Quatrième réunion annuelle du GDR Architecture et Dynamique du Noyau et des Génomes (ADN&G)

Rapport sur les contributions

https://indico.in2p3.fr/e/32585

ID de Contribution: **1** Type: **Non spécifié**

Study of the role of the bacteriophage T4 protein, Ndd in the disorganization of the bacterial nucleoid in E. coli

Study of the role of the bacteriophage T4 protein, Ndd in the disorganization of the bacterial nucleoid in E. coli

Justine Groseille1, 2 (groseillejustine@gmail.com),Cristian Ilioaia4, Agnès Thierry2, Cristian Ilioaia4, Romain Koszul2, Olivier Espéli1

1 Dynamique des chromosomes - CIRB, College de de France, PARIS, France
2 UMR3525- Regulation spatiale des génomes, Institut Pasteur, PARIS, France
4 Atomic Energy and Alternative Energies Commission, CEA ·Institute of Biology and Technology Saclay, Saclay, France

Introduction and objectives

In bacteria, chromosomes are packed in a sub-cellular compartment called the nucleoid, providing rapid access to genetic information for the molecular machineries operating on DNA. Bacteriophages, viruses of bacteria, have selected various mechanisms to specifically hijack key metabolic functions of their bacterial host. Our work focuses on the mechanism by which the T4 lytic phage disorganizes the nucleoid of its host, Escherichia coli. Studies have shown that the expression of a single gene in its genome, the Nucleoid Disorganization Deficient (ndd) gene, is necessary and sufficient to induce this disorganization phenotype and subsequent bacterial death of the bacteria within minutes. The high toxicity associated with Ndd expression prevented researchers in the 1990s from investigating the molecular mechanism behind this intriguing phenomenon. The aim of this project is to study the role of Ndd on bacterial nucleoid disorganization and its relevance for the phage lytic cycle.

Materials and methods

We have developed an easily manipulated inducible system enabling us to study changes in nucleoid organization upon Ndd production using advanced genomic technologies (Chip Seq or, chromosome conformation capture, Hi-C) and cell biology (high-resolution microscopy) approaches.

Results, discussion and conclusion

These experiments confirmed some observations of previous work, but also showed that ndd expression a disruption of the nucleoid at different scale level. These observations led to the hypothesis that Ndd binding to the genome is at the origin of these perturbations. ChIP-seq experiments revealed a strong association of Ndd at several loci in the bacterial genome. High-resolution microscopy and Hi-C enabled us to characterize in detail the internal disorganization of the nucleoid, observing that the long-range organization and the gene organization of the E. coli genome into domains was impacted. Nucleoid of Ndd treated cells form small globules expulsed from cell center and laying on the membrane. These results open the way to the study of Ndd function during lytic T4 phage cycle.

Mots clés : E.coli - Ndd - nucleoide - phage.

Auteur principal: GROSEILLE, Justine (1 Dynamique des chromosomes - CIRB, College de de France et Institut Pasteur)

Co-auteurs: Mme THIERRY, Agnès (2 UMR3525- Regulation spatiale des génomes, Institut Pasteur, PARIS, France); M. ILIOAIA, Cristian (tomic Energy and Alternative Energies Commission, CEA ·Institute of Biology and Technology Saclay, Saclay, France); M. ESPÉLI, Olivier (Dynamique des chromosomes - CIRB, College de de France, PARIS, France); M. KOSZUL, Romain (2 UMR3525- Regulation spatiale des génomes, Institut Pasteur, PARIS, France)

Orateur: GROSEILLE, Justine (1 Dynamique des chromosomes - CIRB, College de de France et Institut Pasteur)

Classification de Session: Oral

ID de Contribution: **2** Type: **Non spécifié**

Exploring uracil base opening dynamics in dsDNA through enhanced molecular dynamics simulations

