

2019

3rd Scientific Retreat
Department of Biology
January 30 - February 1

Sala delle Colonne del Giardino della Biodiversità,
Orto Botanico, Padova

BOOK OF ABSTRACTS



UNIVERSITÀ
DEGLI STUDI
DI PADOVA



ORAL PRESENTATIONS

ORAL PRESENTATIONS – TIMETABLE

Wednesday, January 30

| | |
|---|---|
| 14.00 Keynote Lecture: Prof. Stefano Piccolo | 4 |
| Tumors as wounds that never heal: insights from YAP biology | |

15.00-16.40 Session 1: Metabolic signals

| | |
|---|---|
| 15.00 Roxana Oberkersch | |
| Role of isopentenyl-diphosphate isomerase 1 in tumor angiogenesis | 4 |
| 15.20 Stéphanie Herkenne | |
| Developmental and tumor angiogenesis requires changes in endothelial cell mitochondrial shape | 4 |
| 15.40 Margherita Peron | |
| Mito-Stat3 regulates cell proliferation by promoting mitochondrial gene expression | 5 |
| 16.00 Stefano Cagnin | |
| Non-coding RNAs are involved in metabolic regulation of skeletal muscle fibers | 5 |
| 16.20 Camilla Bean | |
| The mitochondrial fusion protein Opa1 recruits the chromatin remodeling protein Kdm3 to promote adipose tissue browning | 6 |

17.40-19.20 Session 2: Signals in disease

| | |
|---|---|
| 17.40 Elena Ziviani | |
| Ubiquitination/Deubiquitination and mitochondria quality control in neurodegeneration | 6 |
| 18.00 Nicoletta Plotegher | |
| GBA and neurodegeneration: gain or loss of function? | 7 |
| 18.20 Paola Cusumano | |
| MiR-210: a link between the circadian clock and sleep disorders? | 7 |
| 18.40 Claudia Sacchetto | |
| Novel genes and molecular mechanisms involved in arrhythmogenic cardiomyopathy | 8 |
| 19.00 Gaia Codolo | |
| The immune receptor CD300e negatively regulates T cell activation by impairing the expression of MHC-II molecules in antigen-presenting cells | 8 |

Thursday, January 31

9.00-10.40 Session 3: Signals in Plants and Microorganisms

| | |
|--|----|
| 09.00 Elide Formentin | |
| From single cell to the whole plant: ROS and calcium signaling in rice salt acclimation | 9 |
| 09.20 Enrico Cortese | |
| The emerging role of chloroplasts and endoplasmic reticulum in plant organellar calcium signaling | 9 |
| 09.40 Luca Carraretto | |
| Chloroplast-localized mitochondrial calcium uniporter cMCU transduces specific environmental signals and mediates drought tolerance in Arabidopsis | 10 |

| | |
|---|----|
| 10.00 Mattia Storti | |
| Regulation of photosynthetic electron transport to respond to light dynamics in the moss <i>Physcomitrella patens</i> | 10 |
| 10.20 Laura Treu | |
| Deciphering complex microbial interactions in biogas upgrading systems by genome-centric meta-transcriptomics | 10 |
| 11.40-13.20 Session 4: Signals in organisms, evolution and environment | |
| 11.40 Lorenzo Zane | |
| Connectivity signals as the key for networks of MPAs | 11 |
| 12.00 Luca Pagani | |
| Ancestry-specific analyses reveal differential demographic histories and opposite selective pressures in modern South Asian human populations | 11 |
| 12.20 Chiara Anselmi | |
| Molecular signals of chordate development: two disparate pathways, one tunicate | 12 |
| 12.20 Umberto Rosani | |
| Invertebrate herpesviruses as a tool to unlock antiviral pathways in marine mollusks | 12 |
| 13.00 Carlotta Mazzoldi | |
| The Marine Biology Centre in Chioggia: the new facilities | 12 |
| 14.20-15.40 Session 5: Mitochondrial biology | |
| 14.20 Roberto Costa | |
| Impaired mitochondrial energetic metabolism down-regulates Wnt signaling | 13 |
| 14.40 Samantha Corrà | |
| Drosophila melanogaster as a model to investigate the role of Mpv17 in mitochondrial DNA maintenance and metabolism | 13 |
| 15.00 Libero Vitiello | |
| Drug repurposing for Duchenne muscular dystrophy: monoamine oxidase B inhibitors can ameliorate the pathological phenotype in mdx mice and in myogenic cultures from DMD patients | 14 |
| 15.20 Vanessa Checchetto | |
| Identification of an ATP-sensitive potassium channel in the inner mitochondrial membrane | 14 |
| 16.40-18.20 Session 6: Plants and Biotechnology | |
| 16.40 Lorenzo Maso | |
| Novel strategies to contrast antimicrobials resistance: old friends and new weapons | 15 |
| 17.00 Sebastiano Nigris | |
| MADS-box genes and development of flower and fruit in <i>Nymphaea caerulea</i> : modern traits and old inventions | 15 |
| 17.20 Michela Zottini | |
| WHIRLY2, a mitochondrial DNA binding protein, required for efficient mitochondrial functionality in seed development and germination | 16 |
| 17.40 Barbara Gris | |
| Cyanobacteria biodiversity, maturation process and high value compounds of the Euganean thermal muds: revealing the scientific basis of an ancient therapeutic biotechnological product | 16 |

| | | |
|--|--|----|
| 18.00 Marina Simona Robescu | | |
| Adding a new regeneration enzyme to the biocatalysis toolbox: discovery/identification and rational engineering of a new formate dehydrogenase | | 17 |

Friday, February 1

9.20-11.00 Session 7: Disease: from bioinformatics to in vivo models

| | | |
|---|--|----|
| 09.20 Enrica Calura | | |
| Multi-omics data integration for the identification of survival-associated cancer mechanisms | | 17 |
| 09.40 Fabio De Pascale | | |
| Analysis of recurrent variants could reveal inconsistencies in the human reference genome | | 17 |
| 10.00 Maurizio David Baroni | | |
| Control of cell quiescence and survival in yeast by a cancer chemoprevention drug | | 18 |
| 10.20 Laura Civiero | | |
| Glial phagocytic clearance in Parkinson's disease | | 18 |
| 10.40 Giacomo Meneghetti | | |
| The epg5 knockout zebrafish line: a model for Vici syndrome and to screen autophagy-stimulating drugs | | 19 |

11.40-13.00 Session 8: Evolution and Environment

| | | |
|--|--|----|
| 11.40 Loriano Ballarin | | |
| Complement-mediated cooperation between immunocytes in the compound ascidian <i>Botryllus schlosseri</i> | | 19 |
| 12.00 Lucio Bonato | | |
| Unveiling organism intraspecific diversity: multitechnique investigations on terrestrial animals across the Southern Prealps | | 20 |
| 12.20 Carlotta Mazzoldi | | |
| Elasmobranch conservation: interaction between use of space, behavior and fishery | | 20 |
| 12.40 Marco Bonato | | |
| Persistent Mobile Organic Compounds: LIFE PHOENIX Project experience | | 20 |

ORAL PRESENTATIONS - ABSTRACTS

Keynote Lecture

Stefano Piccolo

Department of Molecular Medicine, University of Padova

Tumors as wounds that never heal: insights from YAP biology

Enhanced YAP/TAZ activity is emerging as common trait of multiple solid tumors in humans. Strikingly, in mouse models, adult organs lacking YAP/TAZ are unable to develop tumors without overt side effects for normal tissue homeostasis, making YAP/TAZ prime candidates for cancer therapy. That said, YAP/TAZ have physiological functions during tissue repair, being essential for organ regeneration after injury. YAP/TAZ are typically inactive in normal tissues but potently induced by mechanical and physical cues that the cell receives from its microenvironment, such as extracellular matrix stiffness and topology, and 3D architectural features of the tissue. I will focus on new discoveries on the mechanisms by which YAP/TAZ are controlled by mechanotransduction, and on YAP/TAZ as reprogramming factors in epithelial cells. Indeed, in normal tissues YAP/TAZ activation turns more differentiated normal cells into cells endowed with stem-like properties; in tumors, YAP/TAZ convert non-stem tumor cells into cancer-stem cells, increasing tumor aggressiveness and fueling metastasis. I will also expand on these mechanisms, providing new hints for therapeutic intervention.

Session 1: Metabolic signals. Chair: Luiqi Bubacco

Roxana E. Oberkersch*

**Unit of Cell Biology and Developmental Genetics*

Role of isopentenyl-diphosphate isomerase 1 in tumor angiogenesis

Angiogenesis is the formation of new blood vessels from the pre-existing vasculature and a key component in tumor development. Isoprenoids, middle products of mevalonate pathway, play essential roles in endothelial cells including membrane structure, signal transduction, and redox chemistry. Although many reports have shown deregulation of mevalonate pathway in cancer, little is still known about the role of isopentenyl-diphosphate isomerase 1 (IDI1), key rate-limiting step of the isoprenoids biosynthesis, in tumor angiogenesis. The aim of the present study is to investigate the role of IDI1 as possible target in tumor angiogenesis. First, we determined whether different tumor conditions were able to regulate the expression of IDI1 in endothelial cells (ECs). Our results showed a significant increase of IDI1 levels at low pH, whereas no differences were observed in hypoxia and in presence of VEGF. When we knocked down IDI1 using shRNAs an inhibition of proliferation and induction of cell death were detected in ECs, accompanied by a decrease of tRNA^{Adimethylallyltransferase 1 (TRIT1)}. Interestingly, deferoxamine, an inhibitor of ferroptosis, rescued the lethal phenotype. In parallel, we have generated a lox allele of the IDI1 locus in mice and we are currently investigating tumor angiogenesis in VEC-CreERT2; IDIfl/fl1 mice.

Stéphanie Herkenne*, Olivier Ek, Maya Chergova, Gabriele Sales, Eliška Novotná, Andrielly Agnelli, Natascia Tiso, Antonella Viola, Francesco Argenton, Marta Giacomello, Elena Ziviani, and Luca Scorrano

**Unit of Bioenergetic Organelles*

Developmental and tumor angiogenesis requires changes in endothelial cell mitochondrial shape

Despite the impact of mitochondrial fatty acid and amino acid oxidation on endothelial cell (EC) function, mitochondria are thought to be accessory organelles in angiogenesis, the new blood vessel formation from existing vasculature. Here we show that the inner mitochondrial membrane

mitochondrial fusion protein Optic Atrophy 1 (OPA1) is a required component of the angiogenesis program. Upon angiogenic stimulation, mitochondria elongate in vitro and in vivo, concomitant with a rapid OPA1 levels increase. Consistently, angiogenesis is curtailed by genetic Opa1 ablation in zebrafish and mouse, where endothelial OPA1 is also required for tumor growth and lymphangiogenesis-mediated tumor metastatization. A combination of gene expression profiling and functional studies reveals that endothelial Opa1 orchestrates angiogenesis by limiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) signaling to allow angiogenic genes expression. Accordingly, developmental and tumor angiogenesis is unaffected in cells and mice where NF κ B is deleted with Opa1 in ECs. Our data indicate that mitochondrial morphology is a crucial, targetable component of physiological and tumor angiogenesis program.

Margherita Peron*

**Unit of Cell Biology and Developmental Genetics*

Mito-Stat3 regulates cell proliferation by promoting mitochondrial gene expression

STAT3 is a transcription factor that, through its role of nuclear transcriptional regulator, has been demonstrated to play a pivotal role in many cellular processes including oncogenesis, tumor growth and progression, and stemness. Classically, when phosphorylated in Y705, activated STAT3 translocate into the cell nucleus and binds to the interferon-gamma activated sequence (GAS) within its target gene promoters. This STAT3 canonical pathway is frequently found to be constitutively activated in many human cancers and linked to more malignant cancer behaviors, tumor survival and therapeutic resistance. Nevertheless, evidences suggest that a second non-canonical Stat3 pathway, which acts through Ser727 phosphorylation, is also implied in tumor cells transformation and radio resistance. Interestingly, a body of evidence implicates that while phospho-Stat3Y705 contributes to its nucleic translocation, phospho-Stat3S727 leads to the accumulation in mitochondria, where Stat3 seems to be regulating mitochondrial genes transcription.

We demonstrated in vivo in zebrafish embryos, that Stat3-dependent mitochondrial gene expression requires both Y705 and S727 phosphorylation, for mitochondrial import and mitoStat3 transcriptional activity respectively. Moreover, using an inhibitor of Y705 phosphorylation, we have observed the absolute requirement of mitochondrial Stat3 for normal mitochondrial gene expression. Notably, by injecting zebrafish embryos with a mitochondria targeted form of Stat3 we could also observe an activation of cell proliferation that is blocked when Stat3 injected embryos are treated with a known inhibitor of mitochondrial transcription. Consistently, inhibition of Y705 phosphorylation leads to reduced cell proliferation. Moreover, embryos injected with mitochondrial Stat3 do not show any increase in mtDNA copy number, thus revealing that mitoStat3 promotes cell proliferation by activating mtDNA transcription, independently from mtDNA replication.

These data together imply the need to investigate the dependence of tumor cell proliferation on mitoStat3-dependent gene expression in zebrafish cancer models. If so, mitochondrial STAT3 transcriptional activity could provide an attractive target for therapeutic approaches to cancer.

Enrico Alessio, Francesco Chemello, Lisa Buson, Caterina Peggion, Francesca Grespi, Paolo Martini, Alessandra Zulian, Pasqua Cancellara, Paolo Martini, Camilla Bean, Etienne Hebert-Chatelain, Andrea Armani, Maria Lina Massimino, Beniamina Pacchioni, Caterina Millino, Marco Sandri, Ruggero Ferrazza, Paolo Laveder, Graziano Guella, Carlo Reggiani, Chiara Romualdi, Alessandro Bertoli, Paolo Bernardi, Luca Scorrano, Gerolamo Lanfranchi and **Stefano Cagnin***

**Unit of Human Molecular Genetics and Functional Genomics*

Non-coding RNAs are involved in metabolic regulation of skeletal muscle fibers

Skeletal muscle is a complex tissue composed by different type of cells. The major component is made by elongated and multinucleated contractile units called myofibers. Myofibers exist with different structural, physiological, metabolic and transcriptional profiles. The myofiber-type composition explains the diversity in contraction velocity, metabolism and the plastic response and adaptation to internal and external stimuli of muscle tissue. How non-coding RNAs (ncRNAs) expressed in different myofibers participate to metabolism regulation and pathophysiology and, in general, to the physiological trait of whole muscle is still largely debated. We compiled a comprehensive catalog of ncRNAs expressed at the level of single myofiber, demonstrating that many specific miRNAs and lncRNAs can be involved in the biological processes hampered in metabolic pathologies and muscle atrophy. In particular, we established the function of miR-27a-3p and miR-142-3p revealing their regulatory function of lipid utilization in skeletal muscle. Moreover, we evidenced that the long non-coding RNA Pvt1 impacts mitochondrial respiration and morphology and affects mito/autophagy, apoptosis, and myofiber size *in vivo*. This work describes the role and action of ncRNAs in signaling pathways regulating metabolism and pathological traits of skeletal muscle and offers a valuable resource to study metabolism at a single-cell level, in a complex tissue characterized by pronounced plasticity.

Camilla Bean*, Tatiana Varanita, Francesca Favaretto, Marta Medaglia, Marco Gerdol, Lena Pernas, Alberto Pallavicini, Nico Mitro, Kirsil Pielilainen, Roberto Vettor and Luca Scorrano

**Unit of Bioenergetic Organelles*

The mitochondrial fusion protein Opa1 recruits the chromatin remodeling protein Kdm3 to promote adipose tissue browning

Under specific conditions, white adipocytes can be converted into brown and beige adipocytes that engage mitochondria to dissipate chemical energy into heat. However, it is unclear if mitochondrial factors can per se favor white adipocyte browning. Here, we show that the mitochondrial fusion and cristae biogenesis protein Opa1 contributes to cell autonomous fate specification of adipose tissue. In adipose tissue biopsies of several cohorts of obese individuals, levels of Opa1 are decreased proportionally to the disease severity. By integrating *in vivo* models, genomic, and metabolomic approaches, we show that Opa1 improves adipocyte function, ameliorating energy balance and glucose homeostasis. Mechanistically, Opa1 increases the level of fumarate that drives a phenotypic switch from white to thermogenic beige adipocytes via the chromatin-remodeling protein Kdm3a. These data reveal a new relationship of mitochondrial dynamics with obesity and insulin resistance and identify a cristae shape-dependent mitochondria-to-nucleus signaling pathway, paving the way for developing novel therapies.

Session 2: Signals in disease. Chair: Marina de Bernard

Sophia von Stockum, Joy Chakraborty, Elena Marchesan, and **Elena Ziviani***

**Unit of Bioenergetic Organelles*

Ubiquitination/Deubiquitination and mitochondria quality control in neurodegeneration

Mitochondrial dysfunction and quality control has become a central theme in neurodegenerative diseases, Parkinson's Disease (PD) in particular. Ubiquitination of mitochondrial membrane proteins is a critical step preceding mitochondria quality control, and in the process of Autophagy-dependent degradation of mitochondria (also known as Mitophagy). Mitophagy is generally driven by ubiquitin ligases that reside on or are recruited to mitochondria to ubiquitinate mitochondrial membrane resident proteins, like the E3 Ubiquitin ligase Parkin. Mitochondrial quality control is compromised in the absence of the Parkin-dependent mitophagy pathway. Since a single Parkin substrate that is required for mitochondrial clearance has not been identified, mitophagy appears to be driven by accumulation of ubiquitinated targets on mitochondria, induced by ubiquitin

ligases other than Parkin. These ligases are effectively antagonized by specific Deubiquitinating enzymes (DUBs); therefore specific DUBs inhibitors are beneficial in this context, presumably by up-regulating Parkin-independent mitochondria quality control. With that in mind our lab aims at identifying novel DUBs that affect mitochondria quality control. We recently discovered that mitochondria quality control can be enhanced *in vivo* in flies and in PD patient cells by reducing either the levels or activity of the proteasome-associated deubiquitinating enzyme Usp14 (Chakraborty et al., EMBO Mol Med 2018) or the endosome-associated deubiquitinating enzyme Usp8 (von Stockum S. et al., under revision Life Science Alliance). We knocked down Usp14 or Usp8 in two established fly models of impaired mitophagy, and this restored mitochondria function and ultrastructure, and brain dopamine levels. Remarkably, at the systemic level it extended the flies' lifespan and rescued climbing behavior. An orally bioavailable small-molecule inhibitor of these DUBs had similarly beneficial effects on these mutant flies. Further studies will clarify whether specific DUBs inhibition is protective in mammalian models of PD, and may bring DUBs targets forward as candidate therapeutic avenues for PD.

Nicoletta Plotegher*

**Unit of Molecular & Cellular Physiology and Biophysics*

GBA and neurodegeneration: gain or loss of function?

