12th PhD Workshop

October 12th-13th 2023

PhD School in Molecular and Cellular Biology

Talks: Department of Biosciences, G24 - Via Golgi, 19

Posters: Orto Botanico - Via Golgi, 18

DAY 1

14.00 Welcome

TALK SESSION I

14.15 – *Buratti Stefano*
14.35 – *Chirivì Daniele*
14.55 – *Cornaro Letizia*

15.15 Coffee Break

TALK SESSION II

15.45 – *Rondelli Diego*
16.05 – *Bernini Giulia Maria*
16.25 – *Di Terlizzi Matteo*

POSTER SESSION I

16.45 - *Astori C., Banfi C., Bono G.A., Cesare G., Ferrario C.C., Marone Fassolo E., Orozco Arroyo G., Paleni C., Ravishankar S., Torricella V.*

Join online:

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DAY 2

TALK SESSION I

14.15 – *Bibi Alessia*
14.35 – *Cospito Alessandro*
14.55 – *Gallo Alberto*

15.15 Coffee Break

TALK SESSION II

15.45 – *Londero Michela*
16.05 – *Fagnani Elisa*
16.25 – *Smeele Paulien Hermine*

POSTER SESSION II

16.45 – *Ballabio F., Burattin F.V., Pagani G., Palloni L.M.G., Pizzoccheri R., Polettini S., Scolz A., Tiberi M., Zaccaria M.*

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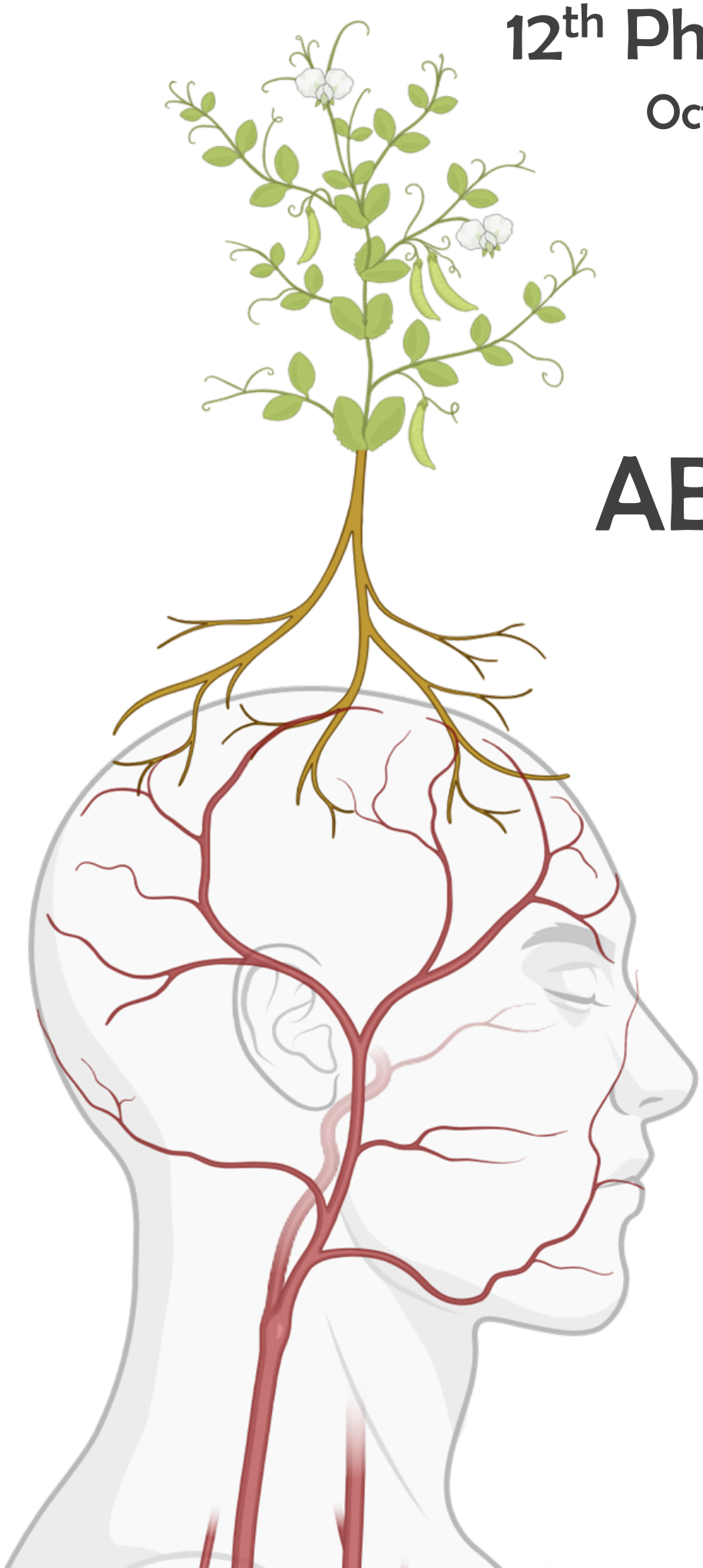

ABSTRACT BOOK

DOTTORATO DI RICERCA
DBMC
IN BIOLOGIA MOLECOLARE E CELLULARE

PhD School
in
Molecular and
Cellular Biology

Day 1: 12th October 2023

14.00 Welcome

TALK SESSION I

- 14.15 **"**GLR3.7 as negative regulator of GLR3.3 mediated stress responses" **Buratti Stefano**
- 14.35 "Interactomics for Rice Flowering: a Proximity Labelling Approach" **Chirivì Daniele**
- 14.55 "Characterization of diplospory in *Taraxacum officinale* L.: the role of the lipid transporter VACUOLAR PROTEIN SORTING-ASSOCIATED 13" **Cornaro Letizia**

15.15 Coffee Break

TALK SESSION II

- 15.45 "The response to DNA damage in non-proliferating cells: mechanisms and pathologies" **Rondelli Diego**
- 16.05 "Unveiling the role of Vid22 in genome protection: insights from mutational analysis in budding yeast" **Bernini Giulia**
- 16.25 "Roles of polo kinase CDC5 in DNA double strand breaks processing and repair" **Di Terlizzi Matteo**

POSTER SESSION

- 16.45 **(Astori, C., Banfi, C., Bono G.A., Cesare G., Ferrario, C. C., Marone Fassolo E., Orozco Arroyo, G., Paleni, C., Ravishankar S., Torricella V.)**

Talk sessions day 1

Talk 1 - Buratti Stefano

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Alex Costa

GLR3.7 as negative regulator of GLR3.3 mediated stress responses

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During evolution, plants have developed sophisticated mechanisms to perceive and respond to the environment. Adaptability and responsivity to biotic and abiotic stresses is a key mechanism that involves multiple factors: second messengers, phytohormones, metabolites and a whole variety of molecules involved into the perception, distribution, and transduction of stress signals. Recent works showed that wounding-driven long-distance propagation of Ca^{2+} waves depend on the activity of the cation permeable channel Glutamate Like Receptor 3.3 (GLR3.3), but further information on how this key factor is regulated is still scarce.
GLRs are homologous to the animals' ionotropic glutamate receptor channels (iGluRs), which can form both homo and hetero-tetramer. This allows us to hypothesize that different assemblies of plant GLRs as well can bring to the formation of channels with different properties.
Here we present multiple lines of evidence supporting the molecular and functional interdependency between GLR3.3 and another relatively uncharacterized GLR isoform, GLR3.7.

Talk 2 – Chirivì Daniele

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Fabio Fornara

Interactomics for Rice Flowering: a Proximity Labelling Approach

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Variability in flowering-controlling genes has been fundamental over history for adapting rice cultivation to different latitudes. The molecular network controlling floral induction in rice is highly complex and it is the result of a stratification of interactions: transcriptional, epigenetic and post-translational. A relatively new technique called Proximity Labelling (PL) allows a high-throughput identification of protein interactors by molecular engineering, and was never tested in rice plants before. The goal of this research was to implement PL in rice, establishing efficient vectors and protocols while applying it to the identification of the interactors of two rice flowering regulators: OsFT-L1 and Hd1. The application of PL to OsFT-L1 and Hd1 will deliver a list of the proteins laying in their contiguity. The method exploits a biotin-ligase fused to the the two proteins of interest: exposure of tissues to biotin leads to biotinylation of OsFT-L1- and Hd1- interacting proteins; biotin tags allow for selective precipitation of the proximal proteome followed by mass spectroscopy analysis.