Uracil can exist in dsDNA by spontaneous cytosine deamination or by misincorporation of dUMP instead of dTMP during the replication process [1]. A highly specific Uracil-DNA glycosylase (UDG) enzyme recognizes and excise the flipped uracil base from the dsDNA helix; this mechanism is part of the base-excision repair (BER) pathway [2]. There remains a lack of complete understanding in the debate over the DNA base flipping mechanism—whether base flip happens due to the DNA helix's distortion or if it is induced by the UDG enzyme during BER. However, the dynamic mechanism of uracil base flipping at an atomic level is still not understood. To gain an insightful understanding, we perform metadynamics (MtD) simulations to generate free energy profiles for uracil base flipping out of its helical stack in the dsDNA in this work. The uracil base causes a slight perturbation (intrahelical) towards the major groove side initially, and the opening angle is ~50° with an energy barrier of ~6 kcal/mol. The energy barrier difference from the intrahelical to extrahelical state is about ~2kcal/mol. We docked intrahelical and extrahelical poses of uracil base in dsDNA with the UDG enzyme using molecular docking tools to understand the UDG enzyme activity in the base flipping mechanism. The obtained docking confirmation indicated that the extrahelical state of uracil in dsDNA fits well into the catalytic pocket of the UDG enzyme. Our simulations suggest that uracil base flipping may be the key step that permits UDG to recognize and excise uracil from the dsDNA.

References:

1. Vassylyev, D. G. & Morikawa, K. Precluding uracil from DNA. Structure 4, 1381–1385 (1996).
2. Hegde, M. L., Hazra, T. K. & Mitra, S. Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res. 18, 27–47 (2008).

Auteurs principaux: Dr SARAVANAN, Vinnarasi (University of Lille); Dr BLOSSEY, Ralf (University of Lille)

Co-auteur: M. RAOURAOUA, Nessim (University of Lille)

Orateur: Dr SARAVANAN, Vinnarasi (University of Lille)

Classification de Session: Oral

ID de Contribution: **3** Type: **Non spécifié**

Controlled liquid-like droplet formation of ParBS complexes through CTP binding and hydrolysis during bacterial DNA segregation

The bacterial DNA segregation is mainly performed with the ParABS system. It is composed of ParB, a binding protein, ParA, an ATPase and parS, a specific binding DNA sequence that ParB binds parS with high affinity. Hundreds of ParB are recruited around parS into a complex, called ParBS, which displays liquid-like properties.

Recently, it has been shown that ParB is using energy stored as CTP in the cells according to the clamping and sliding model. Upon specific binding onto parS, ParB binds CTP that is used to switch the ParB into a clamp that is then released DNA to perform transient 1D diffusion until it detaches and unbinds CTP. The role of the CTP is not completely understood: neither the time scale of the ParBS formation nor the total number of ParB into ParBS could be accounted for with this model.

We hypothesize that the ParB clamping leads to an increase of the ParB-ParB interactions, leading to an increased speed and specificity of the ParBS formation during a liquid-like phase separation. This claim is supported by numerical simulations of ParB via a Lattice Gas combined with dedicated experiments of ParB mutants in CTP binding and hydrolysis.

Auteurs principaux: Mlle DELIMI, Linda (Laboratoire Charles Coulomb); REVOIL, Perrine (Université Toulpuse III Paul Sabatier - Laboratoire de Microbiologie et Génétique Moléculaires); BOUET, Jean-Yves (CNRS); Dr WALTER, Jean-Charles (L2C, CNRS & Montpellier Université)

Orateur: Mlle DELIMI, Linda (Laboratoire Charles Coulomb)