GBA gene encodes the lysosomal enzyme glucocerebrosidase, which hydrolyses glucosylceramide to glucose and ceramides but can also have transglucosylation activity: the glucose moiety is cleaved from glucosylceramide and transferred to cholesterol, to form glucosylcholesterol. GBA homozygous mutations cause the lysosomal storage disorder Gaucher disease, while heterozygous mutations are the most common genetic risk factor for Parkinson's disease (PD). Most of the identified mutations impact on the enzymatic activity of GBA, but a clear correlation between specific mutations, the loss of the enzymatic activity and the disease phenotype is still missing. One of the options is that the phenotype is the result of a more complex effect of the mutations, having both a loss of function in term of enzymatic activity and a gain of function still to be investigated. In this frame, our goal is to provide a better understanding of the function of GBA in the central nervous system (CNS). Starting from the involvement of GBA dysfunction in the etiopathogenesis of sporadic and familial PD, we are now characterizing the functional and structural effects of the common L444P and N370S GBA mutations, in the hope of identifying new molecular chaperones able to stabilize the mutant protein. We are also investigating the role of the newly identified product glucosylcholesterol that may be responsible for aberrant signaling to neurons in PD. More general, the function of glucocerebrosidases in the CNS, not only GBA, but also GBA2 and GBA3, and the effects of changes in the relative levels of their products and substrates will deserve further studies. This will allow correlating these features with the pathophysiological consequences in brain cells, focusing on the autophagy-lysosomal pathway deregulation, on mitochondrial dysfunction and on ER stress.

Paola Cusumano*

**Unit of Neurogenetics and Chronobiology*

MiR-210: a link between the circadian clock and sleep disorders?

Flies have preferred times of sleep and wake. Over the past few years, the role of *Drosophila* micro RNAs in modulating such 24h circadian rhythmicity has been recognized. We have recently demonstrated that depletion or over-expression of miR-210 in clock neurons alters the circadian locomotion phase of flies under light-dark cycles, suggesting a contribution of this miRNA in adjusting the temporal niche of flies. miR-210 is expressed in the Pigment Dispersing Factor (PDF) positive clock neurons, in the retina and in the circadian extra-retinal photoreceptors (H-B eyelets). Both the circadian pacemaker and environmental factors such as light are fundamental in

shaping and confining sleep-activity episodes into the correct time of the day. But is miR-210 also involved in sleep regulation? The up-regulation of miR-210 in clock cells dramatically alters the PDF positive clock neurons morphology. In turn, this affects total sleep length and the temporal distribution of sleep episodes, pointing to a sleep regulatory role for miR-210. Moreover, our preliminary results suggest that the inhibition of neurotransmitters release in miR-210-expressing structures (retinal and extra-retinal photoreceptors) does indeed affect sleep, supporting our working hypothesis that miR-210 modulates the circadian activity phase and contributes to setting sleep timing in flies.

Claudia Sacchetto*

**Unit of Human Molecular Genetics and Functional Genomics*

Novel genes and molecular mechanisms involved in arrhythmogenic cardiomyopathy

Arrhythmogenic cardiomyopathy (ACM) is an inherited cardiac disease characterized by progressive loss of cardiomyocytes, fibro-fatty replacement of the myocardium, ventricular arrhythmias, and sudden death. Among the disease genes identified so far, the most frequently mutated are those encoding for the cardiac intercalated disc proteins. Most recently, our group identified two novel genes: TJP1 encoding zonula occludens-1, an intercalated disc protein interacting with proteins of gap junctions and area composita, and TP63, encoding p63 protein, a member of the p53 family of transcription factors.

To investigate the molecular mechanisms underlying the pathogenesis of ACM, we generated transgenic mice with cardiomyocyte-specific overexpression of a FLAG-tagged human desmoglein-2 harboring the Q558* nonsense mutation found in an ACM patient. The hearts of these mice showed signs of fibrosis, decrease in desmosomal size and number and reduction of the Wnt/β-catenin signaling. Genome-wide RNA-Seq performed in Tg-hQ hearts and non-transgenic hearts revealed that 24 miRNAs were dysregulated in transgenic animals. Further bioinformatic analyses for selected miRNAs suggested that miR-217-5p, miR-499-5p, and miR-708-5p might be involved in ACM pathogenesis.

In conclusion, our new transgenic model further supports the role of Wnt/β-catenin signaling in ACM pathogenesis and the contribution of dysregulated miRNAs in the development of this disease.

Gaia Codolo*

**Unit of Cell Biology and Developmental Genetics*

The immune receptor CD300e negatively regulates T cell activation by impairing the expression of MHC-II molecules in antigen-presenting cells

CD300e is a glycosylated surface receptor belonging to a family of immune receptors that includes 8 members either activating or inhibitory which are expressed on myeloid cells, on lymphoid cells or on both compartments. Since the ligand of CD300e is still unknown, its function was studied by using an agonistic anti-CD300e monoclonal antibody (clone UP-H2). The evidence that the engagement of CD300e in human monocytes and myeloid dendritic cells provided the cells with survival signals and triggered the expression of the activation markers and the release of pro-inflammatory cytokines, led to the conclusion that CD300e is an immune-activating receptor. The same conclusion was reached studying the CD300e ortholog in mice.

However, we got evidence that identify CD300e as an uncommon immune receptor. In fact, despite its activation triggers in monocytes a signaling cascade that culminates in the release of pro-inflammatory cytokines and in the up-regulation of co-stimulatory molecules, in parallel it compromises the antigen presenting capacity toward CD4+ T lymphocytes. This occurs because the synthesis of MHC-II molecules is hampered, but also because the internalization rate of

peptide-MHC-II complexes from the cell surface is increased, even if the second process is expected to contribute little.

In tumors, tumor-associated macrophages (TAMs) are among the most abundant immune cells to be recruited. The majority of studies referring to carcinomas, including the colorectal cancer (CRC), show that greater infiltration of TAMs is associated with poor prognosis. TAM in CRC typically show an immunosuppressive activity accompanied by an impaired antigen-presenting capability. The expression of CD300e in these cells was never assessed, leaving open the possibility of an involvement of the immune receptor in MHCII down-modulation. Preliminary immune histochemistry analysis performed on human CRC specimens showed a clear-cut staining for CD300e in a good proportion of TAMs infiltrating CRC. This was mirrored by a lowered expression of MHC-II, compared to macrophages not expressing CD300e. Furthermore, we observed that monocytes, exposed to a conditioned medium of human colorectal cancer cells acquired a macrophage-like phenotype with an anti-inflammatory profile and showed an increased exposure of CD300e on the plasma membrane paralleled by a decrease of the MHC-II antigen.

Overall, our data support the notion that the immune receptor CD300e might be a novel immune check point that contributes to the regulation of the physiological as well as pathological expansions of T cell-mediated responses and pave the way for a promising and very challenging research line.

Session 3: Signals in Plant and Microorganisms. Chair: Tomas Morosinotto

Elide Formentin*

*Unit of Plant Physiology and Molecular Biology

From single cell to the whole plant: ROS and calcium signaling in rice salt acclimation

Salinity tolerance is a complex trait. Despite many efforts to obtain salt-resistant rice plants, few results have been achieved so far and a deeper understanding of the tolerance mechanisms is needed.

By studying two Italian rice varieties showing contrasting salt responses, we unveiled mechanisms required both at cell and whole-plant level to achieve salt tolerance.

Plants adopted morphological, hormonal, and gene regulation changes to set up an adaptive program for the survival. In presence of salt, specific H₂O₂ profile and calcium signatures defined a tolerant response.

Our results indicate that the acclimation mechanism adopted by tolerant plants is triggered by specific ROS and calcium-mediated pathways that lead to osmotic compensation, photosynthesis preservation and phenotypic plasticity needed for the survival of plants.

Enrico Cortese*

*Unit of Plant Biology

The emerging role of chloroplasts and endoplasmic reticulum in plant organelle calcium signaling

Calcium is a fundamental intracellular messenger involved in a wide range of different signaling pathways in all eukaryotes. In plants, Ca²⁺ has been shown to participate in the transduction of a large plethora of environmental stimuli of both abiotic and biotic nature. A complex Ca²⁺ homeostatic and signaling machinery allows for a tight regulation of the intracellular concentration of the ion ([Ca²⁺]) and its variations during signal transduction. Plant organellar Ca²⁺ signaling is a rapidly expanding field of investigation that has recently benefited from the availability of novel specifically targeted reporters useful to monitor intracellular Ca²⁺ dynamics. In particular, the setup of a toolkit of aequorin-based probes targeted to the different subcompartments of chloroplasts (envelope, stroma, thylakoids) has allowed for the elucidation of stimulus-specific intra-organelar Ca²⁺ signals and their contribution to shaping cytosolic Ca²⁺ signatures. Moreover,

the recent design of an aequorin chimera targeted to the endoplasmic reticulum (ER) has provided accurate measurements of $[Ca^{2+}]$ in the plant ER, highlighting significant differences with respect to the animal compartment in overall Ca^{2+} handling. The comparative analysis of Ca^{2+} dynamics in chloroplasts and ER will help unravel their functional interplay and integration in the plant Ca^{2+} signaling network.

Enrico Teardo, Luca Carraretto*, Roberto Moscatiello, Enrico Cortese, Mattia Vicario, Margherita Festa, Sara De Bortoli, Tito Calli, Ute Vothknecht, Elide Formentin, Laura Cendron, Lorella Navazio and Ildiko Szabo

**Unit of Bioenergetic Organelles*

Chloroplast-localized mitochondrial calcium uniporter cMCU transduces specific environmental signals and mediates drought tolerance in *Arabidopsis*

Chloroplasts are integral to sensing biotic and abiotic stress in plants, but the role of chloroplasts in transducing Ca^{2+} mediated stress signals is still poorly understood. Here we identify cMCU, a member of the mitochondrial calcium uniporter (MCU) family, as the ion channel mediating Ca^{2+} flux into chloroplast *in vivo*. Using a toolkit of aequorin reporters targeted to chloroplast stroma and the cytosol in wild-type and knock-out lines for cMCU, we provide evidence that stress stimulus-specific Ca^{2+} dynamics in the chloroplast stroma correlate with expression of the channel. Drought-triggered fast downstream signaling events involving MAPK3/6 kinase activation and MYB60 and ERF6 transcription factors are influenced by cMCU activity. Relative to wild-type plants, knock-outs lacking cMCU display an increased resistance to long-term water deficit and recover upon re-watering. Modulation of stromal Ca^{2+} in specific processing of stress signals identifies cMCU as a novel component of plant environmental sensing.

Mattia Storti*

**Unit of Plant Biology*

Regulation of photosynthetic electron transport to respond to light dynamics in the moss *Physcomitrella patens*

Photosynthetic organisms support cell metabolism by harvesting sunlight to fuel the electron transport chain at the level of thylakoid membranes. The flow of electrons in the photosynthetic apparatus needs to be continuously modulated to respond to natural dynamic environmental conditions. Multiple mechanisms for regulation of electron transport are present and they are differently distributed in photosynthetic organisms. Bryophytes hold one of the most complete set of mechanisms regulating photosynthetic light reactions. They are also an interesting model to study how these adapted upon land colonization. Cyclic (CEF) and pseudo-cyclic electron flow (PCEF) around photosystem I have been shown to contribute to photo-protection by mitigating electron chain over-reduction.

To understand the connection between CEF and PCEF, we generated *Physcomitrella patens* knock-out for the three main pathways involved in CEF and PCEF. When KO mutations are combined a synergy between these pathways emerged. Lack of these pathways leads to severe growth phenotype and decreased photosynthetic performance mainly due to the inability to regulate electron transport and protect PSI from photodamage.

Laura Treu*

**Unit of Genomics and Bioinformatics*

Deciphering complex microbial interactions in biogas upgrading systems by genome-centric metatranscriptomics

Currently, the increasing global demand for energy and the limited availability of fossil fuels has dramatic impact on the environment. There is an imperative need for development of renewable

energy forms and for effective solutions for storage of surplus electricity. The biologically mediated CO₂ methanation through H₂ addition has been proven an effective technology. Hydrogen-fueled microbial pathways in biogas upgrading systems have been studied for deciphering intracellular and extracellular metabolic fluxes. Genome-centric metagenomics and metatranscriptomic analyses helped to understand the dynamics associated with H₂ injection in different anaerobic digestion systems and to elucidate the complex microbial interactions. At present, the metabolic profiles of more than 5000 metagenome-assembled-genomes (MAGs), belonging to more than 1600 different species, have been determined; among them ~200 uncultivated MAGs are involved in H₂-assisted methanogenesis. To make optimal use of microbial community driven processes, integrating multi -omics data with Flux-Balance-Analysis modeling to resolve intracellular activity is crucial for monitoring and control the system. The metabolic models will aid the investigation of syntrophic interactions occurring between bacteria and archaea. The elucidation of syntropy is pivotal to derive the role of different shuttle compounds on syntrophic associations and subsequently to estimate the effects on CH₄ production.

Session 4: Signals in organisms, evolution and environment. Chair: Andrea Pilastro

Lorenzo Zane*

*Unit of Ecology

Connectivity signals as the key for networks of MPAs

Marine Protected Area (MPA) networks provide more protection than a set of individual, unconnected protected areas. COCONET FP7 project focused on how to obtain useful measures of connectivity that can be combined to improve the design and the management of MPA networks. Dedicated work has been performed in Adriatic and Black Sea pilot areas to obtain measures of connectivity based on genetic approaches. Genetic approaches can provide indirect measures of connectivity, allowing identifying genetically differentiated - unconnected - populations. Genetic data were collected for 13 species from a wide taxonomic range, sampled at 6-8 equally spaced sites for each pilot area.

Most of the species analyzed in the Adriatic are not genetically homogenous, indicating isolation among at least some sites. The majority of the species analysed in the Black Sea are also not genetically homogenous, but they show a much lower level of differentiation than in the Adriatic. The level and pattern of differentiation varies among species and area. It is not possible to identify sites differentiated/isolated for all the species and there is not a simple relation between isolation and geographic distance. The genetic results clearly show that sites in the pilot areas are not fully connected with sharp breaks in genetic connectivity. Many populations at the sampling sites can be self-recruiting or receiving recruits from a scale smaller than the sampling scale, thus deserving dedicated management strategies. MPA networking should potentially focus on a very small geographic scale.

Luca Pagani*

*Unit of Evolutionary Biology

Ancestry-specific analyses reveal differential demographic histories and opposite selective pressures in modern South Asian human populations

Genetic variation in contemporary South Asian human populations follows a northwest to southeast decreasing cline of shared West Eurasian ancestry. A growing body of ancient DNA evidence is being used to build increasingly more realistic models of demographic changes in the last few thousand years. Through high quality modern genomes, these models can be tested for gene and genome level deviations. Using local ancestry deconvolution and masking, we reconstructed population-specific surrogates of the two main ancestral components for more than

500 samples from 25 South Asian populations, and showed our approach to be robust via coalescent simulations.

Our allele sharing estimates reveal the reconstructed haplotypes to be good proxies for the source populations that admixed in the area, and point to complex inter-population relationships within the West Eurasian component, compatible with multiple waves of arrival. Furthermore, the local ancestry deconvolution in South Asians reveals opposite selective pressures on two pigmentation genes (*SLC45A2* and *SLC24A5*) that are common or fixed in West Eurasians, suggesting post-admixture purifying and positive selection signals, respectively.

Chiara Anselmi*, Mark Kowarsky, Kohji Hotta, Katherine J. Ishizuka, Karla J. Palmeri, Stephen R. Quake, Irving L. Weissman, Ayelet Voskoboinik and Lucia Manni

**Unit of Developmental Biology and Morphogenesis*

Molecular signals of chordate development: two disparate pathways, one tunicate

Colonial tunicates are chordates able to reproduce both sexually and asexually. These two developmental pathways, that are characterized by different starting points (a zygote vs a bud), result in an almost identical individual. Combining transcriptome sequencing with confocal and electron microscopy of major embryogenesis and budding developmental stages, we characterized the molecular and morphological signatures along both the developmental pathways. We generated a complete atlas of gene profile and ontogenesis, describing the entire embryogenesis and blastogenesis processes, and linked them to morphological events. We also identified the developmental origin of some organs, including the central nervous system, uncovering the exact time when tissue specific precursor cells emerge in both developmental pathways. This study demonstrates that multiple molecular signals can lead to the same outcome, and reveals the basic principles and evolutionary conserved elements of chordate development.

Umberto Rosani*

**Unit of Human Molecular Genetics and Functional Genomics*

Invertebrate herpesviruses as a tool to unlock antiviral pathways in marine mollusks

The class of Bivalvia includes thousands of species and, in particular, economically relevant oysters, mussels and clams. Bivalve genomics started to develop from 2000 onwards, mainly investigating the molecular mechanisms of host responses to bacteria and eukaryotic parasites, whereas the genetic basis of antiviral immunity in bivalve species is still mostly unexplored due to the elusive nature of circulating viruses and difficulties related to their isolation and propagation. Since few members of the Malacoherpesviridae family are causing heavy mass mortalities in bivalves and gastropods worldwide, we focused on molecular host-pathogen interactions as a tool to unveil the antiviral host response as well as the genomic variability and transcriptome landscapes of Ostreid herpesvirus type 1 and related genotypes. Among other findings, we demonstrated that an Interferon Stimulated Gene of *Crassostrea gigas* (bivalve) and *Haliotid diversicolor supertexta* (abalone) mediates a significant editing of viral dsRNAs. In the evolutionary time, such activity shaped the genomes of these Malacoherpesviruses.

Carlotta Mazzoldi*

**Unit of Evolutionary Biology*

The Marine Biology Center in Chioggia: the new facilities

The Marine Biology Center in Chioggia started in 1941, with the Hydrobiological Station funded by Prof. Umberto D'Ancona. Since its establishment, this field station hosted several research groups of the Institute of Zoology and then Department of Biology, as well as visiting scientists, that carried out researches focused on the marine organisms and environment of the Venetian Lagoon and coastal waters of the north-western Adriatic Sea. The Hydrobiological Station provides the

facilities for field work and the basic laboratory work, however most of the sample analyses is still carried out in the Department of Biology.

The ongoing project of development of the Marine Biology Center will provide new facilities and laboratories thanks to the restoration of two buildings located nearby the field station, with a total area of 610 m². The project includes the setting up of large animal facilities with tanks of different sizes, rooms at controlled temperature and a system to precisely control the running seawater temperature, molecular biology and cell biology laboratories, offices and a meeting room. The new facilities will allow performing elaborate experiments on marine organisms, completing the analyses directly in the Marine Center, hosting visiting scientists, and will integrate with the field station and the teaching facilities in Palazzo Grassi (Chioggia).

Session 5: Mitochondrial biology. Chair: Luca Scorrano

Roberto Costa*, Roberta Peruzzo, Magdalena Bachmann, Giulia Dalla Montà, Mattia Vicario, Giulia Santinon, Andrea Mattarei, Enrico Moro, Ruben Quintana-Cabrera, Luca Scorrano, Massimo Zeviani, Mario Zoratti, Cristina Paradisi, Francesco Argenton, Marisa Brini, Tito Calì, Sírio Dupont, Ildiko Szabo and Luigi Leanza

*Unit of Bioenergetic Organelles

Impaired mitochondrial energetic metabolism down-regulates Wnt signaling

Wnt signaling affects fundamental development pathways by regulating cell differentiation and proliferation and, when aberrantly activated, it promotes the development of several cancers. Wnt signaling is modulated by different factors, but whether mitochondrial energetic state affects Wnt signaling is unknown. Here we show that sub-lethal concentration of eight different compounds that decrease mitochondrial ATP production down-regulate Wnt/β-catenin signaling in HEK293 cells, colon cancer lines and in vivo in zebrafish reporter lines. Accordingly, impaired respiratory chain complex III function in human fibroblasts from a GRACILE syndrome patient and in a respective, newly generated zebrafish model led to reduced Wnt signaling. Other signaling pathways were unaffected even in vivo, indicating specificity of the mitochondria-Wnt signaling axis. We identified a mechanism whereby a decrease in mitochondrial ATP reduced uptake of calcium into the endoplasmatic reticulum (ER), leading to ER stress and to inhibited Wnt signaling. In accordance, reduction of ATP export from mitochondria as well as ER-stress inducers tunicamycin and thapsigargin equally led to inhibition of Wnt signaling. In turn, recovery of ATP level using creatine and phosphoenolpyruvate or an ER stress inhibitor restored Wnt activity, even in GRACILE fibroblasts. These findings reveal an unexpected mechanism that links mitochondrial energetic metabolism to the control of Wnt pathway, indicating that agents affecting mitochondrial ATP production may be beneficial against several pathologies ranging from cancer to neurological disorders that are related to altered Wnt signaling.

