
I2BC
YOUR I2BC
YOUng Researchers of I2BC
PHD DAYS
2023
POSTER SESSION & ORAL PRESENTATIONS
25 & 26 MAY
9 am to 5 pm
bldg 21
Auditorium
Gif-sur-Yvette

Planning PhD days 25th may 2023

9h15-9h45: Entrance / coffee

9h45-10h00: Welcome talk

10h-10h30: Keynote 1 – **Valérie OUARY**, project coordinator at C2R

Epidemiology 10h30-10h45: Coffee break

10h45-12h15: Session 1

- **Ilaria PONTISSO** (team TRAN VAN NHIEU) : Combining experimental and modeling approaches to explore the interplay between moderate ER CA2+ depletion and activation of Unfolded Protein Response C18
- **Pierre DELAMOTTE** (team MONTAGNE) : Investigating metabolic heterogeneity in genetically induced tumors in Drosophila midgut
- **Lucas LEVERNE** (team LEIBL / KRIEGER-LISZKAY) : State transition mutation increased drought resistance
- **Yijie ZHANG** (team BAYAN) : Identification of the mycoloylome in *C. glutamicum*

12h15-13h30: LUNCH (cafeteria Gif)

13h30-15h: Poster session 1

15h-16h30: Session 2

- **Mengyuan LI** (team MALAGNAC) : Deciphering the roles of jumonji domain containing proteins in *Podospora anserina*
- **Taher YACOUB** (team GAUTHERET) : Design of aptamers against BACE1 enzyme involved in Alzheimer's disease

-**Tamara YEHOUESSI** (team DELERIS): Determinants of PRC2 and H3K27me3 recruitment on Transposable Elements (TE) in the flowering plant Arabidopsis thaliana

- **Perla AKIKI** (team MONTAGNE) : Understanding the physiological basis linking social environment to tumor growth in *Drosophila melanogaster*
- **Vahiniaina ANDRIAMANGA** (Team LESPINET) : Exploring the evolution of metabolic networks in fungi

16h30-17h: Goûter

17h-18h: Keynote 2 – **Elisabeth BIK**, expert on science integrity

Planning PhD days 26th may 2023

10h15-10h45: Entrance / coffee

10h45-12h15: Session 3

- **Valentin GUYARD** (team GIORDANO) : Functional crosstalk between ORP5/8 and seipin at ER mitochondria contact sites involved in lipid droplet biogenesis
- **Sara DENDENE** (team FAURE / MERGAERT) : FcrX, a new global regulator of cell cycle in free living conditions and during symbiosis in *Sinorhizobium meliloti*
- **Anastasiia SKOBELKINA** (team CHARBONNIER / ZINN-JUSTIN) : BRCA2 interactions through intrinsically disordered regions: focus on the role of phosphorylation and condensate formation in mitosis
- **Tianyue WEI** (Team BAYAN) : Surface functionalization of non-woven polypropylene (PP) using plasma treatment: chemical and biological approaches to induce antimicrobial properties

12h15-13h30: LUNCH

13h30-15h: Poster session 2

15h-16h30: Session 4

- **Romain JOUAN** (team FAURE / MERGAERT) : The fitness landscape of the insect gut symbiont *Caballeronia insecticola* in diverse environments
- **Rémi RUEDAS** (team BRESSANELLI) : Integrative approach for cryo-EM structural study of flexible antibody-antigen complex
- **Maxime BARRAULT** (team BOULOC) : sRNA-mediated control of citrate metabolism upon iron starvation in *Staphylococcus aureus*
- **Logan GREIBILL** (team TASSIN) : Functional analysis of MAP250, a novel partner of the deubiquitinase CYLD, during ciliogenesis.
- **Murielle SEIF EL DAHAN** (team CHARBONNIER) : Molecular machineries of the human DNA double-strand break repair pathway : Towards the assembly of the Ku-DNA-PAXX complex

16h30: End of the seminar / cocktail

25th may Poster session 1

1. **Amélie Besombes**, “Bidirectional replication initiation prevents chromosome degradation”, *Genome Biology*
2. **Liza Boeffard**, “Identifying interactions between proteins and excipients using NMR, and their consequences on viscosity/injectability” *Biochemistry, Biophysics & Structural Biology*
3. **Julia Buggiani**, “Impact of protein acetylation on nuclear protein quality control and biocondensate”, *Genome Biology*
4. **François Charon**, “Organization of the 3D chromatin structure & nuclear lamina association of the Dlk1-Dio3 imprinted domain in mouse”, *Genome Biology*
5. **Hélène Chérot**, “Structural characterization of disordered proteins by In-cell NMR”, *Biochemistry, Biophysics & Structural Biology*
6. **Lucie Gomes**, “Genetic study of a specialized bacterial group II intron retrotransposon”, *Genome Biology*
7. **Umama Hani**, “Study of Photosynthetic Electron Flow under Mn deficiency in *Marchantia polymorpha*”, *Biochemistry, Biophysics & Structural Biology*
8. **Estelle Leroy**, “Antagonism between small and long non-coding RNAs tunes the mitosis to meiosis transition in fission yeast”, *Genome Biology*
9. **Yingyue Luo**, “Characterization of the phospho-dependent interactions between full-length Mdm2 and p53”, *Biochemistry, Biophysics & Structural Biology*
10. **Vincent Morin,** “Molecular mechanisms of the main DNA double-strand break reparation pathways”, *Biochemistry, Biophysics & Structural Biology*
11. **Alexia Royer**, “Specificity of interactions between the *Clostridioides difficile* SlpA protein and its bacteriophages”, Microbiology
12. **Rémi Ruedas**, “Integrative approach for cryo-EM structural study of flexible antibody-antigen complex”, *Biochemistry, Biophysics & Structural Biology*
13. **Xiaofen Wu**, “Pathogenic virus control of host viability at the level of the ribosome exit tunnel”, *Genome Biology*
14. **Dong Xie**, “Challenging the unique N-terminal modification pathway of plant RuBisCO”, *Genome Biology*

26th may Poster session 2

15. **Adriana Badilla**, “Lobo Characterization of a family of small proteins regulated by second messenger-binding riboswitches in *Clostridioides difficile*”, Microbiology
16. **Clément Fauchereau**, “Classification of anesthesia state using cardiac features and machine learning” *Genome Biology*
17. **Aurelie Favarin**, “In vitro reconstitution of the mechanosensitive α-catenin-vinculin complex of adherens junctions”, *Cell biology*
18. **Armelle Gesnik**, “Structural study of gene rearrangement mechanisms driven by transposases in collaboration with the c-nhej pathway in *Paramecium tetraurelia*”, *Biochemistry, Biophysics & Structural Biology*
19. **Mechri Hanane**, “Characterization of secreted bacterial factors involved in Pneumococcal meningitis”, *Cell biology*
20. **Tom Mariotte**, “Thermococcales Iron sulfide Biomineralization”, *Microbiology*
21. **Guillaume Martin**, “How do growth responses to elevated temperatures affect nitrate uptake, transport and assimilation in *Arabidopsis thaliana*?”, *Cell biology*
22. **Roza Mohammedi**, “Role of the CckA-ChpT-DivL complex in the phosphorylation of the master regulator CtrA during the cell cycle and nitrogen-fixing symbiosis in *Sinorhizobium meliloti*”, *Microbiology*
23. **Orlando Moranchel,** “Acquisition of the cholera toxin is favored by the presence of the TLC prophage in *Vibrio cholerae*”, *Genome Biology*
24. **Sokrich Ponndara**, “Unraveling the Interplay between the ter region of the chromosome and Virulence Plasmids in *Salmonella*”, *Genome Biology*
25. **Manon Soleil**, “Nucleosomal and subnucleosomal organisation of gene promoters in mammalian cells”, *Genome Biology*
26. **Kristian Want**, “Unravelling the Complex Role of Ferredoxin-2 and Frataxin in Iron-Sulfur Cluster Biosynthesis: New Insights from Protein Binding and Functional Studies”, *Biochemistry, Biophysics & Structural Biology*
27. **Dong Xie**, “Challenging the unique N-terminal modification pathway of plant RuBisCO”, *Genome Biology*
28. **Kenza Yefsah**, “The role of SUMO Targeted Ubiquitin Ligases in meiosis, in Sordaria macrospora”, *Genome Biology*

Ilaria PONTISSO[1], Roberto ORNELAS-GUEVARA[2], Eric CHEVETC[3], Geneviève DUPONT[2], Laurent COMBETTES[1]

[1]U1282 "Calcium signaling and microbial infections", Institut de Biologie Intégrative de la Cellule (I2BC) - Université Paris-Saclay, Gif-Sur-Yvette, 91190, France. [2]- Unit of Theoretical Chronobiology, Université Libre de Bruxelles (ULB), 1050 Brussels, Belgium c- Inserm U1242 Université de Rennes-1, 35042, Rennes, France

Combining experimental and modeling approaches to explore the interplay between moderate ER CA2+ depletion and activation of Unfolded Protein Response C18

The Endoplasmic Reticulum (ER) is the primary site of folding and quality control of many cellular proteins and the major intracellular Ca2+ store. Depletion of luminal Ca2+ disrupts the correct folding environment leading to an alteration of ER homeostasis and accumulation of misfolded proteins inside the lumen. To restore ER proteostasis and normal cellular functions, cells have developed an adaptive mechanism referred to as the Unfolded Protein Response (UPR). This leads to an increase of the protein folding capacity of the ER and to homeostasis restoration. Although long-term and strong UPR activation are much studied, the consequences of small amplitude, more physiological, luminal Ca2+ depletions on the early activation of UPR remain largely unexplored. In this study, we investigate how moderate Ca2+ depletion impacts on the activation of the signaling pathways of the UPR. Combination of Ca2+ imaging and UPR activation experiments with a data-driven computational model allows us to decipher, formalize, and quantify these complex signaling pathways. The model in turn predicted the possibility or UPR reversion, which was validated experimentally. This better understanding of the reciprocal crosstalk between Ca2+ and UPR will provide insight into the mechanisms of progression of the many diseases associated with slight ER stress.

Pierre DELAMOTTE [1], M. POIDEVIN [1], P. AKIKi [1], J. MONTAGNE [1]

[1]I2BC/CNRS Paris Saclay

Investigating metabolic heterogeneity in genetically induced tumors in Drosophila midgut

Cancers are usually considered of monoclonal origin, i.e arising from a unique cell, and their energy metabolism considered relying on glucose fermentation to lactate rather than on mitochondrial respiration. Either characteristic has been challenged by a number of studies. The goal of my PhD project was to decipher metabolic needs of a Drosophila midgut tumor model and to demonstrate their polyclonality. These tumors are induced at a given time and express GFP, facilitating their monitoring. They result from the loss of the tumor suppressor Apc (Adenomatous polyposis coli) and the ectopic expression of an oncogenic form of Ras (RasV12). These two genetic events are typically found in human colorectal cancers. By RNA-interference knockdown targeted to the tumor cells, we have evaluated the requirement of 60 metabolic enzymes and follow the tumor polyclonality. Our results suggest that despite their genetic identity these tumors are always polyclonal and exhibit a metabolic heterogeneity. We propose that these intestinal tumors arise from several clones that fulfill different metabolic functions, thereby allowing tumor progression.

Lucas LEVERNE [1], Thomas ROACH [2], François PERREAU [3], Fabienne MAIGNAN [4], Anja KRIEGER-LISZKAY [1] cedex, France)

[1]Université Paris-Saclay, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, 91198 Gif-sur-Yvette cedex, France [2] University of Innsbruck, Department of Botany, Innsbruck, 6020, Austria [3]Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (IJPB), 78000, Versailles,France. [4] Laboratoire des Sciences du Climat et de l'Environnement, LSCE/IPSL, CEA-CNRS-UVSQ, Université Paris-Saclay, Gif-sur-Yvette, France

State transition mutation increased drought resistance

Climate change is inducing an increase in extreme events such as high temperatures and droughts. According to the 2021 IPCC report, unlike heatwaves, droughts can last for several years, destroying entire crop yields and discouraging farmers from replanting. Therefore, it is important to understand the mechanisms of plant response to drought stress. In this study, we used the model plant A.thaliana to study drought stress. Mutants were studied which are mutated in state transitions, a mechanism allowing to adjust the antenna size by migration of LHCII in between photosystems. The mutations of the kinase stn7 and chloroplast acetylase (nsi1 & nsi2 [1]) disturb the transfer of LHCII from PSII to PSI. As a consequence, this leads to a modification of the reduction of the plastoquinone pool as shown by chlorophyll fluorescence. Higher levels of singlet oxygen were generated in the light in the mutants compared with the wild type. Surprisingly, we observed better resistance to moderate drought stress and faster root development in the state transition mutants. Through pharmacological approaches, we were able to demonstrate the importance of the redox state of the plastoquinone for root development. The signaling pathway and molecules involved in signaling between chloroplasts and roots have not yet been identified. Like singlet oxygen and singlet oxygen derived carotenoid oxidation products are involved in the signalling pathway. β-cyclocitral, a product of β-carotene oxidation by singlet oxygen and known for promoting root growth [2], is suspected to be an important signal promoting root development.