Classification de Session: Oral

ID de Contribution: **4** Type: **Non spécifié**

Mechanism of bacterial DNA segregation: LLPS behavior, beyond trend and reality

In bacteria, low-copy-number replicons carry self-specific partition systems to ensure their faithful segregation. Among these systems, ParABS partition systems, consisting of a Walker-type ATPase (ParA) and a DNA-binding protein (ParB) along with parS centromere sites, are the most prevalent on plasmids and the only one present on chromosomes. ParB proteins, recently shown to belong to a novel class of CTP-dependent molecular switches, self-assemble into partition complexes nucleated from parS sites, with over 90% of intracellular ParB concentrated within these clusters. It has been proposed that partition complexes behave as bio-molecular condensates through liquid-liquid phase separation (LLPS). This physical principle describes the behavior of biomolecules in solution that auto-assemble in condensates, with general properties such as a spherical shape, a different mobility of molecules inside and outside the droplet and the ability of the droplets to fuse. To elucidate the assembly dynamics of partition complexes/condensates, we investigated the fusion behavior of ParB condensates in vivo. Such fusion behavior is hindered by the intrinsic function of partition, through ParA ATPases, which separate and localize ParB condensates. To observe ParB condensates fusion, we disrupted ParA activity by removing the matrix over which ParA is mediating their anchoring, i.e. the nucleoid. We developed an inducible, chromosome specific DNA degradation system that occurs rapidly. We showed that in absence of nucleoid, ParB condensates still form on plasmids carrying parS centromere sites, and rapidly fuse, irrespective of the initial number of complexes present in cells. In addition, we found that fusion strictly depends on ParB proteins and conservatively clusters all ParB proteins in a single condensate per cell.
Moreover, we have also demonstrated that hexanediol, a disruptor of weak hydrophobic protein–protein interactions, rapidly induces the disassembly of ParB condensates within tens of seconds. This effect is reversible. It thus indicates that weak ParB-ParB interactions are central to the assembly of ParB condensates. Lastly, we further characterized the fusion/fission dynamics of some condensates and found that fusions occur within seconds, independent of the presence of ParA. These rapid dynamics reinforce the notion that the assembly of partition complexes is mediated by LLPS. Our results underscore the critical role of ParA activity in counteracting the intrinsic merging properties of the LLPS-driven ParB condensates.

Auteurs principaux: BOUET, Jean-Yves (CNRS); REVOIL, Perrine (Université Toulpuse III Paul Sabatier - Laboratoire de Microbiologie et Génétique Moléculaires)

Orateur: REVOIL, Perrine (Université Toulpuse III Paul Sabatier - Laboratoire de Microbiologie et Génétique Moléculaires)

Classification de Session: Oral

ID de Contribution: **5** Type: **Non spécifié**

An in vivo degradation system to study cross-talk between topoisomerases in E. coli

During the E. coli cell cycle, DNA is exposed to coiling variations induced by biological processes such as replication or transcription. Four topoisomerases contribute to the maintenance of DNA homeostasis (TopoI, Gyrase, TopoIII and TopoIV). Among them, TopoI which function appeared to be mostly linked to the relaxation of negative supercoils induced by transcription and TopoIV that promotes replication and segregation by removing positive supercoils and catenanes might be functionally linked. For instance, TopoI mutants are suppressed by duplication of the Topo IV genes. Until now, the study of functional interactions between these topoisomerases has been limited by the difficulty to combine thermosensitive mutants of TopoIV and TopoI mutants that contain different suppressor mutations. To investigate this cross-talk, an inducible degradation system was used that allow rapid depletion of TopoI, TopoIV or both and to monitor cell physiology and DNA topology at low or null TopoI and TopoIV concentration .
Topoisomerase degradation relies on the induction of an exogenous Lon protease that specifically targets the protein(s) of interest fused to a degradation tag. First of all, I validated the degradation system for TopoI and TopoIV; it enabled a drastic reduction of each topoisomerase in 2 hours. Second, I measured consequences of TopoI depletion: although, it rapidly induces over-supercoiling of plasmid DNA, it did not imply growth nor viability defects of the bacteria. Surprisingly, the amount of other topoisomerases (Gyrase and TopoIV) was unchanged and TopoIII was not required for viability. These results suggest that over a short period of time, E. coli can fully tolerate a low amount of TopoI and the associated topological changes. In contrast, degradation of ParE, one of the TopoIV subunits, reduced growth rate and cell viability and induced a well- characterized par phenotype with filamentous and anucleate cells. Surprisingly, when TopoI and ParE were depleted simultaneously, the growth defect associated with ParE degradation is suppressed. These results highlight a novel antagonistic effect between TopoI and TopoIV and further support the utilization of this system to enhance our comprehension of the interaction between topoisomerases.
(1) Cameron and Collins. 2014 Nat Biotechnol