Talk 3 – Cornaro Letizia

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Lucia Colombo

Characterization of diplospory in *Taraxacum officinale* L.: the role of the lipid transporter VACUOLAR PROTEIN SORTING-ASSOCIATED 13

Letizia Cornaro[1], Cucinotta M.[1], Petrella R.[1], Banfi C.[1], Balanzá V.[1], van Dijk P.J.[2], Rigola D.[2], Op den Camp R.H.M.[2], Radoeva T.[2], van Tunen A.[2], Colombo L.[1]

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Diplospory is a type of apomictic reproduction characterized by the lack of a proper meiotic process, which results in the generation of an unreduced egg cell that develops into an embryo by parthenogenesis. Therefore, diplosporous plants produce a clonal progeny identical to the mother. The introduction of this system in sexually reproducing crops could have a huge impact on the ability to fix valuable and complex traits in plant breeding programs.

Taraxacum officinale L., the common dandelion, is characterized by sexual diploid and apomictic polyploid genotypes. The analysis of a *loss-of-diplospory* mutant, generated by gamma-irradiation from a 3x apomictic plant, revealed a reversion to normal meiosis. This mutant allowed the identification of the putative *DIPLOSPORY* locus that controls the trait in dandelions. One of the genes present in the locus and gene for the regulation of diplospory is *VACUOLAR PROTEIN SORTING-ASSOCIATED 13* (*VPS13*). The *VPS13* gene family is conserved across all eukaryotes and encodes large proteins involved in the tethering between different organelles to transfer lipids within the cell. These proteins are well studied in humans and yeast, but there is scarce information about VPS13 in plants.

Here, we characterize the role of VPS13S during female germline progression and a possible involvement of microRNA in this process.

Talk 4 – Rondelli Diego

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Marco Muzi Falconi

The response to DNA damage in non-proliferating cells: mechanisms and pathologies

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Nucleotide Excision Repair (NER) is the DNA damage repair pathway responsible for the removal of bulky lesions in DNA, including those induced by UV light and specific chemical compounds like BPDE, the active metabolite of benzo[a]pyrene, the prototype of polycyclic aromatic hydrocarbons. Mutations in NER give rise to genetic disorders such as xeroderma pigmentosum, Cockayne Syndrome and Trichothiodistrophy. It has been shown that following initial processing by NER of the so called Closely-spaced Opposing Lesions (COLs) a failure to complete the repair reaction can generate EXO1-dependent double strand breaks in non-cycling cells. The repair of such difficult lesions requires NER activity for the removal of a bulky lesion on one strand, but also the recruitment of translesion synthesis (TLS) DNA polymerases for the bypass of the lesion present in close proximity on the opposite strand. During my PhD, I have been investigating potential proteins and novel mechanisms that can take part in this process. Parkin, an E3-Uiquitin Ligase frequently mutated in Parkinson's Disease, was shown in literature to be potentially involved in the ubiquitination of PCNA necessary for lesion bypass. However, studies done so far seem to indicate that the protein has an indirect effect on PCNA expression due to a regulatory activity of the cell cycle, while it might be possible that the protein also protects DNA from the insurgency of oxidative damage (which contribute to the formation of COLs) by its mitochondrial quality control activity. RecQ helicases could also be involved in the resolution of COLs, and indeed we managed to demonstrate Bloom syndrome protein EXO1-dependent localization at Local UV Damage (LUDs) sites, which have been previously demonstrated to be COLs hotspots. Finally, I am investigating the role of EXO1 and SNM1 proteins in the processing of COLs in NER defective cells, where double strand breaks are not expected as the initial incision cannot be performed, but the activation of the DNA damage signalling pathway has been observed at much later time points.

Talk 5 – Bernini Giulia Maria

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Federico Lazzaro

Unveiling the role of Vid22 in genome protection: insights from mutational analysis in budding yeast

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Genomic stability is essential for cellular health, and its disruption is linked to aging, cancer, and various human disorders. Therefore, understanding the molecular mechanisms responsible for maintaining genome stability from endogenous and exogenous perturbations is of paramount importance.
Vid22 has been implicated in genome protection, particularly at the level of non-canonical DNA secondary structures as G-quadruplexes, in the budding yeast model system. However, its precise molecular function and its interacting partners remain elusive. Vid22 is categorized within the Hermes transposase-like protein family due to the computational prediction of an N-terminal BED-type Zn-finger, an RNH-like fold, and a hAT C-terminal dimerization region. The protein is known to physically interact with its uncharacterized paralog Env11 and the essential Tbf1 protein, participating in various nuclear processes.
In this study, we conducted a comprehensive analysis of Vid22 mutants altered in conserved residues. Our investigation revealed that the evolutionarily conserved hAT-like C-terminus of *S. cerevisiae*Vid22 plays a crucial role in Vid22 self-interaction and quaternary structure formation, DNA binding, and function in genome stability maintenance. These findings indicate that the DNA-bound Vid22-containing complex ultimately maintains genome stability and suggest that Vid22 may have originated from transposase domestication during evolution.
Further exploration of Vid22 mutants promises insights into its molecular mechanisms in maintaining genome stability. These insights may provide new evidence rof genome evolution through domestication of transposable elements, aid in identifying potential human homologs and developing therapeutic strategies for genome instability-related diseases.

Talk 6 - Di Terlizzi Matteo

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Achille Pelliccioli

Roles of polo kinase CDC5 in DNA double strand breaks processing and repair

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Among all types of DNA damage that threaten genome stability, Double-Strand Breaks (DSBs) are the most deleterious ones: failure in repairing one single DSB can lead to chromosome loss, translocations, and apoptosis. DSBs are repaired by either direct religation of the broken ends (via Non-Homologous End Joining; NHEJ) or by invasion of a homologous sequence in the genome, which acts as a template for DNA synthesis (Homologous Recombination; HR). Repair by HR requires nucleolitic processing (resection) of the broken ends, generating long stretches of single-stranded DNA (ssDNA), which are coated by Rad51 to direct invasion of the homology and formation of a D-loop with the donor. Regulation of DSB response requires activation of a subset of protein kinases to drive proper processing and repair of the lesion. Polo-Like Kinases (PLKs), key regulators of cell cycle progression, have been shown to phosphorylate different DNA repair factors. Moreover, human PLK1 is overexpressed in different types of tumors and it is considered as a target in anti-cancer therapy.
Cdc5 is the only PLK in S. cerevisiae and we found that Cdc5 kinase activity is critical for Break-Induced Replication (BIR), a DSB repair pathway where DNA synthesis is completed till the end of the donor chromosome. Of note, BIR is associated with mutagenesis and loss of heterozygosity and drives Alternative Lengthening of the Telomeres (ALT) in tumor cells, a mechanism that allows them to escape senescence.
In our assays in yeast, loss of Cdc5 activity leads to reduced levels of Rad51 at the donor locus and inefficient D-loop formation. Interestingly, deletion of Srs2 helicase and translocase, but not its helicase-dead mutation, suppresses D-loop defect of cdc5 mutant cells while, on the other hand, reduced Cdc5 kinase activity ameliorates srs2Δ toxicity during recombination repair. Current experiments are investigating the molecular mechanisms underlying Cdc5 phenotypes in DSB response and Homology-Directed Repair, focusing on the formation and maturation of the Rad51-ssDNA nucleofilament controlled by Srs2 with the cooperation of Rdh54, Sgs1 and Mph1 helicases/translocases. To this aim, we are also setting the conditions to test DSB processing in the context of different genomic sites by the conditional expression of Cas9/gRNAs. Our results obtained in budding yeast may be useful for further analysis in human cells and the definition of specific targets for cancer therapy.