1 Koskela MM, Brünje A, Ivanauskaite A, Grabsztunowicz M, Lassowskat I, Neumann U, Dinh TV, Sindlinger J, Schwarzer D, Wirtz M, Tyystjärvi E, Finkemeier I, Mulo P (2018) Chloroplast Acetyltransferase NSI Is Required for State Transitions in Arabidopsis thaliana. Plant Cell 30:1695-1709.

2 Dickinson AJ, Lehner K, Mi J, Jia KP, Mijar M, Dinneny J, Al-Babili S, Benfey PN (2019) β-Cyclocitral is a conserved root growth regulator. Proc Natl Acad Sci U S A 116:10563-10567

Yijie ZHANG[1], Cecile LABARRE[1], Yann BOURDREUX[2], Emilie LESUR[2], Dominique GUIANVARC'H[2], Christiane DIETRICH[1], Laila SAGO[1], David CORNU[1], and Nicolas BAYAN[1]

[1]Institute for Integrative Biology of the Cell (I2BC), Universite Paris-Sud, Universite Paris-Saclay, CEA, CNRS, Gif-sur-Yvette Cedex, France [2]Institut de Chimie Moleculaire et des Materiaux d'Orsay (ICMMO), UMR 8182, Universite Paris-Sud, Universite Paris-Saclay, CNRS, F-91405 Orsay, France.

Identification of the mycoloylome in *C. glutamicum*

Corynebacteriales is an order of bacteria that comprises several human pathogens, including Mycobacterium tuberculosis and Corynebacterium diphtheriae, as well as economically valuable species like Corynebacterium glutamicum. All these species share a common very atypical cell envelope composed of arabinogalactan-modified peptidoglycan and an outer membrane mainly composed of mycolic acid-derived glycolipids. In 2010, Huc et al. discovered the presence of protein O-mycoloylation in C. glutamicum, a post-translational modification catalyzed by the enzyme mycoloyltransferase C (MytC). MytC has been shown to transfer a mycolate chain from trehalose mono mycolate (TMM) to the serine of unusual porins, namely PorAH and PorBC. An additional unknown protein, ProtX, was also reported to be mycoloylated. (Issa et al. 2017 and Carel et al. 2017). In order to gain a more in-depth understanding of protein mycoloylation, we undertook a proteomic global approach to identify the mycoloylome of C. glutamicum. For this, we performed specific metabolic labelling of mycoloylated proteins using a unique synthetic alkyne-tagged TMM (alk-TMM) analogue as a mycoloyl donor. Potential candidates were identified by mass spectrometry. The results were further refined using a MytC knockout mutant, which led us to identify 14 new high-confidence candidates in fine. Interestingly, all these candidates are predicted to be exported in the cell envelope and do contain an unstructured region (Alphafold prediction) with a serine-rich sequence. We are currently engaged in the biochemical validation of protein mycoloylation candidates. This involves determining their molecular mass and localization and identifying the mycoloylated sites through mass spectrometry analysis. By employing these methodologies, we aim to uncover further insights into the occurrence and importance of mycoloylated proteins in C. glutamicum, and to explore the specific function of this unusual post-translational modification, compared to the coexisting N-acylation, in cell envelope protein trafficking. Additionally, this work opens the possibility of using our validated protocol to identify mycolated proteins in other genera and unravel their fundamental importance more generally in the order of Corynebacteriales.

Mengyuan LI[1], Pierre GROGNET[1] and Fabienne MALAGNAC[1]

[1] FED

Deciphering the roles of jumonji domain containing proteins in *Podospora anserine*

In eukaryotic organisms, histone proteins are associated with DNA inside the nucleus. This structure called chromatin is associated with the epigenetic component of gene regulation. Indeed, epigenetic histone post-translational modifications or histone marks are set up by specialized enzymes, which lead to either an open conformation of chromatin, namely euchromatin, in which transcription occurs; or a close conformation of chromatin, namely heterochromatin, blocking transcription. However, the chromatin conformation needs to be dynamics to allow a fine regulation. Thus, these histone marks have to be periodically removed for other marks to be deposited. The methylation of histone lysine is performed by histone methyltransferases (HMTs), whereas the removal of methyl group, or demethylation, is performed by histone demethylases (HDMs). HDMs containing a JmjC domain are widely conserved among Eukaryotes. In the model fungus Podospora anserina, it has been shown that the deletion of HMT coding genes caused defects in many aspects of the life cycle such as growth, differentiation, gamete production, sexual development, etc. (Carlier et al., 2021). However, compared to HMTs, there are only few studies on HDMs in fungi. We searched P. anserina's genome for genes predicted to encode a JmjC domain containing proteins. We detected 12 candidates for which knock-out mutants have been constructed. I present here the analysis of this gene family and describe the phenotypes of the deletion mutants.

1 *Carlier, F., Li, M., Maroc, L. et al. Loss of EZH2-like or SU(VAR)3–9-like proteins causes simultaneous perturbations in H3K27 and H3K9 tri-methylation and associated developmental defects in the fungus Podospora anserina. Epigenetics & Chromatin 14, 22 (2021).*

Taher YACOUB [1,2], Roy GONZÁLEZ-ALEMÁN [3], Yann PONTY [2], Fabrice LECLERC [1]

[1]Institute for Integrative Biology of the Cell (I2BC), France [2]Laboratoire d'Informatique de l'X (LIX), France [3]Laboratorio de Química Computacional y Teórica, Cuba

Design of aptamers against BACE1 enzyme involved in Alzheimer's disease

Alzheimer's disease is the most common type of dementia, and remains seldom addressed with only six treatments approved by the FDA. Among the hypotheses explaining this disease, the "Amyloid Cascade" hypothesis highlights the role of amyloid plaques in the cognitive decline. To prevent their outbreak, many molecules attempt to inhibit an enzyme called "Beta-Secretase 1" (BACE1). However, no inhibitors have been approved yet, partly due to the prevalence of off-target effects. Aptamers (sequence of oligonucleotides) represent a good alternative to monoclonal antibody, and can interact with proteins which are not physiologically bound to RNA. One aptamer was approved by FDA in 2004, and others are suspected to bind to BACE1, although structural evidences are still lacking. We propose an in-silico method, using a fragment-based approach, to guide the design of specific RNA aptamers with chemical modifications and illustrate its capacity to design ligands for BACE1. We selected 4 conformations of BACE1, on which 444 different nucleotides were docked. For each conformation, we showed differences of spots depending on the modification and/or the solvation around BACE1. A similar work was carried out for a homologous enzyme called BACE2 in order to ensure specificity of the designed aptamer, and thus limit off-target effects. In parallel, we develop a fragment-based graph method for RNA design which groups a set of individually-docked nucleotides, establish their connectivity, and computes either the minimum-free energy conformation (docking), ligand having optimal affinity to the target (design), or statistical properties at the thermodynamic equilibrium. This method uses a computational technique called color-coding approach, allowing for an efficient (parameterized) and exact resolution of a notoriously hard (NP-complete) computational problem. Our work provides insights into the favorable regions involved in contacts between BACE1 and nucleotides, and a general approach to guide the design of aptamers for therapeutic purposes.

Tamara YEHOUESSI[1], Valentin HURE[1],Florence PIRON-PRUNIER[1], Gabrièle ADAM[2], and Angélique DELERIS[1]

[1] Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France, [2]Saclay Plant Sciences, Institute of Plant Sciences Paris-Saclay (IPS2), Batiment 630, Avenue des Sciences, Plateau du Moulon, 91190, Gif-sur-Yvette, France

Determinants of PRC2 and H3K27me3 recruitment on Transposable Elements (TE) in the flowering plant *Arabidopsis thaliana*

Transposable Elements (TE) are repeated DNA sequences that can potentially move in the genome, thus inducing mutations that can be deleterious or adaptive. TE can also regulate nearby gene expression and play an important role in the genome. In many organisms, TE are targeted by DNA methylation (5-Methylcytosine), which, together with the di-methylation of H3K9me2 (di-methylation of lysine 9 of histone 3) leads to the stable repression of their transcription and contributes to maintain genome integrity. Another hallmark of the epigenetic transcriptional silencing is the deposition of the histone mark H3K27me3 (tri-methylation of lysine 27 of histone 3) by PRC2 (Polycomb Repressive Complex 2) at developmental genes, leading to their dynamic transcriptional repression. DNA methylation and H3K27me3 have thus been considered as specific of TEs and genes respectively. However, H3K27me3 can be observed at many TEs in the absence of DNA methylation raising questions about the biological significance and determinants of PRC2 recruitment to TE.

In this project, we want to understand what underlies PRC2 recruitment at TEs and what molecular determinants, favor it, sometimes over DNA methylation, we are using a transgenic approach to test whether PcG proteins can be recruited, de novo, at TEs. We also explore whether TEs can recruit H3K27me3 through the same molecular determinants than have previously been described in genes; Polycomb responsive-like elements and long (non-coding) RNAs. Combination of molecular experiments and bioinformatics analyses have shown that PRC2 can be recruited on neo-inserted TE sequences and is dependent on intrinsic features of the TE-sequence.

P. AKIKI[1], P. DELAMOTTE[1] , M. POIDEVIN[1], A. LEROUZIC[1], F. MERY[1], F. MARION-POLL[1], J. MONTAGNE[1]

[1]Institut for Integrative Biology of the Cell (I2BC), CNRS, Université Paris-Sud, CEA, UMR 9198, 91190 Gif-sur-Yvette, France.

Understanding the physiological basis linking social environment to tumor growth in *Drosophila melanogaster*

Social interactions reduce the growth of genetically induced intestinal tumors in Drosophila females. The aim of my PhD project is to decipher the physiological processes that link social context to tumor growth. We decided to do RNA sequencing in order to identify the potential underlying physiological functions. Using flow cytometry, we have shown that tumor growth is reduced when the tumorous flies are maintained in a homogenous group in comparison to tumorous flies maintained isolated or alone in a heterogeneous group with healthy ones. Surprisingly, this effect operates in virgin but not mated females. However, it is restored in females fertilized by males devoid of sex peptide, knowing that the sex peptide transferred from the male ejaculate stimulates aggression between female flies. The RNAseq revealed that numerous genes implicated in innate immunity, nervous system, cell signaling and metabolism are either down or upregulated depending on the social context of virgin tumorous flies bearing genetic identity. We have shown as well that the effect depends on apoptosis, but not on the microbiota.

Vahiniaina ANDRIAMANGA[1], Anne LOPES[1] and Olivier LESPINET[1]

[1] Institute for Integrative Biology of the Cell (I2BC), 1 avenue de la terrasse bâtiment 21, 91190, Gif-Sur-Yvette, France

Exploring the evolution of metabolic networks in fungi

The metabolic network represents the relationships between all biochemical reactions. It defines the metabolic capacity of the organism to use compounds available in the environment and to synthesize new products. Consequently, the environment plays a role in constraining the evolution of metabolic networks. To unravel the evolution of the metabolic network, we investigated the evolution of the 910 enzyme activities in 174 species of fungi using a unique combination of phylogenetic profiles and graph-based analysis. The enzyme activities were divided based on their conservation, with the first half (454) of the enzyme activities present in all the species studied, while the second half (456) is associated with specific clades or species. Using a phylostratigraphy approach, we reconstructed the evolutionary history (loss and gain) of enzyme activities related to specific clades or species. We showed that 406 of these enzyme activities were already present in fungal ancestors and subsequently lost during evolution, while 50 were novel fungal-specific enzyme activities. Regarding the location of these enzyme activities in the metabolic network, lineage-specific enzyme activities appear on the periphery. They are less connected within the metabolic network than common enzyme activities and are usually alternatives to common ones. In addition, grouping the enzyme activities according to the similarity of their phylogenetic profile showed that the enzyme activities with similar profiles are closer to each other in the network. We also observe that network-breaking enzyme activity losses are tolerated for two reasons: if part of the subnetwork is an accessory or an alternative enzyme activity exists in the other species.