Samantha Corrà*

*Unit of Neurogenetics and Chronobiology

Drosophila melanogaster as a model to investigate the role of Mpv17 in mitochondrial DNA maintenance and metabolism

Mutations in the human MPV17 nuclear gene, which encodes a small hydrophobic mitochondrial inner membrane protein, cause a pediatric hepatocerebral form of mitochondrial DNA (mtDNA) depletion syndrome (MDS). Patients develop steatosis, liver failure, hypoglycemia, and neurological symptoms, leading to death in the first year of life. Although a role for MPV17 in mtDNA maintenance has been proposed, the function of this protein remains poorly understood. By using a fly model of the disease, we have demonstrated that knock-out (KO) of the Drosophila orthologue of MPV17 (dMpv17) causes profound mtDNA depletion in the fat body, an analogous

of the human liver and white adipose tissue. However, *in vitro* analysis shows that mitochondrial dNTP pools are unaffected by dMpv17 down-regulation. Rather, we have demonstrated that the Drosophila protein forms a channel after reconstitution into planar lipid bilayers in physiological conditions. Electron microscopy of fat body tissue in dMpv17 KO flies has revealed striking morphological changes in mitochondria. Moreover, we have shown dMpv17 KO to affect several metabolic pathways leading to a decrease in lipid content, hypersensitization to starvation stress, up-regulation of genes involved in insulin signaling, lipid and glucose metabolism. Taken together, our results highlight the importance of dMpv17 for the maintenance of metabolism and mitochondrial function, suggesting a possible role for dMpv17 in energy homeostasis.

Libero Vitiello*

**Unit of Human Molecular Genetics and Functional Genomics*

Drug repurposing for Duchenne muscular dystrophy: monoamine oxidase B inhibitors can ameliorate the pathological phenotype in mdx mice and in myogenic cultures from DMD patients

Oxidative stress and mitochondrial dysfunction play a crucial role in the pathophysiology of muscular dystrophies. Using *in vivo* and *in vitro* models we have recently demonstrated that safinamide, a reversible MAO-B inhibitor, has considerable potential as a DMD therapeutic molecule. Specifically, we found that administration of safinamide in 3-month old mdx mice reduced myofiber damage and oxidative stress, and improved muscle functionality. *In vitro* studies with myogenic cultures from DMD patients showed that cultured dystrophic myoblasts were more susceptible to oxidative stress than matching cells from healthy donors. Indeed, upon exposure to the MAO substrate tyramine or to hydrogen peroxide, DMD muscle cells displayed a rise in ROS levels and a consequent mitochondrial depolarization. Remarkably, both phenotypes normalized when cultures were treated with safinamide. We have also obtained preliminary evidences indicating that exposure to glucocorticoids, the only pharmacological therapy currently available for DMD, could actually induce a MAO-mediated increase in ROS accumulation in DMD but not in normal myotubes. For this reason, we are now investigating the effect of glucocorticoids on MAO expression and activity in the context of DMD myogenic cultures, by determining if and to what extent GCs cause excessive MAO-dependent ROS formation with consequent oxidative stress and mitochondrial dysfunction.

Angela Paggio, **Vanessa Checchetto***, Antonio Campo, Roberta Menabò, Fabio di Lisa, Ildiko Szabo, Rosario Rizzato and Diego De Stefanì

**Unit of Bioenergetic Organelles*

Identification of an ATP-sensitive potassium channel in the inner mitochondrial membrane

ATP-sensitive potassium channels (KATP) are widely distributed ion channels acting as sensors of cellular metabolism. In the plasma membrane (pmKATP) they couple cell excitability with energy availability. They are also reported to be located on intracellular membranes, in particular in mitochondria, but in this context even their existence is still a matter of debate. Here, we unambiguously identify a mitochondria-localized protein complex that mediates ATP-sensitive potassium currents, referred to as mitoKATP. We show that similarly to its plasma membrane counterpart, mitoKATP is composed of a pore-forming subunit (mitoK) and an ATP-binding subunit (mitoSUR). *In vitro* reconstitution of mitoK together with mitoSUR recapitulates the main electrophysiological properties and pharmacological profile of mitoKATP. Overexpression of mitoK alone causes loss of mitochondrial membrane potential ($\Delta\psi_m$), decrease of mitochondrial Ca^{2+} uptake, organelle fragmentation and disruption of cristae, in line with an increased cation permeability of the inner mitochondrial membrane. However, the concomitant overexpression of the mitoSUR subunit restores the correct channel gating and rescues organelle injury. Conversely,

mitoK ablation causes mitochondrial dysfunction characterized by instability of mitochondrial membrane potential, widening of intracristae space and a decrease of oxidative phosphorylation performance. Overall, our data indicate that the hereby-identified novel components forming mitoKATP control organelle volume, thereby representing key players in shaping mitochondrial physiology with a potential impact on several pathological processes. mitoK-KO animals are viable with no overt phenotype. However, heart from KO mice is more sensitive to ischemia/reperfusion injury, indicating that mitoKATP is cytoprotective. Most importantly, loss of mitoK abolishes cardioprotection induced by pharmacological preconditioning with diazoxide, thus indicating that mitoK is the genetic target of diazoxide mediating cardiac preservation.

Session 6: Plants and Biotechnology. Chair: Barbara Baldan

Lorenzo Maso* and Laura Cendron

*Unit of Synthetic Biology and Biotechnology

Novel strategies to contrast antimicrobials resistance: old friends and new weapons

Abstract: Multi-Drug Resistant Bacteria represent a global emergency, limiting the effective treatment of bacterial infections. The situation is even more dramatic if we consider the limited number of new antimicrobial agents currently in clinical phase. SOS response, orchestrated and triggered by LexA-RecA (ssDNA-bound) interaction, has been recently validated as a key target for combating the evolution of antibiotic resistance. Blocking and/or modulation of RecA/LexA pathway have a direct effect on the expression of genes involved in SOS response and thus on the development of intrinsic resistance and acquisition of genes of resistance. The project, based on a multidisciplinary approach, focuses on the development of therapeutic agents blocking SOS activation, through the inhibition of LexA self-cleavage and/or interference with RecA/LexA interactions. Most promising molecules we are currently testing belongs to both boronic acids derivatives, identified in silico, and natural-compounds derived macrocycles. Furthermore, encouraging results have been obtained very recently in the development and screening of a nanobodies library targeting LexA-RecA complex. This option will be adopted both to rigidify the activated SOS-machinery, for single particle cryo-EM studies, as well as to investigate the minimal peptide scaffold able to block LexA active site, using nanobodies as molecular probes. Such results will pave the way for peptide-based macrocycles building and optimization.

Sebastiano Nigris*

*Unit of Plant Biology

MADS-box genes and development of flower and fruit in *Nymphaea caerulea*: modern traits and old inventions

The invention of the seed boosted the evolutionary success of land plants. Seeds indeed contain the embryo that may remain viable for a long time after being shed, thus having the possibility to colonize new territories and exploit better environmental conditions to germinate. Specialized structures devoted to seed dispersal have been invented quite soon, as for instance fleshy structures to accompany the seeds can be found in all the extant orders of Gymnosperms. This suggests that the “fleshy fruit habit” for seed dispersal is very ancient and has been retained and optimized by Angiosperms. In addition, plant reproduction became particularly efficient thanks to the appearance of flowers. Angiosperms enclose and protect their seeds in the fruit, an ovary derived structure that also facilitate the seed dispersal. The development of all these reproductive structures is mainly regulated by MADS-box transcription factors. MADS-box genes are present also in non-vascular plants like mosses, but their number has greatly expanded in higher plants where their functions range from root development to floral organ specification and to fruit development. The study of these processes in the basal angiosperm *Nymphaea caerulea* stands as

crucial for the complexity of their reproductive structures compared with those of the canonical model plants well studied so far. Our analysis confirms that the flower development of *Nymphaea caerulea* does not fit the ABC(D)E model because the expression pattern of the MADS-box genes is not as well defined as it is in the Eudicots. The fruit development was also considered and a detailed study of the MADS-box gene expression pattern in different tissues of the fruit is provided. We evaluated their involvement in the capsule development and in the mechanism of seed dispersal: the fruit opening is highly regulated and occurs by spatially defined tissue separation events, collectively called abscission. In conclusion, our research describes extensively the involvement of MADS-box genes in the biology of the reproduction of *Nymphaea caerulea*, adding new clues in the study of flower and fruit evolution.

Michela Zottini*

**Unit of Plant Physiology and Molecular Biology*

WHIRLY2, a mitochondrial DNA binding protein, required for efficient mitochondrial functionality in seed development and germination

Whirly proteins belong to a small family of plant specific proteins characterized by the presence of a conserved DNA binding domain, shown to play a role in organellar DNA maintenance and organization. Most flowering plants contain two Whirly proteins, some species, including *Arabidopsis thaliana*, have three WHIRLY proteins, that show specific sub-cellular localization: Whirly1 and Whirly 3 are localized in the chloroplasts while Whirly2 into the mitochondria. A T-DNA insertional knock-out mutant for the WHIRLY2 gene shows altered mitochondria impaired in functionality, morphology and dynamics. A lack of Whirly2 protein affects the early stage of plant development, but not an obvious phenotype is observed in the adult phase. The number of seeds present in the siliques and the percentage of seed germination are indeed strongly compromised, suggesting a key role of Whirly 2 in these phases. The highest gene expression of Whirly 2 occurs in imbibed seeds characterized by the restoring of metabolic activity, in shoot and root apex, where a high rate of cell division occurs, very low expression was observed instead in leaf and other tissues of the adult plants. Interestingly, we have been recently observed that Whirly 2 play also a crucial role in response of plants to various abiotic stresses.

Barbara Gris*

**Unit of Plant Biology*

Cyanobacteria biodiversity, maturation process and high value compounds of the Euganean thermal muds: revealing the scientific basis of an ancient therapeutic biotechnological product

The Euganean Thermal District is the oldest and largest thermal center in Europe and represents a reference in mud-based thermal treatments. The mature mud, utilized in therapies, is obtained by the application of a traditional biotechnological method, handed down over the centuries. The process is characterized by the formation, on the mud surface, of a biofilm mainly consisting of cyanobacteria, a class of photosynthetic bacteria known for their ability to produce bioactive molecules. Among Euganean endemic species, *Phormidium* sp. ETS-05 is already known for the production of anti-inflammatory molecules. However, the biodiversity of cyanobacteria growing on mud is high and potentially other species can contribute to the final beneficial effects of mud-based thermal treatments. In order to unravel all elements influencing the mature mud production and to give scientific basis to its therapeutic properties: i) we checked the *Phormidium* sp. presence and the total cyanobacteria biodiversity and growth in the mature muds of 33 SPAs of Abano and Montegrotto; ii) we investigated cyanobacteria biodiversity, their community changes and some high values compounds production along the mud maturation process (2 months) of 4 thermal SPA, correlating the results with operational and environmental parameters; iii) we

compared the cyanobacteria biodiversity of SPA mature muds with that of natural thermal springs of Euganean Hills showing the importance to preserve this territory resource.

Rudy Rubini, Marina Simona Robescu*, Francesco Filippini, Laura Cendron and Elisabetta Bergantino

**Unit of Synthetic Biology and Biotechnology*

Adding a new regeneration enzyme to the biocatalysis toolbox: discovery/identification and rational engineering of a new formate dehydrogenase

The regeneration of expensive cofactors is crucial to allow application of redox biocatalysts at the industrial scale. Therefore, NAD(P)-dependent formate dehydrogenases (FDHs) are biotechnologically relevant to regenerate nicotinamide cofactors. However, most characterized FDHs are strictly specific for NAD⁺ and this hampers their applicability with reactions requiring NADPH, the cost of which is much higher than NADH. The identification/engineering of NADPH regenerating enzymes is thus vital for the pharmaceutical and chemical industries. We report here the identification and characterization of GraFDH2, a novel FDH from the acidobacterium *Granulicella mallensis* MP5ACTX8, which proved to be a promising catalyst, though classically NAD⁺-dependent. Based on functional and deep structural characterization of GraFDH2 and on computational design, we applied a rational engineering approach to switch its cofactor preference. One out of the mutants produced so far, D222S/Q223R displays a 10-fold activity improvement over the wt enzyme with NADP⁺, together with the highest affinity and biocatalytic efficiency for formate among NADP⁺-dependent FDHs reported so far.

Session 7: Disease: from bioinformatics to in vivo models. Chair: Alessandra Rampazzo

Enrica Calura*

**Unit of Human Molecular Genetics and Functional Genomics*

Multi-omics data integration for the identification of survival-associated cancer mechanisms

Survival analyses of gene expression data for biomarker identification have been a useful and widely used approach. But, especially in complex diseases such as cancers, the efficacy of survival prediction increases when the combination of multiple biomarkers is considered. Genome mutations, copy-number variations, deregulated methylation, aberrant gene expressions can contribute in synergy to the pathological process, and their measurements can improve patient's outcome prognostications, helping in diagnosis and treatment decisions.

The next-generation sequence technologies have boosted cancer studies. In the last decade, we gained the access to an increasing number of multi-omics measurements of cancer cell aberrations opening methodological challenges during analyses and result interpretations.

We present MOSClip, a new topological pathway analysis method to integrate multi-omics data and look for survival-associated pathway modules.

Using MOSClip we studied the multi-omics cancer datasets available of The Cancer Genome Atlas. We showed survival-associated tumor cell processes elicited by the synergistic work of different omics. Moreover, we showed how MOSClip can be used to candidate effective multi-omics survival signature.

Fabio De Pascale*

**Unit of Genomics and Bioinformatics*

Analysis of recurrent variants could reveal inconsistencies in the human reference genome

In human genetics several problems are responsible for the call of false-positive variants occurring with high frequency in exome and genome analyses. It is known that the reference genome does not always represent the real consensus sequence of the human population, due to the inclusion

of rare alleles and sequencing errors. In particular, genomic duplications are often misassembled and as a result they may be found in the reference genome as a collapsed consensus, thus generating false variants. We performed a thorough search for conflicting information between the human reference genome (GRCh37 and GRCh38) and some of the most popular human genetic resources such as the 1000 Genomes Project, to disclose genetic inconsistencies. We found that inaccuracies and errors are much higher than expected: minor alleles occurring with a frequency <10% are found on average every ~7,000 bases, producing high numbers of false positives as well as possible false negatives; ~86,000 variants are likely the result of unreported genomic duplications.

Maurizio David Baroni*

**Unit of Cell Proliferation*

Control of cell quiescence and survival in yeast by a cancer chemoprevention drug

Several cancer prevention properties of Aspirin are mediated by specific activities of salicylate affecting cell processes conserved in eukaryotes. Some of salicylate targets, e.g. AMPK, GCN2, mTORC1 and HMGB1, reveal key biological functions from yeast to man. Cell quiescence can be crucial for maintenance and activation of tumor initiating cells, disseminated tumor cells and dormant micro metastases. Cancer mortality predominantly results from fatal outgrowths derived from these dormant elements. So, targeting quiescence of cancer cells with stem-like properties and their growth recovery from dormancy are major challenges in cancer therapy. In our yeast model salicylate showed its most dramatic effects during the exit from and the entry into a cellular quiescence state. Indeed, the growth recovery of long-term stationary phase cells was strongly inhibited by salicylate. At high salicylate concentration, growth reactivation was completely repressed and associated with cell death. Both phenotypes were fully suppressed by activating the RAS-cAMP-PKA pathway. In contrast, upon nutrient exhaustion the lethal cell cycle arrest in the budded-G2/M phase induced by salicylate cannot be suppressed by PKA activation. This antagonism between cAMP and salicylate might be conserved in yeast and humans. The mechanisms underlying the salicylate inhibitory activities might have significant therapeutic implications towards the selective sensitization of silent cancer cells to ad hoc therapeutic agents.

Laura Civiero*

**Unit of Molecular and Cellular Physiology & Biophysics*

Glial phagocytic clearance in Parkinson's disease

An emerging picture suggests that reactive gliosis and loss of glial cells functions can contribute or trigger neurodegenerative conditions. Among glial cells, microglia and astrocytes have been shown to play phagocytic roles by engulfing synapses, apoptotic cells, cell debris, and released toxic proteins. As pathogenic protein accumulation is a key feature in Parkinson's disease (PD), compromised phagocytic clearance might participate in PD pathogenesis. On the other hand, enhanced, uncontrolled and potentially toxic glial clearance capacity could contribute to synaptic degeneration. My current research is focused in the understanding of the molecular mechanisms underlying astroglial phagocytosis, focusing on the possible implication of phagocytic dysfunction in neuronal degeneration. Several endolysosomal proteins encoded by genes implicated in genetic PD are highly expressed by microglia and astrocytes. Here, I provide some preliminary evidence that lysosomal and/or membrane trafficking defects can affect phagocytic clearance and I discuss whether the restoring or the enhancement of lysosomal function might be therapeutically relevant in PD. Since undigested or partially processed materials in glial cells could be eliminated through the release of extracellular vesicles, glia-modified circulating a-syn might be proposed as biomarker in PD and, therefore, I am currently evaluating this novel possibility.

Giacomo Meneghetti*

**Unit of Cell Biology and Developmental Genetics*

The epg5 knockout zebrafish line: a model for Vici syndrome and to screen autophagy-stimulating drugs

EPG5 protein is a RAB7A effector involved in fusion specificity between autophagosomes and late endosomes/lysosomes during autophagy. Mutations in the human EPG5 gene cause a rare and severe multisystem disorder called Vici syndrome. Our work with zebrafish model shows that epg5-/- mutants are viable and can develop to the age of sexual maturity without conspicuous defects in external appearance. In agreement with the dysfunctional autophagy of Vici syndrome, western blot revealed higher levels of the Lc3-II autophagy marker in epg5-/- mutants with respect to wild type controls. Moreover, starvation elicited higher accumulation of Lc3-II in epg5-/- than in wild type larvae, together with a significant reduction of skeletal muscle birefringence and accumulation of degradation-defective autolysosomes as revealed by electron microscopy analysis of muscle. Epg5 mutants present also a reduced number of putative intestinal stem cells and defective goblet cell maturation, pointing at a possible impairment in intestinal functioning and leading to reduced growth during larval stages.

By aging, epg5-/- mutants showed impaired motility and muscle thinning, together with accumulation of non-degradative autophagic vacuoles. Furthermore, epg5-/- adults displayed morphological alterations in heart and gonads and impaired reproductive capabilities. These findings point at the zebrafish epg5 mutant as a valuable model for EPG5-related disorders, thus providing a new tool for dissecting the contribution of EPG5 on the onset and progression of Vici syndrome as well as for the screening of autophagy-stimulating drugs.