Poster session day 1

Poster 1 – Astori Chiara

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Lucia Colombo

NZZ/SPL regulates MMC differentiation by supporting auxin signalling in ovule primordia

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In plants, the specification of the female germline requires the differentiation of one cell, among the somatic cells within the ovule, named Megaspore Mother Cell (MMC). The MMC, upon meiosis, forms four haploid megaspores; three of which degenerate, and one enlarges to become the functional megaspore (FM), throughout a developmental process named megasporogenesis. Then, the FM in turn will form the female gametophyte. Even though MMC specification is crucial to ensure plant fertility, very little is known about the regulation of this process. NOZZLE/SPOROCYTELESS (NZZ/SPL) is considered a master regulator of MMC formation, since in the nzz/spl mutant the MMC does not develop. It has been shown that during ovule development NZZ/SPL plays a central role in auxin-cytokinin crosstalk; in particular, we observed, in nzz/spl ovules, a reduction in an auxin-transporter expression, PIN1. To better characterize SPL/NZZ mechanism of action and how it sustains auxin signalling, we performed Co-IP and ChIPseq. In parallel, we studied SPL/NZZ evolutionary conservation by comparing its sequences of Arabidopsis thaliana to the ones of other species across the evolution, and by complementing spl/nzz phenotype in A. thaliana by expressing the Marchantia polymorpha SPL/NZZ sequence.

Poster 2 – Banfi Camilla

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Lucia Colombo

Unveil the molecular mechanisms regulating apomixis in dandelion

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Apomixis is an asexual reproductive mechanism in which the embryo forms without fertilization. The clonal seed propagation allows the fixation of a given genotype through generations, saving costs and efforts that characterize plant breeding. During the last decades, many researchers have been working on this topic, but the molecular mechanism regulating apomixis it still under investigation. *Taraxacum officinale* – the common dandelion – is a natural apomict species with diploid sexual and triploid apomictic populations which reproduces through diplospory. In this type of apomixis, the pairing of chromosomes during meiosis is impaired, leading to the formation of an unreduced megaspore that develops in an unreduced female gametophyte. Eventually this gives rise to an embryo through parthenogenesis. By confocal imaging, I characterized the female germline progression in apomictic and sexual plants at high resolution level, providing a detailed morphological atlas of diplospory. Furthermore, I have demonstrated that in *loss-of-diplospory* (*lod*) mutant the meiotic process is impaired, causing apomictic-to-sexual transition. I have used different approaches to unveil the genes potentially involved in diplospory and their network. By RNA-seq I have identified several genes deregulated in the apomictic plant respect to the sexual. Further analysis of the experiment results is ongoing.

Poster 3 – Bono Giulia Ave

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Fabio Fornara

Defining the function of rice florigenic proteins during the reproductive transition and inflorescence development

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Rice is an herbaceous annual plant of Asian origin, whose flowering is accelerated under short-day (SD) conditions. Its importance as a staple crop makes the study of rice flowering regulation and inflorescence development essential to meet the growing demand for food. Florigens, which are small molecules produced in response to the correct daylength for flowering, in rice are encoded by *Hd3a* and *RFT1*, both homologs of *A. thaliana FT* [1]. These proteins are synthesized in the leaves and then transported to the Shoot Apical Meristem[2] (SAM), where they trigger flowering, activating the expression of genes involved in the vegetative-to-reproductive transition[3–5]. Transcriptomic analysis at the SAM allowed the identification of a florigen-like gene, *FT-L1*, the closest homolog of *Hd3a*, whose expression is strongly induced by rice florigens[6]. Its transcription at the SAM distinguishes it from traditional florigens, whose transcription is restricted to leaves, indicating non-canonical regulation and function in rice flowering. FT-L1 transcript and protein are present in the SAM at various stages of early panicle development and persist also in flowers[7]. Genetic analysis using both chemically induced and CRISPR mutations indicates that loss of *FT-L1* causes a delay in flowering time, an increase in the number of secondary or higher order branches, but also a reduction in flower fertility; both phenotypes are exacerbated when *ft-l1* mutants are combined with *hd3a* or *rft1*. According to these studies, FT-L1 may play a role as a positive regulator of the vegetative-to-reproductive transition and of the shift from indeterminate branch meristem to determinate spikelet meristem fate, proposing the existence of a triple system based on florigens and a florigen-like protein determining reproductive commitment.
Current research is focusing on the molecular antagonism between florigenic and antflorigenic proteins, to understand how their activities are integrated into the complex regulation of inflorescence architecture, using different CRISPR-Cas9 and overexpression approaches; moreover, the problem of flower sterility in the presence of *ft-l1* mutations is under study, thanks to the analysis of gene expression in floral organs and through the observation of mutant flowers.

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2. Tamaki, S. et al., Hd3a Protein Is a Mobile Flowering Signal in Rice. *Science (1979)* 316, 1033–1036 (2007).
3. Taoka, K. I. *et al.* 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. *Nature* 476, 332–335 (2011).
4. Zhao, J. *et al.* Genetic interactions between diverged alleles of Early heading date 1 (Ehd1) and Heading date 3a (Hd3a)/ RICE FLOWERING LOCUS T1 (RFT1) control differential heading and contribute to regional adaptation in rice (Oryza sativa). *New Phytologist* 208, 936–948 (2015).
5. Kobayashi, K. *et al.* Inflorescence meristem identity in rice is specified by overlapping functions of three AP1/FUL-Like MADS box genes and PAP2, a SEPALLATA MADS Box gene. *Plant Cell* 24, 1848–1859 (2012).
6. Gómez-Ariza, J. *et al.* A transcription factor coordinating internode elongation and photoperiodic signals in rice. *Nat Plants* 5, 358–362 (2019).
7. Francesca Giaume *et al.* Two florigens and a florigen-like protein form a triple regulatory module at the shoot apical meristem to promote reproductive transitions in rice. *Nat Plants 9, 525 - 534 (2023)*.

Poster 4 – Cesare Giuliana

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Thomas Vaccari

The role of the E3 ubiquitin ligase Hecw in autophagy

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Autophagy is a crucial catabolic process aiming to recycle cytosolic material to maintain cellular homeostasis. During autophagy, dysfunctional organelles and macromolecules are captured by newly formed double-membrane organelles, named autophagosomes, and delivered to lysosomes for degradation. Impairments in the autophagic pathway are associated with a wide number of pathologies, including cancer and neurodegeneration. Nonetheless, despite the wide effort in finding new components of this pathway, we are far from fully elucidating the molecular mechanisms behind autophagy. Recent studies support a role for E3 ubiquitin ligases in autophagy, highlighting the importance of ubiquitination in tightly controlling the progression of this pathway. We recently characterized the function of Hecw, the Drosophila melanogaster ortholog of human HECW1. Hecw is essential to maintain the liquid-like state of ribonucleoprotein particles (RNPs). In its absence, flies show defective oogenesis as well as neurodegenerative-like phenotypes. While it is possible that the function of Hecw in RNPs biology might support both egg development and prevent neurodegeneration, the onset of the neurodegeneration-like phenotypes in flies lacking Hecw remains unexplained. Our data indicates Hecw may act in TFEB/Mitf mediated-autophagy as we find that Hecw is essential for the correct engagement of starvation-induced autophagy in larval and adult tissues. Moreover, aging Hecw mutant flies present a strong reduction of TFEB/Mitf protein levels, possibly leading to impaired autophagy as shown by reduced levels of the autophagic marker Ref2P. Furthermore, Hecw interacts in vivo with the autophagy chaperone mediators 14-3-3, that bind phosphorylated TFEB/Mitf in the cytoplasm to escort it to degradation. Since neuronal cells strongly rely on autophagy for their survival, these results might be important to find new actors in the autophagic process and elucidate the genetics of neurodegeneration.

Poster 5 – Ferrario Carlotta Claudia

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Martin Kater

Modifying phyllotaxis in Brassica seed crop species for yield improvement

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To feed the growing world population and not devolve further land to agriculture, it is desirable to develop plant genotypes of increase yield. In our lab, in the context of studying genes involved in inflorescence architecture, *Arabidopsis* mutants in *PHY1*, *PHY2* and *PHY3* were developed. They display phenotypes worthy to be transferred to crops: indeed, they have a different phyllotactic pattern respect the WT, associated with an increased number of siliques and a reduced plastochron. Since canola (*Brassica napus*) is evolutionary close to *Arabidopsis* and one of the main oil crops in the world, it was selected as species where to develop similar mutants.
The putative homologous genes in *Brassica napus* were identified by bioinformatic tools, resulting in a 10 genes pool of which some placed in linkage. The number of the genes is coherent with the genome evolution and hybridization happened after *Arabidopsis-Brassica* split and *napus* species birth. The genes expression was studied by real time PCR and *in situ hybridization*, and it shows a pattern resembling the *Arabidopsis* one. They are expressed in the inflorescence and floral meristems and in floral reproductive organs of different stages. The functional conservation of some of the candidate genes was studied by a complementation test and Y2H assay. Indeed, it is known that in *Arabidopsis* PHY2 can interact with itself and PHY1. While the homologs' expression pattern is similar to the *Arabidopsis* one, the protein interactions differ, showing different homo- and hetero-dimers formation both in intraspecific and interspecific combinations. For the complementation test, the selected genes were cloned under the 35S constitutive promoter and 3 lines expressing the construct at different levels were used. The same 35S overexpression of the *Arabidopsis* genes themselves were used as a control. The phyllotactic pattern of the lines was considered for the analysis. The genes homologous to *PHY2*, but not to *PHY1*, show a partial complementation.
As a final goal we want to create mutants in *Brassica napus* which could recall the phenotype observed in *Arabidopsis*. 3 gRNAs were designed for each candidate gene, preferring gRNAs targeting more than one gene at the same time. The efficiency of the 21 gRNAs designed was tested by a protoplast destructive assay and the most efficient have been cloned in PTG constructs.
For developing the mutants, we are optimizing several transformation protocols for *B. napus*. We are testing both protoplast transient transformation by Cas9-gRNA constructs as well as *Agrobacterium* mediated transformation by floral dip and in vitro protocols. The faster and more efficient approach will be selected.