Valentin GUYARD [1, 2], VF. MONTEIRO-CARDOSO [1, 2] , M. OMRANE [3] , C. SAUVANET [1,2], A. HOUCINE [4], C. BOULOGNE [5], K. BEN MBAREK [3], N. VITALE [6], O. FAKLARIS [7], N. EL KHALLOUKI [1,2], AR. THIAM [3], F. GIORDANO [1,2]

[1]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France. [2] Inserm U1280, Gif-sur-Yvette, France. [3] Laboratoire de Physique de l'École Normale Supérieure, ENS, Université PSL, CNRS, Sorbonne Université, Université Paris Cité, Paris, France. [4] Institut Jacques Monod, CNRS, UMR7592, Université Paris Diderot, Sorbonne Paris Cité, Paris, France. [5] Imagerie-Gif, Electron Microscopy Facility, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette, France. [6] Centre National de la Recherche Scientifique, Université de Strasbourg, Institut des Neurosciences Cellulaires et Intégratives, UPR-321267000 Strasbourg, France. [7] MRI, BioCampus Montpellier, CRBM, Univ. Montpellier, CNRS, Montpellier, France.

Functional crosstalk between ORP5/8 and seipin at ER mitochondria contact sites involved in lipid droplet biogenesis

Eukaryotic cells have the ability to store large amount of neutral lipids in the lipid droplets (LDs), specialized organelles composed by a core of neutral lipid surrounded by a monolayer of phospholipids. LDs are very dynamic organelles that constantly remodel in response to metabolic changes and allow the cell to respond to energy fluctuations. They arise from the endoplasmic reticulum (ER) at sites that are physically marked by seipin, an integral ER protein whose mutations result in lipodystrophies and neurological disorders. Seipin localizes to ER-LD neck in both yeast and mammals and absence of seipin from these sites results in alteration of LD formation and growth. However, the molecular mechanisms underlying LDs biogenesis and maintenance within the cell remain poorly understood. Also, where LDs originate from the ER is still unclear. We have recently shown that ER subdomains in contact with mitochondria, also called Mitochondria Associated Membranes (MAMs), are hotspots for the formation of LDs. We have also shown that two lipid transfer proteins, ORP5 and ORP8, localize at MAMs and play a key role in regulating LD biogenesis at these sites. Oleic acid treatment induced a massive enrichment of ORP5-labeled MAM to nascent LDs and KD of ORP5/8 in HeLa cells decreased LD biogenesis at MAMs. Moreover, we have found that ORP5 interact with seipin and regulates its targeting to MAM-LD contact sites. We are currently investigating ORP5-seipin interaction and reciprocal regulation at MAM-LD contact sites as well as the functional relevance of MAM-LD contact sites for lipid transfer and storage in the cell. Our study brings novel insights into the metabolic crosstalk between mitochondria, ER, and LDs at membrane contact sites.

Sara DENDENE[1], Shuanghong XUE[2], Quentin NICOUD[1], Odile VALETTE[2], Angela FRASCELLA[2], Anna BONNARDEL[2], Peter MERGAERT[2], Benoit ALUNNI[1], Emanuele G. BIONDI[1,2]

[1]I2BC, Gif sur Yvette, France [2]LCB, Marseille, France

FcrX, a new global regulator of cell cycle in free living conditions and during symbiosis in *Sinorhizobium meliloti*

Sinorhizobium meliloti is a soil bacterium that establishes a symbiosis with *Medicago sativa*, where it fixes the atmospheric nitrogen into ammonia and in return the plant shares carbon sources with bacteria. In this symbiosis, S. meliloti undergoes a drastic cellular change leading to an intracellular terminal differentiation (bacteroid) characterized by genome endoreduplication, cell enlargement and high membrane permeability. The bacterial cell cycle regulation is closely implicated in this process of differentiation. Indeed, in free living cells, the bacterial regulator, CtrA, among other functions, activates cell division (controlled by constriction ring forming FtsZ), and inhibits DNA replication, while during symbiosis CtrA and FtsZ downregulations are essential for bacteroid differentiation. So far little is known about regulators of CtrA and FtsZ in S. meliloti that may play a role during bacteroid development. This study focuses on a new factor, FcrX, that controls both CtrA and FtsZ. Depletion of the essential gene fcrX lead to minicells formation in which levels of FtsZ and CtrA are abnormally high. Using several techniques we showed that FcrX (a alpha-helix-rich protein) is able to interact with FtsZ and CtrA via a still unknown mechanism. Further we showed that, despite a weak homology with FliJ-like proteins, only closely-related species FcrXs are able to complement S. meliloti fcrX deletion. Finally mutants of FcrX showed abnormal symbiotic behaviors in plants suggesting a putative role of this factor during bacteroid differentiation.

Anastasiia SKOBELKINA[1], M. JULIEN[1], R. GHOUIL[1], S. MIRON[1], FX. THEILLET[1], R. LEBARS[1], S. JEANNIN[1], A. CARREIRA[2], A. CONSTANTINOU[2], J. BASBOUS[3], S. ZINN-JUSTIN[1]

[1]Institut de Biologie Intégrative de la Cellule (I 2 BC), CEA, CNRS, Uni Paris Sud, Uni Paris Saclay, Gif sur Yvette, France [2] Institut Curie, Unit eé Intégrit eé du Génome ARN et Cancer, CNRS, Uni Paris Sciences Lettres, Orsay, France [3] Institut de Génétique Humaine, Montpellier, France

BRCA2 interactions through intrinsically disordered regions: focus on the role of phosphorylation and condensate formation in mitosis

The BRCA2 tumor suppressor protein is involved in the maintenance of genome integrity through its role in homologous recombination. In mitosis, BRCA2 is phosphorylated by Polo-like kinase 1 (PLK1). In collaboration with the teams of A. Carreira (CBM, Madrid) and A. Constantinou (IGH, Montpellier), we aim at describing how this phosphorylation contributes to the control of mitosis. We previously identified a conserved phosphorylation site at T207 of BRCA2 that constitutes a docking site for PLK1 (Ehlen et al., Nat Commun 2020; Julien et al., Biomolecules 2021). We showed by NMR that PLK1 phosphorylates in vitro several conserved sites in BRCA2, including T207 its-self, as well as S1115 and S1121. We demonstrated that, when PLK1 phosphorylates T207, it is then able to bind to this phospho-site, and this stimulates phosphorylation of its partner BUBR1 by PLK1. Here, we will describe our search for new phospho-dependent partners of BRCA2, using NMR, affinity chromatography and mass spectrometry. We will focus on the microtubule depolymerase KIF2C, which is also an important regulator of mitosis. We will show by NMR and ITC that KIF2C possesses an N-terminal domain that is able to bind several phospho-peptides, including BRCA2 phospho-T207. We will also discuss the observation that KIF2C is able to phase-separate in vitro and assemble into condensates in cells. This triggers concentration of PLK1 into KIF2C condensates, and might locally regulate phosphorylation of KIF2C partners, including BRCA2 and BUBR1.

Tianyue WEI [1,2], Caroline AYMES-CHODUR [1*], Christophe REGEARD [2], Nadine AUBRY-BARROCA [1], and Philippe ROGER [1]

[1] Synthèse de Molécules et Macromolécules pour le Vivant et l'Environnement- Institut de Chimie Moléculaire et des Matériaux d'Orsay (SM2ViE – ICMMO), UMR 8182, Université Paris-Saclay, 91405, Orsay, France; [2] Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, 91198, Gif-sur-Yvette, France

Surface functionalization of non-woven polypropylene (PP) using plasma treatment: chemical and biological approaches to induce antimicrobial properties

Because of the rapid spread of infectious disease, polypropylene (PP) medical surgical masks have been produced on a very large scale and this pandemic period has evidenced the need for new medical and health products. Our goal is to use the plasma treatment technology to induce the functionalization of the PP surface with antimicrobial components. Essential oils, which are potential biosourced candidates, are intended to be grafted on PP surface to fight against the bacterial infections, by either restricting the growth of bacteria (bacteriostatic effect) or killing bacterial cells (bactericidal effect) (1). Essential oils are also an alternative to the use of classical antibiotics for which the emergence of resistances is increasing (2). The results presented here show the way we modified the molecules that are intended to be grafted on the activated surface. With the background of the SM2ViE team (3), the chosen essential oils are chemically modified so that they are more prone to react with the target surface. In this study we chose citronellol and geraniol compounds, known for their antimicrobial activity, and we used a chemo enzymatic hydrolysis with the help of a lipase catalysis to transform the vinyl bonds into peroxide ones (4). Microbiological tests were undertaken to estimate the MIC (Minimum concentration to inhibit visible growth) and the MBC (Minimum concentration that results in bacterial death) of citronellol and geraniol before and after modification. Three bacterial species have been used: *Escherichia coli* possessing two membranes (Gram negative), *Staphylococcus aureus* with only one membrane (Gram positive) and Corynebacterium glutamicum that possesses features of both Gram-positive and Gram-negative bacteria.

1 S. Tariq et al., A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens, Microbial Pathogenesis. 134 (2019) 103580.
2 M.E.A. de Kraker, et al., Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050, PLOS Medicine. 13 (2016).
3H. Salmi-Mani et al., Poly(ethylene terephthalate) films modified by UV-induced surface graft polymerization of vanillin derived monomer for antibacterial activity, European Polymer Journal. 103 (2018) 51–58.
4E.G. Ankudey, et al., Lipase-mediated epoxidation utilizing urea–hydrogen peroxide in ethyl acetate, Green Chem. 8 (2006) 923–926.

Romain JOUAN[1], Gaëlle LEXTRAIT[1], Aya YOKOTA[1], Raynald COSSARD[1], Joy LACHAT[1], Léa HUET[1], Yoshitomo KIKUCHI[2], Tsubasa OHBAYASHI[1,3], Peter MERGAERT[1]

[1]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198 Gif-sur-Yvette, France
[2]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Hokkaido Center, 062-8517 Sapporo, Japan [3]Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization (NARO), 305-8604 Tsukuba, Japan

The fitness landscape of the insect gut symbiont *Caballeronia insecticola* in diverse environments

Some bacteria are able to live in diverse environments and more investigation is needed to understand which functions are required for their adaptation to these lifestyles. Transposon mutant libraries combined with high throughput sequencing (Tn-seq) is a method of choice for the identification of essential functions for survival, growth or host colonization of bacteria. *Caballeronia insecticola* is able to colonize soil, the rhizosphere of soybean and the gut of the phytophagous insect *Riptortus pedestris*. A saturated Tn-seq library was built in *C. insecticola* using the Himar1 transposon. By using this library in Tn-seq screens, we identified the ensemble of essential genes in each of the natural environments of this bacterium. Interestingly, these essential gene sets contained besides condition-specific genes, many genes shared between the lifestyles. Furthermore, to deconvolute the complexity of the environmental conditions, Tn-seq experiments were performed in multiple simplified in vitro conditions that mimic specific aspects of the environmental conditions. Finally, the Tn-seq results were validated for several genes of interest by direct mutagenesis and phenotype investigation in in vitro and environmental conditions. Among the insect specific essential functions, we identified motility and chemotaxis-related genes as well as genes involved in resistance to antimicrobial peptides. These resistance functions included the LPS biosynthesis pathway and novel resistance pathways. In the soil, the polyhydroxybutyrate biosynthesis pathway is specifically essential, indicating a nutrient-imbalanced environment. Inactivating the first step in the hopanoid biosynthesis pathway affects the bacterial fitness in the rhizosphere, putting forward the importance of membrane fluidity in the rhizosphere. Among the essential genes found for both soil and rhizosphere, exopolysaccharide biosynthesis pathways are over-represented, highlighting the potential importance of biofilm formation and desiccation protection in these environments. This study will contribute to a better understanding of organisms' ability to adapt to a diversity of environments.

