

# 1<sup>st</sup> PhD Day

February 5th, 2026 | Aula F, Campus Aquae - Pavia

## Book of Abstracts



### Affiliated Departments and Institutes

**Info**  
[phd-day-dbb@unipv.it](mailto:phd-day-dbb@unipv.it)



### **PhD Program Director**

Davide Sassera

### **Organizing Committee**

University of Pavia

Rossella Tricarico

Paola Rebuzzini

Viola Camilla Scoffone

Alberto Azzalin

Chiara Albertini

Nicola Bosco

Carlo Croci

CNR-IGM

Francesca Scolari

Elena Botta

### **Abstract Selection Committee**

University of Pavia

Giulia Barbieri

Claudia Binda

Cinzia Calvio

Claudio Casali

CNR-IGM

Paolo Colombi

Laura Zannini

### **Selection Committee for Talk Prizes**

University of Pavia

Alessandro Achilli

Anca Macovei

Lino Ometto

Fondazione IRCCS Policlinico San Matteo

Aurora Piazza

CNR-IGM

Giorgia Zadra

### **Selection Committee for Poster Prizes**

University of Pavia

Silvia Buroni

Valeria Merico

Natalia Simona Pellegata

Paola Perucca

CNR-IGM

Claudia Ghigna

### **Logo Contest Winners**

PhD Day Logo: Carlo Croci

Book of Abstracts Cover Illustration: Chiara Albertini

# Program

08:30 - 09:00	<b>Registration</b>
09:00 - 09:15	<b>Welcome and Opening Remarks</b> <b>Antonio Torroni</b> (DBB Director) · <b>Davide Sassera</b> (PhD Program Director)
09:15 - 10:00	<b>Keynote Lecture</b> <b>Massimo Delledonne</b> (University of Verona)   talk title TBD Chair: <b>Nicola Bosco</b> (XXXIX Cycle)
10:00 - 11:30	<b>Talks   XXXIX Cycle</b> Chairs: <b>Emma Lugli</b> (XL Cycle) · <b>Nicolò Gennari</b> (XL Cycle)
10:00 - 10:15	<b>T1</b> <b>Francesco Ascione</b>   ITF3912-5: a novel SSTR2-directed cytotoxic somatostatin analogue with potent anti-tumor activity in NETs
10:15 - 10:30	<b>T2</b> <b>Virginia Batignani</b>   Engineering <i>Klebsiella pneumoniae</i> 's outer membrane proteins as novel antigen for therapeutic purposes
10:30 - 10:45	<b>T3</b> <b>Francesca Giammello</b>   Contribution of Na <sup>+</sup> /Ca <sup>2+</sup> exchanger (NCX1) to migration and vasculogenic mimicry in a primary human glioblastoma cell line
10:45 - 11:00	<b>Coffee Break</b>
11:00 - 11:15	<b>T4</b> <b>Nadia Tagliaferri</b>   From repeat expansion to DNA repair defect: unraveling the molecular basis of CANVAS
11:15 - 11:30	<b>T5</b> <b>Gaia Veniali</b>   Tumor-stroma crosstalk: insights from NER deficient disorders
11:30 - 11:45	<b>Talk Nuova Genetica Italiana</b> <b>Giorgio Chiodaroli</b>   A decade of genomics: experiences and outlooks of Nuova Genetica Italiana
11:45 - 13:00	<b>P1-P7</b> <b>Poster Session   XXXIX Cycle</b>
13:00 - 14:00	<b>Lunch Break</b>
14:00 - 14:30	<b>A1-A13</b> <b>Pitch   XLI Cycle</b> Chair: <b>Anna Tommasi</b> (XL Cycle)
14:30 - 15:45	<b>Talks   XL Cycle</b> Chairs: <b>Francesca Brevi</b> (XXXIX Cycle) · <b>Carlo Croci</b> (XXXIX Cycle)
14:30 - 14:45	<b>T6</b> <b>Lorenzo Atzeni</b>   Search of mechanisms of RNA:DNA hybrids driven DNA damage
14:45 - 15:00	<b>T7</b> <b>Elisa Cataudella</b>   A transiently amplifying and self-limiting Nova2-ADAR2 circuit in endothelial cells
15:00 - 15:15	<b>T8</b> <b>Giada Maria Giunta</b>   The role of extracellular DNA traps in autoimmune inflammation of the central nervous system
15:15 - 15:30	<b>T9</b> <b>Aromita Mallik</b>   Novel compounds targeting mitochondrial DNA offer strategies for glioblastoma therapy
15:30 - 15:45	<b>T10</b> <b>Michela Vumbaca</b>   Sweep-metagenomic analysis of <i>Candida spp.</i> illuminates species and strain diversity within and between patients in a large hospital cohort
15:45 - 16:00	<b>Coffee Break</b>
16:00 - 17:15	<b>P9-P15</b> <b>Poster Session   XL Cycle</b>
17:15 - 17:45	<b>Awards ceremony</b> Best Talks and Best Posters Awards <b>Rossella Tricarico</b> (Head of Organizing Committee) · <b>Davide Sassera</b> (PhD Program Director)
17:45 - 19:30	<b>Closing Party</b> Main Hall, Golgi Spallanzani (Botta 2) Building

# Keynote Speaker

## **Massimo Delledonne**



Massimo Delledonne is a Full Professor of Genetics at the University of Verona. Between 1990 and 2000, he carried out several research stays abroad, focusing on molecular genetics, and in particular on the genetic basis of disease resistance. Since 2007, with the advent of new DNA sequencing technologies, he has been working on genome structure and function, establishing DNA analysis platforms at the University of Verona to advance the understanding of plant and human biology.

Since 2011, his work has focused primarily on human genome sequencing and interpretation for clinical applications, and he has been actively engaged in science communication, aiming to explain how human genome interpretation is profoundly transforming medicine, which increasingly integrates genetic data with clinical data to improve diagnosis, care, and therapeutic strategies.

Since 2017, he has been a member of Taxon Expedition, an organization dedicated to fostering scientific discovery through expeditions to remote areas of the planet in search of new species.

In 2020, together with his colleagues Marzia Rossato and Alessandro Salviati, he founded Genartis, a spin-off of the University of Verona that operates in the field of personalized genomics and DNA sequencing.

# Abstracts



## **Oral Presentations**

### PhD Students of the XXXIX cycle

Ascione Francesco	<b>T1</b>
Batignani Virginia	<b>T2</b>
Giammello Francesca	<b>T3</b>
Tagliaferri Nadia	<b>T4</b>
Veniali Gaia	<b>T5</b>

### PhD Students of the XL cycle

Atzeni Lorenzo	<b>T6</b>
Cataudella Elisa	<b>T7</b>
Giunta Giada Maria	<b>T8</b>
Mallik Aromita	<b>T9</b>
Vumbaca Michela	<b>T10</b>

## **Poster Presentations**

### PhD Students of the XXXIX cycle

Bahrami Romina	<b>P1</b>
Biundo Marialaura	<b>P2</b>
Banella Venere	<b>P3</b>
Brevi Francesca	<b>P4</b>
Bosco Nicola	<b>P5</b>
Croci Carlo	<b>P6</b>
Mottola Maria Chiara	<b>P7</b>

### PhD Students of the XL cycle

Albertini Chiara	<b>P8</b>
Gennari Nicolò	<b>P9</b>
Ghosh Priyam	<b>P10</b>
Lugli Emma	<b>P11</b>
Petrizzi Ilaria	<b>P12</b>
Riccardi Adelaide	<b>P13</b>
Soldano Sara	<b>P14</b>
Tommasi Sara	<b>P15</b>

## **Pitch**

### PhD Students of the XLI cycle

Arshard Arooba	<b>A1</b>
Bedotto Nicolò	<b>A2</b>
Diaf Adel	<b>A3</b>
Diaguna Ridwan	<b>A4</b>
Erriquez Luca	<b>A5</b>
Flacchi Carolina	<b>A6</b>
Guzzi Fabio Luca	<b>A7</b>
Mayorga Churion Daniela	<b>A8</b>
Nicolini Valeria	<b>A9</b>
Pavesi Simone	<b>A10</b>
Pezzini Davide	<b>A11</b>
Rapisarda Edoardo	<b>A12</b>
Zaffaroni Ottavia	<b>A13</b>

## **Abstract-only (students abroad)**

### **Not presented on site**

Bistika Margarita	<b>B1</b>
Brazzale Giulia	<b>B2</b>
Cavallo Margherita	<b>B3</b>
Villani Giacomo	<b>B4</b>

## **ITF3912-5: a novel SSTR2-directed cytotoxic somatostatin analogue with potent anti-tumor activity in NETs**

Francesco Ascione<sup>1</sup>, Fabio Luca Guzzi<sup>1</sup>, Maria Vlada Reabco<sup>1</sup>, Natalia Simona Pellegata<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology, University of Pavia, Pavia, Italy

### Background

Somatostatin receptors (SSTRs), particularly subtype 2 (SSTR2), are frequently overexpressed in neuroendocrine tumors (NETs) and represent valuable targets for selective drug delivery. Conventional somatostatin analogues (SSAs) such as octreotide exert mainly cytostatic effects, limiting their therapeutic efficacy. ITF3912-5, a novel octreotide-derived analogue conjugated to a cytotoxic moiety, was developed to achieve receptor-targeted cytotoxic activity.

### Methods

SSTR2 expression was analyzed by immunofluorescence and western blot in AtT-20, HEK293 hSSTR2, HEK293, HFF, and NT18-P (human pancreatic NET) cells. Antiproliferative effects were evaluated by WST-1 assay, and IC<sub>50</sub> values were derived from dose-response curves. Apoptosis and DNA damage were assessed through Caspase-Glo® 3/7 and γH2AX assays. NT18-P spheroids were used to examine 3D structural changes after treatment.

### Results

SSTR2 expression was confirmed in AtT-20, HEK293 hSSTR2, and NT18-P cells. ITF3912-5 inhibited proliferation in a receptor-dependent manner, with low-nanomolar IC<sub>50</sub> values in SSTR2-positive lines and up to eightfold higher values in negative controls. In NT18-P cells, ITF3912-5 IC<sub>50</sub> significantly increased caspase-3/7 activity and γH2AX levels, indicating apoptosis and DNA damage. In 3D NT18-P spheroids, treatment induced progressive structural disintegration and necrotic core formation.

### Conclusions

ITF3912-5 exerts strong, SSTR2-dependent antiproliferative and pro-apoptotic effects in 2D and 3D NET models. Its mechanism involves DNA damage leading to apoptotic cell death. These findings highlight ITF3912-5 as a promising next-generation cytotoxic somatostatin analogue for targeted therapy of SSTR2-expressing neuroendocrine tumors.



# T2

## Engineering *Klebsiella pneumoniae*'s outer membrane proteins as novel antigen for therapeutic purposes

Virginia Batignani<sup>1</sup>, Andrea Spitaleri<sup>2</sup>, Rita Sorrentino<sup>1</sup>, Mirco Toccafondi<sup>3</sup>, Elisa Pesce<sup>4</sup>, Andrea Lombardi<sup>5</sup>, Angelo Maccaro<sup>5</sup>, Alessandra Bandera<sup>5</sup>, Renata Grifantini<sup>3</sup>, Daniela Maria Cirillo<sup>1</sup>

<sup>1</sup>Emerging bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy

<sup>2</sup>Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy

<sup>3</sup>Istituto Nazionale Genetica Molecolare "Romeo ed Enrica Invernizzi", Milan, Italy

<sup>4</sup>Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

<sup>5</sup>Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy

### Background

The rise of multidrug-resistant *Klebsiella pneumoniae* (KP) presents a serious health threat, with limited treatment options and high mortality. Alternative approaches like immunotherapy are urgently needed. This study focuses on the engineering of KP's Outer Membrane Proteins (OMPs) to elicit functional antibodies in KP-colonized and infected patients, aiming to identify human therapeutic monoclonal antibodies.

### Methods

Using RFdiffusion, we targeted external epitopes of OmpK35, OmpK36, and OmpK37, generating three antigen candidates: An35p3b, An36p3, and An37p3. Epitope prediction (BepiPred-3.0) and 100 ns molecular dynamics simulations confirmed stability. The designed proteins, tagged with 6XHistag and AviTag, were cloned into an IPTG-inducible pET-28a vector and subcloned by ThermoFisher. Protein expression was carried out in the BL21(DE3) *E. coli* strain using Express™ Autoinduction System 1 (Novagen), followed by Ni-NTA affinity chromatography purification. The purified antigens (2.5 µg/ml) were coated onto 96-well plates (overnight at 4°C) for ELISA assays using sera from 136 patients (49 KP-colonized, 87 KP-infected) collected at one (T1), three (T2), and six months (T3) post-diagnosis.

### Results

All antigens were successfully expressed and purified, as confirmed by Coomassie Blue-stained SDS-PAGE gel. ELISA revealed specific antibody responses, with >50% recognition by KP patient sera. An35p3b and An37p3 reactivity decreased over time, while An36p3 remained stable. Infected patient sera showed higher signal intensity than colonized ones.