Auteur principal: BORDE, Céline (CIRB CDF)

Co-auteurs: BRUNO, Lisa (Collège de France); ESPELI, Olivier (CGM CNRS)

Orateur: BORDE, Céline (CIRB CDF)

Classification de Session: Oral

ID de Contribution: **6** Type: **Non spécifié**

RNA profiling of molecular crowed nuclear micro-environments

Studying nuclear micro-environments, particularly membrane-less organelles (MLO) like Cajal-bodies, PML-bodies, speckles or paraspeckles, has always been a challenge. Indeed, such MLO constitute micro-environments with high molecular crowding which typically result from liquid-liquid phase separation. However, the isolation of such liquid-like droplets and the characterization of their internal components is key to understand their roles.

In a previous study, our team showed that such nuclear micro-environments with high molecular crowding can be insolubilized using high-salt concentrations, allowing their isolation from the rest of the nuclear components that remains soluble. It is then possible to identify the so-called "High-salt Recovered Sequences"(HRS) as genomic DNA sequences that are enriched in the insolubilized nuclear micro-environments compared to the soluble fraction. In mouse Embryonic Stem cells, these sequences have been found to be associated with the active A chromosomal compartment, including transcription start sites and enhancers of highly expressed genes, but also with known MLO, like the Cajal-bodies, the speckles and the paraspeckles (Baudement et al. 2018, Genome Research 28:1733-1746).

Here, using an evolution of this method that allows the recovery of RNA in addition to the genomic DNA on IMR-90 cells (human embryonic lung fibroblasts), we performed the first global profiling of RNA transcripts associated with such highly crowded nuclear micro-environments. We found that such transcripts largely correspond to specific long non-coding RNAs (lncRNAs), some of which are already known to be associated with specific nuclear bodies such as Neat1_2, an architectural RNA of the paraspeckles. We also observed that, globally, premature RNA transcripts are more enriched in this insoluble RNA fraction than their mature counterparts. Finally, some transcripts with specific intron retention events are also enriched in such nuclear micro-environments, including one that depends on paraspeckle integrity.

We now plan to combine an RNA interference approach in IMR-90 cells to disrupt the paraspeckles, and then exploit this original way of observing the distribution of RNA in the nucleus to identify the global RNA content, as well as the genomic DNA content of this MLO. In the future, this approach could be extended to a broad range of MLO to better understand their role inside the nucleus.

Auteur principal: LECOUVREUR, Nathan (IGMM)

Co-auteurs: LESNE, Annick (CNRS); Mme REBOUISSOU, Cosette (IGMM); FORNÉ, Thierry (CNRS)

Orateur: LECOUVREUR, Nathan (IGMM)

Classification de Session: Oral

ID de Contribution: **7** Type: **Non spécifié**

Polymer physics to investigate the elastic properties of bundled hydrogels : applications to collagen gels.

Collagen gels are ubiquitous materials found in biological tissues, serving as structural support, regulating cellular processes, or contributing to wound healing.
Multiple models elucidate their tensile behavior through the lens of polymer physics \[1\].
Nevertheless, the characteristics of these gels under hydrodynamic pressure remain largely unexplored. Our recent findings highlight the varied behavior of these gels, dependent on whether they experience tensile or compressive stress. \[2\]
Here we report a poroelastic response model for collagen gels, integrating large-scale hyperelastic models with polymer deformation at the microscale.
Our proposal suggests that the asymmetric elasticity arises from the interplay of physical and chemical interactions within the gel, attributable to cross-links and self-avoidance mechanisms, respectively. Thus, the elastic properties of soft hydrogels seem to be highly adaptable, contingent upon their microscopic architecture. We further propose that this model holds promise for extension to a wide range of hydrogels, including those composed of DNA.