Rémi RUEDAS [1,2] Magali MATHIEU [2,] Stéphane BRESSANELLI [3]

[1] Université Paris-Saclay, CEA, CNRS - Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France [2] Sanofi, Integrated Drug Discovery, 13, quai Jules Guesde, 94403 Vitry-sur-Seine, France

Integrative approach for cryo-EM structural study of flexible antibody-antigen complex

Single particle analysis from cryogenic transmission electron microscopy nowadays allows structure solution for relatively large protein complexes in the native state. It is particularly attractive for complexes for which structure prediction remains intractable, such as antibody-antigen complexes. We set up a robust workflow with an integrative approach for such complexes. It includes several validation points at pre-microscope, in-microscope, and post-microscope stages and led to the high-resolution structure of a particularly difficult antibody-antigen complex. The first two parts include fast biochemical validation points through accessible biophysical measures (DLS, nano-DSF, SEC-MALS, Mass Photometry) and grid screening focusing on how we manage to handle particle misdistribution and vitrification deterioration. The last part focuses on data processing and allows to appreciate the strength of current software as well as software currently under development in dealing with various kinds of heterogeneities, particularly continuous conformational heterogeneity. We will thus present the structure of the ternary complex of the extracellular part of the ErbB2 receptor bound with two different fab from the two monoclonal antibodies pertuzumab and trastuzumab. This structure provides a much more detailed view than those available for this ternary complex. This allowed us to pinpoint a previously overlooked interaction that may contribute to explain the synergistic anticancer effect of the two antibodies. Beyond that, we provide a dataset of a smallish protein complex of 162kDa with which to further develop software accounting for continuous conformational heterogeneity in cryo-EM images.

Maxime BARRAULT[1,2] Rodrigo CORONEL-TELLEZ[1], Philippe BOULOC[1]

[1]SRRB [2] Ecole doctorale Structure et Dynamique des systèmes Vivants

sRNA-mediated control of citrate metabolism upon iron starvation in *Staphylococcus aureus*

S. aureus is a commensal bacterium, present in the nose of about 30% of the population. In favor of an immunodepression, it can become an opportunistic pathogen and trigger severe and sometimes lethal infections. Like most pathogenic bacteria, *S. aureus* relies on iron to grow and colonize its host. However, in response to that, the host is able to sequester it thanks to the process of nutritional immunity. In order to survive in this iron-depleted environment, S. aureus has developed several mechanisms that allows it to survive. Using fitness experiments, a sRNA involved in the response to iron starvation has been isolated and named IsrR (Iron-sparing response regulator) (Coronel-Tellez et al. 2022). After a bioinformatic analysis, we obtained a list of the potential targets of IsrR, in which three genes encoding for proteins involved in citrate metabolism were found, more specifically the aconitase (citB) and its transcriptional activator CcpE. Interestingly, besides its role as an intermediate of the TCA cycle, citrate is also necessary for the synthesis of two siderophores of *S. aureus*, staphyloferrin A and B. Therefore, we hypothesized that IsrR could reroute citrate from the TCA cycle in order to use it for siderophore production. Bioinformatic predictions suggests that IsrR binds on the 5' UTR of the target mRNAs, more precisely on the RBS (Ribosome Binding Site), thus probably alleviating their translation. To test this hypothesis, we monitored CitB and CcpE quantities in presence or absence of IsrR by Western-Blot and we confirmed that expression of IsrR led to a significant decrease in CitB and CcpE production. To confirm that the effect of IsrR on CitB was direct and not mediated via CcpE, we constructed translational reporter fusions between the 5' UTR of our targets and a fluorescent protein under the control of a constitutive promoter. We inserted these fusions on the chromosome of WT and ΔisrR strains and confirmed that citB and ccpE regulation by IsrR is indeed translational. Using different versions of IsrR on plasmids, we also showed that the activity of IsrR on citB and ccpE is done through its C-rich regions. Finally, we quantified siderophore production in a ΔisrR mutant and showed that IsrR is involved in siderophore production during iron starvation. Taken together, these results suggests that IsrR has a dual role in *S. aureus*, by limiting iron utilization in the cell while promoting its import.

Coronel-Tellez, Rodrigo H, Mateusz Pospiech, Maxime Barrault, Wenfeng Liu, Valérie Bordeau,Christelle Vasnier, Brice Felden, Bruno Sargueil, et Philippe Bouloc. 2022. « SRNA-Controlled Iron Sparing Response in Staphylococci ». Nucleic Acids Research 50 (15): 8529-46. https://doi.org/10.1093/nar/gkac648.

Logan GREIBILL [1] , K. BOUHOUCH[1] and A.M TASSIN[1]

[1] Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris Sud, Université Paris-Saclay, Gif-sur-Yvette France

Functional analysis of MAP250, a novel partner of the deubiquitinase CYLD, during ciliogenesis.

The cilium is a microtubule-based organelle found on the surface of most human cells. This cell antenna is involved in development and in many physiological processes thanks to its various motile and/or sensory functions. A defect in one of these functions leads to severe genetic pathologies with a wide phenotypic range called ciliopathies. Some of them are caused by a deficiency in one of the crucial steps required for cilium assembly called ciliogenesis. This complex mechanism requires a remodeling of the actin and microtubule (MT) cytoskeletons to ensure the fine coordination of all these steps. Previously, the team showed that the deubiquitinase CYLD is localized to basal bodies (BBs) and involved in a specific step of ciliogenesis, BB anchoring. However, the molecular mechanism by which CYLD regulates ciliogenesis remains unknown. Therefore, a search for new candidates involved in BB anchoring was developed using CYLD as bait. Among them, MAP250 was identified. This protein, with still unknown functions, is associated in humans with vertebral malformations from birth, suggesting ciliopathies phenotypes in agreements of CYLD involvement in ciliogenesis . After confirming its interaction with CYLD, I showed that MAP250 localizes to the centrosome and BBs, but also to the actin and MT cytoskeletons by interacting with their MT plus tips end. I have shown that MAP250 is a positive regulator of ciliogenesis. My goal is now to find the molecular mechanisms underlying the function of MAP250, to position its action in the hierarchy allowing the formation of cilia and to explore its regulation by deubiquitination through CYLD. Thus, this project brings the first functional and molecular description of the MAP250 protein proposing for the first time to link the associated pathology to a ciliary defect.

Murielle SEIF EL DAHAN [1], Antonia KEFALA STAVRIDI [2], Philippe FRIT [3], Tom BLUNDELL [2], Patrick CALSOU [3], Amanda CHAPLIN [4],* Virginie ROPARS [1] & *Jean-Baptiste CHARBONNIER [1]

[1]Institute for Integrative Biology of the Cell (I2BC), Institute Joliot, CEA, CNRS, Univ.Paris-Sud, Université Paris-Saclay, France [2]. Department of Biochemistry, University of Cambridge, United Kingdom [3]. Institut of Pharmacology and Structural Biology, IPBS, Université de Toulouse, [4]. Department of Biochemistry, University of Leicester, United Kingdom* corresponding authors

Molecular machineries of the human DNA double-strand break repair pathway : Towards the assembly of the Ku-DNA-PAXX complex

DNA Double Strand Breaks (DSBs) are among the most toxic DNA lesions. Unrepaired DSBs result in cells undergoing in apoptosis or senescence whereas a default during the repair process can lead to chromosomic aberrations, genes rearrangements and cancers. There are two main ways to repair DSBs. Recombinational DNA repair is accurate but it relies on the presence of an unbroken homologous chromosome. Without sister chromatid, the non-homologous end-joining (NHEJ), on the other hand represents the major pathway of DSB repair in eukaryote cells. This pathway involves the ring-shaped heterodimer Ku70/Ku80 (Ku) that binds rapidly to DNA double-strand breaks (DSBs) and recruits several factors of the classical non-homologous end-joining pathway (c-NHEJ) through a specific binding motif (Ku binding motif, KBM) already characterized for two Ku's partners, XLF and APLF1. During my PhD, I studied the molecular mechanism of the PAXX protein 2,3,4 at DSBs: how it is recruited by Ku bound on DNA and how it contributes to NHEJ super complex formation. Our laboratory has previously shown that PAXX interacts with Ku through the Ku70 subunit via a P-KBM and needs a bare DNA extension5. My aim was to determine the 3D structure of the Ku-DNA-PAXX complex by X-ray crystallography and/or cryo-EM and to characterize the interactions of this complex by biophysical methods. I determined the X-ray structure of the PAXX peptide (including the P-KBM) bound to a Ku-DNA complex at 2,98 Å resolution. The PAXX peptide binds to an internal position of Ku70 causing an outward rotation of the vWA domain of Ku70. I performed interaction measurements by isothermal titration calorimetry between Ku-DNA complex and several PAXX peptides (wild type and mutants) to evaluate their affinities and to delineate the most important residues involved in the interaction between Ku and PAXX peptide. I extended this approach, in collaboration with A Chaplin's Lab (Leicester University, UK) to larger complexes including other c-NHEJ factors: XRCC4-Lig4, and XLF to reconstitute the structure of the main multiprotein complex of the c-NHEJ pathway as recently performed with a first super-complex6,7.

1 Nemoz C. & al. (2018) Nat Struct Mol Biol; 25(10) : 971-980. [2] Xing M. & al. (2015) Nat Commun; 6: 6233. [3] Ochi T. & al. (2015) Science; 347(6218): 185-188. [4] Craxton A. & al. (2015) Cell Death Differ; 22(6): 890-7. [5] Tadi SK. & al. (2016) Cell Rep; 17(2): 541-555. [6] Chaplin A. K. & al. (2021) Molecular Cell; 81(16): 3400-3409. [7] Seif el Dahan M. & al., Science advances, accepted

25th may Poster session 1

1- Amélie BESOMBES [1,2], Yazid ADAM [2], Christophe POSSOZ [1], Francois-Xavier BARRE [1], Jean-Luc FERAT [2],

[1] Institute for Integrative Biology of the Cell, Gif-sur-Yvettes, France, [2] Institute Jacques Monod, Paris, France

Bidirectional replication initiation prevents chromosome degradation,

The reason for replication to be initiated bidirectionally in the 3 domains of life has never been experimentally addressed due to the lack of an appropriate biological system. Using genetic and genomic approaches, we show that bidirectional replication initiation is no longer operated upon depletion of the co-helicase DciA in *Vibrio cholerae*. Instead, replication is initiated unidirectionally. We found that this unidirectional replication initiation leads to the degradation of nascent DNA strands. Our results suggest that this degradation results from the annealing of the nascent strands free extremities into a free double stranded DNA end. Thus, bidirectional replication initiation is essential in double stranded DNA replication to handcuff nascent DNA ends to prevent their regression and the subsequent pathological degradation of the chromosome.

2- Liza BOEFFARD[1], Camille DAGALIER[2], Géraldine EUDIER[2], François-Xavier THEILLET [1] & Sophie ZINN-JUSTIN[1]

[1]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS UMR9198, Université Paris-Saclay, Gif-sur-Yvette, France. [2] Formulation and Process Development lab, Sanofi, Vitry-Sur-Seine , France

Identifying interactions between proteins and excipients using NMR, and their consequences on viscosity/injectability

Higher concentrated forms of biopharmaceutical treatments allow for better and faster injection deliveries, hence helping in delivery practicability and patients' quality of life. However, high concentrations of recombinant proteins have consequences on the stability and properties of the treatment preparation. It comes inevitably with increased viscosities, which impacts injection. Small molecular excipients are commonly used to moderate this phenomenon, but in an empirical fashion. The knowledge is very limited on how the most common excipients stabilize and fluidify highly concentrated therapeutic proteins. In this context, we sought to examine the correlation between the strength of excipients' interactions with concentrated proteins and viscosity. NMR spectroscopy appears as an excellentte chnique in this context, because of its ability to report low affinity interactions. Different NMR methods exist for this, based on non-redundant physical mechanisms related to weak interactions (e.g. STD, WaterLogsy, T2/T1rho, DOSY, …). These approaches are commonly used for structural biology purposes, but pharmaceutical formulations are conspicuously different from the standard solutions used in academic labs. Here, we present the first results of our work, testing the adaptation of various pulse sequences to fit our need and biopharmaceutical samples. We will show how the NMR observables can complement each other, and which pulse sequences look promising. We will finally discuss the complementarity with other techniques, using size-exclusion chromatography, light-scattering methods and capillary electrophoresis.

3- Julia BUGGIANI[1], Thierry MEINNEL[1], Carmela GIGLIONE[1], Fréderic FROTTIN[1]

[1]PROMTI

Impact of protein acetylation on nuclear protein quality control and biocondensate

Protein homeostasis (the state of proteome balance or proteostasis) is fundamental to preserve cellular functions. Its disruption is associated with numerous diseases. The accumulation of misfolded proteins and aggregates is the hallmark of many neurodegenerative disorders, cancers, and aging. To maintain and restore proteostasis, cells possess a protein quality control network which combines a multitude of activities and cellular strategies. Recently, it has been discovered that an integral part of the cellular management of nuclear misfolded proteins is their reversible sequestration into the nucleolus upon proteotoxic stress. Therefore, the nucleolus prevents irreversible and toxic protein aggregation highlighting a novel chaperone-like function. The nucleolus is a liquid-like phase separated compartment, also called biomolecular condensate, that is formed through multivalent interactions of its constituents. The liquid-like nature of the organelle ensures its quality control function. However, the regulation of this pathway remains largely unknown, as well as the factors required for nucleolar translocation of misfolded proteins. The aim of the project is to discover new players involved in the regulation of the nucleolar protein quality control pathway. In particular I will discuss the potential role of protein modifications on nuclear protein quality control and biocondensate properties.