Session 8: Evolution and Environment. Chair: Maria Berica Rasotto

Anna Peronato, Nicola Franchi and **Loriano Ballarin***

**Unit of Developmental Biology and Morphogenesis*

Complement-mediated cooperation between immunocytes in the compound ascidian *Botryllus schlosseri*

Two main kinds of innate immune responses are present in ascidians: phagocytosis and cytotoxicity. They are mediated by two different types of circulating immunocytes: phagocytes and cytotoxic morula cells (MCs). MCs, once activated by non-self-recognition, can stimulate phagocytosis by the release of soluble factors able to act as opsonins. BsC3, the complement C3 homologue, like mammalian C3, contains the thioester bond required to split the molecule into BsC3a and BsC3b. BsC3b likely represents the MC opsonin as it can enhance phagocytosis. The tenet is supported by the observed reduction in phagocytosing cells after exposure of hemocytes to compstatin, a drug preventing C3 activation, or after the bsc3 knockdown by iRNA injection. In addition, the transcript for BsCR1, homologous to mammalian CR1, is present in *Botryllus* phagocytes and the transcription is modulated during the blastogenetic cycle. MCs also release cytokines (chemokines) able to recruit immunocytes to the infection site. The activity is inhibited by antibodies raised against human TNF α . Since no genes for TNF α are present in the *Botryllus* genome, the observed activity is probably related to a TNF-domain containing protein, member of the *Botryllus* complement system. Conversely, activated phagocytes release a rhamnose-binding lectin able to interact with microbial surfaces and act as opsonin. It can also activate MCs by inducing the release of the reported cytokine and stimulate their degranulation.

Overall, the results obtained so far indicate the presence of a well-defined cross-talk between the two types of immunocytes during the immune responses of *B. schlosseri*.

Lucio Bonato*, Francesca Bortolin, Emiliano Peretti and Giuseppe Fusco

**Unit of Evolutionary Biology of Arthropods*

Unveiling organism intraspecific diversity: multitechnique investigations on terrestrial animals across the Southern Prealps

It is largely recognized that human well-being depends on a wise management of ecosystems, which in turn requires an accurate understanding of the diversity of organisms and the ecological processes. This urgent task is particularly challenging when dealing with the geographical differentiation of terrestrial species with low dispersal ability, within areas with heterogenous environmental conditions and complex climatic history. We are currently exploring morphological, genetic and ecological differentiation of selected distantly related animals across the Southern Prealps, including fully terrestrial salamanders (*Salamandra atra*), endogeic centipedes (*Clinopodes carinthiacus*, *Strigamia acuminata*) and strictly hygrophilous butterflies (*Caenonympha oedippus*). This is carried out by integrating different modern techniques encompassing GIS-based analysis of field records, geometric morphometrics, genetic and phylogeographic analysis of molecular markers and species distribution modelling, within the conceptual framework of population evolutionary biology and the operational framework of integrative taxonomy. Results are unveiling a largely overlooked intraspecific differentiation in genetic, anatomical and ecological features at a scale of hundreds of kilometers.

Carlotta Mazzoldi*

**Unit of Evolutionary Biology*

Elasmobranch conservation: interaction between use of space, behavior and fishery

Elasmobranchs are known to display an array of behaviors that, coupled with their life history traits, make them particularly vulnerable to direct and indirect fishing. The occurrence of aggregations, sexual segregation, philopatry and extensive migrations may indeed influence connectivity and genetic diversity, on one side, and catchability, on the other one. The decline of several elasmobranch populations worldwide pushes towards the development of effective management plans. Those plans should include, to be effective, not only fishery data and life history traits, but also behavioral characteristics, including the use of space that may be differential according to the life stage and sex.

This study carried out in Chioggia (Hydrobiological Station “Umberto D’Ancona”) aims at disclosing the use of space, movements and connectivity of elasmobranch species in the Adriatic Sea, with a special focus on the most common species, the smooth-hounds, *Mustelus mustelus* and *M. punctulatus*. The use of an integrated approach of analyses of fishery data, tagging, recovery of traditional ecological knowledge of fishermen, and genetic analyses revealed the use of the north-western coastal areas of the Adriatic Sea as parturition and nursery areas.

Marco Bonato*

**Unit of Environmental Physiology and Experimental Zoology*

Persistent Mobile Organic Compounds: LIFE PHOENIX Project experience

“Hydric pollution” is defined by legislative decree 152/1999 as “direct or indirect discharge by human activity into water ecosystem of substances or energy whose consequences are such to put at risk human health, cause damage to natural resources, to water ecosystem and to compromise normal use of water resource. Water resource pollution occurs when contaminated water is spilled into the surrounding environment without proper treatment for the removal of hazardous substances. The direct or indirect discharge of substances into the aquatic environment evidently involves serious risks or damage to the water resource with potential heavy dangers to the ecosystem and human health. Among the most worrying emerging pollutants, of particular interest, are the mobile and persistent organic contaminants (PMOC, Persistent Mobile Organic

Compounds). PMOCs are pollutants that present considerable persistence within the water cycle (for example in drinking water and for irrigation), degrade slowly and are very mobile in the water matrix and often in biological tissues. Exposure to PMOCs can lead to serious health effects which, in many cases, cannot be adequately and effectively assessed due to lack of monitoring data, adequate knowledge of the properties of new substances and difficulties in managing the emergency situation. PHOENIX, starting from a specific subclass of PMOC as the short chain Perfluoroalkyl substances (PFAS), aims to be a strategic project aimed at timely, effective and efficient action in case of pollution, replicable and transferable also in other European geographical contexts.

POSTER PRESENTATIONS

POSTER PRESENTATIONS - TIMETABLE

Departmental Research Facilities

- A. The Fly Facility and The Zebrafish Facility
- B. The IT Office and The Goods Supply
- C. The Next Generation Sequencing Facility
- D. The Radioisotope Facility, The Cytofluorimetry Facility and The BSL2 Virus Facility
- E. The Experimental Greenhouse Facility, The Pure & Ultrapure Water Service and The Centrifugation Facility
- F. The Gene Expression Facility *MicroCribi*
- G. The Departmental Security Service and The Technological Laboratory
- H. The DeBio Imaging facility

Wednesday, January 30

16.40-17.40 Poster Session I

| | | |
|-----|--|----|
| 1. | Enrico Alessio | |
| | Single cell analysis reveals the involvement of the long non-coding RNA Pvt1 in the modulation of muscle atrophy and mitochondrial network | 28 |
| 2. | Giorgia Beffagna | |
| | A conserved signaling signature in arrhythmogenic cardiomyopathy | 28 |
| 3. | Elisa Boscarì | |
| | Population genomics by 2b-RAD sequencing provides clear signals of differentiation among populations of the sunset cup coral (<i>Leptopsammia pruvoti</i>) from different Mediterranean bioconstructions | 29 |
| 4. | Elisa Boscarì | |
| | Signals of return of the critically endangered Adriatic sturgeon in the Po river basin. Checking for the presence of mature individuals 25 years after the first restocking | 29 |
| 5. | Francesca Bucci | |
| | Genetic engineering of <i>Nannochloropsis gaditana</i> for the response to fluctuating light | 30 |
| 6. | Federica De Lazzari | |
| | Alterations in SOD1-mediated redox homeostasis induces locomotion dysfunction in <i>Drosophila Melanogaster</i> | 30 |
| 7. | Davide Doni | |
| | Exploring the role of mitochondria in the pathogenesis of Friedreich's ataxia, a neurodegenerative Disease | 31 |
| 8. | Nicola Facchinello | |
| | Role of pentose phosphate pathway in angiogenesis and vascular myogenesis | 31 |
| 9. | Elide Formentin | |
| | From single cell to the whole plant: ROS and calcium signaling in rice salt acclimation | 32 |
| 10. | Marta Giacomello | |
| | A genome wide screening unveils the mitochondria-endoplasmic reticulum contacts machinery | 32 |
| 11. | Isotta Lorenzi | |
| | The role of DMPK in regulating ER-mitochondria contact sites | 33 |
| 12. | Elena Marchesan | |
| | Calcineurin regulates Parkin-translocation to mitochondria and mitophagy | |

33

| | | |
|---------------------------------|--|----|
| 13. Déborah Naón | Mitofusin 2, mutated in Charcot-Marie-Tooth type IIa, is alternatively spliced in endoplasmic reticulum specific variants controlling organelle morphology, Ca ²⁺ content and tethering to mitochondria | 33 |
| 14. Gianluca Occhi | Silent gonadotroph pituitary neuroendocrine tumor in a patient with Tuberous Sclerosis Complex: evaluation of a possible molecular link | 34 |
| 15. Roberta Peruzzo | A soluble derivative of a Kv1.3 potassium channel inhibitor unveils interaction of mitochondrial Kv1.3 with respiratory chain Complex I | 34 |
| 16. Giovanna Pontarin | The role of glutaminases in endothelial cells | 35 |
| 17. Roberta Provvedi | The integral membrane protein Rv1619 is required for virulence in <i>Mycobacterium tuberculosis</i> | 35 |
| 18. Gianfranco Santovito | Too warm or not too warm: is the antioxidant system of Antarctic fish ready to face climate changes? | 36 |
| 19. Martina Semenzato | Opa1 is oxidized during cardiac ischemia reperfusion injury | 36 |
| 20. Margherita Zamberlan | The Epac1/Rap1 pathway retrogradely signals changes in mitochondrial morphology | 37 |

Thursday, January 31

10.40-11.40 Poster Session II

| | | |
|----------------------------------|--|----|
| 21. Lukas Alan | Dynamic complexomic analysis reveals a role for Von Willebrand Domain-containing Protein 8 (Vwa8) in mitochondrial morphology and physiology | 37 |
| 22. Davide Asnicar | Within- and transgenerational effects of ocean acidification and other environmental stressors in marine Invertebrates | 37 |
| 23. Tiago Fonseca Branco | Unravelling the roles of Fission Protein 1: a forgotten mitochondrial fission factor with pleiotropic functions | 38 |
| 24. Stefano Cagnin | MiRNAs as regulators of metabolism in skeletal muscle fibers | 38 |
| 25. Gaia Codolo | <i>Helicobacter pylori</i> affects the antigen presentation activity of macrophages | 38 |
| 26. Gaia Codolo | The <i>Helicobacter pylori</i> protein HP-NAP: a novel treatment for metastatic melanoma | 39 |
| 27. Susanna Cogo | Exploring the importance of GTPase activity in regulating the autophagic function of LRRK2 | 40 |
| 28. Camilla Maria Fontana | Roles of the autophagic-related proteins Ambra1 on zebrafish gonadal development | 40 |
| 29. Giuseppe Fusco | Effects of phenotypic robustness on adaptive evolutionary dynamics and evolvability | 41 |
| 30. Luigi Leanza | PAPTP leads to neoplastic cell apoptosis in the E6-E7-Tcl1 chronic lymphocytic leukemia mouse model | 41 |
| 31. Paolo Martini | How do cells communicate in muscle regeneration? | 42 |

| | | |
|--|--|----|
| 32. Anna Masato | Altered dopamine metabolism leads to a unique impaired α Synuclein proteostasis in Parkinson's disease | 42 |
| 33. Maddalena Mognato | Functional validation of miRNAs targeting genes of DNA double-strand break repair to radiosensitize non-small lung cancer cells | 42 |
| 34. Akiko Omori | Opa1 is crucial for melanocyte stem cell and melanoma maintenance | 43 |
| 35. Anna Pellattiero | A high throughput screening identifies a small molecule inhibitor of the GTPase activity of OPA1 that enhances apoptotic release of cytochrome c | 43 |
| 36. Paula Rebelo | A new class of contact sites modulators: the mitochondria-ER Spacers | 44 |
| 37. Giovanni Rigoni | Dissecting the dual role of ATAD3A: the link between nucleoid stability and cristae ultrastructure | 44 |
| 38. Giovanni Tosi | Involvement of UBIAD1 in tumor angiogenesis and cancer growth | 44 |
| 39. Alessandro Vezzi | Metagenomic exploration of marine microbial communities | 45 |
| 40. Raffaella Margherita Zampieri | Anti-inflammatory and antioxidant activity of released polysaccharides from cyanobacteria of the Euganean Thermal Mud | 45 |

15.40-16.40 Poster Session III

| | | |
|--------------------------------|--|----|
| 41. Mariano Battistuzzi | An experimental setup to study by remote sensing analyses cyanobacteria growth and photosynthetic performances under non-terrestrial simulated environments | 46 |
| 42. Giuseppe Benvenuto | An integrated computational approach to define transcriptome alterations in platinum resistance | 46 |
| 43. Cristina Catoni | Development of a split-GFP tool to investigate the localization of PKA at sub-mitochondrial level | 47 |
| 44. Gaia Codolo | Cinaedi Atherosclerosis Inflammatory Protein (CAIP)-induced ROS production contributes in sustaining the atherosclerotic process in <i>Helicobacter cinaedi</i> -infected patients | 48 |
| 45. Olivier Ek | Role of Opa1 in zebrafish dopaminergic neurons | 48 |
| 46. Niccolò Forin | The importance of molecular characterization of ancient fungal type specimens for a correct taxonomic reclassification | 48 |
| 47. Elisa Gaio | Keratin nanoparticles co-delivering Docetaxel and Chlorin e6 promote synergic interaction between chemo- and photo-dynamic therapies | 49 |
| 48. Laura Guidolin | Effects of PFOA and PFBS exposure in the soil invertebrate <i>Dendrobaena veneta</i> (Annelida) | 49 |
| 49. Keiko Iwata | Exploring the novel role of mitochondrial dynamics in schizophrenia | 50 |
| 50. Marta Medaglia | The mitochondria shaping protein Opa1 controls adipocyte size | 50 |

| | | |
|---|--|----|
| 51. Masafumi Noguchi | | |
| Exploring the role of mitochondrial dynamics in tracheal stem cells | | 51 |
| 52. Chiara Rampazzo | | |
| SAMHD1 deficiency affects DNA replication fidelity and DNA repair efficiency in human fibroblasts | | 51 |
| 53. Paula Rebelo | | |
| A High Throughput Screen reveals chemical modulators of mitochondria- ER contact sites | | 51 |
| 54. Erwan A. Rivière | | |
| A RNAi-genome wide screening for mitochondrial fission factors | | 52 |
| 55. Cecilia Salvoro | | |
| Systematic evaluation of eye color prediction models for forensic application in Italy | | 52 |
| 56. Diana Simionato | | |
| Microalgae biomass: green power for carbon sequestration and production of high value molecules | | 52 |
| 57. Lorenza Iolanda Tsansizi | | |
| Genetic inactivation of USP8 in the heart, a deubiquitinase hyperactive in Cushing's syndrome adenomas, causes mitochondrial dysfunction and cardiomyopathy | | 53 |
| 58. Giorgio Valle | | |
| Overview of the main topics developed by genomics and bioinformatics research unit | | 53 |

POSTER PRESENTATIONS - ABSTRACTS

Poster Session I

Enrico Alessio*¹, Lisa Buson¹, Francesco Chemello¹, Caterina Peggion², Francesca Grespi¹, Paolo Martini¹, Maria Lina Massimino³, Beniamina Pacchioni^{1,7}, Caterina Millino^{1,7}, Chiara Romualdi¹, Alessandro Bertoli^{2,4}, Luca Scorrano^{1,5}, Gerolamo Lanfranchi^{1,6,7} and Stefano Cagnin^{1,6,7}

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Single cell analysis reveals the involvement of the long non-coding RNA Pvt1 in the modulation of muscle atrophy and mitochondrial network

Long non-coding RNAs (lncRNAs) are emerging as important players in the regulation of several aspects of cellular biology. For a better comprehension of their function, it is fundamental to determine their tissue or cell specificity and to identify their subcellular localization. In fact, the activity of lncRNAs may vary according to cell and tissue specificity and subcellular compartmentalization. Myofibers are the smallest complete contractile system of skeletal muscle influencing its contraction velocity and metabolism. How lncRNAs are expressed in different myofibers, participate in metabolism regulation and muscle atrophy, or how they are compartmentalized within a single myofiber is still unknown. We compiled a comprehensive catalog of lncRNAs expressed in skeletal muscle, associating the fiber-type specificity and subcellular location to each of them, and demonstrating that many lncRNAs can be involved in the biological processes de-regulated during muscle atrophy. We demonstrated that the lncRNA Pvt1, activated early during muscle atrophy, impacts mitochondrial respiration and morphology and affects mito/autophagy, apoptosis, and myofiber size in vivo. This work corroborates the importance of lncRNAs in the regulation of metabolism and neuromuscular pathologies and offers a valuable resource to study the metabolism in single cells characterized by pronounced plasticity.

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A conserved signaling signature in arrhythmogenic cardiomyopathy

Arrhythmogenic Cardiomyopathy (AC) is a heritable heart muscle disease characterized by fibro-fatty replacement, leading to ventricular arrhythmias at risk of sudden death, particularly in the young and athletes. Despite the discovery of several causative genes, early molecular events leading to tissue damage and arrhythmias remain elusive. The AC8 form, linked to the junctional protein Desmoplakin, is the most challenging AC type, being less easily identifiable using the classical ECG and echocardiographic tools, due to a high prevalence of left dominant forms. Hypothesis: Considering the conservation of cardiac organization and function from fish to humans, our hypothesis is that DSP-associated AC8 can be successfully modeled in zebrafish, using both knock-down and knock-out gene targeting approaches. Methods: A morpholino-based antisense strategy was used to obtain the knockdown of zebrafish dspa and dsbp genes, both orthologous to human DSP. Moreover, we have analyzed a zebrafish Desmoplakin-a mutant line obtained by ENU-induced mutagenesis, and a Desmoplakin-b mutant line, locally generated by CRISPR/Cas9 strategy. All AC8 zebrafish models were morphologically characterized by classical, confocal and transmission electron microscopy (TEM), and, subsequently, functionally tested for alterations in different pathways, using signaling reporter

lines. Results: At embryonic and larval stages, Dspa and Dspb knock-down conditions, as well as homozygous and heterozygous Dspa/Dspb mutants, show developmental delay, microcephaly, pericardial effusion and altered heart rate. During early adulthood, Dsp mutant fish exhibit mild bradycardia, cardiomegaly and peripheral effusion; moreover, some animals die suddenly starting from 3 months of age. TEM analysis of heart tissues shows cell detachments and highly disorganized junctions, resembling "pale" desmosomes identified in human AC patients. Moreover, the analysis of signaling pathways detects a cardiac-specific reduction of Wnt signaling responsiveness in all AC8 models, which can be rescued by both genetic and pharmacological approach. Conclusions: Our AC8 transient and stable zebrafish models recapitulate some AC key features, pointing to zebrafish as a suitable system for the *in vivo* screening of molecularly-targeted drugs. The observed reduction of canonical Wnt signaling suggests that this pathway could be a general mechanism involved in the pathogenesis of desmosomal-associated AC forms, and, thus, a promising target for AC therapy.