Auteurs principaux: M. BANCAUD, Aurélien (LAAS); LE FLOCH, Erwan (Université PAUL SABATIER)

Orateur: LE FLOCH, Erwan (Université PAUL SABATIER)

Classification de Session: Posters

ID de Contribution: **8** Type: **Non spécifié**

Rules of engagement for condensins and cohesins guide mitotic chromosome formation

During mitosis, interphase chromatin is rapidly converted into rod-shaped mitotic chromosomes. Using Hi-C, imaging, proteomics and polymer modeling, we determine how the activity and interplay between loop-extruding SMC motors accomplishes this dramatic transition. Our work reveals rules of engagement for SMC complexes that are critical for allowing cells to refold interphase chromatin into mitotic chromosomes. We find that condensin disassembles interphase chromatin loop organization by evicting or displacing extrusive cohesin. In contrast, condensin bypasses cohesive cohesins, thereby maintaining sister chromatid cohesion while separating the sisters. Studies of mitotic chromosomes formed by cohesin, condensin II and condensin I alone or in combination allow us to develop new models of mitotic chromosome conformation. In these models, loops are consecutive and not overlapping, implying that condensins do not freely pass one another but stall upon encountering each other. The dynamics of Hi-C interactions and chromosome morphology reveal that during prophase loops are extruded in vivo at ~1-3 kb/sec by condensins as they form a disordered discontinuous helical scaffold within individual chromatids.

Auteur principal: MIRNY, Leonid (MIT and Institut Curie)

Orateur: MIRNY, Leonid (MIT and Institut Curie)

Classification de Session: Oral

ID de Contribution: **9** Type: **Non spécifié**

Histone ADP-ribosylation controls early multi-scale chromatin dynamics upon DNA damage

ADP-ribosylation signaling by PARP1 is a key early event of the DNA damage response (DDR). PARP1 recruitment occurs within seconds upon DNA damage, triggering the accumulation of ADP-ribose binding repair factors and regulating chromatin architecture at sites of DNA damage. Histones, which are the second main target of this signaling pathway after PARP1 itself, are ADP-ribosylated, causing a rapid and transient relaxation of damaged chromatin. Although it has recently been proposed that this first remodeling event favors the access of further repair proteins to DNA lesions, it has not been characterized yet how exactly the ADP-ribosyl signaling shapes chromatin during the DDR at a nucleosome scale. Furthermore, new findings have described mono-ADP ribosylation as a second wave of PARP signaling, however its role in chromatin remodeling remains unclear.

In this project, we aim to uncover how ADP-ribosylation modulates chromatin folding immediately after DNA damage nearby lesions and to unveil its potential functions in the DDR. To this end, we characterize chromatin architecture at multi- scale levels in live human cells: from the fiber itself to single histones. We use an innovative set-up based on single-molecule imaging combined with laser-micro- irradiation. We develop machine learning approaches to extract diffusion parameters from our experimental single particle tracking data. Our results show that chromatin motion is dramatically increased as early as 30 seconds after irradiation. Interestingly, this effect is transient as chromatin recovers its initial mobility 10 minutes after irradiation. This increased dynamic is restricted to chromatin located within the irradiated area, suggesting a specific response to DNA damage. Notably, these changes in chromatin dynamics occur at both the chromatin fiber and nucleosome scales and correlate with PARP1-dependent chromatin remodeling. We have therefore characterized chromatin dynamics in conditions where ADP-ribosylation is inhibited (PARPi), persistent (PARGi, ARH3KO) or when PARP1 is fully or partially suppressed (PARP1 mutants). We demonstrate that multi-scale dynamic changes of damaged chromatin depend on mono- and poly ADP-ribosylation of histones and play an essential role in the choice of repair pathway.

Auteur principal: GARCIA FERNANDEZ, fabiola (INSERM)

Co-auteurs: CHAPUIS, Catherine (Université de Rennes, CNRS, IGDR (Institut de Génétique et Développement de Rennes, Rennes, France.); HUET, Sébastien (Institut de Génétique et du Développement de Rennes); MINE-HATTAB, judith (LCQB UMR7238)

Orateur: GARCIA FERNANDEZ, fabiola (INSERM)

Classification de Session: Oral

ID de Contribution: **10** Type: **Non spécifié**

Exploring model of chromosome in vivo : ribosomal DNA and nucleolus

Claudie Carron1, Carine Dominique1, Nana Kadidia Maiga 1, Mickaël Lelek2, Thomas Mangeat3, Frédéric Beckouët1, Christian Rouvière3, Isabelle Léger-Silvestre1, Sylvain Cantaloube3, Christophe Zimmer2, Anthony K. Henras1, Benjamin Albert1and Olivier Gadal1.