### Conclusions

In conclusion, we engineered novel antigens derived from the most immunogenic regions of KP OMPs. These proteins triggered significant antibody responses, especially in infected individuals. The findings support their potential for developing therapeutic monoclonal antibodies against KP.

## **Contribution of Na<sup>+</sup>/Ca<sup>2+</sup> Exchanger (NCX1) to migration and vasculogenic mimicry in a primary human glioblastoma cell line**

Francesca Giammello<sup>1</sup>, Erica Cecilia Priori<sup>1</sup>, Francesca Dalle Sasse<sup>1</sup>, Daniela Ratto<sup>1</sup>,  
Elisa Roda<sup>2</sup>, Federico Brandalise<sup>3</sup>, Paola Rossi<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy

<sup>2</sup>Laboratory of Clinical & Experimental Toxicology, Pavia Poison Centre, National Toxicology Information Centre, Toxicology Unit, Istituti Clinici Scientifici Maugeri IRCCS Pavia, Pavia, Italy

<sup>3</sup>Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Cittadella Universitaria di Monserrato, Monserrato, Italy

### **Background**

Glioblastoma (GBM) is the most aggressive brain tumor, characterized by high invasiveness and by vasculogenic mimicry (VM), a process through which tumor cells form vessel-like structures. The Na<sup>+</sup>/Ca<sup>2+</sup> exchanger 1 (NCX1) has been implicated in tumor cell migration; however, its role in primary GBM cells remains unclear. This study aimed to characterize NCX1 involvement in migration and VM of a primary human GBM cell line (GBM19).

### **Methods**

GBM19 cells were analyzed during migration using wound healing and time-lapse assays. Electrophysiological recordings assessed NCX-associated ionic currents, while immunocytochemistry evaluated VE-cadherin and NCX1 expression. Low bepridil concentration (6.25 μM), a selective NCX1 inhibitor, was applied to examine its effects on migration and loops (VM-like) formation.

### **Results**

GBM19 cells formed circular loops expressing high levels of VE-cadherin on the migratory front, confirming their involvement in VM. Electrophysiology revealed an inward NCX-mediated current in leading edge and loop cells on the migratory front, abolished by bepridil.

Furthermore, NCX1 protein and functional expression was higher in migrating cells than in extra-migration regions. Bepridil abolished scratch closure and impaired loop formation and closure, indicating NCX1's dual role in migration and VM.

### **Conclusions**

NCX1 critically regulates both migration and vasculogenic mimicry in GBM19 cells by modulating calcium-dependent signalling and cytoskeletal dynamics. Its inhibition impairs wound closure and loop formation, suggesting NCX1 as a potential therapeutic target in aggressive GBM subtypes.

# T4

## **From repeat expansion to DNA repair defect: unraveling the molecular basis of CANVAS**

Nadia Tagliaferri<sup>1</sup>, Riccardo Currò<sup>2</sup>, Glenda Grupelli<sup>1</sup>, Cecilia Perini<sup>1</sup>, Andrea Cortese<sup>2,3</sup>, Emmanuele Crespan<sup>1</sup>

<sup>1</sup>Institute of Molecular Genetics, Pavia, Italy

<sup>2</sup>Department of Neuromuscular Diseases, UCL, London, United Kingdom

<sup>3</sup>Department of Brain Sciences, University of Pavia, Italy

Cerebellar Ataxia, Neuropathy, and Vestibular Areflexia Syndrome (CANVAS) is a rare neurodegenerative disorder caused by a pentanucleotide repeat expansion in intron 2 of the RFC1 gene. RFC1 encodes the large subunit of Replication Factor C, essential for PCNA loading and, consequently, for DNA replication and repair. Although CANVAS follows a strict recessive inheritance, patients do not exhibit clear alterations in RFC1 mRNA or protein levels.

To investigate the molecular mechanisms underlying disease onset and progression, DNA damage repair was evaluated in patient-derived cells, including isogenic patient-derived iPSCs in which the pathological expansion was removed by CRISPR-Cas9 editing. CANVAS cells are more sensitive to platinum-based drugs, which induce DNA adducts that are repaired by the PCNA-dependent Nucleotide Excision Repair. Both cisplatin and oxaliplatin impaired cell viability, eliciting prolonged  $\gamma$ H2A.X and p53 activation and enhanced apoptosis compared to controls. The kinetics of DNA damage repair were evaluated using antibodies specifically recognizing cisplatin adducts, with CANVAS cells clearly showing delayed lesion clearance compared to control cells. Lastly, the presence of the pathological expansion in homozygosity in iPSC-derived neurons correlated with a slight decrease in RFC1 mRNA levels.

These findings support the hypothesis that PCNA-dependent DNA repair pathways are impaired in specific neuronal populations of CANVAS patients, probably due to defects in the modulation of RFC1 expression upon DNA damage caused by the expanded sequence.

## **Tumor-stroma crosstalk: insights from NER deficient disorders**

Gaia Veniali<sup>1</sup>, Anita Lombardi<sup>1</sup>, Massimo Teson<sup>2</sup>, Tiziana Nardo<sup>1</sup>, Elena Botta<sup>1</sup>, Elena Dellambra<sup>2</sup>, Donata Orioli<sup>1</sup>, Manuela Lanzafame<sup>1</sup>

<sup>1</sup>CNR – Istituto di Genetica Molecolare, Pavia, Italy

<sup>2</sup>Istituto Dermatopatico dell'Immacolata (IDI), Rome, Italy

### Background

Skin cancers are among the most common malignancies, primarily driven by ultraviolet (UV)-induced DNA damage. Development and progression depend on bidirectional crosstalk between tumor cells and stroma: fibroblasts and keratinocytes regulate extracellular matrix remodeling and soluble signals that govern growth and invasion. The nucleotide excision repair (NER) pathway safeguards genomes from UV radiation; mutations in the *XPD* subunits of the transcription/repair factor TFIIH cause the NER-defective disorders xeroderma pigmentosum (XP) and trichothiodystrophy (TTD). Despite sharing the same repair defect, XP is cancer-prone whereas TTD is not, offering a natural model to dissect how reciprocal tumor-stroma signaling modulates cutaneous carcinogenesis.

### Methods

We profiled whole-transcriptome of primary *XPD*-mutant fibroblasts and keratinocytes from XP and TTD patients. Functional validations were performed by using 3D skin cancer models, including spheroids and tumor-stroma co-cultures, combining patient-derived cells with skin cancer cell lines, plus loss-of-function perturbations of deregulated candidates by gene silencing.

### Results

Transcriptomes indicated a pro-tumorigenic profile in XP and an anti-oncogenic signature in TTD cells. In 3D cultures TTD fibroblasts were refractory to tumor-induced activation and failed to generate a compact core in multicellular tumor spheroids. This is consistent with a reduced structural/paracrine support to tumor growth. Importantly, targeted silencing of selected deregulated factors reduced skin-cancer cell invasion in 3D assays.

### Conclusions

Divergent cancer predisposition in XP versus TTD reflects different tumor-stroma crosstalk. These NER-defective syndromes provide a powerful platform to uncover mechanisms promoting or counteracting skin carcinogenesis, and to prioritize stromal/paracrine targets for therapy.

## Search of mechanisms of RNA:DNA hybrids driven DNA damage

Lorenzo Atzeni<sup>1,2</sup>, Luca Zardoni<sup>2</sup>, Giordano Liberi<sup>2</sup>

<sup>1</sup>Università degli Studi di Pavia, Pavia, Italy

<sup>2</sup>Istituto di Genetica Molecolare “Luigi Luca Cavalli-Sforza”, CNR, Pavia, Italy

### Background

Unscheduled accumulation of R-loops, which are structures containing stable RNA:DNA hybrids, can promote DNA damage and gene silencing. The evolutionarily conserved DNA/RNA helicase Senataxin, whose budding yeast orthologue is called Sen1, is among factors that removes such structures.

### Methods

We aim at using yeast *sen1* mutant cells as a tool to identify novel mechanisms of RNA:DNA hybrid-driven DNA damage and gene silencing. To find and characterize factors and pathways implicated in the management of such mechanisms, we are searching for genetic suppressors of *sen1* mutant defects by either high-throughput genetic screenings or gene candidate approach.

### Results

Using gene targeting and crossing procedures, we have obtained a query *sen1* mutant strain that will be employed in a Synthetic Genetic Array (SGA) robotic-automated genetic screening. This genetic screening aims at identifying factors preventing RNA:DNA hybrid-mediated gene silencing associated with a specific drug sensitivity in *sen1* mutant. Through drug sensitivity tests and RNA:DNA hybrid accumulation analysis (DRIP), we found that the loss of the m<sup>6</sup>A RNA methylase Ime4, is able to rescue the DNA damage and gene silencing in *sen1* mutants possibly by modulating RNA:DNA hybrid toxicity.

### Conclusions

Our preliminary data suggest that Ime4-dependent RNA chemical modification of RNA:DNA hybrid could drive DNA damage/gene silencing and that synthetic rescue screens of certain *sen1* mutant defects can be exploited as a strategy to identify novel mechanisms underlying R-loop toxicity.

## **A transiently amplifying and self-limiting Nova2-ADAR2 circuit in endothelial cells**

Cataudella Elisa<sup>1,2</sup>, Anna Di Matteo<sup>2</sup>, Elisa Belloni<sup>2</sup>, Mariagiulia Spazzapan<sup>2,3</sup>, Marina Maurizio<sup>3</sup>, Serena Zacchigna<sup>3</sup>, Alessandro Barbon<sup>4</sup>, Claudia Ghigna<sup>2</sup>

<sup>1</sup>Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy

<sup>2</sup>Institute of Molecular Genetics IGM-CNR "Luigi Luca Cavalli-Sforza", Pavia, Italy

<sup>3</sup>International Centre for Genetic Engineering and Biotechnology, ICGEB, Trieste, Italy

<sup>4</sup>Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

### **Background**

Adenosine-to-inosine (A-to-I) RNA editing, catalyzed by ADAR1 and ADAR2, is an epitranscriptional modification essential for life. RNA editing can affect RNA secondary structure, localization, processing and interaction with proteins, leading to a plethora of biological functions. Despite brain and vasculature are among the most highly edited tissues, so far, research has mostly focused on the consequence of A-to-I RNA editing in the brain and neurological diseases, while its role in the vasculature is poorly understood.

Our previous studies demonstrated that the alternative splicing (AS) factor Nova2, in addition to neurons, is also expressed in endothelial cells (ECs), where it controls angiogenesis at transcriptional level.

### **Methods**

By integrating bioinformatic analyses, molecular/cellular biology techniques and *in vivo* studies, we demonstrated that Nova2 regulates ADAR2 (but not ADAR1) in ECs at multiple levels.

### **Results**

We found that Nova2 promotes the expression an AS isoform of the E2F co-activator Tfdp2, which moves to the nucleus and stimulates E2F-dependent *ADAR2* transcription. Nova2 is also part of a negative autoregulatory circuit through which ADAR2 regulates its own levels. Indeed, ADAR2 can edit its own pre-mRNA creating a *de novo* Nova2 binding site leading to the generation of a Nova2-dependent AS isoform of ADAR2 containing a premature translation termination codon. Consequently, we found that ADAR2 and Nova2 protein levels are inversely correlated in ECs.

### **Conclusions**

Collectively, our data suggest that the interplay between different aspects of the RNA metabolism orchestrates a transiently amplifying and self-limiting Nova2-ADAR2 circuit, which could be relevant for physiological and pathological angiogenesis.

# T8

## **The role of extracellular DNA traps in autoimmune inflammation of the central nervous system**

Giada Maria Giunta<sup>1,2</sup>, Francesca Colciaghi<sup>3</sup>, Arianna Ciotti<sup>3</sup>, Elisa Maffioli<sup>4</sup>, Italia Bongarzone<sup>5</sup>, Gabriella Tedeschi<sup>4</sup>, Emilio Ciusani<sup>6</sup>, Maria Grazia Bottone<sup>1</sup>, Massimo Costanza<sup>2</sup>

<sup>1</sup>Department of Biology and Biotechnology "L.Spallanzani", University of Pavia, Pavia, Italy

<sup>2</sup>Neuroimmunology and Neuromuscular Diseases Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

<sup>3</sup>Epilepsy Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

<sup>4</sup>MALDI-imaging laboratory, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

<sup>5</sup>Department of Veterinary Medicine and Animal Sciences, University of Milan, Milan, Italy

<sup>6</sup>Laboratory of Neurological Biochemistry and Neuropharmacology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

### Background

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS), whose pathogenic mechanisms are still incompletely understood. Recent studies have shown that innate immune cells can eject chromatin fibers associated with proteases named extracellular traps (ETs), to entrap invading pathogens or to boost sterile inflammation. The release of ETs by adaptive immune cells is poorly characterized. In this project, we aim to verify whether CD4<sup>+</sup> T cells, key orchestrators of CNS autoimmunity, release ETs and their potential involvement in the pathogenesis of experimental MS.