Elisa Boscari*, Leonardo Congiu, Ilaria A. Marino, Chiara Papetti and Lorenzo Zane

**Unit of Ecology*

Population genomics by 2b-RAD sequencing provides clear signals of differentiation among populations of the sunset cup coral (*Leptopsammia pruvoti*) from different Mediterranean bioconstructions

Marine bioconstructions such as coralligenous formations are hot spot of biodiversity and play a relevant ecological role in the preservation of biodiversity by providing carbon regulation, protection and nursery areas for several marine species. For this reason, the European Union Habitat Directive included them among priority habitats to be preserved. Despite their ecological role is well-established, connectivity patterns are still poorly investigated, representing a limit in conservation planning. The present study pioneers a novel approach for the analysis of connectivity in marine bioconstructor species, which often lack suitable genetic markers, by taking advantage of next generation sequencing techniques. We applied the restriction site associated 2b-RAD approach to genotype over 1,000 high quality and filtered Single Nucleotide Polymorphisms in 10 population samples. The results revealed the existence of a strongly supported genetic structure, with highly significant pairwise FST values between all the population samples, including those collected few kilometers apart from each other.

All in one, our results highlight the importance of assessing connectivity in species belonging to coralligenous habitats as, due to their limited dispersal ability, they might require specific spatial conservation measures.

Elisa Boscari* and Leonardo Congiu

**Unit of Ecology*

Signals of return of the critically endangered Adriatic sturgeon in the Po river basin. Checking for the presence of mature individuals 25 years after the first restocking

The Adriatic sturgeon (*Acipenser naccarii*) is an anadromous species endemic of the Adriatic region. During the last 30 years, this species experienced a dramatic decrease, reaching the brink of extinction. In 2010, the IUCN updated its status to "Critically endangered and possibly extinct in the wild" since no reproduction had been officially registered in the previous 20 years. The existence of this species is strictly tied to the release of individuals produced from a single captive broodstock descending from 50 individuals collected in the wild (F0) in 1977. Starting from the 90ies, over 250.000 F1 juveniles were released within different restocking programs. Nowadays, if survived, they should have reached the maturity. The present project aimed at assessing the presence of adult individuals in the Po River through catches, visual census and eco-sonar surveys and to investigate their origin (released versus wild) through genetic analyses. More than 30 adults were totally counted, largely exceeding our expectancies. Out of the 10 animals for which genetic analysis were possible 4 resulted to be compatible with the released stocks and 6 were probably of wild origin. These results represent the first evidence that the Adriatic sturgeon, even if generated in captivity can survive in the Po River for over 20 years and reach the age of maturity. Additionally, the presence of individuals not

genetically compatible with the parental stock suggests that at least some individuals of wild origin are still present in the Po River.

Francesca Bucci*, Alessandra Bellan and Tomas Morosinotto

**Unit of Plant Biology*

Genetic engineering of *Nannochloropsis gaditana* for the response to fluctuating light

Microalgae exploitation in food and energy industry is gaining increasing interest. Despite the potential of microalgae cultivation, the economic feasibility of the system is still far from the expected. In the industrial scale the main limitation to biomass productivity is the light exploitation from the culture. Indeed, in the artificial environment of photobioreactors or ponds microalgae are exposed to light intensity variations, named as fluctuating light conditions, that consist in dark-light cycles of variable length. Our work is focalized on *Nannochloropsis gaditana*, which is an interesting microalga for its high lipid content and thus seems to be a promising candidate for biodiesel production. We verified that fluctuating light conditions severely reduce the culture growth and affect negatively the biomass productivity. Analyzing the photosynthetic apparatus, we found a strong decrease in the PSI content per cell. We attributed this phenotype to the lack of several components, like Flavodiiron (FLV) proteins, that in other photosynthetic organisms are involved in alternative electron transports that avoid PSI over-reduction. The purpose of our work is verifying if the heterologous-expression of FLV proteins could alleviate the phenotype and increase the growth of *N. gaditana* in fluctuating light.

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Alterations in SOD1-mediated redox homeostasis induces locomotion dysfunction in *Drosophila melanogaster*

Reactive oxygen species (ROS) exert an important role in several signaling pathways and in host defense mechanisms associated to inflammation. However, an excessive unbalanced accumulation of ROS, has been proposed as one of the major pathological mechanisms underlying diverse neurodegenerative disorders including amyotrophic lateral sclerosis (ALS). In this frame, the protein superoxide dismutase 1 (SOD1) seems to play a central role in the disease as more than 170 mutations in the gene coding for SOD1 have been associated to familial forms of ALS.

Through its ability to disproportionate the superoxide radical into hydrogen peroxide and molecular oxygen, SOD1 plays a pivotal role in redox homeostasis. The activity of SOD1 relies on the presence of a copper ion in its active site, which is inserted through the action of a dedicated copper chaperone (CCS). However, in the absence of CCS, SOD1 maturation relies on an alternative pathway, which accounts for approximately 15% of SOD1 activity.

Therefore, we evaluated how alterations in redox homeostasis, due to a reduced expression of SOD1 affect locomotion. As the SOD1 maturation pathway is well conserved between humans and flies, we exploited *Drosophila melanogaster* as model organism, utilizing three different fly lines expressing different levels of functional Sod1, the fly orthologous to human SOD1. Individuals were evaluated for resistance to oxidative insults and subsequently assessed for locomotion deficits, analyzing climbing ability and complex locomotion behaviors. Our results indicate an association between ROS dyshomeostasis and locomotion defects which are dependent on the loss of SOD1-mediated antioxidant defense.

Davide Doni* and Paola Costantini

*Unit of Bioenergetic Organelles

Exploring the role of mitochondria in the pathogenesis of Friedreich's ataxia, a neurodegenerative disease

Friedreich's ataxia (FRDA) is the most common autosomal recessive inherited ataxia, clinically characterized by a severe impairment of muscle coordination associated with cardiomyopathy and diabetes. This disease results from an abnormally expanded GAA triplet repeat in the first intron of the nuclear FXN gene leading to a reduced expression of frataxin (FXN), an ubiquitous and highly conserved mitochondrial protein. Several evidences support a role of frataxin in the mitochondrial FeS clusters biogenesis but its specific function in this pathway, and more in general in the cell (patho)physiology, is still under investigation. A definite relationship between frataxin decrease, FeS clusters assembly dysregulation and mitochondrial bioenergetics failure has not been established, and it is not exactly known which metabolic consequences primarily occur after frataxin depletion and would be most relevant for disease therapy strategies.

The research topic of our group is focused on a multidisciplinary approach ranging from in vitro biochemical and structural studies to genetics and cell imaging, to explore how FXN deficiency could be involved in FRDA pathogenesis. Since the cardinal downstream biochemical feature of FRDA cells is an impaired mitochondrial respiration, the effects in the FeS proteins biogenesis due to frataxin decrease could potentially contribute to the FRDA onset and progression by impairing oxidative phosphorylation, which relies on FeS clusters at respiratory complexes I, II and III. For this reason, we have recently investigated mitochondrial morphology and functionality in FRDA patients-derived cells to gain new insights on the possible pathophysiological role(s) of frataxin. We observed that, in specific pathological conditions, the mitochondrial ultrastructure is severely damaged, with extended cristae disruption and a reduced capability to assemble them in supercomplexes (RSC), quaternary structures which in healthy cells improve the overall mitochondrial respiratory efficiency. Interestingly, we also found that a transient mild overexpression of human OPA1 (a mitochondrial protein with a key role in cristae shape and assembly of RSC) in FRDA lymphoblasts corrects mitochondrial fragmentation, restoring the normal mitochondrial morphology. We thus provide the evidence that mitochondrial morphodynamics dysregulation could be an early key event in the pathogenesis of FRDA rather than a late, secondary consequence of the bioenergetic failure. In the next future, we will move a different cellular model for our studies, i.e. FRDA induced Pluripotent Stem Cell-derived cardiomyocytes, which is a model of choice to interpret the obtained results in the context of the disease.

Nicola Facchinello* and Massimo Santoro

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Role of pentose phosphate pathway in angiogenesis and vascular myogenesis

Pentose phosphate pathway (PPP) is a shunt of the glycolysis that produces NADPH as reducing equivalents and ribulose-5-phosphate as essential component of the synthesis of nucleotides. The role of PPP in angiogenesis is not well known. To this end we aim to define the function of PPP in the angiogenic processes using both *in vivo* and *in vitro* models. Preliminary data show that inhibition of PPP enzymes, including glucose-6- phosphate dehydrogenase (G6PD) and phosphogluconate dehydrogenase (PGD), strongly affects cell proliferation, cell migration and apoptosis in HUVEC cell line. We used RNA-seq to compare the transcriptomes of G6PD- and PGD-silenced HUVECs. RNA-seq analysis revealed a decreased level for gene of ECM and an increase level in the genes related to inflammation. We then generated a g6pd-null zebrafish line that show brisk hemolysis, reduced viability, decrease in fitness and reduction in normal growth rates. By using zebrafish models that carry endothelial-specific g6pd and pgd gene deletion we found a crucial role of PPP for the crosstalk between EC (endothelial cell) and MC (mural cell). So far, our results indicate that PPP inhibition leads to a strong inhibition of MC recruitment to the dorsal aorta, which in turn results in enhanced dorsal aorta vessel elasticity and failure to restrict aortic diameter. A better understanding of what downstream of this metabolic pathways is needed to identify new targets that could contribute to the development of new therapy in the treatment of vascular diseases.

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**Unit of Plant Physiology and Molecular Biology*

From single cell to the whole plant: ROS and calcium signaling in rice salt acclimation

Salinity tolerance is a complex trait. Despite many efforts to obtain salt-resistant rice plants, few results have been achieved so far and a deeper understanding of the tolerance mechanisms is needed.

By studying two Italian rice varieties showing contrasting salt responses, we unveiled mechanisms required both at cell and whole-plant level to achieve salt tolerance.

Plants adopted morphological, hormonal, and gene regulation changes to set up an adaptive program for the survival. In presence of salt, specific H₂O₂ profile and calcium signatures defined a tolerant response.

Our results indicate that the acclimation mechanism adopted by tolerant plants is triggered by specific ROS and calcium-mediated pathways that lead to osmotic compensation, photosynthesis preservation and phenotypic plasticity needed for the survival of plants.

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**Unit of Bioenergetic Organelles*

A genome wide screening unveils the mitochondria-endoplasmic reticulum contacts machinery

The outer mitochondrial membrane and endoplasmic reticulum (ER) are physically tethered and properly spaced at the Mitochondria-Endoplasmic Reticulum (ER) contacts (MERCs) by proteinaceous bridges whose nature is largely unknown.

Here we provide a molecular MERCs atlas with a genome wide short hairpin RNA (shRNA) screens coupled to high content, ratiometric, quantitative microscopy of a FRET ER-mitochondria proximity probe (FEMP). Automated image and cellHTS2 statistical analysis of the screening iterations yielded 193 gene candidates classified as tethers (i.e., genes whose ablation increases ER-mitochondria distance) and 303 genes as Spacers (i.e., genes whose ablation decreases ER-mitochondria distance).

Gene candidates were enriched in lipid metabolism, apoptosis signaling and metabolic response pathways. Subcellular localization analysis of the identified hits revealed about 20 proteins predicted to be present in both ER and outer mitochondrial membrane. Low-throughput assays as well as mass spectrometry analysis of

ER associated mitochondrial membranes (i.e. the biochemical counterpart of MERCs) validated some of the identified hits.

Overall, our screen provides a rich resource to understand the molecular anatomy of the ER-mitochondria interface and unveils novel functions for disease-associated genes.

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The role of DMPK in regulating ER-mitochondria contact sites

Mitochondria form a highly dynamic network that undergoes constant reorganization. This network interfaces with other organelles by physical contacts. Important examples are the endoplasmic reticulum (ER)-mitochondria contact sites (MERCs), also known as mitochondria-associated membranes (MAMs), which provide a platform for the control and regulation of various cellular processes. We developed a FRET (fluorescence resonance energy transfer) based approach to investigate the juxtaposition between the ER and mitochondria (Naon et al., 2016 PNAS). Using this method, we performed a FRET-based genome wide screen to identify regulators of ER-mitochondria contact sites. Over 200 candidate genes were found that act as tethers (i.e. their ablation increases the distance between ER and mitochondria) or spacers (their ablation decreases the distance between ER and mitochondria). Among the candidates were known ER-mitochondrial tethers such as Mfn2 and PACS-2. One of the most intriguing hits was the myotonic dystrophy protein kinase, DMPK, whose ablation was found to increase ER-mitochondria distance. Indeed, DMPK was retrieved biochemically in MAMs and in a secondary screening its ablation by siRNA reduced ER-mitochondria juxtaposition measured using the FRET sensor. We are currently investigating the mechanism by which DMPK regulates ER-mitochondria contact sites.

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Calcineurin regulates Parkin-translocation to mitochondria and mitophagy

The selective removal of dysfunctional mitochondria via autophagy, named mitophagy, is crucial for the maintenance of cellular homeostasis. This event is initiated by the translocation of the E3 ubiquitin ligase Parkin to CCCP intoxicated mitochondria, and it requires phosphorylation by the kinase PINK1. Damaged mitochondria priming mediated by the Pink1-Parkin signaling pathway is not completely unraveled, notably the molecular mechanism that controls Parkin translocation to mitochondria remains elusive. In this study we investigate the role of the Ca^{2+} -dependent phosphatase Calcineurin (CaN) in the regulation of Parkin translocation and Parkin-dependent mitophagy. CaN downregulation prevents translocation of Parkin to intoxicated mitochondria, and stress induced mitophagy. Furthermore, CaN constitutive activation leads to exacerbated mitophagy, and affects Parkin thermal stability and translocation.

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Mitofusin 2, mutated in Charcot-Marie-Tooth type IIa, is alternatively spliced in endoplasmic reticulum specific variants controlling organelle morphology, Ca^{2+} content and tethering to mitochondria

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Mitofusin 2, mutated in Charcot-Marie-Tooth type IIa, is alternatively spliced in endoplasmic reticulum specific variants controlling organelle morphology, Ca^{2+} content and tethering to mitochondria Mitofusin 2,

mutated in Charcot-Marie-Tooth type 2A (CMTIIa) neuropathy, is a large mitochondrial GTPase involved in mitochondrial fusion, regulation of the shape of the endoplasmic reticulum (ER) and in tethering of the two organelles, impacting on Ca^{2+} transfer between the two. Here we report the existence of different splice variants of Mitofusin 2 expressed in human tissues. Splice variants lacking part of the GTPase domain are localized in ER and can be also found enriched in the interface between ER and mitochondria. The mitochondrial targeting of full length Mfn2 requires the integrity of coiled-coils 1 and 2, while the transmembrane domain alone is sufficient to target the Mfn2 variants to the ER. Re-expression of ER-specific Mfn2 variants in Mfn2^{-/-} cells rescues ER morphology corrects ER-mitochondrial tethering and normalizes ER Ca^{2+} levels, without rescuing mitochondrial morphology and function. The discovery of ER-specific Mitofusin 2 variants reveal the existence of entirely extramitochondrial MFN2 functions that are likely to contribute to the pathogenesis of CMTIIa.

Gianluca Occhi*

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Silent gonadotroph pituitary neuroendocrine tumor in a patient with Tuberous sclerosis complex: evaluation of a possible molecular link

Background: Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem hereditary condition, characterized by multiple hamartomas. In rare cases, pituitary neuroendocrine tumor (PitNET) have been described in patients with TSC, but the causal relationship between these two diseases is still under debate. TSC is mostly caused by mutations of two tumor suppressor genes, encoding for hamartin (TSC1) and tuberin (TSC2), involved in intracellular signaling pathways controlling cell growth and proliferation, including the mammalian target of rapamycin (mTOR) cascade. Therapeutic approaches based on mTOR signaling (everolimus) has been successfully used in patients with TSC and tested in non-functioning PitNET cellular models with promising results.

Case presentation: A 62 years old Caucasian woman with diagnosis of TSC presented with headache and a MRI scan showed a macroadenoma with suprasellar extension, bilateral cavernous sinus invasion and left optic nerve compression. After 4 months she underwent transsphenoidal endoscopic neurosurgery with complete tumor resection. Clinical and histological findings were consistent with a silent gonadotroph PitNET.

Investigation: To search for the TSC disease-causative variant, and possibly associate it with PitNET development, the entire TSC1 and TSC2 coding sequence and intronic boundaries were analyzed in patient's germline DNA. No disease-associated changes have been found with the exception of the heterozygous intronic variant c.4006-71C>T in TSC2, not present in publicly accessible genomic databases. A computational tools analysis predicted a gain of a new hypothetical splice site with consequent intron retention that was, however, not confirmed by an in-vitro analysis of patient's lymphocyte derived RNA. Molecular analysis of pituitary tumoral tissue failed to identify both loss of heterozygosity in TSC2 locus, and protein expression reduction, as expected if this would have been a loss of function mutation involved in the pathogenesis of this silent gonadotroph PitNET.

Primary cells from her pituitary adenoma were cultured in vitro and treated with everolimus 0.1 and 1 μM . After 72 hours the highest concentration of everolimus induced a significant 20% decrease in cell viability ($p<0.05$).

Conclusions: Our data although not conclusive for establishing unequivocally the causal nature of the association between TSC and PitNET further support previous observations of an antiproliferative effect of everolimus on PitNET.

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A soluble derivative of a Kv1.3 potassium channel inhibitor unveils interaction of mitochondrial Kv1.3 with respiratory chain Complex I

One of the most specific inhibitors of the Kv1.3 potassium channel, crucial for the proliferation of lymphocytes and different types of cancer cells is PAP-1, a psoralen derivative. In order to render PAP-1 more soluble, we generated PEGME, a derivative of the molecule by adding polyethylene glycol moieties to PAP-1. PEGME and different parts of the molecule were able to induce apoptosis, to reduce respiration and to inhibit Kv1.3 to different extent. PEGME was also able to reduce Complex I activity, while leaving unaffected other respiratory chain complexes. BN-PAGE experiments revealed co-migration of complex I subunits and of mitoKv1.3, indicating that the channel protein might functionally interact with Complex I. PEGME was able to induce apoptosis of cancer cells and to reduce tumor growth *in vivo*.

Giovanna Pontarin*, Roxana Oberkersch and Massimo Santoro

**Unit of Cell Biology and Developmental Genetics*

The role of glutaminases in endothelial cells

Metabolism is emerging as an important regulator in co-determining endothelial cell (EC) behavior and function. Little is known about a possible role of glutamine metabolism during new vessel sprouting in normal and pathological conditions. The key rate-limiting step of glutaminolysis is the conversion of glutamine to glutamate by the mitochondrial glutaminases GLS1 and GLS2. Given the very low expression level of GLS2 in ECs, in the present study we investigate the role of GLS1 at cellular level and how it affects angiogenesis *in vivo*.

At first we characterized the effect of GLS1 on EC behavior. The absence of glutaminase activity by GLS1 specific blocker CB-839 or expression by shRNA alters cell cycle progression and ultimately impairs proliferation, with no cytotoxicity. In addition, we found that the absence of GLS1 induces a stress response via ATF4 which in turns triggers the expression of the enzymes of the trans-sulfuration pathway (TSP) with concomitant production of the proangiogenic gas H2S and the VEGF A, resembling the aminoacid starvation response. Interestingly we noticed that the total level of VEGF R2 receptor and its activation after stimulation with VEGF A, is affected by GLS1 ablation. This seems to be independent of ATF4 induction suggesting a more specific response and linking glutamine metabolism to VEGF R2 signaling pathway. To determine whether GLS1 plays a role during angiogenesis *in vivo*, we generated endothelial conditional GLS1 knockout mice by crossing floxed Gls1 mice (Gls1^{flox/flox}) with VE-cadherin CreERT2 transgenic mice. In xenograft tumors we found that knock-out of GLS1 *in vivo* significantly inhibited the tumor growth and vascular vessels formation. These findings underscore the importance of glutamine metabolism in ECs and during angiogenesis *in vivo*.