4- François CHARON[1], Sabina FARHADOVA[2], Robert FEIL[2], Daan NOORDERMEER[1], Benoît MOINDROT[1]

[1]CHRODY Team, I2BC, Université Paris-Saclay; [2]IGMM, CNRS, Montpellier

Organization of the 3D chromatin structure & nuclear lamina association of the Dlk1-Dio3 imprinted domain in mouse

Genomic imprinting is an epigenetic mechanism that controls the expression of around 200 genes, clustered in about 20 loci. During development, the deregulation of imprinted genes expression causes “Imprinted Disorders” (IDs). Imprinted genes are expressed in a parent-of-origin dependent manner, from a single parental allele only. In the imprinted Dlk1-Dio3 locus, the maternal allele expresses the long non-coding polycistronic Meg3-Rian-Mirg gene, while the paternal allele expresses protein coding genes Dlk1 and Rtl1. The in-cis retention of the Meg3 ncRNA prevents the expression of Dlk1 gene from the maternal allele. The allele-specific regulation of imprinted genes is largely dictated by the DNA methylation status of Differentially Methylated Regions (DMRs). Within the Dlk1-Dio3 locus, the methylation level at the Meg3¬¬-DMR, located in the promoter of Meg3, controls the Meg3-Rian-Mirg polycistron expression. The maternal Meg3-DMR, hypomethylated, allows for Meg3-Rian-Mirg expression while the paternal Meg3-DMR, hypermethylated, abolishes Meg3 expression. In addition, the Meg3¬¬-DMR overlaps with binding sites of the CTCF protein, an insulator protein largely involved in the organization of the genome into functional Topologically Associated Domains (TADs). CTCF binding is sensitive to DNA methylation. Its binding at the Meg3-DMR is thus restricted to the maternal hypomethylated Meg3-DMR while no binding happens to the paternal hypermethylated Meg3-DMR. To better understand to what extent the methylation levels of the Meg3-DMR influence local allelic chromatin organization of the Dlk1-Dio3 locus, we developed allele-specific Capture-Hi-C and 4C-seq assays. This reveals the formation of two maternal-specific sub-TADs that are hinged by the unmethylated CTCF-bound Meg3-DMR. Local loss of methylation at the paternal Meg3-DMR alters interaction frequencies, with the paternal chromosome adopting a more maternal-like sub-TAD organization. Besides TAD organization, mammalian chromosomes adopt non-random radial organization in the nucleus, notably by contacts between chromatin regions and the nuclear lamina. To determine the allelic relationship between the Dlk1-Dio3 locus and the nuclear lamina, we developed Capture MadID-seq assays (derived from DamID). This revealed that, in mouse embryonic stem cells, the paternal Dlk1-Dio3 locus associates more with the nuclear lamina than the maternal allele does. Altogether, our results highlight parental tridimensional organization of the Dlk1-DIo3 locus, with the formation of allelic sub-TADs and allelic association with the nuclear lamina.

5- Hélène CHÉROT[1,2], François Xavier THEILLET[2]

[1]CEA-Saclay, Gif-sur-Yvette, France; [2] Institute for Integrative Biology of the Cell (I2BC, Univ. Paris-Saclay/CEA/CNRS), Gif sur Yvette, France

Structural characterization of disordered proteins by In-cell NMR

In-cell structural biology by NMR is appealing in many regards: It proposes, among others, to investigate conformational equilibria or ligand binding in very relevant conditions, i.e in cells1.The approach has often been limited in its capacities: by the many difficulties in sample production, and by important signal losses due to promiscuous, transient interactions with cellular entities, which, in turn, urges to use (too) high concentrations of the studied proteins. Establishing simpler protocols and improved labeling schemes for enhancing S/N would help. In an attempt to facilitate in-cell NMR studies along these two dimensions, we initiated an advanced evaluation of in situ protein production in mammalian cells, using either transient or permanent transfection, in home-made culture media supplemented with isotope-labeled amino-acids. Here, we report the early results of this program applied to disordered proteins: α-synuclein, Tau and TDP-43. Among others, we will show the first spectra ever recorded of a disordered protein at 37C in human cells.

1 Theillet, F.-X. In-Cell Structural Biology by NMR: The Benefits of the Atomic-Scale. Chem. Rev. 2022, in press. https://doi. org/10.1021/acs.chemrev.1c00937.

6- Lucie GOMES[1] and Maria COSTA[1]

[1]SSFA

Genetic study of a specialized bacterial group II intron retrotransposon

Group II introns are large catalytic RNAs (ribozymes) and retrotransposable elements of bacterial origin. They invade genomes by a highly site-specific mobility pathway called ‘retrohoming’ that is operated both by the intron ribozyme and the intron-encoded reverse transcriptase. Most often, mobile group II introns insert themselves into intergenic regions of the genome, which is consistent with the ‘selfish’ behavior of these mobile elements. In contrast with this trend, some classes of group II introns target particular structures or genetic signals involved in gene expression. This is the case of some particular group II introns, forming a monophyletic family, that are always associated with 'start' and 'stop' codons of specific genes. Interestingly, most of the genes colonized by these introns are implicated in either repair or replication of bacterial DNA or in stress-response. These 'specialized' introns also share peculiar structural features and are highly abundant in Gram-negative bacteria. To study the biology of these retrotransposable elements, we use as the model system, the 'groEL KTE56' retrotransposon which is a group II intron naturally present in the ‘stop’ codon of the heat-shock gene groEL in the pathogen *Escherichia coli* KTE56 strain. Using genetic approaches, we have shown that the 'groEL KTE56' intron is highly mobile and specifically invades the stop codon of the groEL gene. We also constructed several laboratory *E.coli* strains carrying a genomic copy of this retrotransposon inserted into its groEL gene in different genetic context. We are characterized phenotypically these strains in order to study the potential impact of this intron on its host. We also plan an RNA-seq to study the potential impact of the 'groEL KTE56' intron at transcriptional level. To further understand the mobility mechanism of this lineage of specialized retrotransposons, we are currently testing whether this intron needs a specific genetic context for its insertion. We are also developing genomic approaches that will allow us to test, for example, if the ‘groEL KTE56’ retrotransposon only ‘survives’ in genomes if inserted into the ‘stop’ codon of coding genes. Moreover, other genetic systems able to report on translation impact will be used to explore the possibility that the presence of the retrotransposon affects in some way the translation termination of its host groEL gene leading to the synthesis of a new isoform of the GroEL protein.

7- Umama HANI[1], Anja KRIEGER LISZKAY[1]

[1]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, 91198 Gif-sur-Yvette cedex, France

Study of Photosynthetic Electron Flow under Mn deficiency in *Marchantia polymorpha*

Photosynthesis is the only biochemical process that converts sunlight energy into chemical energy in the form of ATP and NADPH. Under stress conditions or when ATP/NADPH demand is altered, plants switch to Cyclic Electron Flow (CEF). CEF reinjects electrons from PSI via Ferredoxin into the plastoquinone pool leading to ATP formation without NADPH production. We study the effect of Manganese deficiency on CEF in liverwort *Marchantia polymorpha*. In this condition, the ratio of PSII/PSI is lower. We hypothesize that under Manganese deficiency, CEF is enhanced, thus protecting Photosystem II. In this context, light-induced P700 and Plastocyanin (Pc) absorbance changes were measured via DUAL KLAS_NIR. Slow oxidation of P700, faster re-reduction of P700+ and significant decrease in Pc/P700 ratio under Mn deficiency points to the fact that pool size of electrons that can be donated to P700+ is higher, thus indicating higher CEF and possibility of super complex formation around PSI.

8- Estelle LEROY[1], Drice CHALLAL[1], Alexandra MENANT[1], Stéphane PELLETIER[1], Jessica ANDREANI[2], Mathieu ROUGEMAILLE[1]

[1]RNA-binding proteins in gene expression and cell differentiation (GEXDIF) - Genome Biology Department - I2BC [2]Molecular Assemblies and Genome Integrity - Biochemistry, Biophysics and Structural Biology Department - I2BC

Antagonism between small and long non-coding RNAs tunes the mitosis to meiosis transition in fission yeast

In the fission yeast *Schizosaccharomyces pombe*, sexual differentiation is under the control of post-transcriptional mechanisms and involves long non-coding RNA (lncRNA) species. During vegetative growth, a subset of meiosis-specific transcripts is selectively eliminated by an RNA degradation system involving the YTH-family RNA-binding protein Mmi1. Upon meiosis onset, Mmi1 is sequestered in a ribonucleoparticle (RNP) complex comprising the RNA-binding protein Mei2 and the lncRNA meiRNA, thereby allowing expression of the meiotic program. The lab previously showed that during mitosis, Mmi1 associates with another lncRNA, termed mamRNA, to limit the accumulation of its own inhibitor Mei2 and hence preserve its activity in meiotic RNA degradation. However, the precise role of mamRNA and its functional relationship with meiRNA during the mitosis to meiosis transition have remained elusive. To determine the role of mamRNA and meiRNA on meiotic gene expression profiles, we performed RNA-seq experiments in wild type cells and mutants lacking either lncRNA upon synchronized meiosis induction. Computational analyses unveiled the precise timing of induction of Mmi1-targeted genes in the different strains. Remarkably, while deletion of meiRNA abolished their expression, the absence of mamRNA resulted in their strong accumulation, exceeding the levels observed in wild type cells. meiRNA and mamRNA thus exert antagonistic roles on meiotic gene expression by virtue of their opposite function in the regulation of Mmi1 activity. We further dissected the cis-acting elements within both lncRNAs and uncovered i) specific hexanucleotide motifs in meiRNA responsible for the binding of Mei2 and proper meiosis progression, and ii) an intron-encoded small nucleolar RNA (snoRNA) within mamRNA that is sufficient to restrict Mei2 levels during mitosis and expression of Mmi1 target genes upon meiosis onset. Together, our results shed light on an intricate regulatory network involving RNA-binding proteins and their non-coding RNA partners in the control of sexual differentiation, and expand our vision on the non-canonical functions exerted by snoRNAs.

9-Yingyue LUO[1], Chafiaa BOUGUECHTOULI[1], Ania ALIK[1], Francois-Xavier THEILLET[1]

[1]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France.

Characterization of the phospho-dependent interactions between full-length Mdm2 and p53

The transcription factor p53 coordinates the cellular response to DNA damage. P53 protein level and activity are controlled by a signaling network comprising notably the E3-ubiquitin ligases Mdm2 and MdmX. p53, Mdm2 and MdmX show long disordered regions, carrying abundant, clustered (de)phosphorylation sites of DNA-damage response (DDR) kinases ATM/ATR/DNA-PK (PIKKs), Chk2, and Wip1. The role of the resulting various phosphorylation schemes is poorly understood. We sought to use NMR to monitor the phosphorylation in a site-specific fashion, and to delineate the (phospho-dependent) interactions, either intramolecular for Mdm2 isolated, or intermolecular between Mdm2 and p53. Here, we present the NMR-monitored intramolecular interactions and dynamics of Mdm2, and the characterization of the phospho-dependent Mdm2:p53 complexes using NMR and ITC. This required intense efforts to produce the corresponding full-length proteins, which alternate folded domains and long disordered regions, and the active kinases that modify them. At the fundamental level, we show to which extent disordered regions can impact protein:protein affinities. Finally, this helps to better understand the interaction between Mdm2 and p53, which may support the design of new inhibitors for cancer strategy.