### Methods

Primary human memory CD4<sup>+</sup> T lymphocytes were obtained by immunomagnetic separation from healthy donors and stimulated *in vitro* with anti-CD3/anti-CD28 antibodies. Gene profile was measured by RT-qPCR. Protein composition of ETs has been analyzed by confocal microscopy and nano LC-MS/MS. T cell functional profile was evaluated by flow cytometry.

### Results

CD4<sup>+</sup> T cells express at both transcript and protein levels protein-arginine deiminase (PAD)4, a crucial enzyme regulating the release of ETs. Extracellular DNA fibers can be detected in cultures of human memory CD4<sup>+</sup> T cells even in the absence of any stimulus. Upon activation, CD4<sup>+</sup> T cells release numerous DNA fibers associated with citrullinated histone H3 (CitH3), a canonical marker of ETs. Treatment of CD4<sup>+</sup> T cells with DNase or a PAD4 inhibitor impairs ETs formation, significantly reduces T cell aggregation, and hampers the production of pro-inflammatory cytokines.

### Conclusions

CD4<sup>+</sup> T cells express PAD4 and release ETs. These fibers promote cell aggregation and support T cell inflammatory activity.

# T9

## **Novel Compounds Targeting Mitochondrial DNA Offer Strategies for Glioblastoma Therapy**

Aromita Mallik<sup>1</sup>, Paola Corna<sup>2</sup>, Elisa Maniscalco<sup>1</sup>, Carlotta Bernardi<sup>1</sup>, Emma Galeotti<sup>1</sup>, Vasco De Maio<sup>1</sup>, Giorgia Scibilia<sup>1</sup>, Eleonora Formigoni<sup>1</sup>, Sergio Comincini<sup>1</sup>, Lorenzo Magrassi<sup>2</sup>, Concettina La Motta<sup>3</sup>

<sup>1</sup>Department of Biology and Biotechnology, University of Pavia, Pavia, Italy

<sup>2</sup>Department of Clinical Surgical Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

<sup>3</sup>Department of Pharmacy, University of Pisa, Pisa, Italy

### Background

Glioblastomas are Grade IV astrocytomas that have high biological complexity, leading to their aggressive nature and chemoresistance. Mitochondria can reprogram the metabolism of glioma cells, leading to rapid proliferation and an enhanced oxidative stress response. This work aims to explore the properties of multiple newly designed molecules to target mitochondrial DNA, namely PV1-PV7.

### Methods

Confocal Microscopy was used to analyze the in vivo and ex vivo localization of the molecules, following which qPCR and 4-NBP assays demonstrated their ability to target and mutate mitochondrial DNA. MTT assay measured the effects on cell viability, while the Amnis Flow Cytometer measured internalization. qPCR analysis of exosomal DNA was used to showcase mitochondrial release. Mice were injected with GL261 intracranially and PV3 intraperitoneally, and subsequent MRIs were done.

### Results

Quantitative PCR implied mutations in mitochondrial DNA. All the compounds, especially PV3, showed high alkylation potential and affected the viability of cancer cells but not that of normal cells, with similar trend for internalization. Exosomal DNA following the treatment, especially with PV3, shows the presence of mutated mitochondrial DNA. PV3 readily localized the meningioma patient-derived organoids and primary cells. Finally, PV3 reduced the tumor in GL261-treated mice in preliminary in vivo.

### Conclusions

The compounds selectively internalize and show cytotoxicity in cancer cells. They are capable of mutating mitochondrial DNA and also enter other tumor cells, such as meningioma. PV3 is capable of crossing the BBB and internalizing selectively in tumor cells, as well as reducing the tumor following intraperitoneal injections.



## **Sweep-metagenomic analysis of *Candida* spp. illuminates species and strain diversity within and between patients in a large hospital cohort**

Michela Vumbaca<sup>1</sup>, Gherard Batisti Biffignandi<sup>2</sup>, Caterina Cavanna<sup>2</sup>, Marta Corbella<sup>2</sup>, Cristina Merla<sup>2</sup>, Stefano Gaiarsa<sup>2</sup>, Harry Thorpe<sup>3</sup>, Johanna Rhodes<sup>4</sup>, Jukka Corander<sup>3,5,6</sup>, Davide Sassera<sup>1,7</sup>

<sup>1</sup>University of Pavia, Italy, Department of Biology and Biotechnology

<sup>2</sup>Microbiology and Virology Unit, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy

<sup>3</sup>Department of Biostatistics, University of Oslo, Norway

<sup>4</sup>MRC GIDA, Imperial College London, London, UK

<sup>5</sup>Helsinki Institute for Information Technology HIIT, Department of Mathematics and Statistics, University of Helsinki, Finland

<sup>6</sup>Parasites and Microbes, Wellcome Sanger Institute, Hinxton, UK

<sup>7</sup>Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

### Background

*Candida* is a leading cause of hospital-acquired fungal infections, with multiple species causing localized and systemic infection and increasing prevalences of resistant strains. Our study investigates *Candida* transmission and genomic diversity within the study hospital through an innovative sweep-metagenomic approach. This method enables to capture the diversity present in clinical samples, characterizing it at strain level, moving beyond the classical approach focused on single colony samples.

### Methods

We collected respiratory, nasal and rectal samples from inpatients at San Matteo Hospital. Samples were cultured on CHROMID *Candida* agar and plates were swept to recover all species grown. DNA was extracted and Illumina sequenced. Bioinformatic analyses allowed fine strain assignment, establishment of hospital transmission and reconstruction of within patient genomic evolution.

### Results

We analyzed 601 samples, detecting 17 fungal species, with *Candida parapsilosis*, *C. albicans*, and *C. glabrata* being the most common. *C. parapsilosis* was unique, being represented by a single dominant azole-resistant persistent strain circulating in the hospital since 2018. We are applying a phylodynamic approach to estimate the date of emergence of this clone, to infer transmission rates across patients and assess carriage duration. Furthermore, leveraging longitudinal sampling from long-term patients we are exploring intra-patient genomic variation to assess microevolution of clusters and patterns of translocation across body sites.

### Conclusions

We characterized fungal species diversity across samples with high-resolution methods that identify different clusters for each species, improving pathogen surveillance, understanding of colonisation patterns and detection of intra- and inter-patient transmission in a high-risk hospital environment.

**Friend or Foe? *Asaia* as a Para transgenic tool in *Aedes koreicus***

Romina Barhami<sup>1</sup>, Sofia Di Castri<sup>2</sup>, Sara Repetto<sup>1</sup>, Daniel Gingel<sup>1</sup>, Alessia Cappelli<sup>2</sup>,  
Mariangela Bonizzoni<sup>1</sup>, Claudia Damiani<sup>2</sup>, Maria Vittoria Mancini<sup>1</sup>

<sup>1</sup>Dept. Biology and Biotechnology “L. Spallanzani”, University of Pavia, Pavia, Italy

<sup>2</sup>Scuola di Bioscienze e Medicina Veterinaria, University of Camerino, Camerino, Italy

Symbiotic bacteria are increasingly explored as Para transgenic tools for mosquito control. *Asaia*, a Gram-negative bacterium, is a promising candidate due to its ability to colonize multiple mosquito species. In the invasive mosquito *Aedes koreicus*, *Asaia* is the predominant bacterial associate, yet its colonization dynamics, interactions with the native microbiota, and effects on pathogen transmission remain poorly understood.

We investigated *Asaia* colonization in laboratory-reared *Ae. koreicus* at two temperatures (24°C and 28°C), assessing tissue distribution and temporal dynamics. We tested whether *Asaia* isolated from *Ae. koreicus* could establish infection via adult feeding and examined its competition with resident microbiota using axenic mosquitoes. Additionally, we evaluated how *Asaia* colonization affects vector competence.

Our results show age- and tissue-dependent colonization, with stable persistence even under thermal stress. Infection via adult feeding occurred at low prevalence, suggesting interference from native microbiota, whereas axenic mosquitoes exhibited higher colonization, confirming microbial competition. Notably, *Asaia* altered vector competence, indicating a potential modulatory role on pathogen transmission.

These findings suggest that while *Asaia* can persist under diverse conditions, its establishment is influenced by microbial interactions. Such dynamics should be considered when evaluating *Asaia* as a Para transgenic tool for mosquito control.

## **Unconventional centromere architectures in *Tapirus indicus* reveal hotspots for satellite-free centromere formation in Perissodactyla**

Marialaura Biundo<sup>1</sup>, Francesca Maria Piras<sup>2</sup>, Edoardo Rapisarda<sup>1</sup>, Solomon Nergadze<sup>1</sup>, Oliver Ryder<sup>3</sup>, Elena Giulotto<sup>1</sup>, Eleonora Cappelletti<sup>2</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

<sup>2</sup>Unit of Anatomic Pathology, IRCCS San Matteo Hospital Foundation, Pavia, Italy

<sup>3</sup>Conservation Science Wildlife Health, San Diego Zoo Wildlife Alliance, Escondido, CA, USA

### Background

Centromeres, the nucleoprotein structures required for the faithful sister chromatids segregation, are epigenetically specified by the histone H3 variant CENP-A. In mammals, centromeres are typically embedded within extended arrays of satellite DNA, that include binding sites for the centromeric protein CENP-B. In equids, our group has identified several satellite-free centromeres that originated through centromere repositioning or chromosome fusion. Given that *Tapirus indicus* - a member of the order Perissodactyla, like equids- exhibits a large number of chromosome rearrangements relative to the inferred ancestral karyotype, we sought to investigate the organization of its centromeres.

### Methods

Leveraging a T2T genome assembly and a cell line from the same individual, we characterized centromeres by ChIP-seq using an anti-CENP-A antibody. Satellite DNA organization and comparative sequence analyses were performed through bioinformatic approaches (TAREAN, ModDotPlot and Chromeister). CENP-B binding was assessed by immunofluorescence.

### Results

The 23 satellite-based centromeres are embedded within extended arrays of the major satellite TINSat1. CENP-A binds the most homogeneous satellite regions, suggesting that sequence uniformity promotes its incorporation. Chromosomes 4 and 15 carry completely satellite-free centromeres, arisen after centromere repositioning in genomic hotspots for neocentromere formation among Perissodactyla. The chromosome 18 centromere contains sparse satellite repeats, likely representing an intermediate stage in satellite DNA acquisition. CENP-B binds only the centromere of chromosome 9, indicating an uncoupling between CENP-A and CENP-B.

### Conclusions

The presence of satellite-free centromeres is not restricted to equids. The presence of hotspots for centromere formation in Perissodactyla suggests that peculiar chromatin contexts may predispose certain genomic regions to neocentromere emergence.

## **Phycoremediation potential of *Chlorella vulgaris* and *Tetradesmus obliquus* under heavy metals and acid mine drainage stress**

Venere Banella<sup>1</sup>, Alberta Pinnola<sup>1</sup>, Elisabetta Franchi<sup>2</sup>, Giovanna Carpani<sup>2</sup>

<sup>1</sup>Department of Biology and Biotechnology, AlgaePlant Lab, University of Pavia, Pavia, Italy

<sup>2</sup>Eni S.p.A.

### Background

Heavy metals (HMs) and acid mine drainage (AMD) are one of the main environmental pollutants according to their toxic and persistent nature. Phycoremediation provides a sustainable method for HMs removal. This study focused on the identification of tolerant microalgal strains that could be applied on an industrial scale.

### Methods

*Chlorella vulgaris* and *Tetradesmus obliquus* were screened for tolerance to mixtures of HMs and AMD. The EC<sub>50</sub> values of individual metals and AMD were determined. Once the strains were tested in the Multi-Cultivator system with HMs mixtures.

### Results

EC<sub>50</sub> values were determined for individual metals, mercury as the most toxic for *C. vulgaris* (12.8 mg L<sup>-1</sup>) and cadmium for *T. obliquus* (3.59 mg L<sup>-1</sup>). Growth of *C. vulgaris* and *T. obliquus* was completely inhibited by AMD at a concentration greater than 3.5% with EC<sub>50</sub> of 2.10% and 2.35%, respectively. In the Multi-Cultivator system *C. vulgaris* showed higher resistance to the combined heavy metal exposure, maintaining biomass levels comparable to the control. In contrast, *T. obliquus* experienced a 39% biomass reduction due to the combined heavy metal toxicity.

### Conclusions

Tolerance to synthetic HMs is impressive, but AMD was toxic even at lower concentrations, supporting the need to develop improved strategies. Next steps will involve adaptive laboratory evolution and mutagenesis, as well as co-cultivation with other strains and the introduction of acidophilic strains to help improve remediation efficiency and further development of the circular bioeconomy.