Roberta Provvedi*

**Unit of Genomics and Bioinformatics*

The integral membrane protein Rv1619 is required for virulence in *Mycobacterium tuberculosis*

Abstract: MprF is a multi-peptide resistance factor characterized in *Staphylococcus aureus* as a defense mechanism against the action of cationic antimicrobial peptides (CAMPs). MprF is an integral membrane protein that modifies anionic phospholipids such as phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) with L-lysine, thereby introducing positive charges into the membrane surface and reducing the affinity for CAMPs. In *Mycobacterium tuberculosis* (MTB) an MprF-like protein named LysX was demonstrated to be required for the production of Lys-PG and confer resistance to CAMPs. In addition to LysX, the genome of MTB contains the gene rv1619 encoding an integral membrane protein with a MprF-like domain localized in the extracytoplasmic region rather than in the cytoplasm as for LysX and MprF of *S. aureus*. Rv1619 is absent in the saprophyte *Mycobacterium smegmatis* and upon introduction it increases resistance to the human

beta-defensins HBD-1 and HBD-2. Additionally, mice infected with a rv1619 deletion mutant survived better than those infected with the parent strain H37Rv, suggesting that Rv1619 plays a role in establishing a successful infection.

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**Unit of Environmental Physiology and Experimental Zoology*

Too warm or not too warm: is the antioxidant system of Antarctic fish ready to face climate changes?

Antarctic fish, due to the short- and long-term stability of thermal conditions in the Southern Ocean over the last million years, are considered highly stenothermal with limited evolutionary potential to cope with climate changes. Recently, warm acclimation experiments performed on some notothenioid species have indicated a significant capacity for these fish to elevate their heat tolerance, over the environmental CTMax, through acclimation. This result suggests that thermal plasticity may be universal throughout the Antarctic ichthyofauna. However, the physiological and genetic bases of their heat tolerance have been poorly studied. In the present work we described molecular characterization of mitochondrial peroxiredoxins in the Antarctic emerald rockcod *Trematomus bernacchii* and gene expression of this antioxidant enzyme in varius tissues, in response to short-term thermal stress. The obtained results suggest antioxidant physiological responses and genetic features that can contribute to limit the stenothermy of *T. bernacchii* and other Antarctic fish species. These data represent the starting point for a research project, recently funded by the Italian Program for Antarctic Research (PNRA), which is aimed to investigate the physiological responses of these organisms to temperature changes, in a perspective of climate changes. This project involves seven Italian research institutions (University of Padova, University of Trieste, University of Tuscia, University of Camerino, University of Pisa, University of Macerata and IBP-CNR center of Naples) and two foreign ones (Northeastern University Marine Science Center - USA and Nanyang Technological University - Singapore).

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**Unit of Bioenergetic Organelles*

Opa1 is oxidized during cardiac ischemia reperfusion injury

Mitochondrial reactive oxygen species (ROS) generation is accompanied by morphological changes of the organelle, which is controlled by fusion and fission processes regulated by a growing family of mitochondria-shaping proteins. The inner membrane mitochondria-shaping protein Opa1 regulates not only the fusion of the organelle, but also cristae remodeling during apoptosis, hampering cell death through cytochrome c release.

Here we show that Opa1 is a target of ROS since it is oxidized during ischemia-reperfusion injury at conserved Cys residues, causing an impairment of its antiapoptotic function. Upon ischemia reperfusion or oxidative stress of the heart, cardiomyocytes or mouse embryonic fibroblasts, Opa1 undergoes oxidative oligomerization and forms insoluble aggregates involving disulphide bonds formation. Through Opa1 homology modeling we identified four Cys residues which are exposed on a surface region of the protein likely required for protein protein-interaction. These residues represent natural candidates to mediate the aggregation of Opa1 upon oxidation.

In conclusion, using a genetic approach we demonstrate that preventing Opa1 oxidation, cells are protected from cytochrome c release and consequent cell death mediated by oxidative stress.

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*Unit of Bioenergetic Organelles

The Epac1/Rap1 pathway retrogradely signals changes in mitochondrial morphology

Mitochondrial dynamics, the regulated processes of fusion and fission of the organelle controlled by a set of mitochondria-shaping proteins, is essential for metabolism, cell proliferation, differentiation and death. However, how cells sense and respond to changes in mitochondrial shape is unclear. Here we show that the Epac1/Rap1 pathway is an unexpected sensor of mitochondrial morphology to coordinate nuclear gene expression. A deep sequencing analysis across cellular and *in vivo* models of mitochondrial shape alteration revealed a common signature pointing to the Ras-like GTPase Rap1 and to its cyclic AMP (cAMP)-activated nucleotide exchange factor Epac1. Epac1 is retrieved on mitochondria upon genetic ablation of the key mitochondrial fusion gene Opa1, or after its chemical activation and Epac1 genetic or pharmacological inhibition can curtail the effect of Opa1 deletion on integrated, complex cellular responses such as angiogenesis. Our results suggest that Epac1/Rap1 pathway can sense and rely to the nucleus changes in mitochondrial morphology.

Poster Session II

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Dynamic complexomic analysis reveals a role for Von Willebrand Domain-containing Protein 8 (Vwa8) in mitochondrial morphology and physiology

How changes in mitochondrial morphology influence the organization of membrane protein complexes and how this reverberates on mitochondrial function is unclear. Here, we report that Von Willebrand Domain-containing Protein 8 (Vwa8), an uncharacterized mitochondrial protein, links mitochondrial dynamics and metabolism. By combining 1 and 2-dimension blue native gel electrophoresis with proteomics and bioinformatics we compiled an online searchable catalogue of protein composition of native mitochondrial complexes in mitochondria from different tissues undergoing membrane remodeling. This approach allowed the identification of a strong correlation between the key cristae biogenesis protein Opa1 that influences mitochondrial respiration and Vwa8, a putative AAA+ ATPase with a dynein related domain, and a Von Willebrand factor type A domain. Vwa8 localizes in the mitochondrial intermembrane space where it forms discrete spots. Deletion of Vwa8 impairs mitochondrial bioenergetics and cellular growth in galactose where cells rely on amino acid oxidation but allows a more efficient utilization of fatty acids. In conclusion, Vwa8 links mitochondrial morphology to substrate preference.

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*Unit of Ecology

Within- and transgenerational effects of ocean acidification and other environmental stressors in marine invertebrates

There is growing concern about the capability of marine invertebrates, such as echinoderms and mollusks, to cope with environmental changes that are occurring much faster than in the past. To investigate the adaptive potential to environmental stressors, ocean acidification in particular, parents of the sea urchin *Paracentrotus lividus* are exposed to differing environmental conditions in both the laboratory and the field. Parental

responses (physiological, biochemical, cellular and behavioral) and offspring performances (gamete and larval features) are assessed to shed light on the presence of phenotypic plasticity and transgenerational effects.

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Unravelling the roles of Fission Protein 1: a forgotten mitochondrial fission factor with pleiotropic functions.

Mitochondrial fission regulates a myriad of cellular functions, including mitochondrial clearance, and its ultimate physiological importance is underscored in devastating human disorders that arise from mutations in mitochondrial fission proteins. The machinery driving mitochondrial fission consists of the master regulator Dynamin-related protein 1 (Drp1) and its outer mitochondrial membrane adaptors, namely Fission Protein 1 (Fis1). Despite being the first proposed Drp1 receptor, Fis1 role in recruiting and regulating Drp1-mediated mitochondrial fission is highly controversial. To elucidate Fis1 role in mitochondrial dynamics, we generated a Fis1 hypomorphic mouse model, in which the levels of Fis1 are constitutively downregulated. Fis1 hypomorphic mice die at weaning age, due to a pleiotropic phenotype of defective growth, muscular atrophy and disseminated haemorrhages. Using a conditionally ablation system to deplete Fis1 from endothelial cells or from specific tissues, such as the brain, will help us pinpoint Fis1 essential roles in mammals physiology.

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MiRNAs as regulators of metabolism in skeletal muscle fibers

Skeletal muscle is composed by different myofiber types that can preferentially use glucose or lipids for ATP production. How fuel preference is regulated in these post-mitotic cells is a point of interest in the field of muscle and whole-body metabolism. Here we show that miRNAs are important players in defining the myofiber metabolic profile. mRNA and miRNA signatures of all myofiber types obtained at single cell level unveiled fiber-specific regulatory networks and identified two master miRNAs that coordinately control myofiber fuel preference and mitochondrial morphology. Our work provides a complete and integrated mouse myofiber type-specific catalogue of genes and miRNAs expressed and establishes miR-27a-3p and miR-142-3p as key regulators of lipid utilization in skeletal muscle.

Gaia Codolo*, Sara Coletta, Marina de Bernard and Stefano Cagnin

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***Helicobacter pylori* affects the antigen presentation activity of macrophages**

Helicobacter pylori (Hp) is a Gram-negative bacterium that infects the human gastric mucosa, leading to chronic inflammation. If not eradicated with antibiotic treatment, the bacterium persists in the human stomach for decades. Hp has developed a number of strategies to escape recognition and to dampen immune responses; as result, the bacterium can persist lifelong in the host, thus leading to the chronicization of the

inflammatory status that pays the way for the development of the more severe gastric disorders, such as gastric cancer.

It has been recently shown that during Hp infection phagocytic cells promote high Hp loads rather than contributing to bacterial clearance. Within these cells Hp survives in megasomes, but the mechanism that Hp employs to avoid phagocytic killing is not completely understood.

In a recent work, we demonstrated that macrophages infected with *H. pylori* strongly reduce the exposure of MHC-II molecules on the plasma membrane compromising the bacterial antigen presentation towards Th lymphocytes. Ultimately, this would impair the local proliferation of Th cells and the possibility for them to exert the effector function. These evidences support the notion that macrophages may represent a survival niche for the bacterium in the stomach and emphasize the key role of these professional phagocytes in *H. pylori*-related disorders. The mechanism by which the bacterium manipulates the antigen-presentation pathway of macrophages remained an issue to be addressed.

In this work, we demonstrated that *H. pylori* down-regulates the expression of the class II major histocompatibility complex transactivator (CIITA), the master regulator for the expression of MHC class II genes, and also the genes encoding for the alpha and beta chain of MHC-II complex. We evidenced that this effect relies on the up-regulation of several miRNAs (miR146b, miR Let7f1, miRLet7i and miR185) targeting CIITA; accordingly, the overexpression of these miRNAs in macrophages decreases the expression of MHC-II and impairs the ability of the cells to present antigens to T lymphocytes.

Taken together, our data shed light on one of the mechanism of immune evasion adopted by *Helicobacter pylori* to survive within the host, allowing the establishment of a life-long relationship between Hp and the human host, leading to the chronicization of the infection.

Gaia Codolo*, Marina de Bernard, Nicola Facchinello and Natasca Tiso

**Unit of Cell Biology and Developmental Genetics*

The *Helicobacter pylori* protein HP-NAP: a novel treatment for metastatic melanoma

Melanoma is the most dangerous common skin cancer. Early diagnosis offers the best chance of cure. Nevertheless, when a primary melanoma is detected at a later stage, there is a risk of disease spreading to the nearest lymph nodes and distant sites, such as the lungs, liver, bone and brain. In this case, systemic chemotherapy and biochemotherapy have been the main treatments for over three decades. Unfortunately, only few people experience tumor regression, and the chance of being cured is lower than 10% after five years from diagnosis of metastatic disease. Recently, new classes of drugs (immune checkpoint inhibitors and small-molecule targeted drugs) have significantly improved patient prognosis; however, the toxicity is severe, and patients nearly invariably engender resistance at some stage. This disappointing situation underscores the urgency of developing new therapeutic strategies to treat metastatic melanoma.

A protein produced by *Helicobacter pylori*, HP-NAP, used in animal models *in vivo* to treat different types of cancers, has shown promising results, in virtue of its immune stimulating activity.

A zebrafish model of metastatic human melanoma has been set up in order to study the therapeutic efficacy of HP-NAP and to define the mechanisms and pathways involved. At day 3 post fertilization (dpf) 60 zebrafish embryos were microinjected with A375 melanoma cells previously stained with VybrantDil. After 3 days (6 dpf) fishes were randomly divided into 2 groups and treated with of HP-NAP or with vehicle alone as negative controls. The results obtained demonstrate that after 3 days from HP-NAP injection the protein strongly counteracts tumor growth and spreading in zebrafishes injected with A375 melanoma cells. Moreover, in zebrafishes treated with HP-NAP we evidenced by qRT-PCR and *in situ* hybridization a strong expression of IFN- γ which, in turn, inhibits tumor cells proliferation via the down-regulation of cyclins A1, A2 and E, as well as cyclin-dependent kinase 2(Cdk2) production in melanoma cells.

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Exploring the importance of GTPase activity in regulating the autophagic function of LRRK2

After fifteen years of research on leucine-rich repeat kinase 2 (LRRK2), a kinase mutated in familial and idiopathic forms of Parkinson's disease, the role of this protein in health and disease is still incompletely understood. Disease-associated mutations cluster within the catalytic core, which contains dual GTPase (Roc) and kinase activities. The kinase activity has been the major focus of LRRK2 research since mutations increase activity, which is toxic in cellular and animal models, a phenotype that can be reverted by pharmacological inhibition. Conversely, the Roc/GTPase domain has received less attention, possibly due to the challenges in measuring GTPase activity in vitro and in the cellular context. Roc is predicted to be a signaling output through the binding with multiple partners, including p21-activated kinase 6 (PAK6) that we recently described, as well as in the process of dimerization. Given the poorly characterized role of Roc in LRRK2 pathobiology, we set out to dissect its impact on LRRK2 biochemical properties and cellular functions, taking advantage of murine RAW264.7 macrophage knock-in cell lines harboring the GTP-binding deficient T1348N mutation within the endogenous locus. We evaluated how the ability to bind GTP affects the steady-state levels of endogenous Lrrk2. In addition, we characterized the consequence of impaired GTPase activity on basal and induced autophagy. Our data indicate a significant impact of the mutation on Lrrk2 steady state levels, together with a basal accumulation of p62 – an autophagic cargo protein and a recently described LRRK2 kinase substrate – in the T1348N cell line. Electron microscopy analysis revealed an aberrant accumulation of endolysosomal and autophagic vesicles, along with multilamellar bodies, in the mutant cell line as compared to the WT, possibly suggestive of impairment in autophagy-dependent degradative pathways. In addition, T1348N-Lrrk2 cells displayed alterations in the ability to respond to autophagy induction (via mTOR). Although further investigations are required to understand the exact mechanisms as to how GTPase activity affects the autophagic pathway, our present data suggest that the lack of GTPase activity is associated with an impairment of the autophagic flux. Overall, the T1348N mutation in Lrrk2 impacts on the biochemical properties of the protein, with predicted consequences on its downstream signaling and cellular functions.

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Roles of the autophagic-related proteins Ambra1 on zebrafish gonadal development

Ambra1 is a positive regulator of the Beclin1-dependent autophagic pathway and is also involved in the regulation of cell proliferation and apoptosis. The zebrafish genome contains two ambra1 paralogous genes, ambra1a and 1b, both involved in development. Differently from knockdown results, ambra1a-/- and ambra1b-/- mutant embryos do not display overt developmental defects, due to compensatory effects of the paralogous genes remaining active. On the contrary, generation of a stable double ambra1 mutant line was not possible as double mutants do not survive after larval stages. Silencing of the zebrafish ambra1b gene lead to all-male individuals as demonstrated by visual analysis of secondary sexual features and reproductive behavioral of these mutants. Moreover, analysis of the gonadal morphology of 35 dpf larvae confirmed the lack of female only in ambra1b-/- mutant but also suggested a general delay in gonadal maturation with respect to WT. As signaling from primordial germ cells (PGCs) are considered to be important for sex determination in zebrafish, we analyzed the number of PGC in 10-hpf embryos and found a statistically significant reduction of PGC's number in ambra1b-/-, compared to sibling ambra1b+/- and ambra1b+/+.

Reduction of PGCs was further confirmed with ATG and splicing ambra1b MOs. On the contrary, analysis of 10-hpf ambra1a-/- embryos as well as silencing of both maternal and zygotic ambra1a transcripts had no effects on PGCs number. These results underline the sub-functionalization of the two paralogous genes, favoring their maintaining in zebrafish genome and suggest a specific Ambra1b function on sex determination and gonadal development, likely by means of regulation of PGCs survival.

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Effects of phenotypic robustness on adaptive evolutionary dynamics and evolvability

Theoretical and experimental studies have provided evidence for a positive role of phenotype resistance to genetic mutation, or “phenotype mutational robustness,” in fostering evolvability by enhancing adaptation. However, the mechanisms by which robustness might be established during evolution are far from clear and overall little explored. With the aim of contributing to an understanding of the origin and evolution of phenotypic robustness in living systems, we adopted a theoretical approach, elaborating on a standard population genetic model of evolutionary dynamics, complemented by computer simulations. Results show that, under common selective regimes, there is a minimum level of phenotypic robustness below which evolution by natural selection cannot occur, even in the case of sizable selection coefficient and in the absence of any drift effects. Phenotypic robustness qualifies as major quantitative determinant of biological system’s evolvability, a key feature of the genotype-phenotype map which would deserve to be formally integrated into a more inclusive explanatory framework of the evolutionary theory.

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**Unit of Bioenergetic Organelles*

PAPTP leads to neoplastic cell apoptosis in the Eμ-Tcl1 chronic lymphocytic leukemia mouse model

The potassium channel Kv1.3 is highly expressed in CLL cell mitochondria. We previously demonstrated that direct inhibition of Kv1.3 using mitochondria-targeted inhibitors alters mitochondrial function and leads to ROS mediated death of even chemoresistant cells. The inhibitor PAPTP killed 98% of ex vivo primary CLL cells while sparing healthy B cells. With this background we aimed to evaluate effectiveness and toxicity of PAPTP in the Eμ-TCL1 CLL murine model, which is characterized by a high expression of TCL1 protein in B cells leading to a CLL-like lymphoproliferative disease development. Once we assessed the effectiveness of PAPTP in murine cells, we performed in vivo experiments. After 2 weeks of therapy, we observed an improvement of treated mice in term of appearance (posture and weight gain) with respect to controls. We also demonstrated a decrease in absolute total lymphocyte number after PAPTP administration and a higher reduction of pathological B cells (CD19+CD5+) in spleen and bone marrow of treated, with respect to untreated, mice. Moreover, hematoxylin and eosin stain shows that untreated mice had enlarged spleen and liver, with evidence of CLL infiltration in both organs. Treated mice had smaller spleens and livers, with minimal (if any) CLL infiltration. In particular, the spleens disclosed well preserved with pulp architecture with clearcut follicles and marginal zones. In human CLL patients after treatment an imbalance of CD4:CD8 ratio was described due to the increase of CD8+ T lymphocytes. Interestingly, in mice after nocodazole administration we confirmed a similar expansion of cytotoxic T cells. The high selectivity of PAPTP and its ability to induce

apoptosis in CLL B cells also in the E μ -TCL1 mouse model, may suggest the use of this inhibitor for designing new therapeutic strategies.

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How do cells communicate in muscle regeneration?