10- Vincent MORIN[1], Paloma Fernandez VARELA[2], Jean-Baptiste CHARBONNIER[3]

[1]CEA PhD ; [2]IR, CNRS - supervisor ; [3] CEA researcher - PhD director

Molecular mechanisms of the main DNA double-strand break reparation pathways

DNA double strand breaks (DSB) are one of the most deleterous damage among the molecule. A loss of genetic information can potentially lead to mutations, gene deletions or many other misfunctions in cell's life. Therefore, it is essential to maintain the DNA quality with various reparation pathways. Our lab is focusing on the understanding of molecular mechanisms in one of these DSB reparation pathways, the Non-Homologous-End-Joining (NHEJ). It's a well-known system based on the recognition of DNA ends by fast interaction with the heterodimer Ku70-Ku80 (named after Ku) and then the recruitment of NHEJ actors in order to process the damage if necessary and then proceed to the ligation of the two extremities. My PhD project is mainly divided in two directions. One consists on the understanding of an interaction between Ku protein and an endogenous ligand (Inositol-6-phosphate) in Homo sapiens. We highlighted various effects either on Ku itself or on the Ku-DNA interaction such as an increased thermal stability or a favoured equilibrium of Ku/Ku-DNA complex respectively. Structures have also been characterized by X-ray diffraction or CryoEM and in-vivo experiments were achieved to obtain a well view of this question. Moreover, it seems that this regulation can also have influence on the recruitment of NHEJ factors, such as XLF (protein that can forms the "bridge" on DNA end synapsis. This project was a strong example of how a multi-disciplinary approach can provide maximum information of a biological study ! Secondly, we started to manipulate the NHEJ in a more simple organisms which is *Saccharomyces cerevisiae*. Where in human, much has been done, it is not the case for other species and we are still lacking of information of this system, even about basic steps of the NHEJ. Globally, we want to obtain detailed mechanisms in each of these steps and structures of complexes or the complete synapsis. These can potentially provide information about how the absence of DNA-PKcs is processed on yeast or how MRX is acting or not in the system. Ku-DNA interaction is also different comparing in human form. To resume, NHEJ is a tough system with many aspects to analyse where it is mandatory to have a super strong multi-disciplinary method to have all views of the molecular mechanisms.

11- Alexia L. M. ROYER[1,2] Andrew A. UMANSKY[1], Marie-Maude ALLEN[1], Julian R. GARNEAU[1], Maicol OSPINA-BEDOYA[1], Joseph A. KIRK[3], Gregory GOVONI[4], Robert P. FAGAN[3], Olga SOUTOURINA[2], Louis-Charles FORTIER[1]

[1]Department of Microbiology and Infectious Diseases, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Québec, Canada [2]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France [3] Molecular Microbiology, School of Biosciences, University of Sheffield, Sheffield, United Kingdom [4]AvidBiotics, South San Francisco, California, USA

Specificity of interactions between the *Clostridioides difficile* SlpA protein and its bacteriophages

Clostridioides difficile (CD) is a Gram-positive, spore forming bacterium which is the leading cause of intestinal post-antibiotic nosocomial infection in industrialized countries. Antibiotherapy is the standard treatment to fight against CD infections, but also leads to gut microbiota dysbiosis that favours recurrences. Therefore, new therapies are explored like phage therapy, which is a promising approach consisting of using bacteriophages (phages), i.e. bacterial viruses, specifically targeting bacteria. A large panel of CD bacteriophages has been characterized and found to be species specific, making them a promising “microbiota-friendly” therapy. To develop phage therapy, interactions between CD and its phages need to be better understood. The CD surface is made of a proteinaceous coat, named “S-layer”, mainly composed of SlpA proteins. SlpA is formed of the assemblage of two subunits, one of high molecular weight (HWM) and another of low molecular weight (LMW). Fourteen different isoforms have been described to date, and the variations are mainly attributed to the LMW subunit. The LMW subunit is the most exposed on the cell surface and possesses two subdomains (D1 and D2). We demonstrated that SlpA is used as a phage receptor and that the interaction specificity is driven by the different isoforms expressed at the cell surface. Also, our experiments have revealed that D2 is essential for infection by some phages from our collection (Royer et al, Microbiol. Spectr. 2023). Thus, these observations reveal that phage interactions occur through different SlpA subdomains, possibly including the D1 subdomain of the LMW subunit and/or the HMW subunit. A collection of SlpA mutant strains expressing modified SlpA isoforms is being created to better understand which region(s) of the protein is involved in interaction with different phages. Also, the work will focus on the phage receptor binding proteins (RBP) known to interact with the bacterial host. Directed mutagenesis and interaction tests will be done to refine our understanding of these interactions. In conclusion, our work will provide detailed information about interactions between phages and their SlpA receptor. This will allow the design of recombinant phage cocktails targeting different SlpA isoforms to improve phage host range in future phage therapy applications against CD infections

12- Rémi RUEDAS[1,2] Magali MATHIEU[2], Stéphane BRESSANELLI[3]

[1]Université Paris-Saclay, CEA, CNRS - Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France [2]Sanofi, Integrated Drug Discovery, 13, quai Jules Guesde, 94403 Vitry-sur-Seine, France

Integrative approach for cryo-EM structural study of flexible antibody-antigen complex

Single particle analysis from cryogenic transmission electron microscopy nowadays allows structure solution for relatively large protein complexes in the native state. It is particularly attractive for complexes for which structure prediction remains intractable, such as antibody-antigen complexes. We set up a robust workflow with an integrative approach for such complexes. It includes several validation points at pre-microscope, in-microscope, and post-microscope stages and led to the high-resolution structure of a particularly difficult antibody-antigen complex. The first two parts include fast biochemical validation points through accessible biophysical measures (DLS, nano-DSF, SEC-MALS, Mass Photometry) and grid screening focusing on how we manage to handle particle misdistribution and vitrification deterioration. The last part focuses on data processing and allows to appreciate the strength of current software as well as software currently under development in dealing with various kinds of heterogeneities, particularly continuous conformational heterogeneity. We will thus present the structure of the ternary complex of the extracellular part of the ErbB2 receptor bound with two different fab from the two monoclonal antibodies pertuzumab and trastuzumab. This structure provides a much more detailed view than those available for this ternary complex. This allowed us to pinpoint a previously overlooked interaction that may contribute to explain the synergistic anticancer effect of the two antibodies. Beyond that, we provide a dataset of a smallish protein complex of 162kDa with which to further develop software accounting for continuous conformational heterogeneity in cryo-EM images.

13- Xiaofen WU[1], Vincent GRELAt[1], Francesco LAVECCHIA[1], Renata GRZELA[1], Julien NUSBAUM[1], Anca SEGALL[2], Thierry MEINNEL[1] & Carmela GIGLIONE[1]

[1] Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif sur Yvette, cedex France

[2] San Diego State University, San Diego, California, USA

Pathogenic virus control of host viability at the level of the ribosome exit tunnel

All prokaryotic nascent chains must be co-translationally deformylated to allow the further essential removal of the first methionine. This early processing is ensured by peptide deformylase (PDF). Metagenomics analysis of oceanic microbial samples revealed unusual PDF genes as one of marine phage genomes' most abundant gene families. Why so many phages carry PDF genes in their genome remains unknown. Comparisons of sequence, structure and deformylase activity with known PDFs reveal that viral PDFs strongly resemble bacterial PDFs1, despite the absence of the C-terminal extension known to be important in bacterial PDF for ribosome binding and activity2. Here, we show that the shortest known PDF occurring in the genome of Vibrio sp.16-specific bacteriophage Vp16, progressively accumulates during the infection process, reaching a maximal expression at the beginning of cell lysis. Interestingly, preliminary results involving Vp16PDF knockout from the phage genome indicate that the absence of Vp16 PDF inhibits phage release from the host, suggesting an essential function of Vp16PDF in phage infectivity. Accordingly, the expression of Vp16PDF alone in Vibrio sp.16, induces a strong cold-sensitive growth inhibition. This Vp16PDF-dependent cold-sensitive inhibition was also detected when Vp16PDF was expressed in *Escherichia coli*, a phenomenon not observed upon expression of other PDFs. We reveal that Vp16PDF expression causes cell lysis, affects outer membrane integrity, and induces the formation of intracellular aggregates, suggesting that Vp16PDF might specifically interfere with sec-dependent translocation. Unexpectedly, we observed that Vp16PDF can still bind directly to *E. coli* ribosomes via a new dedicated ribosome-binding domain despite lacking the C-terminal extension. Vp16PDF interacts with ribosomes in several distinct regions, including that next to the exit tunnel overlapping the specific binding sites for trigger factor (TF) and SecB. In agreement, overexpression of TF and SecB in both *E. coli* and Vibrio sp.16 overcomes the Vp16PDF inhibitory effect. In conclusion, our findings provide the basis for understanding a previously unrecognized mechanism based on viral control of host viability at the level of the ribosome exit via the action of phage PDF.

1 Grzela R. et al. Sci Rep. 2017 7, 11041 [2] Bingel E. et al. Nature, 2008, 452, 108

14- Dong XIE[1] , Cyril DIAN[1], Jean-Baptiste BOYER[1], Lucile JOMAT[1], Carmela GIGLIONE[1] & Thierry MEINNEL[1]

[1]Promti team, Genome Biology Department, Bat.21, I2BC, UMR9198, 91198 Gif sur Yvette, France.

Challenging the unique N-terminal modification pathway of plant RuBisCO

During the oxygenic photosynthesis in plants, algae and cyanobacteria, atmospheric carbon dioxide (CO2) is assimilated into carbohydrates making photosynthetic organisms autotrophic. This unique step of carbon dioxide and oxygen uptake is catalyzed by the enzyme D-ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO). RuBisCO is the most abundant protein on Earth and its large catalytic subunit (RbcL) is encoded in the chloroplast genome of plants and algae. RbcL is not only the major catalyst ensuring atmospheric CO2 scavenging but it also contributes to maintain oxygen balance in the biosphere and to supply all food sources for life on Earth. Understanding the parameters that alter the catalytic rate and stability of RuBisCO is therefore a key challenge for the survival of humankind in the worrying context of climate changes and global warming. N-terminal protein modifications are major contributors of cellular proteostasis. The large RuBisCO complex (>500 kDa) requires a very specific folding and assembly of its two subunits to gain its unique function and activity. Interestingly, the RbcL subunit of RuBisCO undergoes a unique maturation pathway involving several proteolytic events and unusual N-terminal modifications1. This mechanism is conserved in plants, and results in the formation of an N-terminal acetylated proline. Such acetylation of a proline exclusively occurs on RbcL, which protease(s) and acetyltransferase(s) are involved in this unique pathway is unknown so far, as is the impact of this maturation on RuBisCO. My PhD project is to unravel the dedicated machinery leading to RbcL processing. I have focused first on the removal of residue 2 of RbcL, a conserved serine in land plants. We have identified two open reading frames (ORFs) in *Arabidopsis thaliana* that might contribute to this unique removal. I challenged both conserved aminopeptidase (AtAPP2 and AtAARE) with in vitro experiments from the purified proteins and ad hoc knockout Arabidopsis plant lines. We show that AtAPP2 is in charge of residue 2 release, while AtAARE is involved neither in RbcL maturation nor in any N-terminal protein maturation of the plastid. We also have gained strong data on the final proline acetylation machinery. Next, we have established conditions that allow the production of a range of RbcL N-terminal variants in the presence or absence of the identified enzymes involved in its N-terminal maturation. Together, the unique N-terminal modification machinery involved in RbcL processing relies on three processing enzymes that are only committed to RbcL.

1. Meinnel & Giglione (2022) J Exp Bot 73, 6013-33.

15- Adriana BADILLA LOBO[1], Olga SOUTOURINA[1], Frédéric BARBUT[2] Johann PELTIER[1]

[1]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France. [2]Centre National de Référence C. difficile, Hôpital Saint-Antoine, Assistance Publique-Hôpitaux de Paris et UMR S-1139, 3 PHM, Paris, France.

Characterization of a family of small proteins regulated by second messenger-binding riboswitches in *Clostridioides difficile*

Clostridioides difficile is an anaerobic, spore-forming, Gram-positive bacterium. *C. difficile* infection is the leading cause of antibiotic-associated nosocomial diarrhea in adults. The expression of the different pathophysiological processes in *C. difficile* is controlled by a great diversity of regulatory networks, including RNA-based mechanisms such as riboswitches. Riboswitches are regulatory RNA regions located at the 5' untranslated end of mRNAs that bind small molecules triggering a conformational change, positively or negatively affecting the expression of the downstream coding sequence. Sixteen riboswitches responding to cyclic di-guanosine monophosphate have been identified in C. difficile. C-di-GMP is a second messenger controlling the transition from a free planktonic to a sessile lifestyle formation and virulence factor expression. Among the c-di-GMP-responding riboswitches of C. difficile, 5 are upstream genes encoding nearly identical small proteins. Intriguingly, preliminary experiments revealed that the expression of these genes is repressed by high c-di-GMP levels but is also modulated by the second messenger, c-di-AMP, primarily involved in osmoregulation. The aim of this project is, therefore, to characterize these small proteins and to decipher their regulation by second messengers. Because of their small size, these small proteins are unlikely to have enzymatic activity, and they possibly interact with other proteins to regulate their function. Using affinity chromatography, we identified 5 protein candidates possibly interacting with a tagged version of one of the small proteins. To characterize the small proteins at the phenotypic level, we generated a strain deleted of all 5 genes and a strain overexpressing one of the copies from the chromosome. Overexpression of the target gene was then verified by qRT-PCR. Our preliminary results suggest that toxin production is reduced in the small protein-overexpressing strain. Using an alkaline phosphatase (PhoZ) reporter assay, we confirmed on one hand that c-di-GMP interacts with the riboswitch to control the expression of the downstream gene. However, high c-di-GMP increased PhoZ activity instead of the expected decrease. Further experiments are underway to understand the meaning of this result better. On the other hand, this assay revealed that c-di-AMP regulation is riboswitch-independent and relies on controlling the promoter activity.