## Relevance of proteostasis perturbation in trichothiodystrophy

Francesca Brevi<sup>1</sup>, Arjan Theil<sup>2</sup>, Alan Lehmann<sup>3</sup>, Sebastian Iben<sup>4</sup>, Donata Orioli<sup>1</sup>, Elena Botta<sup>1</sup>

<sup>1</sup>Istituto di Genetica Molecolare (IGM) CNR, Pavia, Italy

<sup>2</sup>Department of Molecular Genetics, Erasmus MC, University Erasmus Medical Center, Rotterdam, The Netherlands

<sup>3</sup>Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton, UK

<sup>4</sup>Department of Dermatology and Allergic Diseases, Ulm University, Ulm, Germany

### Background

Trichothiodystrophy (TTD) is a rare hereditary multisystem disorder showing hair and skin abnormalities associated to neurodevelopmental features. Mutations in a variety of genes have been associated to the disease, including transcription initiation factors, RNA splicing factors and aminoacyl-tRNA synthetases (aaRSs) involved in translation. All TTD-related factors participate in gene expression, thus raising the notion of TTD as gene expression syndrome. However, how the shared clinical features of TTD patients occur and how they can be related to common downstream consequences of perturbed gene expression remains unclear.

### Methods

Taking advantage of the laboratory collection of primary skin fibroblasts from TTD patients representative of the different defects, the pathogenic mechanisms underlying the disease have been investigated by combining cellular assays and molecular approaches.

### Results

We found that TTD cells share as a common feature an elevated translation error rate, indicating translation inaccuracy. In aaRSs-defective cells, impaired translation accuracy appears to be specifically due to impaired editing activity. In general, in the different TTD forms translation infidelity results in cellular accumulation of misfolded proteins, thus indicating a loss of protein homeostasis (proteostasis). This proteostatic imbalance is paralleled by dysregulated integrated stress response (ISR), an intracellular signaling network that helps the cells to adapt to the environment and maintain health. Notably, persistent ISR activation is implicated in disease, including age-related diseases such as neurodegeneration.

### Conclusion

Overall, translation infidelity and consequent loss of proteostasis emerge as a common underlying pathomechanism for the different forms of TTD, potentially contributing to impaired neurodevelopmental features in patients.

## Identification of potential hemp seed quality marker through volatilome profile analyses

Nicola Bosco<sup>1</sup>, Laura Bassolino<sup>2</sup>, Massimo Montanari<sup>2</sup>, Roberta Paris<sup>2</sup>, Giuseppe Mandolino<sup>2</sup>, Alma Balestrazzi<sup>1</sup>, Daniela D'Esposito<sup>3</sup>, Michelina Ruocco<sup>3</sup>, Francesco Loreto<sup>4</sup>, Anca Macovei<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology 'L. Spallanzani', University of Pavia, Pavia, Italy

<sup>2</sup>CREA-CI, Centro di Ricerca per le Colture Industriali, Bologna, Italy

<sup>3</sup>National Research Council – Institute for Sustainable Plant Protection (CNR-IPSP), Portici, Italy

<sup>4</sup>Department of Biology, University of Naples "Federico II", Naples, Italy

### Background

Hemp is a crop with relevant industrial applications in the pharmaceutical, textile and agri-food fields. However, seed germination and seed quality aspects are largely overlooked in this species. Non-invasive techniques can provide a rapid and accurate assessment of hemp seed quality. To this end, volatile organic compounds (VOCs) present a promising target as their emission profiles can be related to seed physiological and quality status. levels and types depend on the seed quality.

### Methods

Volatilome and germination profiles of 12 accessions belonging to four commercial varieties and harvested in different years were investigated. Meteorological data during seed development in the field were also recorded. VOCs profiles were assessed through proton-transfer electron spectroscopy (PTR-Qi-TOF-MS), at the CNR-IPSP INFRA-VOL platform. This work is part of the CaRiFIT project funded by MASAF and coordinated by CREA-CI.

### Results

Correlations among seed germination and meteorologic data, indicate that seed quality is highly influenced by the environmental factors. Among the 73 identified VOCs, 7 represent potential seed quality markers as they are significantly correlated with minimum three germination indices.

### Conclusion

These findings highlight the potential of VOC profiling as a reliable, non-invasive tool for assessing hemp seed quality while considering environmental influences. Future research should validate these markers across diverse cultivars and environments to strengthen their applicability and ecological relevance.

## **Diversity, function, and cytoplasmic incompatibility potential of the bacterial symbiont *Lariskella (Rickettsiales)* across arthropod hosts**

Carlo Croci<sup>1</sup>, Tiago Nardi<sup>2</sup>, Jani Sormunen<sup>3</sup>, Davide Sassera<sup>1,4</sup>, Michele Castelli<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology, University of Pavia, Italy

<sup>2</sup>EPIGET Lab - Department of Clinical Sciences and Community Health, "Dipartimento di Eccellenza 2023-2027", Università degli Studi di Milano, Milan, Italy

<sup>3</sup>Department of Biology, University of Turku, Turku, Finland

<sup>4</sup>Fondazione IRCCS Policlinico San Matteo, Pavia

### Background

*Lariskella arthropodorum (Rickettsiales)* is a maternally inherited, intracellular bacterium associated with diverse arthropods, including vectors of pathogens like ticks and fleas. Despite its widespread occurrence, its role in host biology remains largely unclear. Recent findings suggest that *Lariskella* may induce cytoplasmic incompatibility (CI), similarly to other well-known arthropod-associated bacteria like *Wolbachia*.

### Aim and Methods

Our study aims to screen *Lariskella* distribution and prevalence in ticks and other arthropods using full-length 16S rRNA gene sequencing with Oxford-Nanopore technology, as well as targeted qPCR assays. Subsequently, to better understand their relationship with the host, we aim to sequence the high-quality genomes of selective representative samples, particularly from tick hosts. Thanks to the newly generated data, we further aim to infer the phylogeny of *Lariskella* across hosts. Moreover, we are performing comparative genomic analyses to investigate the functional potential of the bacterium and the interactions with the host.

### Results

In a preliminary dataset of 121 specimens (including 76 ticks) belonging to over 30 different arthropod species, we found a 37% positivity to *Lariskella*. We newly sequenced and assembled two complete *Lariskella* genomes. Phylogenomics indicates that *Lariskella* phylogeny is not strictly correlated with host taxonomy or geographical origin. Preliminary genomics suggests the presence of possible host supportive functions, such as vitamin (biotin, folate) biosynthesis. Further analyses, including on CI genes, are ongoing.

### Conclusions

A deeper understanding of *Lariskella* features will shed light on the evolution of host-symbiont interactions, offering broader insights into arthropod population dynamics, vector biology, possibly supporting new vector control strategies.

## **Characterization of the LINP1/LINC00707 locus in a LigI-defective model of chronic DNA damage**

Maria Chiara Mottola<sup>1</sup>, Silvia Bione<sup>1</sup>, Giuseppe Biamonti<sup>1</sup>, Alessandra Montecucco<sup>1</sup>

<sup>1</sup>Institute of Molecular Genetics CNR, Pavia, Italy

### Background

46BR.1G1 fibroblasts are derived from a patient carrying a biallelic substitution (R771W) in the *LIG1* gene. This defect reduces DNA ligase I (LigI) activity and impairs maturation of newly synthesised DNA, causing accumulation of single- and double-strand breaks. Despite persistent damage, these cells retain proliferative capacity, a feature typical of preneoplastic lesions and tumour cells. Our aim is to explore how chronic DNA damage impacts lncRNA expression.

### Methods

RNA-seq was performed comparing 46BR.1G1 cells with either LigI-rescued cells (7A3) and a wild-type line (GM847). Differential expression analysis revealed several deregulated lncRNAs associated with cancer. Among these, the LINP1 locus (Ensembl v114), now including transcripts previously assigned to LINC00707, was selected for further genomic and functional analysis.

### Results

RNA-seq reads analysis identified four major transcript groups from LINP1 locus upregulated in 46BR.1G1 cells compared to both a transgenic clone expressing wild type LigI cDNA and control cells wild type for *LIG1* gene. The transcripts mapping to region 1 (LINP1\_R1) include the isoform previously characterized for its role in NHEJ. LINP1\_R2 and LINP1\_R3 include transcripts formerly annotated as LINC00707, while transcripts in LINP1\_R4 are still uncharacterized. Silencing of LINP1\_R1 increased γH2AX levels and reduced cell proliferation without altering the expression of other LINP1 transcripts, indicating a specific role of LINP1\_R1 in counteracting replication-induced damage.

### Conclusions

The LINP1 locus is transcriptionally activated by chronic replication stress and may be part of the cellular adaptation to persistent DNA damage, providing new insights on its functional relevance.



**The impact of transposable elements in the genomic plasticity  
of the Asian tiger mosquito *Aedes albopictus***

Chiara Albertini<sup>1</sup>, Alba Marino<sup>1,2</sup>, Mattia Baricordi<sup>3</sup>, Omar Rota-Stabelli<sup>4,5,6</sup>, Andrea Luchetti<sup>3</sup>, Lino Ometto<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology, University of Pavia, Pavia, Italy

<sup>2</sup>ISEM, University of Montpellier, CNRS, IRD, Montpellier, France

<sup>3</sup>Department of Biological, Geological and Environmental Sciences, University of Bologna, Bologna, Italy

<sup>4</sup>Center Agriculture Food Environment (C3A), University of Trento, San Michele all'Adige, Trento, Italy

<sup>5</sup>CIBIO Department, University of Trento, Trento, Italy

<sup>6</sup>Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Trento, Italy

**Background**

Genome plasticity enables structural variation, which is the base on which evolution can act. Transposable elements (TEs), which can replicate and insert into new genomic loci, are major contributors to such plasticity. Their mobilization, which seems to happen more frequently under stress, can influence gene expression, generate novel functions, and potentially foster rapid adaptation. The Asian tiger mosquito *Aedes albopictus*, an invasive species with remarkable environmental tolerance and a TE-rich genome (~55%), represents an ideal model to explore how TE activity contributes to adaptation under thermal stress.

**Methods**

We combined laboratory and population genomics approaches to assess TE mobilization in *Ae. albopictus*. In the lab, larvae of the Foshan strain were reared under heat stress (36 °C day / 32 °C night for seven days) and compared to controls at 28 °C. Adult pairs and offspring were collected for DNA extraction and sequencing. In parallel, wild populations were sampled from six Italian locations spanning different climatic zones. TE insertions were detected using PoPoolationTE2 with a curated TE library.

**Results**

Lab-reared, heat-stressed mosquitoes showed reduced fitness compared to controls (mean pupation = 46% vs. 80%). Preliminary PoPoolationTE2 analyses seem to suggest a higher number of novel TE insertions in heat-stressed families and in mosquitoes from warmer southern populations, suggesting temperature-dependent TE mobilization.

**Conclusions**

These early results seem to support the hypothesis that thermal stress can trigger TE activity, contributing to genome plasticity and potentially facilitating rapid adaptation. Ongoing analyses across populations will further elucidate the evolutionary role of TEs in *Ae. albopictus*.

## Molecular insights into factors mediating vector competence

Nicolò Gennari<sup>1</sup>, Giulia Mancini<sup>1</sup>, Irene Arnoldi<sup>2</sup>, Bruno Arcà<sup>3</sup>, Paolo Gabrieli<sup>2</sup>,  
Federico Forneris<sup>1,4</sup>

<sup>1</sup>The Armenise-Harvard Laboratory of Structural Biology, Dept. Biology and Biotechnology, University of Pavia, Pavia, Italy

<sup>2</sup>Dept. of Biosciences, University of Milan, via Celoria 26, Milan, Italy

<sup>3</sup>Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy

<sup>4</sup>Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

### Background

Vector-borne diseases transmitted by hematophagous insects remain major global health challenges. Understanding the molecular mechanisms underlying vector competence and host-pathogen-symbiont interactions is crucial for developing effective disease control strategies. This work focuses on the biochemical and structural characterization of proteins mediating interspecies interactions in *Aedes albopictus* and *Glossina spp.*

### Methods

Recombinant expression and purification protocols were optimized for four target proteins: mosquito salivary proteins LIPS-2, aaAG5-3 and alb30k-3, and the tsetse symbiont surface protein Spiralin. Expression systems included *E. coli* strains and mammalian HEK293-F cells. Purified proteins were characterized using SEC-MALS, intact MS, biochemical assays and BLI. Crystallization trials were initiated for Spiralin to investigate its structure.

### Results

The proteins have been successfully purified, and initial characterization has been carried out, laying the foundation for functional assays. The established protocols enable the production and initial characterization of key insect and symbiont proteins involved in vector-host interactions.

### Conclusion

These results lay the groundwork for future structural studies and the potential development of biomarkers and molecular probes for biomedical and vector control applications.