Poster 6 – Marone Fassolo Elena

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Paolo Pesaresi

New antimicrobial peptides for a sustainable agriculture

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The protection against fungal plant pathogens is mainly based on the use of fungicides, that creates great concerns for the risks associated to human health, environmental toxicity, or development of resistance in the target pathogen. Several pesticides are currently being banned or included in a list of candidates for substitution by the EU. To avoid implication on food security, safer and novel molecules are needed, having the ability to control existing and emerging pathogens. In my PhD project we focused on three different plant pathogens, *Zymoseptoria tritici*, *Phytophthora infestans* and *Pseudocercospora fijiensis*, that cause dramatic yield and economic losses every year. Our technology is based on the Yeast Two-Hybrid combinatorial library suitable for the identification of small cyclic peptides (8 aa long) able to bind a bait (i.e., target protein) of interest needed from the plant pathogen to infect the plants. This approach aims to increase the specificity toward the targeted protein and, as consequence, for the desired pathogen, thus reducing the risks of negative effects on other organisms and the environment. Some of the identified cyclic and linear peptides showed fungicidal activity in *in vitro* and *ex vivo* assays. Future perspectives will consist in deeper investigations about their effects on the physiology of the target pathogens like interferences with membrane integrity and/or interaction with cellular compartments. the absence of toxicity on non-target organisms will also be investigated. All together, these analyses will conduct to the identification of leads compounds for field trials and industrial scale-up.

Poster 7 – Orozco Arroyo Gregorio

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Martin Kater

Regulating cytokinin metabolism to promote higher rice productivity through changes on plant architecture

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Climate change is challenging plant agriculture and our ability to manage food security. Cultivated rice is a staple crop for more than half of the global population, thus increasing its production is a key step for global food security. Crop growth and yield are controlled by several phytohormones and their overlapping signal networks. Cytokinin homeostasis has a deep impact during rice panicle development, especially on panicle branching which determines grain number and therefore yield.
We generated a multiple knock-out mutant of CKXs (*osckx3 osckx5 osckx9*) rice plant with improved yield using the CRISPR/Cas9 system. The phenotypic analysis of this triple mutant line showed a significant enhancement on total grain yield productivity with minor effects on other agronomical-favourable morphological characters. In greenhouse conditions, the triple mutant plants presented 23% of total grain yield increase in respect to wild type control plants. This increase observed on yield is achieved mainly through the increased number of panicles per plant and more secondary branches produced. To determine the actual contribution of each of the three *OsCKX* genes in yield improvement, we obtained single and double mutant combinations and nowadays we are evaluating the yield components traits of these lines.
In addition, we performed a transcriptome analysis on early stages of inflorescence meristem development to identify genes with significantly altered expression in the *osckx3 osckx5 osckx9* triple mutant. This data will help us to clarify the precise role of CK homeostasis during inflorescence meristem and panicle development.
Taken together, our findings will provide new insights into the manipulation of endogenous CKs homeostasis in rice growth and may provide the foundations for future studies aimed to improve growth and increase grain yield in rice as a direct consequence of changes on the rice panicle complexity.

Poster 8 – Paleni Chiara

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Cycle: XXXVII PON A.Y. 2021 – 2022

Supervisor: Martin Kater

Salvia pratensis genomic studies for endemic species conservation and flower development

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Salvia is a common taxon in the collections of botanical gardens due to its long history of medicinal and culinary use. Twenty-five species can be found in the wild in Italy; *S. pratensis* is one of the most common and is closely related to some endemic taxa with debated species rank (S*. ceratophylloides, S. saccardiana, S. haematodes*). In the Botanical Garden of Brera, we are studying the distribution of genetic diversity in wild populations of *Salvia pratensis* and related taxa to inform species delineation and guide conservation efforts. We are also interested in genes responsible for the distinctive flower morphology and pollination mechanisms of this species. To reach these goals, we have sequenced the whole genome of *Salvia pratensis* using both short read and long read sequencing technology and assembled the full genomic sequence. The genome will be annotated with RNA-seq data to identify genes and functional elements, with a focus on genes involved in flower development. We are complementing the transcriptomic data with RNA *in situ* hybridization experiments on genes of the MADS-BOX family to assess their expression in female and hermaphrodite flowers. Secondly, we are using the genome as a reference to investigate the distribution of genetic diversity of *S. pratensis* populations with a population genomics approach. Thanks to a network of collaborators, we have gathered a collection of samples from wild populations across a large part of the Italian territory and we are characterizing it with Genotyping-By-Sequencing (GBS). We will analyse genomic variation in the collection by combining a population structure approach with phylogenomic analysis to investigate separation between endemic taxa and detect gene flow between populations.

Poster 9 – Ravishankar Srikanth

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Paolo Landini

Anti-biofilm activity of fluorocytosine (5-FC) in *Escherichia coli* requires its conversion to a nucleotide and involves PBP1B

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Anti-virulence agents*, i.e.* drugs that selectively "disarm" pathogenic bacteria are considered as a promising strategy to combat the alarming issue of antibiotics resistance in acute and chronic infection causing-opportunistic pathogens. Several antimetabolites, like fluorocytosine (5-FC) have been shown to inhibit virulence factors' production in Gram negative bacteria such as *Escherichia coli*, yet their mechanism of action is not understood. In this work, we show that, at subinhibitory concentrations for growth, 5-FC impairs biofilm formation in laboratory E. coli strain MG1655, by reducing the expression of the *csg*BAC operon encoding curli fimbriae subunits. Conversion of 5-FC into fluoronucleotides is necessary for its antibiofilm activity, as inactivation of the *upp* gene, required for UMP synthesis from uracil, prevented 5- FC-dependent inhibition of biofilm formation and curli gene expression. Once turned into F-UMP, 5-FC might affect gene expression either via incorporation into nucleic acids, or by mimicking the allosteric inhibition by UMP of carbamoyl-phosphate synthetase enzyme (CarAB), involved in the first step of *de novo* pyrimidine biosynthesis. This in turn would result in perturbations of the intracellular pyrimidine pool, which has been described to downregulate curli expression. Indeed, exposure to 5-FC resulted in a ca. 2-fold reduction of UMP intracellular levels, while not affecting ATP. Consistently, expression of the de novo pyrimidine biosynthesis genes *car*B and *pyr*B were upregulated in the presence of FC. Thus, our results suggest that the antibiofilm activity of fluoropyrimidines be mediated, at least in part, by perturbation of the pyrimidine nucleotide pool. To identify molecular targets of 5-FC, we screened an *E. coli* genomic overexpression library and found that a short fragment of the *mrc*B gene was able to restore curli production in the presence of 5-FC, suggesting a potential link connecting peptidoglycan biosynthesis with curli production and biofilm formation.