After injury, the skeletal muscle can regenerate within few days. This process is regulated by crosstalk among several cell populations like muscle progenitors (MP), fibro/adipogenic progenitors (FAP), inflammatory cells (INF), pericytes (PER) and endothelial cells (EC).

In an attempt to understand how the cells communicate with each other, we performed time course RNA-Seq over ~10 time points after injury on 5 cell populations and total tissue as well. We created a new analysis pipeline to reconstruct the interactome that regulates muscle regeneration.

Our work can answer to different question like how do the cell talk to each other and how do they react to the external signaling. Our results give an overview of the intercellular cross-talks that occur in muscle regeneration and highlight the importance of the location of redundant ligand-receptor signaling pathways.

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Altered dopamine metabolism leads to a unique impaired α Synuclein proteostasis in Parkinson's Disease

Parkinson's Disease (PD) is pathologically characterized by the progressive loss of nigrostriatal dopaminergic neurons and aberrant accumulation of the presynaptic protein α Synuclein (α Syn). Several factors have been proposed to trigger α Syn aggregation, resulting in α Syn-induced neurotoxicity. One of them is 3,4-dihydroxyphenylacetaldehyde (DOPAL), a toxic dopamine metabolite, which covalently modifies lysine residues of proteins. In vitro and cellular studies demonstrated that DOPAL triggers α S oligomerization, prevents α S association to synaptic vesicle membranes and affects synapse physiology.

On this ground, our aim is to investigate the consequences of DOPAL modification of α Syn lysines on overall α Syn proteostasis, in terms of aggregation, degradation and trafficking.

To address these issues, we conducted ultrastructural analysis by using markers for correlated light and electron microscopy (LM&EM) to investigate DOPAL-induced α Syn accumulation and aggregation, in both time and space resolution. First, we aimed to localize DOPAL- α Syn oligomers in rat primary cortical neurons. By using α S-split miniSOG protein-fragment complementation assay, we specifically detected α Syn oligomers in lysosomal compartments in the soma, at pre-synaptic terminals and multi-vesicular bodies. Secondly, we studied α Syn degradation in catecholaminergic BE(2)-M17 cells. Pulse-chase experiments with HaloTag™ labelling technology revealed significant α Syn build-up, oligomerization and decreased clearance after DOPAL treatment. Finally, live time-lapse imaging on rat primary cortical neurons overexpressing α Syn with TimeStamp tag, revealed impaired α Syn trafficking between soma and periphery in the presence of DOPAL. Concluding, our study combines different biochemical and imaging approaches to shed light on the crucial contribution of altered dopamine metabolism in α Syn-associated neurodegeneration in PD.

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Functional validation of miRNAs targeting genes of DNA double-strand break repair to radiosensitize non-small lung cancer cells

DNA-Double strand breaks (DSBs) generated by radiation therapy represent the most efficient lesions to kill tumor cells, however, the inherent DSB repair efficiency of tumor cells can cause cellular radioresistance and impact on therapeutic outcome. Genes of DSB repair represent a target for cancer therapy since their down-regulation can impair the repair process making the cells more sensitive to radiation. In this study, we analyzed the combination of ionizing radiation (IR) along with microRNA-mediated targeting of genes involved in DSB repair to sensitize human non-small cell lung cancer (NSCLC) cells. MicroRNAs are natural occurring modulators of gene expression and therefore represent an attractive strategy to affect the expression of DSB repair genes. As possible IR- sensitizing targets genes we selected genes of homologous recombination (HR) and non- homologous end joining (NHEJ) pathway (i.e. RAD51, BRCA2, PRKDC, XRCC5, LIG1). We examined these genes to determine whether they may be real targets of selected miRNAs by functional and biological validation. The *in vivo* effectiveness of miRNA treatments has been examined in cells over-expressing miRNAs and treated with IR. Taken together, our results show that hsa-miR-96-5p and hsa-miR-874-3p can directly regulate the expression of target genes. When these miRNAs are combined with IR can decrease the survival of NSCLC cells to a higher extent than that exerted by radiation alone, and similarly to radiation combined with specific chemical inhibitors of HR and NHEJ repair pathway.

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Opa1 is crucial for melanocyte stem cell and melanoma maintenance

Mouse models of hair graying processes can serve as useful systems to uncover mechanisms involved in aging and the maintenance of tissue homeostasis. Despite that mitochondria are involved in ageing, their role in hair follicle genesis and hair cycle processes have not been defined yet. Here we show that Opa1 is essential to provide differentiated melanocyte during the hair follicle cycle. Indeed, Opa1 ablation in the mouse reduced differentiated and TRP2+ melanocytes during hair cycles and resulted in early hair graying, pointing to a critical role for Opa1 in melanocyte stem cell survival. Conversely, Opa1 is increased in melanomas and its expression in NRAS-driven acral melanomas clusters with increased incidence of metastasis. Indeed, in cellular models of NRAS-driven melanomas Opa1 was essential for colony formation in soft agar assays and *in vivo*, deletion of Opa1 in a classic NRAS driven mouse model of melanoma consistently reduced the number of mature melanocyte. In conclusion, we highlight the essential role of Opa1 in the melanocyte compartment and suggest that NRAS driven melanomas depend on Opa1 for survival and proliferation.

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*Unit of Bioenergetic Organelles

A high throughput screening identifies a small molecule inhibitor of the GTPase activity of OPA1 that enhances apoptotic release of cytochrome c

The GTPase activity of OPA1, a dynamin-related mitochondrial protein upregulated in several tumors, controls cristae remodeling, complete cytochrome c release and apoptosis. To pharmacologically target OPA1 in cancer, we setup and performed a high-throughput screening (HTS) of a diversity based chemical library of 10,000 drug-like small molecules on the GTP hydrolysis of recombinant purified OPA1. We obtained 8 confirmed hits that inhibit OPA1 GTPase activity *in vitro* and we characterized the most promising small-

molecule hit, called MYLS22. Saturation transfer difference (STR) NMR showed that MYLS22 binds to recombinant OPA1. MYLS22 was not intrinsically mitochondriotoxic, but it increased OPA1 oligomer disassembly and hence cytochrome c release in response to the proapoptotic Bcl-2 family member BID. Cells exposed to MYLS22 displayed fragmented mitochondria and increased cytochrome c release in response to hydrogen peroxide. MYLS22 also inhibited breast cancer cells migration, an effect that was not additive to OPA1 silencing. Thus, we identified a first-in-kind OPA1 inhibitor with pro-apoptotic and anti-migration effects.

Paula Rebelo*, Caterina Vianello, Alessandro Bregalda, Adriano Gonnelli, Sang Hun Shin and Marta Giacomello

**Unit of Bioenergetic Organelles*

A new class of contact sites modulators: the mitochondria-ER Spacers

The field of inter-organelles communication, since its beginning, has focused on the ability of different subcellular compartments to exchange metabolites and ions, and to coordinate their functions through tethering complexes.

These “molecular bridges” can be highlighted through electron microscopy as electron dense, rod-like structures. The latter have been indeed reported, for example, in case of the mostly studied contact sites, i.e those amongst mitochondria and Endoplasmic Reticulum (ER).

However, the possibility that not only the proximity, but also the distance between the two organelles plays a role in cell physiology has not been yet elucidated. By means of a genome wide high content imaging screen, we have previously identified proteins able to keep apart the two organelles, and we have named them “mitochondria-ER spacers” (MESs). We here provide a preliminary characterization of some MESs candidates, based on bioinformatic, imaging, molecular biology and biochemistry approaches.

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Dissecting the dual role of ATAD3A: the link between nucleoid stability and cristae ultrastructure

**Unit of Bioenergetic Organelles*

The regulation of mitochondrial dynamics and ultrastructure is crucial for cell function and viability. A well-characterized regulator of such processes is the inner mitochondrial membrane GTPaseOPA1, which forms high molecular weight (HMW) complexes that maintain cristae ultrastructure and thus restrict cyt c to the cristae lumen. Our proteomic analysis of OPA1-HMW complexes has enabled the identification of proteins that potentially interact with OPA1 during apoptotic cristae remodeling. Among these, ATAD3A stands out as one of the most promising OPA1 interactors, data confirmed by coimmunoprecipitation experiments. Our studies demonstrate that ATAD3A regulates cristae biogenesis and stability. We demonstrate that ATAD3A oligomers are not required for cristae biogenesis, but instead are likely involved in nucleoid and mtDNA stability. Indeed, mutants in the coiled-coil domains impede the ATAD3A oligomerization but not cristae formation. On the other hand, mutants at the ATPase domain which are still able of oligomerizing do not maintain cristae ultrastructure. Moreover, inhibiting ATAD3 oligomerization results in the specific disruption of protein complexes that we have identified to regulate mtDNA replication and nucleoid stability.

Our studies support that ATAD3A plays a key role in cristae formation and in mtDNA stability, functions that we propose to depend on the ATPase and coiled-coil domain, respectively.

Giovanni Tosi*, and Massimo Santoro

**Unit of Cell Biology and Developmental Genetics*

Involvement of UBIAD1 in tumor angiogenesis and cancer growth

Background: UBIAD1 is a prenyltransferase recently identified as a new antioxidant enzyme. It is important for the synthesis of the antioxidant CoQ10 in the Golgi membrane compartment. Loss of UBIAD1 induces a decrease in the non-mitochondrial pool of CoQ10 that leads to an increase in ROS/RNS-mediated oxidative damage within the cell. Hypothesis: Considering that protection against excessive oxidative damage is essential in cardiovascular system development as well as during tumor progression, our hypothesis is that silencing/deleting UBIAD1 enzyme increase lipid peroxidation and DNA-oxidative damages in cancer cells as well as in tumor-associated endothelial cells. Therefore, we expect a reduction in tumor angiogenesis and inhibition of tumor growth after UBIAD1 deletion/silencing. Preliminary results: UBIAD1 silencing in endothelial cells leads to an increase in RNS (Reactive Nitrogen Species) amount and apoptosis level. Similar results were obtained in different breast cancer cell lines where UBIAD1 silencing stimulates apoptosis. Moreover, UBIAD1 expression changes seem to be associated to cancer cell resistance to different chemotherapy drugs. Future perspectives: Impaired tumor angiogenesis and breast cancer growth will be analyzed *in vivo* with mouse models of breast cancer carrying Ubiad1 gene deletion specifically in endothelial cells or in mammary gland tissues.

Alessandro Vezzi*

**Unit of Genomics and Bioinformatics*

Metagenomic exploration of marine microbial communities

The study of marine microbial communities is a growing field of marine ecology. Marine microbial communities account for the largest part of Ocean global biomass and probably for one-half of the global biogeochemical flux of biologically important elements (e.g. C, N, Fe). We focused our studies on the marine communities of the Venice lagoon and of the Ross Sea (Antarctica). We participate to an ongoing long term sampling campaign focusing on 4 different sites of the Venice lagoon, sampling twice a year at each solstice. We sequenced both the 16S and 18S genes to have a snapshot of the microbial diversity along the years as well as the metagenomes of 2 different time-points. The study of this environment continues with the participation to the monitoring of the effects of the MOSE system on the lagoon. We are also part of two different project founded by PNRA and aimed at the study of Antarctic marine microbial communities. The first project is focused on the study of the microbial communities involved in the degradation of the dissolved organic matter through the water column in the Ross Sea. The second study is aimed to the identification of cold adapted enzymes for PUFA biosynthesis in coastal samples collected during the XXXIII Italian Antarctic Expedition. These enzymes could be used for the set-up of low-temperature bioreactors for the production omega-3 fatty acids.

Raffaella Margherita Zampieri*, Alessia Codato, Luisa Dalla Valle and Nicoletta La Rocca

**Unit of Plant Biology*

Anti-inflammatory and antioxidant activity of released polysaccharides from cyanobacteria of the Euganean Thermal Mud.

The Euganean Thermal Mud has been used since Ancient Rome as an anti-inflammatory treatment and is recognized by the "Servizio Sanitario Nazionale" as a therapy to many arthro-rheumatic pathologies. The effectiveness of the mud is due to the fusion of several elements: heat, presence of electrolytes and presence of a microbiota that grows during the mud maturation process. Among these organisms, a consistent portion is occupied by cyanobacteria, photoautotrophic bacteria able to live in extreme environment such as thermal water. Until now, the therapeutic effect of these organisms has been attributed to produced compounds such as glycolipids, with anti-inflammatory efficacy but no side effects as for traditional drugs (Ulivi, 2011). However, despite their high abundance, polysaccharides released by endemic cyanobacteria have never been analyzed.

We attempted to assess the efficacy of this relevant mud component by *in vivo* analysis with the model organism *Danio rerio*. Released polysaccharides (RPS) obtained from batch cultures of cyanobacteria *C.*

aponinum and *Phromidium sp.*, commonly growing in Euganean muds, were supplied to zebrafish embryos in order to perform preliminary tests on the toxicity of these molecules (Fish Embryo Acute Toxicity Test, OECD TG 236). Furthermore, we tested and demonstrated their anti-inflammatory and antioxidant activity thanks to transgenic zebrafish lines LysC::DsRED2 (Hall et al., 2007) and NFkB:Luc (Kuri et al., 2017), morphometric and qPCR expression analyses.

Poster Session III

Mariano Battistuzzi*, Lorenzo Cocola, Riccardo Claudi, Luca Poletto, Tomas Morosinotto and Nicoletta La Rocca

*Unit of Plant Biology

An experimental setup to study by remote sensing analyses cyanobacteria growth and photosynthetic performances under non-terrestrial simulated environments.

During recent years, by means of space and ground-based observations, more than 3,800 new exoplanets have been discovered, many of which are terrestrial-like planets orbiting the Habitable Zone of their host stars, enhancing our hope to find life elsewhere in the universe. Due to the great distances separating us from these planets, any study regarding their habitability must be made through remote sensing techniques or in laboratory, by means of environmental condition simulations. Thanks to the collaboration between INAF, CNR-IFN and the department of Biology, a Star Light Simulator (SLS) and an Atmosphere Simulator Chamber (ASC) have been built. Using these devices, we study oxygenic photosynthetic microorganisms under simulated environmental conditions of terrestrial-like exoplanets orbiting M-type stars, to understand if they could maintain their oxygenic photosynthetic activity and furthermore impact on primeval atmospheres lacking oxygen. Testing these conditions requires light, temperature, pressure and atmospheric composition conditions extremely different from terrestrial ones and imposes us to seal the ASC and assess the physiological responses of the cultures only at the beginning and at the end of the experiments. To overcome this issue, here we present a novel experimental setup to follow by remote sensing the growth and photosynthetic activity of oxygenic photosynthetic microorganisms by means of reflectivity, spectroscopic and fluorescence measurements, respectively.

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An integrated computational approach to define transcriptome alterations in platinum resistance

Epithelial ovarian cancer (EOC) is the most lethal gynecological malignancy due to its diagnosis at advanced stages. EOC is generally sensitive to first line chemotherapy, and the vast majority of patients respond to platinum (Pt)-based therapy after debulking surgery. Unfortunately, the median progression free survival (PFS) lasts only 18 months, and more than 80% of Pt-responsive patients relapse with a disease that progressively becomes Pt-resistant. The process by which disease relapses is still poorly understood, so the aim is to identify biomarkers of sensitivity to chemotherapy and therapeutic targets in HGS-EOC by integrating transcriptomic data, coding and non-coding RNAs.

To this end, we used matched miRNA and mRNA expression data obtained by both microarray and sequencing technologies of HGS-EOC biopsies (at the time of diagnosis). HGS-EOC tumor samples are divided as platinum-resistant (Pt-r), platinum-sensitive (Pt-s) and platinum-partially sensitive, having a complete follow up of at least five years. After data normalization, we performed differential expression analysis on

coding and non-coding elements, using the two more extreme categories of patients (Pt-r and Pt-s), and we applied an integrated pathway approach to identify circuits associated to the therapy response.

In addition, we used a set of strand specific paired-end RNA-seq experiments, selected from tumor tissue collection, to perform transcriptome reconstruction in order to identify new aberrant splicing such as circular RNAs and to estimate their expressions.

Our findings suggest that the molecular landscape between Pt-s and Pt-r patients at the time of diagnosis is highly complex and heterogeneous. This variability seems to involve mainly novel transcripts, including specific non coding RNAs. In this perspective a two-step approach using classical array-based gene expression and recent NGS technology is the best way to approach the problem. Here we identify a regulatory circuit composed of 127 genes and 5 miRNAs wiring mainly ATP synthesis, calcium channel, HLAs and CREB proteins, and also a list of novel transcripts potentially involved in Pt resistance mechanism.

Specifically using RNA-Seq we report a large number of circRNAs differentially expressed between tumor resistance types. The consistency of circular RNA expression, in conjunction with the regulatory circuit, may offer new candidates for cancer treatment and prognosis, revealing that the circRNA-miRNA-mRNA network may shed light on the biological functions of circRNAs in ovarian cancer.

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**Unit of Bioenergetic Organelles*

Development of a split-GFP tool to investigate the localization of PKA at sub-mitochondrial level

Mutations in the mitochondrial serine–threonine kinase PINK1 are associated with familial forms of Parkinson's disease and mitochondrial Ca²⁺ overload (1,2). The targeting of PKA to mitochondria and its activation rescue functional defects observed in PINK1 deficient neurons (3) and mitochondrial Ca²⁺ overload due to the loss of PINK1 function (4). PINK1 and PKA have been proposed to cooperate at the mitochondria level to prevent neurodegeneration, and we have found that PINK1 was able to reduce mitochondrial Ca²⁺ accumulation. Sustained Ca²⁺ accumulation into the mitochondrial matrix has been shown to correlate with increases of cAMP levels in the same compartment (5). If the localization and the action of PKA at the outer mitochondrial membrane (OMM) are well recognized, its presence in the mitochondrial matrix and in the intramembrane space (IMS) is still amply debated.

We developed a probe based on the split-GFP protein and Bimolecular Fluorescence Complementation (BiFC) to monitor PKA distribution at sub-mitochondrial level in living cells. The non-fluorescent GFP1-10 fragment was targeted to the OMM, the IMS and the mitochondrial matrix by the addition of targeting sequences. The β11 fragment, necessary to reconstitute GFP fluorescence, was fused to two PKA regulatory subunits (RⅠα and RⅡβ) and to PKA catalytic subunit (Cat-α).

The co-transfection of the plasmids encoding the targeted GFP1-10 fragments and the Cat-α β11 or the RⅠα β11or the RⅡβ β11 in HeLa cells revealed the presence of all these subunits at the OMM and IMS. Interestingly, strong GFP fluorescence emission in the presence of GFP1-10 fragment targeted to mitochondrial matrix was observed in the case of Cat-α β11 co-expression, but not of RⅠα β11and RⅡβ β11, suggesting the presence of PKA Cat-α in the mitochondrial matrix. To evaluate whether regulatory subunits could interfere with mitochondrial Cat-α localization, we co-transfected the GFP1-10 fragment targeted to OMM, IMS and mitochondrial matrix with Cat-α β11 and RⅠα or RⅡβ without β11 fragment. In these conditions we still observed fluorescence reconstitution at the OMM and IMS, but not in the mitochondrial matrix when Cat-α β11 was co-expressed together with RⅡβ, suggesting that Cat-α may translocate to this compartment only upon activation and release from its regulatory subunit. Further investigations are necessary to reveal the mechanism and the cellular conditions that favor Cat-α distribution at mitochondrial level and eventually its interplay with mitochondrial PINK1 kinase.

