compared to the expression of the homolog genes in dicots and other monocots. These results suggest that in *O. italica* the flower development follows a "fading borders" model, where the determination of the floral structures is due to a gradient of expression levels of the MADS-box genes across the floral organs.

P22.16 - Strigolactones are involved in the floral induction in tomato

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Strigolactones (SLs) are a class of plant hormones with various functions in plant development and in the interaction with (micro) organisms in the rhizosphere. As developmental regulators the SLs control above- and below-ground morphology, the inhibition of shoot branching, the modulation of the root morphology and the promotion of the shoot secondary growth. It follows that SL-depleted or insensitive plants show an altered morphology (stunted and bushy), a prolongate vegetative development and a delayed in reproductive transition. This latter aspect leads to a reduced number of flowers fruits and seeds in *Solanum lycopersicum*. To know the effects of an excess of SLs on the reproductive phenotype in tomato we analyzed the results of the exogenous treatments with rac-GR24, a synthetic strigolactone. Preliminary data indicate that the treated plants show a preponed flowering and an earlier harvestable fruits compared to mock-treated plants. Moreover the cumulative yield at the end of the season is higher for GR24-treated plants. The integration of these data is currently proceeding by NGS and targeted transcript quantification of the genes involved in floral induction in tomato.

P22.17 - Neofunctionalization of a plant prolamin upon whole genome duplication

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Zeins, the seed storage proteins of maize, assemble into insoluble protein bodies (PB) in the endoplasmic reticulum (ER). The very abundant γ-zein of 27kD (27γz), fundamental for maize protein quality, forms homotypic PBs also when expressed in transgenic plants. 16γz originates

from duplication of the 27γz gene followed by deletion, after maize allotetraplodization. 16γz is mainly characterized by the loss of part of the 27γz Pro-rich repeats and of three of the seven Cys residues necessary for 27γz polymerization, and is located in the PB region between 27γz and α-zeins. We show that, in transgenic Arabidopsis, 27γz forms PBs, but 16γz forms very unusual polymeric filamentous structures that greatly enlarge the ER lumen. ER retention of 27γz depends on disulphide-mediated insolubilization, whereas retention of 16γz depends, at least in part, on a different mechanism. Therefore, a prolamin paralog generated upon maize whole genome duplication has changed its polymerization properties, losing the ability to form homotypic PBs and acquiring a new function in the assembly of maize PB. Co-expression of different zein constructs is undergoing, to define their interactions.

P22.18 - The mitochondrial protein WHIRLY2 is required for efficient functionality of mitochondria during seed development and germination

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Variations in amount and structural integrity of organellar DNA are tightly regulated by nuclear organelle cross-talk. Whirly proteins are DNA binding proteins that were shown to play a role in organellar DNA maintenance and organization. Arabidopsis thaliana has three Whirly proteins with different subcellular localization: Whirly1 and Whirly3 are targeted to chloroplasts, while Whirly2 is targeted to mitochondria. *WHIRLY2* gene expression is related to early plant development, being expressed in imbibed seeds, shoot apex and roots of young seedlings. A T-DNA insertional mutant for the *WHIRLY2* gene shows an obvious phenotype on seeds, germination and early stages of plant growth. At subcellular level WHIRLY2 regulates mitochondria morphology, dynamics and functionality of the electron transport chain.

AUTHOR INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

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