## **Impact of conditioned medium from DDB2<sup>PCNA-</sup> expressing HEK293 cells on macrophage activity**

Priyam Ghosh<sup>1</sup>, Martina Furfaro<sup>1</sup>, Paola Perucca<sup>1</sup>, Anna Tricarico<sup>1</sup>, Ennio Prosperi<sup>2</sup>,  
Lucia Anna Stivala<sup>1</sup>, Ornella Cazzalini<sup>1</sup>

<sup>1</sup>Dipartimento di Medicina molecolare, Unità di Immunologia e Patologia generale, Università degli studi di Pavia, Pavia

<sup>2</sup>Istituto di Genetica Molecolare "Luigi Luca Cavalli-Sforza", Consiglio Nazionale delle Ricerche (CNR), Pavia

### Background

DNA damage-binding protein 2 (DDB2) plays the role of recognizing UV-C-induced DNA lesions. It interacts with PCNA through a conserved PIP-box motif. A mutation in this PIP-box (DDB2<sup>PCNA-</sup>), leading to defective DDB2 degradation and accumulation in cells, drives to reduced NER efficiency, persistent DNA damage, and an aggressive cellular phenotype. This study examines how the mutant DDB2<sup>PCNA-</sup> protein affects macrophage function *in vitro*, potentially linking DNA repair to the tumor microenvironment.

### Methods

THP-1 monocytes were cultured in conditioned medium from HEK293 DDB2<sup>Wt</sup>, and DDB2<sup>PCNA-</sup> cells, collected before and after UV-C (10 J/m<sup>2</sup>). Subsequently, co-culturing was performed with THP-1 as recipient cell and irradiated HEK293 DDB2<sup>Wt</sup> and DDB2<sup>PCNA-</sup> as feeder cells. The THP-1 cell morphology was evaluated using gentian violet staining, while immunofluorescence was performed to examine the expression of CD80. Additionally, western blot analysis was conducted to determine inflammatory protein levels.

### Results

The most significant changes occurred in cells exposed to DDB2<sup>PCNA-</sup> conditioned medium (7d post-UV). Microscopic observations revealed that THP-1 cells exhibit macrophage-like morphology characterized by multiple cellular "pseudopodia", whereas cells exposed to control or DDB2<sup>Wt</sup> medium show minimal morphological changes. Similar results were obtained in co-culture experiments. Immunofluorescence studies demonstrated increased CD80 membrane localization in THP-1 cells exposed to DDB2<sup>PCNA-</sup> conditioned medium. Preliminary Western blot analysis showed elevated IL-6, IL-8, and CD38 levels in UV-treated HEK293 cells stably expressing the DDB2<sup>PCNA-</sup> mutant protein.

### Conclusions

These findings support a potential link between the loss of DDB2-PCNA interaction and macrophage-driven inflammation, relevant in understanding tumour microenvironmental dynamics.

## **Brain vulnerability in Osteogenesis Imperfecta: exploring the striatum in the *Brtl*<sup>+/-</sup> murine model**

Emma Lugli<sup>1</sup>, Roberta Besio<sup>2</sup>, Ludovica Gaiaschi<sup>1</sup>, Federica Gola<sup>1</sup>, Wendy Perez Franco<sup>2</sup>, Antonella Forlino<sup>2</sup>, Maria Grazia Bottone<sup>1</sup>, Fabrizio De Luca<sup>1</sup>

<sup>1</sup>Lab. Biologia Cellulare e Neurobiologia, Dipartimento di Biologia e Biotecnologie 'L. Spallanzani', Università di Pavia, Pavia, Italy

<sup>2</sup>Dipartimento di Medicina Molecolare, Unità Biochimica, Università di Pavia, Pavia, Italy

Osteogenesis imperfecta (OI) is a heterogeneous group of genetic disorders resulting from defects in collagen type I (COL1) synthesis, modifications and secretion. Despite being well known for its skeletal implications, OI encompasses a wide spectrum of extraskeletal symptoms including central nervous system (CNS) implications such as neurocranial malformations and neurovascular impairments. Notably, COL1, a major component of the extracellular matrix, plays crucial roles in the CNS; defining brain architecture, supporting vascularisation and regulating neuronal differentiation, migration and synaptogenesis.

Therefore, the aim of this work was to investigate whether COL1 defects may alter the brain's homeostatic balance by promoting sustained cellular stress and progressively undermining CNS integrity. To do this, we explore possible brain morphology alterations and modulation of specific molecular mechanisms involved in the maintenance of CNS intracellular homeostasis in 18-month-old *Brtl*<sup>+/-</sup> mice, a well-characterised murine model of dominant OI, performing both histological and immunohistochemical assessments, focusing our attention on a specific CNS area, i.e. the striatum (caudate-putamen).

Altogether, our results evidenced striatal morphological and molecular alterations in *Brtl*<sup>+/-</sup> mice, suggesting an altered neurobiological homeostasis within the caudate-putamen region of mutant mice.

This study supports that ECM changes may progressively undermine the brain's integrity and provides new insights into the mechanisms underlying CNS vulnerability in OI.

## **Hypervirulent *Klebsiella quasipneumoniae* subsp. *similipneumoniae* strain causing meningoencephalitis and liver abscess: the role of whole-genome sequencing**

Ilaria Petrizzi<sup>1</sup>, Aurora Piazza<sup>1,2</sup>, Raffaello Umberto Dario Biagini<sup>3</sup>, Elisa Matarazzo<sup>4</sup>, Marco Merli<sup>5</sup>, Livia Tartaglione<sup>4</sup>, Massimo Puoti<sup>5</sup>, Arturo Chiericato<sup>3</sup>, Chiara Vismara<sup>4</sup>, Roberta Migliavacca<sup>1,2</sup>

<sup>1</sup>Dept. of Clinical-Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Pavia

<sup>2</sup>IRCCS Fondazione Policlinico San Matteo, Pavia

<sup>3</sup>Neurointensive Care Unit, ASST Grande Ospedale Metropolitano Niguarda, Milan

<sup>4</sup>Clinical Microbiology Unit, ASST Grande Ospedale Metropolitano Niguarda, Milan

<sup>5</sup>Department of Infectious Diseases, ASST Grande Ospedale Metropolitano Niguarda, Milan

### Background

*Klebsiella quasipneumoniae* subsp. *similipneumoniae* (Kqps) is an emerging antibiotic-resistant pathogen. Aim was the genomic characterization of a Kqps (G1), responsible for meningoencephalitis and liver abscess in a 46-year-old woman.

### Methods

Identification and antimicrobial susceptibility testing were performed using MALDI-TOF MS Biotyper and MicroScan-WalkAway. Whole-genome sequencing (WGS) was performed on Illumina NovaSeq platform and analyzed with Galaxy, TYGS and Center for Genomic Epidemiology tools. Virulence was assessed *in vivo* using the *Galleria mellonella* infection model.

### Results

Culture from enriched broth and MALDI-TOF MS identified an hypermucoviscous *K. pneumoniae* (string test positive). WGS identified the strain as Kqps, belonging to ST3857. G1 showed a fully susceptible phenotype, although resistome analysis (ResFinder) revealed *bla*OKP-B-171, *oqxAB*, and point mutations in *ompK36/ompK37* and *acrR*. Virulome analysis (VFDB) showed virulence genes involved in iron uptake (*fyuA*), aerobactin (*iucA-D*, *iutA*), salmochelin (*iroB-D*, *iroN*), and yersiniabactin (*ybtAEPQSTUX*, *irp1*, *irp2*); fimbriae (*mrkABFI*) and the hypermucoviscous phenotype (*rmpA*, *rmpA2*). Virulence score (Kleborate): 4/5. Plasmid replicons repB, IncHI1B(pNDM-MAR) and IncFIBK (PlasmidFinder) were identified. BLAST analysis showed that the pG1node13 shared >99% similarity with known virulence plasmids of *Klebsiella spp.* *In vivo*, larvae injected with 10<sup>5</sup> CFU/mL, exhibited 50%, 60%, and 70% mortality at 16, 24, and 48 hours, respectively. In contrast, no mortality was observed in control and PBS groups.

### Conclusions

This work highlights the crucial role of genomic approaches for a proper and reliable identification of emergent and underestimated pathogens of clinical relevance.

## **DROSHA, DICER and Damage-Induced long ncRNA control BMI1-dependent transcriptional repression at DNA double-strand break**

Francesca Esposito<sup>1,2\*</sup>, Ilaria Capozzo<sup>1,§\*</sup>, Adelaide Riccardi<sup>1</sup>, Ubaldo Gioia<sup>1,3</sup>, Stefania Modafferi<sup>1,4</sup>, Letizia Manfredi<sup>1,¶</sup>, Fabio Iannelli<sup>3,§</sup>, Alessio Colantoni<sup>5,6</sup>, Alessia di Lillo<sup>3</sup>, Sara Tavella<sup>1,3</sup>, Matteo Cabrini<sup>3</sup>, Fabrizio d'Adda di Fagagna<sup>1,3</sup>, Sofia Francia<sup>1,7</sup>

\*These authors contributed equally to this work

<sup>1</sup>Istituto di Genetica Molecolare, CNR - Consiglio Nazionale delle Ricerche, Pavia, Italy

<sup>2</sup>PhD program in Genetics, Molecular and Cellular Biology (GMCB), University of Pavia, Pavia, Italy

<sup>3</sup>IFOM-ETS- The AIRC Institute of Molecular Oncology Foundation, Milan, Italy

<sup>4</sup>PhD program in Biomolecular Sciences and Biotechnology (SBB), Istituto Universitario di Studi Superiori (IUSS), Pavia, Italy

<sup>5</sup>Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy

<sup>6</sup>Center for Life Nano- and Neuro-Science, RNA Systems Biology Lab, Fondazione Istituto Italiano di Tecnologia (IIT), Rome, Italy

<sup>7</sup>Correspondence to: [sofia.francia@igm.cnr.it](mailto:sofia.francia@igm.cnr.it);

<sup>¶</sup>Present address: University of Zurich, Institute of Molecular Cancer Research, Winterthurerstr. 190, Zürich, Switzerland

<sup>§</sup>Present address: IUSS, Scuola Universitaria Superiore, Palazzo del Broletto, Piazza della Vittoria, n.15, Pavia, Italy

<sup>§</sup>Present address: IEO, Istituto Europeo di Oncologia IRCCS, Via Ripamonti 435, Milan, Italy

### Background

Genome integrity is maintained by the DNA damage response (DDR). Transcriptional repression of genes near DNA double-strand breaks (DSBs), known as DSB-induced silencing in cis (DISC), requires the DDR kinase ATM and the Polycomb Repressive Complex 1 (PRC1) component BMI1. In contrast, DSBs also triggers de novo transcription of damage-induced long non-coding RNAs (dilncRNAs) which are processed by DROSHA and DICER in shorter RNA (DDRNA). Together, DilncRNA and DDRNA enhance DDR signaling and DNA repair. We investigate the relationship between these two opposite transcriptional events.

### Methods

We investigated DISC in cancer cells expressing restriction enzymes or Cas9 to induce DSBs within active genes. Gene expression was quantified before and after DNA damage using RT-qPCR. Recruitment of BMI1 and the PRC1-mediated histone mark H2AK119ub was analyzed by immunofluorescence and ChIP. Antisense oligonucleotides and Cas13 targeted damage-induced ncRNAs were used to assess their role in DISC.

### Results

We demonstrate that dilncRNAs, together with DROSHA and DICER, are essential for RNA-mediated regulation of DISC. DICER nuclease activity is crucial, and its stimulation by Enoxacin enhances DISC and restores it even under ATM inhibition. DROSHA and DICER promote BMI1 recruitment to DSBs, enabling H2AK119 ubiquitination and silencing. DROSHA interacts with BMI1 and mediates its binding to dilncRNAs, while dilncRNA inactivation by ASO or Cas13 reduces BMI1 recruitment and DISC.

### Conclusions

We propose that DROSHA, DICER, and dilncRNAs regulate DISC by mediating BMI1 recruitment to DSBs, revealing how local de novo transcription suppresses canonical gene expression at damaged loci.

## **Larval density shapes reproductive and metabolic traits in the Asian tiger mosquito, *Aedes albopictus***

Sara Soldano<sup>1</sup>, Daniel De Luca<sup>1</sup>, Francesca Scolari<sup>2</sup>, Ludvik Marcus Gomulski<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology, University of Pavia, Pavia, Italy

<sup>2</sup>Institute of Molecular Genetics (IGM), National Research Council (CNR), Pavia, Italy

### Background

The Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), is a global invasive vector of arboviruses such as dengue, Zika and chikungunya, posing a major public health threat. With vaccines still limited, understanding how environmental factors influence mosquito physiology and behaviour is essential for effective vector control strategies. Larval rearing density is a critical but still poorly understood parameter affecting adult fitness, mating competitiveness and metabolism. This is particularly relevant for *Ae. albopictus*, which exploits transient and variable breeding sites typical of urban settings.

### Methods

We investigated the effects of two larval densities (“low” and “high”) on *Ae. albopictus* development, adult physiology, behaviour and metabolism by integrating developmental monitoring, fluorescence microscopy to assess sperm transfer and viability and liquid chromatography-mass spectrometry (LC-MS) targeting the gonads and the fat bodies due to their key roles in energy metabolism.

### Results

Mosquitoes reared at low-density developed more slowly but emerged significantly larger than those from the high-density treatment. Preliminary data suggest that larval density influences adult reproductive traits, including sperm transfer rates. Pilot metabolomic analyses identified key metabolites linked to energy metabolism, such as the pentose phosphate pathway and amino acid biosynthesis. These findings are currently being validated in a larger dataset.