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Gaia Codolo*

**Unit of Cell Biology and Developmental Genetics*

Cinaedi Atherosclerosis Inflammatory Protein (CAIP)-induced ROS production contributes in sustaining the atherosclerotic process in *Helicobacter cinaedi*-infected patients

Recent studies have shown that certain microbial infections participate in atherosclerosis by inducing inflammation and immune reactions, but how the pathogens implicated in this pathology trigger the host responses remains unknown. In this study we show that *Helicobacter cinaedi* (Hc) is a human pathogen linked to atherosclerosis development since at least 27% of sera from atherosclerotic patients specifically recognize the antigen CAIP. The bacterial protein appears to be implicated in this pathology because atheromatous plaques isolated from atherosclerotic patients are enriched in CAIP-specific T cells (10%) which, in turn, drive a Th1 inflammation, an immunopathological response typically associated to atherosclerosis. Recombinant CAIP promotes the differentiation and maintenance of the pro-inflammatory profile of human macrophages and triggers the formation of foam cells, which are a hallmark of atherosclerosis.

This study demonstrates that CAIP induces an oxidative stress both in macrophages and endothelial cells, through which it triggers the production of pro-inflammatory cytokines and chemokines that participate in sustaining the recruitment of circulating inflammatory cells. Moreover, CAIP-induced ROS production is responsible for the over-expression of the scavenger receptor LOX-1, one of the main receptors of oxidized LDL (oxLDL). Accordingly, the blockage of ROS production resulted in the abrogation of LOX-1 expression, paralleled by a reduced uptake of LDL and foam cells formation. Finally, the signaling pathway elicited by CAIP-mediated oxidative stress, led to the phosphorylation of p38 and ERK1/2 MAP kinase as well as to the activation of NF-κB.

This study identifies CAIP as a relevant factor in atherosclerosis inflammation in patients infected by Hc and highlights the role of the CAIP-induced oxidative stress in the process.

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**Unit of Bioenergetic Organelles*

Role of Opa1 in zebrafish dopaminergic neurons

Mitochondria not only synthesize most of the ATP, but they are also involved in TCA, fatty acid β-oxidation, Ca²⁺ signaling, redox homeostasis and apoptosis. Mitochondrial functions are regulated by their dynamic, resulting from a balanced activity of mitochondrial fusion (Optic Atrophy 1 OPA-1, and Mitofusins MFN1 and MFN2) and fission proteins (Fis, Dynamin-related protein-1 DRP-1, and MFF). Mitochondria shape, ultrastructure and function are affected in pathological conditions, including those associated to neurodegenerative diseases such as Parkinson disease (PD). Here we show that mitochondrial fusion protein OPA1 in Zebrafish is a crucial component for the development of dopaminergic neurons, selectively affected in PD. In particular, OPA1 ablation in Zebrafish embryos inhibits dopaminergic neurons development, an event that correlates to activation of the NFκB pathway and diminished protein levels of Parkin, an E3 ubiquitin ligase mutated in familiar PD.

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*Unit of Plant Biology

The importance of molecular characterization of ancient fungal type specimens for a correct taxonomic reclassification

The mycological collections, also known as fungaria, represent a source of molecular information that may be exploited to obtain important DNA sequences; however, these collections, in particular the oldest ones, are an underused resource for this purpose, due to the difficulty to obtain DNA data from ancient biological material. The Saccardo mycological collection, preserved in the herbarium of the Botanical Garden of Padua, has a huge scientific importance due to the presence of over 4,000 type specimens. These specimens have been borrowed by mycologists from all over the world for morphological revisions and consequent taxonomic reclassifications, but they have never been involved in sequencing projects. Using a next-generation sequencing (NGS) to overcome the problems of DNA degradation and exogenous DNA contamination, we have been able to obtain ITS sequences (the consensus barcode for the identification of fungal species) from several types belonging to a specific genus within the collections. The molecular analysis and the new morphological observations done have suggested that there is a need to reclassify some types previously reclassified only on a morphological basis and some types never considered for taxonomic revisions. This demonstrates that in fungi the morphology alone does not always lead to a correct fungal systematic evaluation and therefore it is highly recommended to combine morphological and molecular analyses. In view of these results, the application of an NGS method to recover crucial genetic information from as many as possible of the over 4,000 old type specimens stored in the Saccardo collection could result in a taxonomic reassessment of many fungal species.

Elisa Gaio* and Francesca Moret

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Keratin nanoparticles co-delivering Docetaxel and Chlorin e6 promote synergic interaction between chemo- and photo-dynamic therapies

Several strategies aimed at improving the therapeutic outcome of cancer treatments are being considered and investigated. Among them, combining chemotherapy with photodynamic therapy (PDT) seems to offer the possibility of limiting systemic toxicity of chemotherapeutics through the reduction of the administered dose and by contrasting drug-resistance phenomena. Moreover, the re-formulation of clinically approved pharmaceuticals in biocompatible nanoparticles (NPs), appears particularly appealing for the possibility of co-loading drugs exerting cytotoxic effects by quite different mechanisms, as chemotherapeutics and photosensitizers (PS) with the aim to produce synergic effects. Thus, we exploited wool-extracted keratin NPs for the co-delivery of the antimitotic drug Docetaxel (DTX) and the PS Chlorin e6 (Ce6) for the combination of chemo- and photo-dynamic therapy. The drug-induced aggregation method allowed the in-water formation of monodisperse NPs (DTX/Ce6-KNPs) with an average diameter of 133 nm and with a drug ratio of 1:1.8 of Ce6 vs DTX. The NPs were tested for combination therapy in *in vitro* monolayers and multicellular spheroids of DTX-sensitive HeLa (HeLa-P) and DTX-resistant HeLa (HeLa-R) cells. In HeLa-P monolayers the cytotoxic effects of DTX/Ce6-KNPs were comparable to those induced by DTX + Ce6 delivered in the standard formulation. Interestingly, in HeLa-R monolayers chemo- and photo- dynamic therapy interacted synergistically only delivering DTX and Ce6 co-loaded in DTX/Ce6-KNPs. Moreover, DTX/Ce6-KNPs induced stronger cytotoxicity with respect to the single drug-loaded nanoformulations (i.e. DTX-KNPs and Ce6-KNPs) and significantly reduced the volumes (up to 50%) of spheroids formed of HeLa-P and HeLa-R cells. Overall, the results suggest that these KNPs are very promising for the co-delivery of chemotherapeutics and PSs exploiting synergic interactions of PDT and chemotherapy.

Laura Guidolin*

*Unit of Environmental Physiology and Experimental Zoology

Effects of PFOA and PFBS exposure in the soil invertebrate *Dendrobaena veneta* (Annelida)

The aim of the work is to study in *Dendrobaena veneta* (Annelida), a soil Invertebrate, bioaccumulation patterns and cellular and biochemical responses in coelomocytes (mortality and lysosomal membrane stability), and at tissue level (GPX and MTs), after the exposure to two perfluorinated alkyl acids (PFOA and PFBS) for short (72 h) and longer (14, 28 and 42days) times. The exposures are carried out in soil microcosms prepared with glass containers filled with 300 ml of soil humidified at 30% with PFOA or PFBS spiked water. Different accumulation patterns are observed for PFOA and PFBS both in the soft tissues and in coelomocytes, the main immunodefensive system cells of the organism, with a higher PFBS bioaccumulation than PFOA in both compartments. With both compounds significantly higher coelomocyte mortality than in the controls is detected. Additionally, lysosomal membrane stability significant decreases are observed in these cells and MT total level decrements in soft tissues. Further studies are running to explore the mechanisms

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Exploring the novel role of mitochondrial dynamics in schizophrenia

**Unit of Bioenergetic Organelles*

Abnormal energy metabolism is reported in brains of schizophrenia (SZ) patients. Mitochondria have a central role in energy metabolisms and significant reduction of mitochondria mass in oligodendrocytes has been observed in SZ brains, however the molecular mechanisms behind mitochondrial biogenesis in oligodendrocytes are unclear. Here we show that mitochondrial mass is involved in differentiation of oligodendrocytes. Mitochondrial mass and expression levels of L-PGC-1 α a key regulator of mitochondrial biogenesis, increased during differentiation of an oligodendrocyte cell line and accordingly, L-PGC-1 α specific knockdown suppressed oligodendrocyte differentiation. In mouse brain, white matter specific PGC-1 α expression correlated with MBP expression during brain development. These findings suggest that L-PGC-1 α plays a crucial role in oligodendrocyte differentiation via regulating mitochondrial biogenesis, pointing to its potential role in pathophysiology of SZ.

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**Unit of Bioenergetic Organelles*

The mitochondria shaping protein Opa1 controls adipocyte size

White adipose tissue is specialized in the storage and release of fat, the balance of which is critical to maintain healthy energy homeostasis. Whether mitochondria, crucial in fat oxidation, participate in the biology of these cells is unclear. Here we show that mitochondrial dynamics, the controlled processes by which mitochondria fuse and divide, is fundamental for white adipose tissue function and for its healthy expansion in response to metabolic challenges. *In vivo*, the fusogenic protein Opa1 is required for the ability of adipocytes to expand upon exposure of mice to a high-fat diet. Mechanistically, a deep sequencing analysis revealed that controlled overexpression of Opa1 in pre- and mature mouse adipocytes led to changes in cellular pathways associated to the control of cell size. Metabolomic profiling of these cells unveiled a correlation between Opa1 and polyamines levels, further substantiated by the effect of Opa1 on the expression of key enzymatic regulators of polyamine synthesis in subcutaneous adipose depots from animals exposed to a high fat diet. Because polyamine metabolism has been linked to the regulation of cell growth, our results overall indicate that Opa1 impacts on signaling pathways and metabolic cues that regulate cell size.

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**Unit of Bioenergetic Organelles*

Exploring the role of mitochondrial dynamics in tracheal stem cells

Various signaling pathways regulate somatic stem cells self-renewal and lineage commitment by impinging on a variety of biological signals, many of which are controlled by mitochondrial dynamics. Whether mitochondrial dynamics can regulate states of airway stem cells remains unclear. The dynamin-like GTPase optic atrophy 1 (OPA1) is a master regulator of mitochondrial fusion. To verify if OPA1 dependent mitochondrial regulation participates in the biology of tracheal stem cells (basal cells), we assessed the stemness of OPA1 deleted basal cells by using a tracheosphere assay and primary culture of tracheal epithelial cells (mTEC). Our preliminary findings suggest that OPA1 regulates self-renewal and differentiation of basal cells.

Chiara Rampazzo*

**Unit of Cell Biology and Developmental Genetics*

SAMHD1 deficiency affects DNA replication fidelity and DNA repair efficiency in human fibroblasts

In mammalian cells, SAMHD1 with its important function in dNTP catabolism influences the balance of the four dNTPs and their relative concentrations, both in cycling and in quiescent cells. We have studied the consequences of SAMHD1 deficiency for the dynamic of DNA replication in skin fibroblasts isolated from two unrelated Aicardi Goutieres Syndrome (AGS) patients where SAMHD1 is mutated. In DNA combing experiments we did not detect any difference between AGS fibroblasts and wild type (WT) cells in terms of fork rate, inter origin distances and cluster length. We propose that in AGS fibroblasts the endogenous higher supply of dNTPs is a constitutive condition to which they have adapted. To investigate if dNTP pool expansion in AGS fibroblasts affects mutation rates, we compared de novo mutations in AGS and WT cells by a genomic approach based on next generation sequencing. Somatic variant analyses highlighted a clear cut mutator effect demonstrating that SAMHD1 deficiency is *per se* a mutagenic condition that promotes genome instability in non-transformed cells. In addition, we investigated the involvement of SAMHD1 in DNA repair by analyzing the ability of the mutated fibroblasts to repair DNA after UV damage induced during quiescence. After irradiation the fraction of intact dsDNA was measured by fluorimetric analysis of DNA unwinding (FADU) from the fluorescence of EtBr bound to alkali-treated DNA. Quiescent SAMHD1- mutated cells exhibit a faster re-synthesis step during the excision-repair of UV damaged DNA compared with WT quiescent fibroblasts.

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**Unit of Bioenergetic Organelles*

A High Throughput Screen reveals chemical modulators of mitochondria-ER contact sites

The sites of contact between mitochondria and Endoplasmic Reticulum (ER) are points in which the surfaces of the two organelles run in parallel for several nm in length. In the last decades, the interest on mitochondria-ER contacts (MERCs) has grown exponentially, due to their role in fundamental physiological processes such as lipid and Ca²⁺ homeostasis, autophagosome formation and apoptosis. Moreover, structural and functional MERCs alterations have been reported in cell models of severe pathological conditions, such as Alzheimer's and Parkinson's Disease.

Pharmacological modulation of these interfaces, so far proposed only for anti-diabetic compounds such as sulforaphane and metformin, appears therefore as a potential target for the development of novel therapeutical strategies or to deepen our knowledge on MERCs biology.

We have designed and set up a high content imaging screen on a library of about 600 proprietary compounds, aiming to find molecules that modulate the proximity between the two organelles. Molecular structure and bioinformatic analyses as well as next-generation sequencing and mass spectrometry experiments are undergoing to characterize the effects on the cell physiology and the subcellular targets of the identified hits.

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**Unit of Bioenergetic Organelles*

A RNAi-genome wide screening for mitochondrial fission factors

Mitochondria are dynamic organelles which undergo fission and fusion in response to cellular environment to control apoptosis, energy production, mitochondria quality control and cell cycle. As of today, we know a handful of core mitochondria shaping proteins involved in fusion like mitofusin 1 (MFN1), mitofusin 2 (MFN2) and Optic Atrophy 1 (OPA-1). Remarkably, fission is orchestrated only by dynamin related protein 1 (DRP1). Drp1 is a crucial factor that oligomerize in a ring structure and induces mitochondrial fission downstream of the pre-constriction mediated by the endoplasmic reticulum. Interestingly, in some cellular models, the genetic ablation of Drp1 does not prevent mitochondrial segregation and inheritance. Moreover, fission of the inner mitochondrial membrane can occur independently from that of the outer membrane. Yet, no factors involved in inner mitochondrial membrane fission have been identified in mammals. Finally, it is not clear if Drp1-independent pathways for mitochondrial fission exist. This is why new regulators of the fission machinery need to be identified. To this end, we are taking advantage of an in house devised, imaging based high-throughput genome-wide siRNA screen in cells deficient for mitochondrial fusion to identify new candidate genes involved in the regulation of mitochondrial fission. Our approach is aimed at identifying genes and pathways previously not linked to the control of mitochondrial morphology.

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**Unit of Human Molecular Genetics and Functional Genomics*

Systematic evaluation of eye color prediction models for forensic application in Italy

Forensic DNA phenotyping (FDP) is a branch of forensic genetics aiming at predicting the physical appearance of an unknown person from a biological sample. Recently, the genetic understanding of complex phenotypic traits has given a great impulse to FDP development, by identifying genes and polymorphisms (SNPs) that provide the bases for predictive models. Nowadays, the majority of operable FDP predictive models have been developed for human pigmentation traits including iris, hair, and skin color.

In this study, we investigated an Italian sample of 337 subjects using a custom-designed NGS panel targeting SNPs associated to iris color. Data were used to predict eye color with the four most popular currently available models. Overall, we observed that all the methods performed significantly worse than expected, with major issues in predicting intermediate eye colors. The adjustment of predictive algorithms with Italian allele frequencies gave only minor improvements for intermediate eyes and no remarkable overall changes in performance. An association study revealed only weak association signals with the intermediate phenotype suggesting a still incomplete genetic knowledge of this trait. Considering that intermediate eyes represent 25% of the total Italian sample, a better genetic and phenotypic characterization of this category is required to set up a valuable DNA intelligence tool applicable to Italian forensic caseworks.

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**Unit of Plant Biology*

Microalgae biomass: green power for carbon sequestration and production of high value molecules

TMCI Padovan Group (Vittorio Veneto, TV) and the PARLAB Group of the University of Padova are joint to pursue the common project of optimizing the entire process of microalgae biomass production showing how the industry and the academic world could be synergistic. The potential of microalgae is very high thanks to their ability to exploit light, CO₂, water and few nutrients to accumulate high value molecules such as natural pigments, antioxidants, omega-3, proteins, lipids which already find application in many fields (cosmetics, nutraceutics, food, energy, natural additives, ecc.). This project starts with the cultivation of microalgae at both lab and industrial scales in different pilot photobioreactors (PBRs) made by TMCI (exploiting its expertise in offering process equipment and plants for different applications in the food and beverage industrial sectors) and located at the University of Padova, passing through the collection of biomass (exploiting the specific filtration unit DYNAMOS, property of TMCI, usually employed in the filtration of fruit juices, liquid foods, chemical and pharmaceutical suspensions) and introducing it into the market. Then, among the objectives of this collaboration, there is the one very ambitious of reducing the carbon dioxide emissions in the atmosphere by industry. Since algae have to be provided with carbon dioxide to grow, why not use PBRs to sequester this gas coming from industry to transform it in reusable compounds in a green, sustainable and eco-friendly way?

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**Unit of Bioenergetic Organelles*

Genetic inactivation of USP8 in the heart, a deubiquitinase hyperactive in Cushing's syndrome adenomas, causes mitochondrial dysfunction and cardiomyopathy

Activating mutations in the USP8 gene, coding for ubiquitin-specific protease 8, a deubiquitinase involved in endocytic trafficking and mitophagy, can cause Cushing's syndrome. Usp8 inhibitors are therefore scrutinized to treat Cushing's pituitary adenomas. However, because heart function requires mitophagy, it is unclear if Usp8 inhibitors could be detrimental for the already failing hearts of Cushing's patients. Here we show that acute Usp8 genetic ablation in the mouse heart impairs mitochondrial function and autophagic clearance. Myocardial Usp8 deletion in adult mice results in cardiomyopathy associated with the accumulation of damaged and dysfunctional mitochondria. Mechanistically, we found that USP8 interacts with, and stabilizes PINK1 that senses dysfunctional mitochondria and activates Parkin dependent mitophagy. Consequently, in cardiomyocytes and cells lacking USP8, PINK1 is not stabilized upon mitochondrial dysfunction, mitophagy is not activated in response to mitochondrial depolarization and chemical mitochondrial uncouplers lead to cell death. Our data not only shed light on the mechanisms of mitophagy regulation, but also recommend caution in investigative anti Usp8 therapy for Cushing's syndrome.

Giorgio Valle*

**Unit of Genomics and Bioinformatics*

Overview of the main topics developed by genomics and bioinformatics research unit

The Research Unit (RU) leadeed by Giorgio Valle has been involved in Genomics and Bioinformatics for many years, both in coordination roles and in participation to international projects. Three main areas are now under active development: software and strategies for data analysis, plant genomics and microbial genomics/metagenomics. In the area of bioinformatics applied to personal genomics, software for variants and gene prioritization such as QueryOR and Scuba have been recently released. At present, research is focused on analysis of variants related to the identification of misassembled regions in the human reference

genome. Regarding plant genomics, the Breeding API (BrAPI) Project is a bioinformatics effort to create a RESTful specification to enable interoperability among plant breeding databases. Microbial genomics and metagenomics research is mainly focused on marine microbiology (e.g. Venice lagoon, Antarctica) and on applied microbiology related to the anaerobic digestion system (e.g. biogas production and upgrading). The research unit was recently enlarged and is now in the process of applying next-generation sequencing approaches to the study of *Mycobacterium tuberculosis* gene expression.