Poster 10 – Torricella Viola

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Paolo Pesaresi

Non-Photochemical Quenching (NPQ) barley mutants: a valuable source of allelic variants to improve photosynthesis efficiency and yield in crops

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Increasing crop yield is one of the main challenges for ensuring food supply in a context where the global population is growing, and climate change threatens crop productivity. The manipulation of photosynthetic traits is still a widely unexplored field and shows great potential for crop improvement. When sunlight is absorbed in excess by leaves, defensive mechanisms against photodamage are activated. The non-photochemical quenching of chlorophylls (NPQ) is the faster photoprotective pathway that dissipates excess energy in the form of heat. Since the kinetics of NPQ activation/deactivation are not as prompt as fluctuations in light, the optimization of this process could lead to the increase of CO_2 fixation rate by minimizing the waste of energy through heat. One of the main components of NPQ is the xanthophyll cycle, where energy dissipation involves two special carotenoids named Violaxanthin and Zeaxanthin. The interconversion reaction between Violaxanthin and Zeaxanthin is catalyzed by two enzymes: Violaxanthin de-epoxidase (VDE), located in the thylakoid lumen, and Zeaxanthin epoxidase (ZEP), located in the chloroplast stroma. Allelic variants of these two genes could potentially lead to a faster adaptation to light changes.
Here, we report on the characterization of five barley mutants, isolated from the HorTILLUS mutant population, carrying SNPs in the *VDE* and *ZEP* genes. Mutated lines show no differences in growth and protein accumulation compared to the wild type, while changes in NPQ kinetics and CO_2 uptake were detected using a PAM fluorometer and IRGA gas analyzer. Furthermore, three of these mutants were tested in an automated growth chamber system which can simulate light fluctuations, while measuring the canopy CO_2 assimilation. With this system, we were able to detect differences in light use efficiency under changing light conditions. Furthermore, *in vitro* assays to test the enzymatic activity of the different allelic variants are currently set up to support physiological data, with the final aim to identify novel alleles able to optimize photoprotection and photosynthesis efficiency in crops.

Day 2: 13th October 2023

TALK SESSION I

- 14.15 “Role of circular-PVT1 in ischemic heart failure**” Bibi Alessia**
- 14.35 “Striatin knock out induces a gain of function of I_{Na} current in mESC-derived cardiomyocytes” **Cospito Alessandro**
- 14.55 “NF-YA overexpression and isoforms balance in Stomach Adenocarcinoma Claudin^{low} subtype” **Gallo Alberto**

15.15 Coffee Break

TALK SESSION II

- 15.45 “Role of NF-YA isoforms in breast cancer” **Londero Michela**
- 16.05 “Structural and functional study of AIF:CHCHD4 complex involved in respiratory chain biogenesis regulation” **Fagnani Elisa**
- 16.25 “Investigating the role of autophagy and Snap29 in C9orf72-linked ALS/FTD” **Smeele Paulien Hermine**

POSTER SESSION & AWARDS

- 16.45 **(Ballabio F., Burattin F.V., Pagani G., Palloni L.M.G, Pizzoccheri R., Polettini S., Scolz, A., Tiberi M., Zaccaria, M.)**

Talk sessions day 2

Talk 1 – Bibi Alessia

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Andrea Francesco Barbuti, Fabio Martelli

Role of circular-PVT1 in ischemic heart failure

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Introduction: Circular RNAs (circRNAs) are an emerging class of noncoding RNAs originating from the splicing and circularization of pre-mRNAs and long non-coding RNAs. CircRNAs have been found deregulated in several cardiovascular diseases, including heart failure (HF). However, incomplete and sometimes contradictory results have been reported on their regulation and function in HF, indicating that our understanding of the regulation and role of cardiac circRNAs is still very limited. We aim to identify new circRNAs candidates deregulated in ischemic HF and to functionally characterize them in HF.

Methods and results: We performed a high-depth RNA-seq of left ventricle (LV) samples of 20 ischemic HF patients and matched controls. qRT-PCR results confirmed the differential expression of 3 circRNAs, cSLC6A6, cMLIP and cHDCA9. To complement the unbiased strategy of RNA-seq, we evaluated the modulation of 19 candidate circRNAs identified in the literature as dysregulated in ischemic or not-ischemic cardiomyopathies or in biological mechanisms relevant for ischemic HF. qRT-PCR analysis showed that 6 of them were significantly upregulated in HF compared to controls (cANKRD17, cBPTF, cFBXO7, cNEBL, cPVT1, cSLC8A1) and 6 were significantly down-regulated (cALPK2, cHIPK3, cPCMTD1, cTTN29, cTTN275, cTTN90). Among validated circRNAs, we focused on cPVT1 for further characterization according to the extent of its increase in failing hearts compared to controls and to the circular/linear ratio fold increase in ischemic HF.

RNA-seq analysis of cPVT1-silenced AC16 cells identified differentially expressed genes that are mainly involved in the deposition of extracellular matrix and in cellular senescence, suggesting the involvement of cPVT1 in cardiac remodeling. cPVT1 knockdown in primary adult cardiac fibroblasts attenuated the expression of profibrotic markers upon TGF-β1 treatment, indicating a role of circPVT1 in cardiac fibrosis. We identified several miRNAs interacting with cPVT1. RNA pull down assay confirmed the interaction between cPVT1 and 3 fibrosis-related miRNAs, miR-125b-5p, miR-369-5p and miR-30. The levels of these miRNAs were not altered upon cPVT1 knockdown in the presence or absence of TGF-β1. However, miR-125b-5p, miR-369-5p and miR-30 respective targets were deregulated upon cPVT1 silencing, suggesting that decreased levels of cPVT1 could increase the cellular pool of bioavailable miRNAs.

Conclusions: We identified new deregulated circRNAs in ischemic HF patients that might play a pathogenic role in HF. circPVT1 is a circRNA upregulated in HF and it plays a role in cardiac fibrosis. circPVT1 binds to fibrosis-related miRNAs miR-125b-5p, miR-369-5p and miR-30, and could modulate their cellular bioavailability.

Talk 2 – Cospito Alessandro

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Andrea Barbuti

Striatin knock out induces a gain of function of I_{Na} current in mESC-derived cardiomyocytes

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Striatin (Strn), a scaffold protein expressed also in cardiomyocytes (CMs), whose altered expression has been found in various cardiac diseases. Here we studied the role(s) of cardiac *Strn* by comparing the electrophysiological properties of CMs, generated from Strn-KO and isogenic WT lines. 10-12-day old beating mESC-CMs were analyzed by Patch-clamp, motion video tracking, Ca^{2+} dynamics and immunofluorescence analysis. Strn-KO cells have a higher spontaneous beating rate and faster action potential dV/dt than WT, correlated with a larger fast I_{Na} conductance. Since in HEK cells downregulation of *STRN* was reported to destabilize microtubules and increase I_{Na}, immunofluorescence analysis confirmed the higher Na^+ channel expression and a more dynamic microtubule network in KO CMs. Motion video tracking analysis highlighted an altered contraction in Strn-KO CMs and this was associated with a global increase in intracellular Ca^{2+}. This was likely due to an increased late Na^+ current (I_{NaL}) and a reduction of Ca^{2+}extrusion through the Na^+/Ca^{2+} exchanger (NCX). I_{CaL}, I_f and I_{Kr} were not altered in Strn-KO cells. Incubation of Strn-KO CMs with the microtubule stabilizer Taxol, induced a reduction of I_{Na} conductance toward WT levels. In conclusion, loss of Strn alters CMs electrical and contractile profile and affects cell functionality by a disarrangement of multi-protein complexes leading to the impairment of microtubules dynamics and trafficking of Na^+ channels.

Talk 3 – Gallo Alberto

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Diletta Dolfini

NF-YA overexpression and isoforms balance in Stomach Adenocarcinoma Claudin^{low} subtype

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Claudin^{low} is a recently characterized highly invasive subtype of stomach adenocarcinoma (STAD), associated with a mesenchymal identity, low expression of claudins, and unfavorable patient prognosis. Mesenchymal fate determination in epithelial cancer cells is caused by the activation of Epithelial to Mesenchymal Transition (EMT), in which their phenotype is transformed by multi-layered regulations. Among these we find alternative splicing, where diverse transcripts are generated from the same gene. We previously reported how NF-YA, the DNA-binding subunit of the NF-Y pioneer transcription factor, is widely overexpressed in epithelial tumors. NF-YA major isoforms, NF-YA long (NF-YAl) and short (NF-YAs), differ in the inclusion of exon-3. Similarly to breast triple-negative, mesenchymal tumors, characterized by an increased expression of NF-YAl, gastric cancers with EMT features are consistently associated with higher NF-YAl/NF-YAs ratio (NF-YAr) levels, concomitant with decreased survival rates. In particular, Claudin^{low} showed the highest NF-YAl expression among STAD subtypes, while single cell RNA-seq data deconvolution revealed that primary tumors with high NF-YAr values included a larger proportion of cancer cells undergoing EMT. Additionally, a novel prognostic signature consisting of 158 genes shared between breast and gastric Claudin^{low} tumors was delineated, and we identified several RNA-binding proteins associated to high NF-YAr, validating RBFOX2 as promoting expression of NF-YAl. Collectively, these results confirmed the key role of NF-YAl in driving EMT in gastric cancer cells and shed light on the splicing factors potentially implicated in determining NF-YA isoform selection, and more broadly in Claudin^{low} distinct aggressiveness.