### Conclusions

Our optimized experimental pipeline enables high-throughput assessment of reproductive and metabolic traits in *Ae. albopictus*. Ongoing efforts aim to increase sample size and refine metabolomic analyses to establish links between metabolic profiles, reproductive performance, and environmental adaptation.

### Fifteen millennia of human mitogenome evolution in Sicily

Anna Tommasi<sup>1,†</sup>, Rajiv Boscolo Agostini<sup>2,†</sup>, Giacomo Villani<sup>1,†</sup>, Nicola Rambaldi Migliore<sup>1</sup>, Maria T. Vizzari<sup>2</sup>, Irene Cardinali<sup>3</sup>, Rosalinda Di Gerlando<sup>1,4</sup>, Valeria Nicolini<sup>1</sup>, Gary Sorasio<sup>1</sup>, Patricia Santos<sup>2</sup>, Anna Olivieri<sup>1</sup>, Ugo A. Perego<sup>5</sup>, Giulio Catalano<sup>6</sup>, Nicoletta Volante<sup>7</sup>, Lucia Sarti<sup>7</sup>, David Caramelli<sup>8</sup>, Luca Sineo<sup>6</sup>, Hovirag Lancioni<sup>3</sup>, Alessandra Modi<sup>8</sup>, Silvia Ghirotto<sup>2,\*</sup>, Alessandro Achilli<sup>1,\*</sup>

†These authors contributed equally to this work

\* Corresponding authors

<sup>1</sup>Department of Biology and Biotechnology “L. Spallanzani”, University of Pavia, Pavia, Italy

<sup>2</sup>Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy

<sup>3</sup>Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy

<sup>4</sup>IRCCS Mondino Foundation, Pavia, Italy

<sup>5</sup>Southeastern Community College, West Burlington, Iowa, USA

<sup>6</sup>Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy

<sup>7</sup>Department of History and Cultural Heritage, University of Siena, Siena, Italy

<sup>8</sup>Department of Biology, University of Florence, Florence, Italy

Sicily, situated at the heart of the Mediterranean Sea, has been a crossroads of people of different origins since the Paleolithic. To gain further insight into the genetic history of the island from a matrilineal viewpoint, we investigated fifteen millennia of mitogenome evolution. A unique Sicilian mitochondrial dataset, represented by 116 ancient mitogenomes (including 2 newly sequenced) collected in 16 archaeological sites dating from 14,700 to 545 years before present (BP), was compared with a novel collection of 236 modern mitogenomes covering all districts of the island. By integrating demographic modeling with phylogeographic analyses for the first time, we were able to identify a statistically supported genetic discontinuity between the Paleolithic/Late Mesolithic and Early Neolithic periods, as well as two mtDNA lineages (U5b and U8b/K) that specifically mark this transition, which is characterized by a certain degree of population replacement. Specifically, Paleolithic hunter-gatherer groups carrying U5b1d1, U5b2b, and U5b3 arrived and expanded on the island until the Late Mesolithic. These groups survived in various refugia in western Eurasia and were partially replaced by farming communities migrating from the Near East via the Mediterranean and inland routes. These farming communities introduced the Neolithic lineages U8b1b1, K1a3, and K2b into the island. The extensive variation and lack of genetic structure among modern mitogenomes suggest the presence of a continuous, maternally inherited gene flow from different regions of Western Eurasia (since the Paleolithic) and Africa (since the Bronze Age).



**New antibacterial drugs against the ESKAPE pathogens**

Arooba Arshad<sup>1</sup>, Viola Camilla Scoffone<sup>1</sup>, Gabriele Trespidi<sup>1</sup>, Giulia Barbieri<sup>1</sup>, Silvia Buroni<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

In the 21st century, antibiotic resistance remains an impending doom on global health complicated by bacterial persistence, a phenotypically cunning survival tactic that allows bacterial cells to defy the onslaught of lethal antibiotic exposure. Ploidy, referring to number of chromosome copies nestled within a cell, plays a pivotal role in reshaping this biological landscape. The first goal of my project is to meticulously examine the connection between ploidy variation and persistence in *Escherichia coli*, employing a multidisciplinary approach integrating single-cell ploidy measurement via flow cytometry, persistence assays and -omics analyses. Flow cytometry will uncover growth phase-dependent DNA content patterns within rapidly dividing cells showing multimodal profiles that reflect ploidy heterogeneity. Next, we will isolate these ploidy-defined subpopulations and subject each to antibiotic persistence assays and transcriptomic analysis, enabling us to determine how chromosome copy number, metabolic state, and gene expression collectively influence survival. Expanding beyond Gram-negatives, we further wish to examine how Gram-positive bacteria utilize the stringent response, triggered by nutritional and antibiotic stresses and mediated by the alarmone (p)ppGpp, which reprograms bacterial physiology toward dormancy and reduced metabolic activity, promoting the emergence of persister cells. By combining ploidy dynamics with stringent response signaling, this project aims to uncover novel vulnerabilities to interface the persister pathways. Another aim of my project will be to identify novel antibacterial drugs, focusing the attention on bacterial cell division as new drug target. Taken together, these approaches will lead to the identification of new strategies to fight persistence and drug resistance in MDR bacteria.

## **Determinants of seed quality in crops**

Nicolò Bedotto<sup>1</sup>, Alma Balestrazzi<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

Seed quality is the set of morphological, genetic and physiological characteristics of seeds, reflecting the germination potential. The identification of seed quality hallmarks is essential to define features on which seed companies can rely to assess the overall quality of a seed lot. Transcriptomic, metabolomic, and proteomic analyses are commonly used to study embryo tissues and their development under the various conditions seeds experience during maturation and germination. Although several quality biomarkers have already been proposed, validation of their universality and reliability is still missing.

The present project aims to identify and validate novel determinants of seed quality by analysing multiple rice and orphan legume genotypes in different experimental systems. The purpose of this project is to build a toolbox of reliable indicators that can be used to predict the germination profile of a seed lot through its morphological, biochemical, and molecular features.

Single-seed germination tests will be performed to evaluate possible correlations between seed morphology (length, width, perimeter, area, colour), the reactive oxygen species (ROS) released during imbibition, and the time required to germinate. Additionally, seed priming treatments will be applied to possibly improve seed quality and germination profiles. These will include the use of innovative priming agents obtained through the valorisation of waste products. Omics approaches will be considered to investigate the molecular pathways influenced by seed priming as well as the identification of novel seed quality biomarkers. Using qRT-PCR, changes in the relative expression of genes involved in DNA damage response and antioxidant defense will be assessed.

## **Analysis of chromatin reorganization during differentiation**

Adel Diaf<sup>1</sup>, Margherita Cavallo<sup>1</sup>, Gloria Milanese<sup>1</sup>, Marco Biggiogera<sup>1</sup>, Claudio Casali<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

### Background

Neurogenesis is a process in which extensive epigenetic and structural remodeling occurs, as pluripotent cells acquire a neuronal identity. The embryonic carcinoma cell line P19 represents a versatile in vitro model for studying this transition, as it can differentiate into derivatives of all three germ layers under appropriate conditions. It possesses a normal karyotype without aneuploidy or polyploidy, and following differentiation, it gives rise to non-tumorigenic cells. After exposure to retinoic acid, P19 cells commit to a neuronal lineage, providing a system that allows the study of neuronal differentiation.

### Main goals

This project aims to characterize ultrastructural changes occurring during neuronal differentiation, with particular emphasis on nuclear architecture. Key features to be examined include global morphology, chromatin organization, and the arrangement of nuclear bodies across defined differentiation stages. To verify lineage commitment, the downregulation of pluripotency markers OCT4 and NANOG and the upregulation of the early neuronal marker NEUN and late stage marker MAP2 will be assessed. By integrating ultrastructural analysis with molecular profiling, the study seeks to link transcriptional reprogramming with nuclear and chromatin reorganization at high resolution.

### Methods

Cell morphology and nuclear ultrastructure will be examined using high-resolution optical microscopy and transmission electron microscopy. Differentiation status will be validated by quantitative RT-PCR analysis of OCT4, NANOG, NEUN, and MAP2 expression. This combined approach will allow correlation of molecular marker dynamics with structural hallmarks of neuronal commitment.

**Priming-induced DDR (DNA Damage Response) signatures contributing to heat stress tolerance in tomato (*Solanum lycopersicum* L.)**

Ridwan Diaguna<sup>1</sup>, Alma Balestrazzi<sup>1</sup>, Anca Macovei<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

Tomato is a widely grown vegetable, relevant for human consumption, yet recurrent heat waves place its production at risk. Pre-sown treatments can mitigate high-temperature damage by enhancing the protective mechanisms activated during the seed pre-germinative metabolism. As seed vigour relies on effective DNA repair and antioxidant responses triggered during early imbibition, a deeper understanding of the molecular processes underlying germination is essential for developing more effective priming techniques. The aim is to design optimised, sustainable priming protocols for tomato seeds, minimizing the influence of genotype and seed lot. 20 tomato genotypes will be screened for heat tolerance under continuous and heat-wave regimes. Based on these profiles, two heat-sensitive genotypes and one tolerant genotype will be selected for priming optimization. Hydropriming, spermidine-based chemo-priming, and physical priming (plasma and thermopriming) will be preliminarily assessed for controlled-imbibition time, dose, and specific parameters to generate optimized protocols. Their impact on heat stress tolerance will be evaluated through standard germination tests. Primed and unprimed seeds of sensitive and tolerant tomato genotypes will be analysed in presence/absence of heat stress to uncover DDR mechanisms. Molecular profiling will include quantification of reactive oxygen species and DNA damage, metabolomics, RNA-Seq and ATAC-Seq. This will reveal DDR signatures associated with heat stress tolerance triggered by seed priming and the DDR regulatory networks linked to seed priming. The selected seed-priming protocols will be applied to a wide range of genotypes to identify the most sustainable treatments, showing minimal genotype-dependent effects, combined with low cost, and limited environmental impact.

## Evolution of rickettsial proteins involved in host interactions and pathogenicity

Luca Erriquez<sup>1</sup>, Greta Bellinzona<sup>1</sup>, Carlo Croci<sup>1</sup>, Paolo Losi<sup>1</sup>, Davide Sassera<sup>1,2</sup>,  
Michele Castelli<sup>1</sup>

<sup>1</sup>Midifactory, Department of Biology and Biotechnology, University of Pavia, Pavia, Italy

<sup>2</sup>Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Members of the genus *Rickettsia* are obligate intracellular bacteria, including multiple pathogens vectored by hematophagous arthropods. Several *Rickettsia* species are responsible for human diseases, including epidemic typhus and Rocky Mountain spotted fever. *Rickettsia* spp. deploy numerous proteins involved in host cell adhesion, invasion and evasion of the immune system, with each *Rickettsia* encoding a unique repertoire.

The aim of this project is to comprehensively investigate the presence of these proteins in *Rickettsia* spp. and their relatives, and to understand their evolutionary and functional diversification throughout the evolutionary history of this bacterial lineage.

The genome of a novel *Rickettsia*-related bacterium was sequenced from a mite using short and long reads, selectively assembled and incorporated into a dataset of over 100 *Rickettsiaceae* genomes. For the selected proteins, multiple maximum-likelihood phylogenetic trees were inferred. The trees indicate that these proteins have evolved independently within each lineage, giving rise to distinct copies multiple times throughout evolution. It appears that each genus has independently altered the number of these proteins, and that the rates of duplications and loss vary even among different species of the same genus.

This degree of variation is likely linked to the specialization of their interactions with distinct host organisms, a process that has probably occurred repeatedly across the *Rickettsiaceae* family.

*In silico* structural analyses are ongoing in order to perform protein-protein interaction predictions with putative host targets

The results shed further light on the host-pathogen molecular interplay, on the role of these proteins in host specificity, and on their evolution.

## Identification of the molecular mechanisms underlying neurodegeneration in Cockayne Syndrome

Carolina Flacchi<sup>1</sup>, Tiziana Nardo<sup>1</sup>, Giulia Branca<sup>1</sup>, Manuela Lanzafame<sup>1</sup>, Fiorenzo Peverali<sup>1</sup>, Donata Orioli<sup>1</sup>

<sup>1</sup>Istituto di Genetica Molecolare “Luigi Luca Cavalli Sforza” - CNR, Pavia, Italy

### Background

Nucleotide Excision Repair (NER) is a major DNA repair system that protects cells from ultraviolet (UV) and chemical-induced bulky lesions. Within NER, the transcription-coupled NER (TC-NER) removes lesions on actively transcribed DNA strands. The CSA protein is a key player in TC-NER, and mutations in *ERCC8/CSA* gene are associated to two different disorders: Cockayne Syndrome (CS) and UV-sensitive syndrome (UV<sup>SS</sup>). Although both conditions cause UV hypersensitivity, CS individuals develop neurological and cognitive impairments, photosensitivity and signs of premature aging, whereas UV<sup>SS</sup> patients present only UV hypersensitivity. Notably, while CS cells display strong sensitivity to both UV light and oxidative stress, UV<sup>SS</sup> cells fail at repairing UV-induced DNA lesions but do not exhibit oxidative-stress hypersensitivity. These differences suggest that specific molecular defects underlie the neurological and progeroid features of CS.