16- Clément Fauchereau[1], Fanny Carimalo[2], Axelle Merienne[2], Marc Laffon[2], Emmanuel Godat[2], Jean-Christophe Aude[1]

[1]Université Paris Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France [2]CHRU Tours, Service Anesthésie - Réanimation Bretonneau, Tours, France

Classification of anesthesia state using cardiac features and machine learning

The monitoring of anesthesia is a key element in preventing underdosing and overdosing during surgery which can lead to severe outcomes. Most established monitoring indicators are computed using the electroencephalogram. However, studies have shown that electrocardiograms (ECG) are affected by anesthesia. We propose a machine learning method to distinguished between awake and anesthetized states using cardiac signal. ECGs of 33 patients were recorded before (awake) and during surgery (anesthetized). For each patient, both recordings were processed and segmented to 5min windows. Then, heart rate variability and morphological features were extracted from the ECG to train models to predict anesthesia states (awake or anesthetized). We benchmark standard machine learning methods on this classification task and selected a gradient boosting algorithm. We achieved over 85% accuracy with good generalization on public datasets. Our results suggest that cardiac features are closely linked to anesthesia state. Age is particularly relevant in our model, accounting for the physiological differences between patients. Future research will focus on leveraging the temporal dynamic of the anesthesia and increasing the granularity of the prediction of the consciousness level.

17-Aurélie Favarin*[1], Rayan Said[1], Hemalatha Narassimprakash[1] and Christophe Le Clainche[1]

[1]Institut de Biologie Intégrative de la Cellule – Commissariat à l'énergie atomique et aux énergies alternatives, Université Paris-Saclay, Centre National de la Recherche Scientifique – France

In vitro reconstitution of the mechanosensitive α-catenin-vinculin complex of adherens junctions

Tissues and cells migrating in a collective manner must adapt to mechanical stresses to maintain their architecture and function. The cohesion of tissues requires that cells establish cell-cell junctions. Cells contact each other by forming Arp2/3-dependent lamellipodia before they initiate the formation of cadherin-based adherens junctions (AJs). Maturing AJs then assemble actin under force though the formation of a mechanosensitive complex comprising the actin-binding proteins α-catenin, vinculin and VASP, which individually act on the nucleation, elongation and organization of actin filaments in different ways. Recent results from our group reveal that α-catenin, vinculin and VASP combine their activities to inhibit Arp2/3-mediated branching, stimulate the nucleation and elongation of linear actin filaments from profilin-actin and crosslink these filaments into bundles (1). The reciprocal relationship between actomyosin contractility, which governs the formation of this α-catenin-vinculin-VASP polymerizing machinery, and the polymerization activity of this machinery, which feeds actomyosin contractility by adding actin filaments, is not known. The aim of this project is to decipher the biochemical parameters that govern the crosstalk between actin polymerisation and actomyosin contractility in a reconstituted in vitro system composed of purified proteins immobilised in micropatterns and observed by fluorescence microscopy (2,3).

1The adherens junction proteins α-catenin, vinculin and VASP cooperate to promote actin assembly. Said R., Wang H., Pernier J., Narassimprakash H., Roméro S.,Gautreau A.M., Mège R-M., Le Clainche C.. Biorxiv doi: https://doi.org/10.1101/2022.12.04.518837

2 Actomyosin-dependent formation of the mechanosensitive talin-vinculin complex reinforces actin anchoring. Ciobanasu C, Faivre B, Le Clainche C. Nat Commun. 2014;5:3095. doi: 10.1038/ncomms4095.

3Talin dissociates from RIAM and associates to vinculin sequentially in response to the actomyosin force. Vigouroux C, Henriot V, Le Clainche C. Nat Commun. 2020 Jun 19;11(1):3116. doi: 10.1038/s41467-020-16922-1.

18- A. GESNIK[1], J. ACKER[1], J. BICHEROUR[1], M. BETERMIER[1], V. ROPARS[1], J.B. CHARBONNIER[1]

[1]Institute for Integrative Biology of the Cell (I2BC), Institute Joliot, CEA, CNRS, Université Paris-Saclay - 91198 Gif sur Yvette (France)

Structural study of gene rearrangement mechanisms driven by transposases in collaboration with the c-nhej pathway in *Paramecium tetraurelia*

DNA double-strand breaks (DSBs) are genotoxic lesions that threaten the integrity of the genome. They are repaired either by homologous recombination (HR) or by the non-homologous end-joining pathway (NHEJ) (1). The classical NHEJ pathway is initiated by the binding of the ring-shaped Ku70/Ku80 (Ku) heterodimer to damaged DNA ends. Then the ligation complex is recruited to the Ku-DNA sites to repair the broken ends. Although potentially dangerous, programmed DSBs (prDSBs) are essential for a number of physiological processes. In Paramecium, the PiggyMac (Pgm) transposase and its partners, the PiggyMac-Like (PgmL) proteins precisely excise Internal Eliminated Sequences (IES) during the new differentiation of the macronucleus (2)(3). A transposase domain has been predicted in all five PgmL proteins, from PgmL1 to PgmL5 but they lack the DDD catalytic triad. Therefore, their role in prDSB seems to be architectural to form with Pgm an endonuclease complex (3). The prDSBs generated by Pgm and PgmL at IES sites are subsequently repaired by the NHEJ pathway, suggesting that programmed genome rearrangements require tight coupling between DNA cleavage and DSB repair (4). As the interaction mechanisms of all these factors are still unknown, our aim is to understand how the endonuclease complex forms and binds the Ku heterodimer by performing biophysical interaction measurements and cryo-EM structural studies. First, I successfully expressed the Piggymac interactome in insect cells, using the MultiBac system implemented in the laboratory. I optimized the purification protocol of Pgm, PgmL1, PgmL3 and Ku. Preliminary interaction tests with a non-specific DNA substrate were performed by gel shift assays. I recently initiated the structural study of PgmL1 by negative stained TEM, as well as the structural study by cryo-EM of the Ku heterodimer from Paramecium in presence of DNA substrates.

1 Lieber MR. The Mechanism of Double-Strand DNA Break Repair by the Nonhomologous DNA End-Joining Pathway. Annu Rev Biochem. 7 juin 2010;79(1):181-211.

2. Baudry C, Malinsky S, Restituito M, Kapusta A, Rosa S, Meyer E, et al. PiggyMac, a domesticated piggyBac transposase involved in programmed genome rearrangements in the ciliate Paramecium tetraurelia. Genes Dev. 1 nov 2009;23(21):2478-83.

3. Bischerour J, Bhullar S, Wilkes CD, Mathy N, Dubois E, Singh A, et al. Six domesticated PiggyBac transposases together carry out programmed DNA elimination in Paramecium. :24.

4. Bétermier M, Borde V, de Villartay JP. Coupling DNA Damage and Repair: an Essential Safeguard during Programmed DNA Double-Strand Breaks? Trends in Cell Biology. févr 2020;30(2):87-96

19- Hanane MECHRI[1] -Guy TRAN VAN NHIEU[1]

[1]Université paris saclay, Inserm(U1280)-cnrs(UMR9198)

Characterization of secreted bacterial factors involved in pneumococcal meningitis

Streptococcus pneumoniae or pneumococcus (PN) is a human nasopharyngeal commensal and major bacterial pathogen responsible for meningitis, it occurs when bacteria enter the bloodstream who becomes invaded which makes it easier for the bacteria to cross the blood-brain barrier (BBB) and damage the brain by bacterial virulence factors. Underlying mechanisms of such high mortality are still poorly understood. We investigate the mechanism of PN secreted factors (PSFs) specifically associated with PN strains causing meningitis (MG+) inducing cytotoxicity of in vitro cultured vascular endothelial cells. Physico-chemical characterization indicate that PSFs have low molecular weight and are sensitive to proteinase treatment, suggesting that PSFs correspond to complexes of peptides that induce cytotoxicity associated with the disassembly of filamentous actin, loss of cell adhesions and nuclear shrinkage. PSFs determination was carried out by Liquid chromatography–mass spectrometry (LC–MS) and De novo analysis on Laboratory strains: Serotype 4 (TIGR4). Serotype 2. Recombinant PSFs will be further purified using affinity chromatography and their cytotoxic activity will be tested on human microvascular endothelial cell line (HBMEC) to confirm the functional role of PSFs during blood brain barrier (BBB) destabilization. Keywords: *Streptococcus pneumoniae*, PN secreted factors (PSFs), De novo analysis, TIGR4, Serotype 2, HBMEC cells.

20-Tom MARIOTTE[1], François GUYOT[1], Romain COUDRAY[1], Aurore GORLAS[1]

[1]Université Paris-Saclay, Institut de minéralogie, de physique des matériaux et de cosmochimie (MNHN) Sorbonne université

Thermococcales Iron sulfide Biomineralization

Major inhabitants of the hottest parts of deep-sea hydrothermal vents (Takai et al, 2021), Thermococcales are sulfur-reducing archaea that have adapted to this extreme environment. Characterized by steep physicochemical gradients, these vents release heavy-metal enriched fluids (Holden et al, 2015). One putative survival mechanism involves iron sulfide mineralization by Thermococcales, which could help them deal with iron toxicity (Gorlas, Mariotte et al, 2022).

21- Guillaume MARTIN[1], Christelle ESPAGNE[1], Anne-Sophie FIORUCCI[1]

[1]Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, 91198 Gif-sur-Yvette, France

How do growth responses to elevated temperatures affect nitrate uptake, transport and assimilation in *Arabidopsis thaliana*?

In response to mild warm temperatures, *Arabidopsis thaliana* young seedlings adjust their development by elongating vegetative organs like hypocotyls. These growth responses are called thermomorphogenesis and are mostly allowed by modifications in hormones biosynthesis and/or signalling. But thermomorphogenesis effects on nutrients homeostasis, and especially on nitrogen nutrition are unknown. Our main hypothesis is that higher temperatures could affect nitrate-related actors and/or homeostasis in order to promote cell elongation. Firstly, actors from nitrate homeostasis necessary for thermomorphogenic responses will be identified via mutant phenotyping and transcript levels analyses. Secondly, some steps of nitrate homeostasis will be analysed in response to temperature. This works will set the basis for a better understanding of nitrate utilisation in seedlings subjected to high ambient temperature.

22- Roza MOHAMMEDI[1], Dendene SARA[1], Emanuele BIONDI[1]

[1]I2BC, UMR1918, CNRS/CEA/Paris-saclay university, 91190 Gif sur Yvette, France

Role of the CckA-ChpT-DivL complex in the phosphorylation of the master regulator CtrA during the cell cycle and nitrogen-fixing symbiosis in Sinorhizobium meliloti

Sinorhizobium meliloti is an alphaproteobacterium which is able to live free in the soil or in symbiosis with legumes. During symbiosis, bacteria fix atmospheric nitrogen within symbiotic organs, called nodules, where they undergo extreme cell differentiation into bacteroid. Bacteroids are characterized by genome endoreduplication, cell enlargement and high membrane permeability. The transcription factor CtrA has been shown to be the master regulator of the cell cycle and the transition from a free to a symbiotic lifestyle is accompanied by a gradual disappearance of CtrA during the differentiation of bacteroids, suggesting that the (de-) regulation of the cell cycle by CtrA is a crucial point for the establishment of the symbiosis. In the alphaproteobacterium Caulobacter crescentus, a bacterium related to S. meliloti, cell differentiation is also closely related to the cell cycle via the activity of the master regulator CtrA. CtrA has been shown to be activated by phosphorylation via a phosphorelay system consisting of the two histidine kinases DivL and CckA and the histidine phosphotransferase ChpT. Orthologs of these different regulators are present in S. meliloti, suggesting a conservation of this module in the regulation of CtrA in this bacterium. The objective of this work is to study the functions of the CckA-ChpT-DivL complex and its impact on CtrA in S. meliloti in free and symbiotic life. We first confirmed the essentiality of divL in S. meliloti by transduction of a deletion cassette. The study of a DivL-depletion strain allowed us to demonstrate that DivL is essential for the proper functioning of the cell cycle and that it is involved in the regulation of CtrA. A translational fusion with the fluorescent protein YFP showed that DivL is localized to a single pole. We also purified the phosphorelais proteins and reconstructed a part of the phosphorylation cascade in-vitro. Finally, the DivL-depletion strain is not able to perform an efficient symbiotic relationship with Medicago sativa under the tested conditions.