Talk 4 – Londero Michela

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Roberto Mantovani

Role of NF-YA isoforms in breast cancer

Michela Londero [1], Alberto Gallo [1], Camilla Cattaneo [1], Anna Ghilardi [1], Mirko Ronzio [1], Luca Del Giacco [1], Roberto Mantovani [1] and Diletta Dolfini [1]

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NF-Y is a pioneer transcription factor that has an essential role in the transcriptional regulation of genes involved in metabolism, cell cycle progression, ER stress and differentiation. It is an heterotrimer composed of three subunits: NF-YA, NF-YB and NF-YC. The regulatory subunit NF-YA confers sequence-specificity to the complex, recognizing and binding the CCAAT box element, found enriched in cancer- promoting genes. NF-YA primary transcript matures in two isoforms called NF-YAl (long) and NF-YAs (short); the first retains Exon 3, the latter excides Exon 3. In the literature emerged that NF-YAl/NF-YAs ratio changes in different tissues, cell lines and at distinct differentiational stages. A robust increase in mRNA levels of NF-YA has been observed in different tumors of epithelial origin, among which breast cancer (BRCA). In TCGA breast cancer samples and breast cancer cell lines, NF-YAs isoform is found highly expressed in Luminal, HER2 and Basal-like Claudin^{high} subtypes, while in Basal-like Claudin^{low} (the most aggressive, prone to metastasize and associated to a worse prognosis) NF-YAl isoform is predominant. We focus on the investigation of the NF-YA isoform contribution to the different cancer phenotypes employing two different Basal-like Claudin^{low} cell lines that mainly express NF-YAl and show a mesenchymal identity: SUM159PT and BT549. Through CRISPR/Cas9 nickase genome editing system we force the unique NF-YAs expression by NF-YA Exon 3 DNA-excision; two homozygous Exon 3 deleted clones (NF-YAl-KO) for each cell lines are obtained and validated and analyzed with qualitative observations and quantitative analysis both *in vitro* and *in vivo*. *In vitro* NF-YAl-KO clones' cellular migration and invasion abilities resulted significantly impaired compared to the control samples in both cell lines. In agreement with what is observed *in vitro*, *in vivo* experiments in zebrafish embryos assess a significantly reduced invasiveness and metastatic abilities of edited clones compared to the controls. To investigate the molecular pattern responsible for the altered migratory phenotypes we perform RNA-seq of NF-YAl-KO clones and controls. Collectively downregulated pathways in NF-YAl-KO clones have been identified, among which EMT process, suggesting a less aggressive behavior of edited clones. These results support our hypothesis that correlates NF-YAl expression with invasiveness and metastatic features.

Talk 5 – Fagnani Elisa

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Cycle: XXXVI A.Y. 2020 – 2021

Supervisor: Alessandro Aliverti, Mario Milani

Structural and functional study of AIF:CHCHD4 complex involved in respiratory chain biogenesis regulation

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Apoptosis Inducing Factor (AIF), a highly conserved mitochondrial flavoprotein, is generally known as a caspase-independent effector in the intrinsic apoptosis pathway [1]. Beside this apoptotic function, recent studies demonstrate that AIF is also able to regulate the cell energy homeostasis by promoting the biogenesis and the function of multi-subunit respiratory complexes. Although the underlying molecular mechanisms have not been yet elucidated, it is clear that this role is played thanks to the interaction of AIF with CHCHD4, a soluble inner membrane space (IMS) protein which promotes the entrance in the IMS and the oxidative folding of substrates belonging to the respiratory complexes' subunits [2,3]. Given the interest in deeply understanding the AIF vital role in mitochondria, we decided to investigate the AIF-CHCHD4 interaction from both the functional and the structural point of view. We focused on the study of the possible impact of the 27-residues N-terminal portion of CHCHD4, which effectively mimics its binding site for AIF, on the catalytic activity and NAD+-binding ability of AIF. The peptide turned out to stimulate the DCIP-NADH reductase activity of AIF and the apparent Kd of the AIF-peptide complex was estimated in the sub-micromolar range. Another interesting finding was the ability of AIF to bind NAD+ only in the presence of the peptide. Preliminary experiments indicates that NAD+ complexation is strongly stimulated by lowering the temperature and that such effect is secondary to the AIF-peptide interaction. For the determination of relevant structural features of the complex, we used AlphaFold software to build up a model, which was then experimentally confirmed by mutagenesis experiments on AIF and mass-spectrometry analyses on the crosslinked AIF-CHCHD4 complex. Since peptide and NAD+ bindings display strong positive cooperativity, we used this information to set up the optimal conditions for X-ray diffraction studies through which we obtained the crystal structure of the complex.

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Talk 6 – Smeele Paulien Hermine

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Cycle: XXXV A.Y. 2019 – 2020

Supervisor: Thomas Vaccari

Investigating the role of autophagy and Snap29 in C9orf72-linked ALS/FTD

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Disruptions of autophagy are observed in several neurodegenerative diseases, including *C9*-linked amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD). *C9*-linked ALS/FTD is caused by a *G4C2* hexanucleotide repeat expansion (HRE) in the *C9orf72* locus and constitutes the most common genetic cause of the diseases. Among other disruptions, the RNA and Dipeptide Repeat (DPR) species that are generated by the *G4C2* HRE appears to impair the nuclear pore complex, thereby disrupting the nucleocytoplasmic transport of TFEB, the master regulator of autophagy and lysosomal biogenesis. How TFEB mislocalisation is connected with mTOR signalling and autophagy regulation during *C9*-linked ALS/FTD pathogenesis, however, remains unclear. We therefore aim to address this question using well-established *Drosophila melanogaster* models of *C9*-linked ALS/FTD. In line with previous findings, we observe a reduction in nuclear localisation of Mitf (*Drosophila* TFEB) upon overexpression of *G4C2* repeats, as compared to controls. Surprisingly, in the adult head, Mitf appears to accumulate at the protein level, predominantly in the inactive, cytoplasmic form. These results suggest that proteasomal degradation of inactive Mitf or indeed its inactivation - mediated by mTOR - may additionally be disrupted by *G4C2* toxicity. We are further investigating these possibilities and exploring genetic modulators of Mitf in the context of *G4C2* toxicity. Importantly, we find that reducing levels of the SNARE protein *Snap29* leads to alteration in Mitf localisation and regulation. Further, by performing a genetic interaction in the adult fly eye, we show that reducing *Snap29* levels either by RNAi or by a heterozygosity, strongly suppresses *G4C2* toxicity. We are currently dissecting how Snap29 may prevent *G4C2*-induced toxicity and disruptions of Mitf activity. We are also interested in ascertaining whether such effects involve the regulation of autophagy or mTOR signalling.

Poster session day 2

Poster 1 – Ballabio Federico

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Carlo Camilloni

Integrative modelling for the characterisation of the structure and dynamics of biomolecules

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Combining experimental data with computational methods has emerged as a successful strategy for the characterisation of the structure and dynamics of biomolecules, enhancing the predictive capabilities and the accuracy in the description of molecular mechanisms to the atomistic scale. Here, we present three projects where the synergy of this integration has yielded insights across various disciplines.

Project 1: An accurate and efficient SAXS/SANS implementation including solvation layer effects suitable for molecular simulations.
Combining Small-Angle X-ray and Neutron Scattering (SAXS/SANS) techniques with Molecular Dynamics (MD) simulations has become a powerful approach to increase the resolution of the former and the accuracy of the latter. In particular, this method allows the generation of conformational ensembles or the refinement of structures at atomistic resolution using the experimental data as a restraint. This project addresses the challenge of the high computational cost associated with the *in silico* calculation of scattering intensities. We developed a method that is fast but accurate, compatible with SAXS and SANS, and that can be used for proteins and/or nucleic acids. As case studies, we used SAXS data measured for full-length human gelsolin in closed state and for the UP1-RNA complex to show that such an approach can refine the structure and dynamics of proteins and nucleic acids.