### Methods and Main Goals

To identify these molecular mechanisms, we will generate isogenic cell lines carrying disease-specific CSA mutations. The Cre-Recombinase Mediated Cassette Exchange (RMCE) approach will be used to overcome the genetic variability of patient-derived primary cells. CS-specific (i.e. Q106P) and UV<sup>SS</sup>-specific (W361C) variants of CSA, fused in frame with Flag and HA epitope tags, will be expressed alongside with the wild-type form of CSA as control. All lines will be tested for UV sensitivity and oxidative stress, using potassium bromate or menadione. If they replicate the patient cells' phenotypes, CSA-interacting proteins will be isolated via tandem affinity immunoprecipitation and identified by mass spectrometry. Comparing CS and UV<sup>SS</sup> protein interactome profiles will hopefully uncover the mechanisms contributing to the neurodegenerative phenotypes of CS.

## **An innovative approach to target membrane-bound markers in neuroendocrine tumors (NETs)**

Fabio Luca Guzzi<sup>1</sup>, Francesco Ascione<sup>1</sup>, Natalia Simona Pellegata<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

### Background

Neuroendocrine tumor (NET) cells frequently express receptors and surface markers that can be exploited for therapy and imaging. Among them are the somatostatin receptors (SSTRs), especially the type 2 receptor (SSTR2) which constitute a major therapeutic target for NETs. Other antigens, e.g. GD2 and CD276, are expressed on NET cells but are also present on different tumor types. These membrane-bound molecules represent exploitable entry points for targeted strategies aimed at killing the tumor cells. In this scenario, a promising approach involves the use of Photo-Oncolytic Phages engineered to selectively recognize distinct targets (GD2, CD276 and SSTR2) and induce a localized production of reactive oxygen species (ROS). The rationale is that targeted oxidative stress may compromise NET cell viability, disrupt redox homeostasis and amplify anti-tumor responses by altering key signaling pathways and microenvironmental conditions.

### Main Goals

The aims of this project are: (i) to characterize selected NET cell lines for the expression of these markers; (ii) to validate the binding specificity and selectivity of the engineered phage toward marker-positive cells; (iii) to quantify ROS induction and define its functional contribution to cytotoxicity; and (iv) to assess preliminary anti-tumor activity in advanced in vitro models.

### Methods

To accomplish these objectives, HEK cells engineered to express the candidate surface molecules and NET cell lines expressing them endogenously will be employed alongside marker-negative control cell lines. Marker expression will be assessed by Western blot, immunofluorescence and real-time PCR. Phage binding will be evaluated by flow cytometry and confocal microscopy using fluorescently labelled phages or anti-phage antibodies. The functional impact on cell fate will be examined through viability assays, cell death assays (caspase assays,  $\gamma$ H2AX-based detection of DNA damage). Finally, three-dimensional spheroid models will be used to investigate phage penetration and early therapeutic responses under conditions that better recapitulate tumor architecture.

## **Next-Generation Sterile Insect Technique for the genetic control of *Drosophila suzukii***

Daniela Mayorga Churion<sup>1,2</sup>, Maria Vittoria Mancini<sup>1</sup>, Gerard Terradas<sup>2</sup>, Andrew Hammond<sup>2,3,4</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

<sup>2</sup>Biocentis, London, United Kingdom

<sup>3</sup>Johns Hopkins Bloomberg School of Public Health, Baltimore (MD), United States of America

<sup>4</sup>Imperial College London, London, United Kingdom

### Introduction

*Drosophila suzukii* is a globally invasive fly pest species that causes an annual economic loss estimated at 500M dollars in the US alone. Its unique serrated ovipositor allows egg laying inside of ripening soft-skinned fruits, making it a challenge to target non-adult stages for traditional control methods, as these act only on the fruit's surface. Effective control of *D. suzukii*, by chemical pesticides, is constrained in part due to restrictions on their use, as governments try to limit their negative impact on humans and non-target species. Other control approaches do not cause damage to non-target species, but have limited, variable effectiveness, and high costs of producing and deploying insects at a great scale.

### Main goal

The scope of my PhD project is to generate an environmentally friendly and sustainable pest control solution via fit, competitive, and sterile males. Generated data will characterize the key aspects of their biology that contribute to mating competitiveness. The work also aims to create a method to mass produce sterile males for industrial application.

### Methods

Precision genome engineering will be used to generate specific mutations in essential male fertility genes to obtain male-sterile strains. Fitness assays will ensure that sterile males can compete with their wild-type counterparts for females of the target pest population. Application of such sterile males will decrease the pest insects being born in the release site, as they will mate with wild females, which will not produce any progeny.



### **The genomic identity of unknown museum specimens**

Valeria Nicolini<sup>1</sup>, Nicola Rambaldi Migliore<sup>1</sup>, Massimo Mezzavilla<sup>2</sup>, Giacomo Villani<sup>1</sup>,  
Anna Tommasi<sup>1</sup>, Elisabetta Moroni<sup>1</sup>, Nataliia Kozak<sup>1</sup>, Nicola Carrara<sup>3</sup>, Anna Olivieri<sup>1</sup>,  
Luca Pagani<sup>2</sup>, Alessandro Achilli<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

<sup>2</sup>Department of Biology, University of Padua, Padua, Italy

<sup>3</sup>Museo della Natura e dell’Uomo, University of Padua, Padua, Italy

Over the past two decades, ancient DNA has become a cornerstone in the study of human history, with new advancements in genomic analysis enabling the study of archival specimens through the emerging field of “museomics”.

This study aims to emphasize the value of 23 human individuals from the “Museo della Natura e dell’Uomo” (Padova, Italy). These belong to a historical collection of skulls, for which minimal context is available, except for a few fragmentary information on geographic origin or cultural affiliation. After several attempts of DNA extraction from teeth, we managed to recover genomic data, achieving an endogenous content ranging from 2.63 to 44.58%. Preliminary allele frequency-based analyses confirmed the biogeographic origins recorded by the museum labels for the entire set, except for one African individual labelled as Indian. In the upcoming step, we are focusing on two different subsets: 1) two individuals, labeled as members of the Botocudo culture, one male and one female, who appear to be related, 2) a group of eight individuals, labelled as Saracens, who exhibit a significant mitochondrial diversity without evidence of genetic relatedness and 3) two individuals identified as Etruscan and Pompeian, respectively, who may provide new insights into these two peculiar facets of the cultural and genetic history of the Italian peninsula.

The purpose of this work is to underline the significant contribution of museomics to the renewal of lost museum collections and the importance they might gain in the discovery of our past.

## Tracking Genome-wide Dynamics of DNA Polymerase $\eta$

Samuele Pavesi<sup>1,2</sup>, Lina Cipolla<sup>1</sup>, Simone Sabbioneda<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy

<sup>2</sup>Institute of Molecular Genetics, National Research Council (IGM-CNR), Italy

### Background

Pol $\eta$  (Rad30 in yeast) is a DNA polymerase classically implicated in damage bypass during translesion synthesis following UV damage, yet recent data indicate broader roles in genome maintenance. Pol $\eta$  can traverse G-quadruplex (G4) DNA, incorporate ribonucleotides, and extend RNA primers, while its loss correlates with R-loop accumulation and DNA breaks. This project utilizes genome-wide ChIP-seq and complementary assays in human and *S. cerevisiae* systems to define Pol $\eta$  interactions with DNA G4s, RNA G4s (rG4), DNA:RNA hybrids (R-loops/G-loops), and topoisomerase-induced topological stress across S-phase and after DNA damage.

### Main Goals

- Determine whether Pol $\eta$  is enriched at G4 loci (constitutive versus damage-induced), whether enrichment is topology- or damage-dependent, and how it modulates local transcription across S-phase sub-stages.
- Map Pol $\eta$  localization to high R-loop and G-loops regions, assess how Pol $\eta$  deficiency alters hybrid distribution and transcription, and test its role in G-loop resolution (including PDS perturbation and helicase/RNase H controls).
- Investigate recruitment to rG4s, impacts on RNA processing/translation, and functional interactions with RNA helicases (e.g., DHX9/DHX36).
- Assess Pol $\eta$  association with topoisomerase cleavage sites, its response to topoisomerase inhibitors, and its role in mitigating topological stress and resultant DNA damage.

### Methods

This project employs human and yeast cell lines to conduct comprehensive high-throughput genomic and transcriptomic profiling. Our multi-omics approach integrates ChIP-seq (targeting Pol $\eta$ /Rad30, G4s and topoisomerases), DRIP-seq, rG4-seq, RIP-seq/eCLIP, and DSB mapping. To assess mechanistic roles, we will examine the impact of specific perturbations on genome stability, including UV-C irradiation, G4-stabilizers (PDS), topoisomerase inhibitors, helicase mutants, and RNase H expression.

## **The seed repair response – from models to crops**

Davide Pezzini<sup>1</sup>, Anca Macovei<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

The seed repair response refers to multiple cellular mechanisms activated primarily upon imbibition to counteract the accumulation of DNA damage occurring during maturation and storage. The two key mechanisms involved in the seed repair response are the antioxidant processes and DNA damage response (DDR). While the first includes defense systems that neutralize reactive oxygen species (ROS) to prevent oxidative damage, the latter is a coordinated network that detects DNA lesions, signals their presence, and activates repair pathways to maintain genome integrity.

Because the interplay among these pathways is highly complex, the seed repair response remains only partially understood. Therefore, the main objectives of this PhD thesis are: (1) to investigate the molecular mechanisms involved in the seed repair response in model plants, and (2) to translate this knowledge into agricultural applications for orphan legume crops. The use of diverse *Arabidopsis thaliana* mutants will allow to pinpoint specific gene functions and investigate their roles during seed germination. Subsequently, these genes will be investigated in legume seeds subjected to priming treatments to assess their roles in enhancing repair and germination performance. The methodologies to be used span from morpho-physiological biometrics (seed weight, size, germination profiling), to biochemical (quantification of ROS), and molecular (qRT-PCR, DNA damage level evaluation through ELISA and comet assay) analyses. Omics approaches will be used to identify the molecular pathways influenced by seed priming, while genetically diverse orphan legumes will serve as complementary models to examine how the seed repair response pathways are conserved across plant species.

## **Investigating centromere architecture from evolution to cancer**

Edoardo Rapisarda<sup>1</sup>, Marialaura Biundo<sup>1</sup>, Francesca M. Piras<sup>2</sup>, Eleonora Cappelletti<sup>2</sup>, Ted Kalbfleisch<sup>4</sup>, Oliver A. Ryder<sup>3</sup>, Solomon G. Nergadze<sup>1</sup>, Elena Giulotto<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

<sup>2</sup>Unit of Anatomic Pathology, IRCCS San Matteo Hospital Foundation, Pavia, Italy

<sup>3</sup>Conservation Science Wildlife Health, San Diego Zoo Wildlife Alliance, Escondido, CA, USA

<sup>4</sup>Department of Veterinary Science, Martin-Gatton College of Agriculture, Food, and Environment, University of Kentucky, Lexington, KY, USA

### Background

Centromeres are essential chromosomal loci required for chromosome segregation, epigenetically defined by the histone H3 variant CENP-A. Centromere defects are linked to chromosomal instability and cancer. Because centromeric DNA typically consists of satellite arrays, these regions have been difficult to analyse at the molecular level. Equids carry conventional satellite-based centromeres and naturally occurring satellite-free centromeres, making them valuable models for centromere biology. Equid satellite-free and most satellite-based centromeres are not bound by CENP-B, enigmatic centromeric protein that binds and stabilizes human and mouse centromeres, yet they are fully functional, indicating that satellite DNA and CENP-B binding are not essential for centromere stability.

### Main Goals

1. To expand the study of centromere organization across mammals, testing whether satellite-free centromeres are more widespread than previously thought, and investigating their evolution, organization, and CENP-B binding patterns.
2. To investigate the potential impact of chromosomal rearrangements in cancer on centromere organization, focusing on cell lines with chromothripsis, complex phenomenon involving chromosome fragmentation and reshuffling.

### Methods

The project integrates cytogenetic, molecular, and bioinformatic approaches. Centromeric proteins and satellite DNA will be investigated through immunofluorescence, FISH, and ChIP-seq. Satellite DNA families will be identified and mapped onto T2T reference genomes that we are contributing to assemble. Comparative genomics will be used to reconstruct centromere and karyotype evolution.

### Results

We identified satellite-free centromeres and uncoupling between centromeres and CENP-B in two gazelles and a tapir. These findings suggest that the peculiar centromere organization that appeared to be a peculiarity of equids may be widespread among mammals.