23- Orlando MORANCHEL[1], James PROVAN[1], François-Xavier BARRE[1]

[1]EMC2

Acquisition of the cholera toxin is favored by the presence of the TLC prophage in *Vibrio cholerae*

The ubiquitous aquatic bacterium *Vibrio cholerae* can undergo pathogenic conversion after infection by the integrative bacteriophage CTXΦ which encodes the cholera toxin genes responsible for the deadly diarrheal disease cholera. CTXΦ does not encode its own integrase but instead relies on the host recombinases XerCD for integration of the phage DNA into the bacterial chromosome. The Xer system is ubiquitous in bacteria with circular chromosomes because it serves to resolve chromosome dimers, ensuring correct segregation of the DNA upon cell division. Thus, numerous bacteriophages exploit this mechanism, referred to as Integrative Mobile Elements exploiting Xer (IMEX). IMEX have developed diverse strategies to avoid cellular regulation of their integration and excision reactions, three of which have been described so far. As they integrate into the bacterial cognate Xer-recombination site, dif, multiple bacteriophage integration events lead to the formation of an 'IMEX array'. The sequence of this array is therefore representative of the history of IMEX infection of the strains. Whole-genome sequencing of clinical strains of *Vibrio cholerae* revealed that CTXΦ integration always follows the prior integration of another IMEX called TLCΦ, suggesting a cooperative relationship between the two IMEX. The aim of this work is to decipher the factors involved in this cooperation at the molecular level. To estimate integration efficiency, we transfer phage genomes, including their attachment site, into *V. cholerae* cells by bacterial conjugation. The recipient strains harbor a colorimetric (blue/white) screening construct to observe integration events. Previous work using this method showed that TLCΦ integration requires the activity of a phage-encoded protein, XafT, which enables the reaction of XerCD on the imperfect attachment site of TLCΦ. Our current work demonstrates that the integration rate of CTXΦ is enhanced by the simultaneous expression of XafT. This result suggests that the TLC gene xafT favors the toxigenic conversion of *V. cholerae* by assisting the integration of CTXΦ during infection. However, the precise molecular effect of XafT on the XerCD reaction during CTXΦ integration remains yet unknown. Sequencing data also shows that TLCΦ integration modifies the dif1 site by 2 bp, which could participate in favoring subsequent integration of CTXΦ. This hypothesis is currently being tested. This research intends to understand the acquisition of CTXΦ by environmental *V. cholerae*, which could be crucial to understand the underlying molecular factors leading to cholera outbreaks.

24- Sokrich PONNDARA[1], Mounia KORTEBI[1], Stéphanie Bury-Moné[1], Frédéric BOCCARD[1] and Virginia.S. LIOY[1]

[1]OCB

Unraveling the Interplay between the ter region of the chromosome and Virulence Plasmids in *Salmonella*

Salmonella is a Gram-negative bacterium that belongs to the Enterobacteria family. Its pathogenicity is achieved through the presence of genes located on the Salmonella Pathogenicity Islands (SPIs). SPI-1 encodes a type 3 secretion system (T3SS1) that is essential for the invasion, formation, and survival of the bacterium within the early Salmonella-containing vacuole (SCV), and within the cytosol. On the other hand, SPI-2 encodes a secondary T3SS, which is expressed later during infection to protect the vacuole's integrity and maintain Salmonella survival in a nutrient-deprived acidic environment. Interestingly, SPI-2 is found in the terminus region of the chromosome. This region presents a different structural organization, mainly thanks to the MatP/matS system. This study aims to investigate the role of chromosome conformation on virulence expression under conditions that mimic infection. Here, we constructed a MatP deletion mutant and revealed more anucleated and filamented cells in conditions expressing SPI-1 comparing to the WT. Using chromosome conformation capture (HiC), we show that in the absence of MatP, the ter organization is lost, represented by an increase in long-range DNA contact from ~500 Kb up to ~1 Mb. Due to the positioning of SPI-2 in the ter, we address whether the disturbance of the ter influences SPI-2 expression. Using the promoter of ssaG, one of the genes of SPI-2 fused to a stable green fluorescent protein (GFP), we observed not significant changes in the expression of GFP; indicating that SPI-2 expression is not influence by ter folding. Furthermore, we performed infection studies with Salmonella wt and a matP deletion mutant and we observed that MatP is dispensable for infection. Strikingly, in conditions in which SPI-1 is expressed, our results unveiled a MatP dependent interplay between the ter region and the virulence plasmids pSLT and pCOL1B9. Overall, this study suggests that chromosome structure influences plasmid dynamics in Salmonella.

25- Manon SOLEIL[1], Marina NOCENTE[1], Emilie DROUINEAU[1], Hélène PICAUD[1], Cécile DULARY[2], Sophie CHANTALAT[2], Matthieu GERARD[1]

[1]Mammalian Epigenomics Team, [2]CNRGH, Institut François Jacob, CEA, Evry

Nucleosomal and subnucleosomal organisation of gene promoters in mammalian cells

TBP (TATA box binding protein) is a general transcription factor (GTF) that intervenes in the three transcription machineries: RNA polymerase I for most ribosomal RNAs, RNA pol II for messenger RNAs, and RNA pol III for transfer RNAs and other small non coding RNAs. In the context of pol II transcription, TBP is known to bind to the TATA box (TATAa/tAa/t motif) 30 bp upstream of the transcription start site (TSS). However, only ~15% of pol II promoters have a TATA box. How TBP binds to the TATA-less promoters is not well characterized. We recently identified a new class of subnucleosomal particles containing the four core histones associated with 50-80 bp of DNA, which occupy discrete positions at promoters and enhancers. Our ChIP-seq experiments show that TBP distribution on the genome coincides with the subnucleosomes at TATA-less promoters, suggesting that these subnucleosomal particles represent a new binding substrate for TBP.

26- Kristian WANT[1], Beata MONFORT[1], Remi MOR-GAUTIER[1], Magali NOIRAY[1], Thibault TUBIANA[1] ,Benoit D'AUTREAUX[1].

[1]Université Paris-Saclay, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198 Gif-surYvette, France

Unravelling the Complex Role of Ferredoxin-2 and Frataxin in Iron-Sulfur Cluster Biosynthesis: New Insights from Protein Binding and Functional Studies

Iron-sulfur (FeS) clusters are essential cofactors that play a crucial role in a range of cellular processes across all domains of life. In mammalian cells, [2Fe2S] clusters are synthesized in the mitochondria via the sequential activities of several proteins composing the iron-sulfur cluster (ISC) complex. The assembly of clusters occurs on the scaffold protein ISCU, which requires the insertion of iron followed by the transfer of sulfur in the form of a persulfide from the cysteine desulfurase NIA. Frataxin (FXN) has been shown to play a critical role in the transfer of persulfide from NIA to ISCU, and Ferredoxin-2 (FDX2) provides a source of electrons to generate the cluster. However, the mechanism by which FDX2 interacts and donates electrons is still largely unknown, and the functional role of FXN is debated. Here, we identify new insights into the interactions that coordinate the ISC complex using a combination of ITC, FIDA, and AUC, and expand our functional understanding of FXN and FDX2 by manipulating the kinetics of [2Fe2S] cluster biosynthesis. Our findings reveal a phenomenon of competitive binding between FXN and FDX2 on NIA-ISCU and provide previously undocumented insights into the conformational changes induced by FDX2 binding. These discoveries have important implications for understanding the molecular processes that underlie [2Fe2S] cluster assembly, and provide the structural basis to understand how FDX2 transfers electrons to the ISC complex.

27- Dong Xie[1], Cyril Dian[1], Jean-Baptiste Boyer[1], Lucile Jomat[1], Carmela Giglion[1] & Thierry Meinnel[1]

[1]Promti team, Genome Biology Department, Bat.21, I2BC, UMR9198, 91198 Gif sur Yvette, France.

Challenging the unique N-terminal modification pathway of plant RuBisCO

During the oxygenic photosynthesis in plants, algae and cyanobacteria, atmospheric carbon dioxide (CO2) is assimilated into carbohydrates making photosynthetic organisms autotrophic. This unique step of carbon dioxide and oxygen uptake is catalyzed by the enzyme D-ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO). RuBisCO is the most abundant protein on Earth and its large catalytic subunit (RbcL) is encoded in the chloroplast genome of plants and algae. RbcL is not only the major catalyst ensuring atmospheric CO2 scavenging but it also contributes to maintain oxygen balance in the biosphere and to supply all food sources for life on Earth. Understanding the parameters that alter the catalytic rate and stability of RuBisCO is therefore a key challenge for the survival of humankind in the worrying context of climate changes and global warming. N-terminal protein modifications are major contributors of cellular proteostasis. The large RuBisCO complex (>500 kDa) requires a very specific folding and assembly of its two subunits to gain its unique function and activity. Interestingly, the RbcL subunit of RuBisCO undergoes a unique maturation pathway involving several proteolytic events and unusual N-terminal modifications1. This mechanism is conserved in plants, and results in the formation of an N-terminal acetylated proline. Such acetylation of a proline exclusively occurs on RbcL, which protease(s) and acetyltransferase(s) are involved in this unique pathway is unknown so far, as is the impact of this maturation on RuBisCO. My PhD project is to unravel the dedicated machinery leading to RbcL processing. I have focused first on the removal of residue 2 of RbcL, a conserved serine in land plants. We have identified two open reading frames (ORFs) in *Arabidopsis thaliana* that might contribute to this unique removal. I challenged both conserved aminopeptidase (AtAPP2 and AtAARE) with in vitro experiments from the purified proteins and ad hoc knockout Arabidopsis plant lines. We show that AtAPP2 is in charge of residue 2 release, while AtAARE is involved neither in RbcL maturation nor in any N-terminal protein maturation of the plastid. We also have gained strong data on the final proline acetylation machinery. Next, we have established conditions that allow the production of a range of RbcL N-terminal variants in the presence or absence of the identified enzymes involved in its N-terminal maturation. Together, the unique N-terminal modification machinery involved in RbcL processing relies on three processing enzymes that are only committed to RbcL.

1. Meinnel & Giglione (2022) J Exp Bot 73, 6013-33.

28- Kenza YEFSAH[1], Karine BUDIN[1], Robert DEBUCHY[1], Denise ZICKLER[1], Eric ESPAGNE[1], Stéphanie BOISNARD[1]

[1]-Meiotic Recombination and Pairing team, Institute for Integrative Biology of the Cell, CNRS

The role of SUMO Targeted Ubiquitin Ligases in meiosis, in *Sordaria macrospora*

Crossovers do not occur independently of each other: a crossover at one position interferes with occurrence of another crossover nearby such that crossovers tend to be evenly spaced along chromosomes. We are currently exploiting the power of the Sordaria macrospora as a system for visualizing chromosome dynamics in relation with the recombination process to elucidate the role of the Ubiquitination and SUMOylation pathways in this fungus, particularly in the regulation of crossover interference. We discovered that: (1) Sordaria Slx5 and SLx8 are required for the regulation of CO patterning by decreasing the interference strength like the budding-yeast orthologs (Zhang et al. 2014). (2) Both proteins modulate chromosome-axis lengths plus crossover numbers, patterning and interference when compared to wild type, but differently. (3) While the slx8 null mutant exhibits longer than wild-type axis lengths, the slx5 null mutant exhibits shorter axes, but both mutants show more crossovers than the wild-type strain. This result suggests antagonistic functions for Slx5 and Slx8 despite their well-known function as a heterodimer. (4) We furthermore identified Rrp2, another member of the STUbL family, as a new modulator of crossover interference and axis length in Sordaria. In addition, we analyzed the localization of Slx5, SLx8 and Rrp2 throughout the meiotic prophase 1. To identify the precise role of each of the three proteins for their corresponding localization along chromosomes and for their potential role(s) in crossover interference, we are currently studying their interplay by analyzing all mutant combinations.