Project 2: Structural characterisation of the barley pale green mutant *TM2490.*
The study, conducted in collaboration with the group of Prof. Paolo Pesaresi, focuses on the barley mutant *TM2490*. This population exhibits altered photosynthetic parameters and a pale green colouration but retains wild-type-like growth and morphology. This trait increases the availability of photons in the lower leaf layers and reduces the energy dissipated as heat by leaves exposed to direct sunlight. The group identified the mutation responsible for *TM2490* phenotype in *ChlI* subunit of magnesium chelatase, an enzyme involved in chlorophyll synthesis. Despite the lack of structural information for the barley ChlI subunit, we built an all-atom model using computational tools in combination with experimental data from homologous structures. Furthermore, by extending the analysis to non-photosynthetic organisms, we inferred critical interactions between highly

conserved residues. This allowed us to rationalise the mutation and formulate hypotheses about its role in the context of the observed phenotype.

Project 3: *De novo* binders design for beta-2 microglobulin.

The group of Prof. Stefano Ricagno showed that beta-2 microglobulin (B2M) plays a critical role in the progression of multiple myeloma. B2M is internalised via phagocytosis by tumour-associated macrophages, leading to its accumulation in lysosomes where it aggregates into amyloid fibrils. The resulting damage triggers pro-inflammatory responses that support tumour progression. The group is currently investigating B2M aggregation to explore strategies to inhibit fibril formation. We joined this research with the aim of rationalising potential binding mechanisms to B2M. We employed RoseTTAFold Diffusion (RFD), a deep-learning framework based on denoising diffusion models, capable of generating *de novo* protein binders with high affinity to a specific target. We used RFD to generate 20 binders for B2M, which underwent stability analysis using MD-based approaches. The sequences of the most promising candidates were provided to the group of Prof. Ricagno for the expression, purification, and evaluation of the binders.

Poster 2 – Burattin Filippo Vittorio

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Cycle: XXXVII A.Y. 2021 – 2022

Supervisor: Beatrice Bodega

T cell quiescence is developmentally tuned by mTORC1/LINE1 regulatory axis

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Neonatal T cells are considered a unique developmental layer as they lack memory T lymphocytes and are prevalently composed by naïve T cells. Neonatal naïve T cells upon activation display early entry in the cell cycle and enhanced proliferation respect to adult's, foreseeing an unusual preparedness for activation. However, the molecular mechanisms responsible for this rapid mobilization of T cells to antigens in neonatal life remain obscure. The regulation of adult naive CD4 + T cells quiescence involves the splicing of LINE1 elements (DNA repeats) in novel alternative transcript variants. Upon TCR activation, LINE1 are downregulated under the control of mTORC1 via a splicing suppression mechanism mediated by PTBP1, while the corresponding canonical transcripts are expressed. Here we sought to explore LINE1 expression and function in the regulation of neonatal naïve CD4+ T cells preparedness to activation. We have discovered that neonatal naïve CD4+ T cells show a reduced content of LINE1. We found that tonic, under-threshold signals, transduced from the highly self-reactive TCR found on neonatal naïve T cells, induce basal mTORC1 activity and promote LINE1 splicing suppression. LINE1 downregulation promotes the expression of the corresponding canonical transcripts involved in TCR signaling, sustaining mTORC1 activation and creating a positive feedback loop. Furthermore, we found that LINE1 downregulation supports neonatal naïve CD4+ T cells preparedness to activation by regulating at transcriptional level genes involved in mRNA translation and cell cycle progression. Therefore, neonatal naïve CD4+ T cells quiescence is less enforced, having increased ATP production and basal protein synthesis. Our study identified a novel function for LINE1 in T cell development, by establishing a feedback loop with tonic TCR stimulation to regulate a primed-yet quiescent state instrumental for a rapid immunological response, essential in a naïve – memory free – immune system.

Poster 3 – Pagani Giulia

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Supervisor: Paolo Gandellini

NFYA alternative cleavage and polyadenylation in cancer

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The majority of human genes undergo alternative cleavage and polyadenylation (APA) regulating 3'UTR length and accessibility, thus affecting mRNA stability and protein level by altering microRNA and RNA-binding protein (RBP) activity. *NFYA* is an oncogene encoding for the regulatory subunit of the nuclear transcription factor Y, which controls key transcriptional changes in pathways broadly deregulated in cancer. *NFYA* is strongly overexpressed in human tumors and the poor correlation between NF-YA protein and mRNA in different cancer types suggests that APA may be as relevant as other mechanisms to control NF-YA activity in cancer. Concerning this, our bioinformatic analyses identified multiple APA sites resulting in four possible *NFYA* 3'UTRs, not yet correctly annotated. We observed that tumor and immortalized cells mainly use shorter 3'UTRs compared with normal cells, which prevalently use longer ones. In this regard, we found an increased NF-YA protein/mRNA ratio to be associated with higher usage of shorter 3'UTRs. Moreover, the effect of different *NFYA* 3'UTRs on transcript stability, protein level, and microRNA and RBP binding is under study employing different strategies to alter *NFYA* 3'UTR usage. We found that increased usage of shorter *NFYA* 3'UTRs, induced either exogenously or endogenously, is associated with higher NF-YA protein/mRNA ratio in prostate adenocarcinoma cells. Thus, *NFYA* APA can be a post-transcriptional mechanism important to control NF-YA activity in cancer. Notably, the emerging application of ASOs in the clinics opens the possibility to target/mask polyadenylation signals, microRNA or RBP binding sites to therapeutically manipulate the effects of APA in cancer. Overall, our studies intend to characterize *NFYA* APA in the cancer context from a functional and biological point of view.

Poster 4 – Palloni Luca Maria Giovanni

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Supervisor: Anna Moroni

AMPK modulates HCN4 channels through direct phosphorylation of the serine 1157

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The "funny current" (I_f) is a mixed Na^+/K^+ current expressed in cardiac cells of the sinoatrial node (SAN), inward and activated on hyperpolarization, that contributes essentially to generation of spontaneous pacemaker activity and heart rate regulation. Molecular components of native I_f, representing pore-carrying α-subunits, are the HCN4 channels. While the short-term modulation of HCN4 channels by cAMP has been extensively studied, long-term events that affect HCN4 trafficking and membrane expression are mostly unknown. Recent studies show that the AMP-activated protein kinase (AMPK) is involved in the modulation of mice heart rate due to a decrease in the I_f amplitude, but the mechanism underlying this reduction is still unclear. We identified the serine 1157 as the putative AMPK phosphorylation site on HCN4 and confirmed the presence of phosphate groups on this residue by mass spectrometry analysis. In addition, pharmacological activation of AMPK though the activator AICAR 1 mM (3.5 hours) reduced the amplitude of I_f in mice SAN cells and of I_{HCN4} in HEK293T cells transiently transfected with hHCN4. The same treatment did not affect the current of HEK293T cells transfected with the non-phosphorylatable mutant hHCN4 S1157A, supporting the hypothesis of a direct phosphorylation of this residue.

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Poster 5 – Pizzoccheri Roberto

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Supervisor: Federica Briani

Application of model systems to characterise polynucleotide phosphorylase disease-linked variants

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Polynucleotide phosphorylase (PNPase) is a trimeric phosphorolytic RNA exonuclease highly conserved from Bacteria to Eukarya. In humans, PNPase (hPNPase) localises within mitochondria, and it is implicated in RNA import from cytoplasm, RNA degradation and R-loops processing. Mutations in the hPNPase-encoding gene are associated to disorders with a wide spectrum of symptoms and severities, ranging from hereditary hearing loss to multisystem diseases. Correlation of specific mutations to the protein molecular defects and the clinical symptoms is not an easy task due to the plethora of processes involving hPNPase and the small number of patients and their heterogenous genetic backgrounds.