## Regulatory interactions between mosquito hosts and their inherited symbionts

Ottavia Zaffaroni<sup>1</sup>, Daniel Gingell<sup>1</sup>, Maria Vittoria Mancini<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy

### Background

*Wolbachia* is a widespread maternally inherited endosymbiont that inhabits arthropods and nematodes. It can act as either a parasite or a mutualist to its host, in some cases enhancing the host pathogen resistance while manipulating the host reproduction. These characteristics make *Wolbachia* a well-established tool for reducing arboviral diseases spread by mosquito vectors *Aedes albopictus* and *Aedes aegypti*.

*Wolbachia*'s tissue distribution varies across hosts, with restriction to reproductive tissues in native infections and higher densities in somatic tissues in non-native, artificially introduced infections. Evidence shows that *Wolbachia* density and tissue tropism strongly influence its virus-blocking capacity, highlighting the importance of uncovering the mechanisms underlying its different tissue distributions.

### Main goals

This project aims at understanding the mechanisms driving host-symbiont interactions in native and artificial *Wolbachia* infections in mosquitoes, starting by mapping the spatial-temporal divergent patterns of *Wolbachia* distribution in different host/symbiont strains combinations and investigating the molecular pathways limiting symbiont tropism in native hosts.

### Methods

Using different mosquito-*Wolbachia* strain combinations, I will characterize *Wolbachia* density and distribution across reproductive and somatic tissues through complementary molecular and imaging approaches. Quantitative PCR will be used to measure *Wolbachia* load, while tissue localization will be examined by Fluorescence in situ Hybridization (FISH) targeting the *Wolbachia* 16S rDNA, together with immunostaining using antibodies against tissue-specific protein markers. In addition, RNA-seq on dissected mosquito body parts will be carried out to profile *Wolbachia* gene expression across different host tissues, providing a comprehensive view of symbiont activity and tissue specificity.

## **Cancer-associated fibroblasts in PPGLs: functional and molecular insights into tumor–stroma crosstalk**

Margarita Bistika<sup>1</sup>, Rouaud Ernest<sup>1</sup>, Francesca Amore<sup>2</sup>, Elena Rapizzi<sup>3,4,5</sup>, Natalia Simona Pellegata<sup>1,6</sup>

<sup>1</sup>Department of Biology and Biotechnology, University of Pavia, Pavia, Italy

<sup>2</sup>Department Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy

<sup>3</sup>Department Experimental and Clinical Medicine, University of Florence, Florence, Italy

<sup>4</sup>Centro di Ricerca e Innovazione sulle Patologie Surrenaliche, AOU Careggi, Florence, Italy

<sup>5</sup>ENS@T Center of Excellence, Florence, Italy

<sup>6</sup>Institute for Diabetes and Cancer, Helmholtz Zentrum München, Neuherberg, Germany

### Background

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors with high heritability and metastatic potential, especially in SDHB-mutated cases. The tumor microenvironment (TME), and particularly cancer-associated fibroblasts (CAFs), critically influence extracellular matrix remodeling and tumor aggressiveness. Yet, their contribution to PPGL progression remains poorly defined.

### Methods

We used human PPGL models (hPheo1 parental and SDHB knockout cells) and primary fibroblasts from PPGL patients. Functional assays included wound healing and 3D spheroid invasion in standard medium, CAF-conditioned medium, or direct co-culture. Cytokine profiling was performed with TaqMan™ arrays to evaluate transcriptional changes upon co-culture. Secretome analysis is ongoing, employing TurboID proximity labelling to assign proteins to specific cell types.

### Results

CAF-conditioned medium enhanced migration selectively in SDHB-deficient cells, while parental cells were unresponsive. In 3D assays, both conditioned medium and direct co-culture promoted invasion, with the strongest effect in mixed spheroids where tumor-CAF contact triggered extensive dissemination. Cytokine profiling revealed context-dependent changes, with tumor cells consistently upregulating pro-inflammatory mediators. Among them, IL-8 emerged as a key deregulated cytokine, particularly induced in tumor cells exposed to CAFs.

### Conclusions

CAFs enhance migration and invasion of PPGL cells through mechanisms shaped by tumor genetic background. IL-8 stands out as a potential mediator of tumor–stroma crosstalk, underscoring the role of inflammatory signaling in PPGL progression. Ongoing secretome studies aim to clarify molecular drivers of this interaction, with the potential to identify therapeutic targets that disrupt CAF–tumor communication in aggressive PPGLs.

## **miRNAs from human cumulus cell-derived extracellular vesicles contribute to oocyte developmental competence**

Giulia Brazzale<sup>1</sup>, Giulia Fiorentino<sup>1</sup>, Valeria Merico<sup>1</sup>, Mario Zannoni<sup>1</sup>, Marilena Taggi<sup>2</sup>,  
Federica Innocenti<sup>2</sup>, Fani Konstantinidou<sup>3</sup>, Valentina Gatta<sup>3</sup>, Sergio Comincini<sup>1</sup>,  
Gianluca Nastri<sup>2</sup>, Valentina Casciani<sup>2</sup>, Alberto Vaiarelli<sup>2</sup>, Filippo Maria Ubaldi<sup>2,5</sup>, Laura  
Rienzi<sup>2,4</sup>, Silvia Garagna<sup>1</sup>, Danilo Cimadomo<sup>2</sup>, Maurizio Zuccotti<sup>1</sup>

<sup>1</sup>Department of Biology and Biotechnology, University of Pavia, Italy

<sup>2</sup>IVIRMA Global Research Alliance, Genera, Clinica Valle Giulia, Rome, Italy

<sup>3</sup>Dept of Neuroscience and Unit of Molecular Genetics, University of Chieti, Italy

<sup>4</sup>Dept of Biomolecular Sciences, University of Urbino, Italy

<sup>5</sup>Dept of Pharmacy, Health Sciences and Nutrition, University of Calabria, Italy

The bidirectional communication between the oocyte and its surrounding cumulus cells (CCs) within the cumulus–oocyte complex (COC) is crucial for establishing oocyte quality and developmental competence. In a previous mouse study, we demonstrated that germinal vesicle (GV) oocytes matured to metaphase II (MII) on feeder layers of CCs (FL-CCs) derived from competent oocytes developed to the blastocyst stage, whereas those co-cultured with FL-CCs from incompetent oocytes arrested at the two-cell stage (Cavalera et al., 2019). Both FL-CC types released extracellular vesicles (EVs) carrying 74 differentially expressed miRNAs, 7 of which regulate 71 genes involved in oocyte and follicle development (Fiorentino et al., 2024).

To translate this model to humans, we established feeder layers from CCs collected individually from women undergoing intracytoplasmic sperm injection, related to either competent (BL-hFL-CCs) or incompetent (NoBL-hFL-CCs) oocytes. EVs secreted by both hFL-CC types were isolated and their miRNA content analysed through microarray analysis.

90 differentially expressed miRNAs were identified in EVs when released by BL-hFL-CC vs. NoBL-hFL-CC, with 4 upregulated and 86 downregulated. Among these, 23 miRNAs are known to participate in ovarian functions. Bioinformatic analysis revealed target genes, 55 of which are in common to the 71 previously identified in the mouse model and are involved in meiosis resumption, fertilization, cumulus expansion, and oocyte developmental competence.

Our data demonstrate that CC-derived EVs carry miRNAs associated with oocyte developmental competence. The overlap between human and mouse gene targets supports the existence of conserved molecular mechanisms governing the acquisition of mammalian oocyte developmental competence.

## The Role Of Nuclear Reorganization In Hypoxia Adaptation

Margherita Cavallo<sup>1</sup>, Claudio Casali<sup>1</sup>, Adel Diaf<sup>1</sup>, Davide Tunesi<sup>1</sup>, Gloria Milanese<sup>1</sup>,  
Giuliano Mazzini<sup>2</sup>, Marco Biggiogera<sup>1</sup>

<sup>1</sup>Laboratory of Cell Biology and Neurobiology, Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia; Italy

<sup>2</sup>Institute of Molecular Genetics, National Research Council (IGM-CNR), Italy

### Background

The spatial organization of the cell nucleus bears functional relevance in both maintaining cellular homeostasis and facilitating pathological transformations. Under external stimuli, chromatin undergoes extensive remodelling, leading to the dynamic redistribution of nucleic acids and proteins into specialized subnuclear compartments. Among environmental cues, oxygen availability plays a central role. Hypoxia profoundly reshapes the epigenetic landscape through HIF-1 $\alpha$  activation, yet the mechanisms linking oxygen sensing to nuclear reorganization remain largely unexplored.

### Methods

Mouse hepatocytes were exposed to hypoxia-mimicking conditions followed by reoxygenation. TEM and STED microscopy, combined with various cytochemical approaches, were employed to investigate chromatin architecture and the spatial relocation of RNP complexes. Predictive analyses were performed to assess the LLPS propensity of proteins identified within these nuclear assemblies. Finally, flow cytometry was used to evaluate cell-cycle progression.

### Results

Hypoxia triggered extensive chromatin decondensation, enhancing transcriptional accessibility but potentially increasing DNA fragility, as evidenced by the accumulation of hypoxic cells in G2/M phase. We observed that this arrest coincides with the spatial clustering of subnuclear components, including PG and RNP assemblies. PG-like clusters were enriched in the lncRNA Malat1 and HIF-1 $\alpha$ , indicating a potential cooperation in the spatial regulation of hypoxia-induced transcripts. In contrast, the other class of assemblies appears to be degraded within the perinuclear space, suggesting a previously unrecognized nucleophagy pathway activated under hypoxia.

### Conclusions

These findings provide new insights into hypoxia-driven nuclear reorganization and support a model in which spatial transcript control may contribute to cellular adaptation within hypoxic microenvironments.



### Deciphering the genomic history of the Rendena Valley people

Giacomo Villani<sup>1\*</sup>, Nicola Rambaldi Migliore<sup>1\*</sup>, Anna Tommasi<sup>1</sup>, Irene Cardinali<sup>2</sup>, Elisa Somenzi<sup>3</sup>, Rosalinda Di Gerlando<sup>1,4</sup>, Erika Partel<sup>5</sup>, Ana Maria Chero Osorio<sup>1</sup>, Nicola Martellozzo<sup>6</sup>, Roberta Raffaetà<sup>6</sup>, Licia Colli<sup>3,7</sup>, Anna Olivieri<sup>1,8</sup>, Heidi Christine Hauffe<sup>5,8</sup>, Hovirag Lancioni<sup>2</sup>, Paolo Ajmone Marsan<sup>3</sup>, Antonio Torroni<sup>1</sup>, Alessandro Achilli<sup>1</sup>

\*These authors contributed equally to this work

<sup>1</sup>University of Pavia, Department of Biology and Biotechnology, Pavia, Italy

<sup>2</sup>University of Perugia, Department of Chemistry, Biology and Biotechnology, Perugia, Italy

<sup>3</sup>Catholic University of the Sacred Heart, Department of Animal Science, Food and Nutrition, Piacenza, Italy

<sup>4</sup>IRCCS Mondino Foundation, Molecular Biology and Transcriptomics Unit, Pavia, Italy

<sup>5</sup>Fondazione Edmund Mach, Department of Innovation in Agricultural and Zootechnical Productions, San Michele All'Adige, Trento, Italy

<sup>6</sup>Ca' Foscari University of Venice, Department of Philosophy and Cultural Heritage, Venice, Italy

<sup>7</sup>BioDNA Research Centre on Biodiversity and Ancient DNA, Biodiversity research group, Piacenza, Italy

<sup>8</sup>National Biodiversity Future Center (NBFC), Piazza Marina 61, 90133 Palermo, Italy

Genomic studies have greatly increased our knowledge of the genetic history of human populations. Present-day Italians (i) harbour a higher genetic diversity compared to other European populations, an effect of various genetic contributions, and (ii) cluster into four major groups: Sardinia, Northern, Central and Southern Italy. This diversity was here further explored at the microgeographic level. We analyzed the genomic variation of 125 individuals belonging to the Rendena Valley (Trentino-Alto Adige/Südtirol) community. We assessed their mitochondrial DNA variation by sequencing the complete mitogenomes of 94 maternally unrelated Rendeneri. Haplotype and haplogroup distributions showed high internal variability and differentiation from the northern Italian context. To delve deeper, 48 individuals were genotyped using the Human Origin SNP chip (~600,000 SNPs). Allele frequency methods confirmed the high level of variation in Rendeneri and highlighted a peculiar placement outside the Italian genomic cline of variation in PCA. ADMIXTURE analysis of Western Eurasia detected a specific component maximized in Rendeneri, suggesting genetic isolation of the valley. Runs of homozygosity distribution further hinted towards a small and isolated population, within the context of a general panmictic Italy. Haplotype-based methods reaffirmed these findings, also revealing a much lower effective population size than Italy. Furthermore, ChromoPainter/fineSTRUCTURE analysis unveiled a north-south substructure within the valley that mirrors geography. These results highlighted Rendena Valley as a genetically distinct, sub-structured alpine isolate. Future work will include geographic comparisons with additional alpine valleys and diachronic assessments of ancient genomes from alpine archaeological sites.

# Sponsor

## Acknowledgments



NUOVA GENETICA  
ITALIANA



CeGaT



Promega

CliniSciences



Sial