In this work, we address hPNPase sequence-function correlation *in vitro* and in a bacterial model generated by expressing in *E. coli* hPNPase and the pathological variants P140L, Q387R, E475G and M745T. Preliminary *in vitro* data show that hPNPase binds and degrades ssDNA like its bacterial orthologue. All mutants but M745T have reduced RNA and ssDNA binding, whereas only the Q387R displays reduced RNA degradation activity. In the *E. coli* model*,* hPNPase causes SOS response activation, R-loop accumulation, and increased ROS production; furthermore, the expression of hPNPase worsens phenotypic traits typical of the *E. coli* Δ*pnp* strain lacking the orthologous EcPNPase. Hundreds of *E. coli* RNAs are stabilized in presence of hPNPase, with only few transcripts destabilized, suggesting that in *E. coli,* hPNPase may bind but not degrade the RNA. Strains expressing mutant hPNPases are phenotypically more similar to the Δ*pnp* mutant than to the hPNPase-expressing strain, in agreement with their decreased RNA binding activity observed *in vitro*. Moreover, R-loop levels are reduced in the Q387R and E475G mutants. We are currently testing in a bacterial two-hybrid system whether the mutations interfere with the interaction between hPNPase monomers.

Finally, we are generating custom human cell line models in HEK293-T and SH-SY5Y cells.

Poster 6 – Polettini Sofia

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Supervisor: Diletta Dolfini

Dissecting the Role of NF-YA Transcription Factor in Mesodermal Differentiation of mouse Embryonic Stem Cells

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The balance between pluripotency and differentiation governs embryogenesis and is finely controlled at transcriptional level by a handful of transcription factors (TF). The regulation of stemness maintenance is well characterized, while the molecular mechanisms implied in differentiation remain poorly understood in large part. Our study focuses on the role of NF-Y in these processes. As a pioneer TF, NF-Y promotes chromatin accessibility on genes' promoters and enhancers. The regulatory sequence-specific subunit of this complex, NF-YA, drives its binding to the CCAAT box, a DNA element enriched in genes specifically active in embryonic stem cells (ESCs). Two isoforms of NF-YA are generated by Alternative Splicing of Exon 3: the short (NF-YAs) and the long (NF-YAl) isoforms were demonstrated to display identical DNA-binding properties *in vitro,* but they exert distinct functions in several biological processes. The opposite expression pattern of NF-YAs and NF-YAl in mESCs differentiating through Embryoid Bodies (EBs) confirmed the already known role of NF-YAs in stemness regulation and proposed a novel function of NF-YA long in cell lineage decisions. We generated clones of two mESC lines deleted in Exon 3 of NF-YAl and characterized their capability to differentiate toward mesoderm. The forced expression of NF-YAs in deleted mESCs caused a reduced expression of mesendodermal and mesodermal TFs during differentiation process, as demonstrated by RNAseq. The identification of cells subpopulations differentiating in wt and YAl-KO EBs by scRNA-seq showed a strong under-representation of mesodermal cells with a large number of pluripotent cells in loss of NF-YA long. Finally, thanks to CUT&RUN experiments on mESCs and differentiated EBs, we discovered isoform-specific targets that are regulated by the short or by the long isoform of NF-YA. Overall, our data reveal that the switch in expression of the two isoforms is necessary to induce differentiation: the increase in NF-YAl expression, combined with a decrease in NF-YAs, is critical for early and late mesodermal formation. This shed light on the distinct functions of the two isoforms in the regulation of stemness maintenance and pluripotency exit in favour of cell fate decisions.

Poster 7 – Scolz Andrea

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Supervisor: Elena Cattaneo

ADAM10 (dys)function at the Huntington's Disease cortico-striatal synapse

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Huntington's Disease (HD) is caused by a CAG repeat expansion in the Exon1 of the Huntingtin (HTT) gene. The striatum is the most vulnerable region, but prominent pathological alterations are visible also in the cortex. Indeed, multiple lines of evidence point to cortical dysfunctions and impaired cortico-striatal connection as early events in HD. As a result, presynaptic cortical dysfunction represents a very attractive site of action for developing new therapeutic approaches.

Recent studies from our group have demonstrated that a novel HTT interactor, A disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), is implicated in HD synaptic dysfunction. Indeed, wild-type HTT regulates glutamatergic synapse remodeling by binding to ADAM10 and inhibiting its proteolytic activity on N-Cadherin (N-CAD). Mutant HTT binds less to ADAM10, which accumulates at the synapse weakening synaptic communication *via* excessive N-CAD proteolysis leading to cognitive decline in HD mice. Intriguingly, inhibiting ADAM10 rescues N-CAD proteolysis and prevents synaptic and cognitive defects (*Vezzoli et al., 2019*).

Recently, we revealed that ADAM10's interactome shares presynaptic binding partners with HTT, including vesicle transport/release regulators. These findings suggested a novel and unexpected role of ADAM10 in orchestrating vesicle transport/release at the presynaptic district. We discovered that the level of active ADAM10 increases in the HD cortex's presynaptic fractions, resulting in reduced synaptic vesicles. Notably, synaptic vesicle storage was restored by normalizing ADAM10 activity (*Cozzolino et al., 2021*).

Based on this novel role of ADAM10 at the presynapse, we propose that cortico-striatal circuitry defects, and striatal pathology, could mostly depend on mutant HTT detrimental impact on presynaptic ADAM10 in cortical afferents. To this aim, we reconstructed the cortico-striatal circuitry *on-a-chip* exploiting a microfluidic-based platform that allows to inhibit ADAM10 separately in the pre- (cortical) and post-synaptic (striatal) compartments. The ADAM10 inhibitor GI254023X was administered to cortical and striatal neurons independently, and cortico-striatal functionality was assessed.

Poster 8 – Tiberi Michele

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Supervisor: Marco Nardini

Structural Insights into the Nuclear Factor I X-DNA Complex Revealed by Cryo-Electron Microscopy

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Nuclear Factor I X (NFIX) is a vital transcription factor known for its essential role in various cellular processes, including neural development, skeletal muscle development and regeneration and tissue-specific gene regulation. In the context of muscular dystrophy, recent studies have demonstrated that the absence of NFIX in skeletal muscle leads to a slower degeneration of the dystrophic phenotype. Although the crystal structure of NFIX DNA-binding domain has been previously elucidated, the structural details of its interaction with target DNA remained unknown. In this PhD project, we undertook the challenging task of determining the structure of NFIX in complex with its target DNA. The initial crystal structure of NFIX provided crucial insights into the protein overall fold and conformation, but the absence of DNA limited our understanding of its functional mechanisms. To unravel the structure of the NFIX-DNA complex, we employed cryo-electron microscopy (cryo-EM) techniques, through which we successfully solved the high-resolution structure of the NFIX-DNA complex. Our findings shed light on the molecular basis of NFIX DNA recognition and binding, elucidating the key residues and structural motifs involved in this crucial interaction. The determination of the NFIX-DNA complex structure not only contributes to the fundamental knowledge of transcriptional regulation but also has potential implications for various fields, such as studies aimed at modulating NFIX activity for therapeutic purposes. Our findings provide a solid foundation for further research into the functional mechanisms of NFIX and its role in gene regulation.

Poster 9 – Zaccaria Marta

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Supervisor: Alessandra Polissi

Cell envelope homeostasis: role of peptidoglycan remodelling factors to survive outer membrane stress in *Escherichia coli*

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Gram-negative bacteria have a unique cell envelope consisting of a lipopolysaccharide-containing outer membrane (OM) that is covalently linked to the thin layer of peptidoglycan (PG). The OM serves as a barrier against toxic molecules including many antibiotics and allows the cells to survive in many environmental stress conditions. The growth of OM and PG layers needs to be tightly coordinated. Our laboratory recently found that when the OM biogenesis is compromised a PG remodeling program is required to avoid cell lysis. In *Escherichia coli* cells this modification program relies on the activity of LD-transpeptidase family proteins that introduce the non-canonical 3-3 cross-links in the PG layer to restore the mechanical strength and the overall stability of the bacterial cell envelope. Among the member of this family, DpaA is the enzyme that detaches Lpp from PG. Notably, Lpp is the abundant *E. coli* OM lipoprotein that covalently links the OM to the PG. Previous works of our laboratory have shown that a mutant deleted for ***dpaA*** undergoes lysis when LPS transport to the OM is blocked. However, the lysis phenotype of the lipopolysaccharide defective *dpaA*-deleted mutant is suppressed by the deletion of *actS* which codes for an activator of amidases, the enzymes that hydrolyze septal PG during cell separation. Our goal is to understand the interplay between *dpaA* and *actS* to better define the physiological role of *dpaA* under envelope stress conditions and the biological meaning of Lpp dynamic attachment to the PG.