1: MCD (CBI), Université de Toulouse, CNRS, UPS, 31000, Toulouse, France

2: Imaging and Modeling Unit, Department of Computational Biology, Institut Pasteur, Paris, France

3: Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, 31000, Toulouse, France

Spatial organisation of chromosomes is a key feature for genome stability, transcription and is required for proper mitotic segregation. Using a large panel of technics, ranging from imaging chromatin position and motion in living cells to chromosome capture technologies, underlying mechanisms responsible of genome organization are now actively explored.

In model organism such as Saccharomyces cerevisiae, in silico models of the entire genome have been developed. A model based on passively moving polymer chains and local tethering accounted for a large amount of experimental data. In my presentation, I will rapidly introduce success and limit of in silico model to predict global organisation of chromatin fibers observed by microscopic technics. We applied a multiscale imaging to directly confront in silico model to imaging dataset. Using four imaging technologies of increasing resolution from widefield fluorescent microscopy, random illumination microscopy, single molecule localization microscopy to electron microscopy, we could acquire high resolution imaging of yeast chromatin.

Next, focusing on ribosomal DNA, a genomic region associated with the largest nuclear compartment, the nucleolus, we will discuss how putative liquid-liquid phase separation and condensate formation can affect chromatin properties. The nucleolus is enriched in protein bearing intrinsically disordered regions (IDRs), and we focused on one type of IDR, named KKE/D domains. We will show that KKE/D has a dual function in nucleolar organization depending on transcriptional activities activity.

Auteur principal: GADAL, Olivier (Centre de Biologie Intégrative (CBI), UMR5077 CNRS, bat IBCG)

Orateur: GADAL, Olivier (Centre de Biologie Intégrative (CBI), UMR5077 CNRS, bat IBCG)

Classification de Session: Oral

ID de Contribution: **11** Type: **Non spécifié**

Chromatin and nucleoplasm motions in response to DNA damage

Maelle Locatelli2, Chloé Hommais1, Fadil Iqbal3, Keith Bonin4, Kerry Bloom2, Jing Liu3, and Pierre-Alexandre Vidi1

1 Institut de Cancérologie de l'Ouest, Angers, France
2 Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, USA
3 Department of Physics, Purdue University, West-Lafayette, USA
4 Department of Physics, Wake Forest University, Winston-Salem, USA

Chromatin mobility is influenced by and may regulate genome functions, including the DNA damage response. We mapped the motions of chromatin microdomains in mammalian cells using structured illumination of photoactivatable histone probes and found high levels of heterogeneity between cells and within individual cell nuclei. DNA damage reduces heterogeneity and alters chromatin dynamics: motions are globally reduced but higher mobility is retained at break sites. These effects are driven by context-dependent changes in chromatin compaction. Using an X-ray system integrated to the microscope, we detected a rapid decrease in global chromatin motions, occurring within minutes following break induction. Measurements with nuclear nanoparticles show that nucleoplasmic macromolecule dynamics are also rapidly impacted by DNA damage, with likely consequences on chromatin accessibility. Irradiating cells on the microscope enables us to evaluate DNA damage outcomes relative to chromatin dynamics at the single cell level. The results indicate that the capacity of cells to process radiation-induced DNA damage as well as DNA repair outcomes depend on chromatin mobility. Overall, our data show that chromatin motions are finely tuned after genomic insults and that chromatin motions influence DNA repair.

Auteur principal: VIDI, Pierre-Alexandre (Institut de Cancérologie de l'Ouest)

Orateur: VIDI, Pierre-Alexandre (Institut de Cancérologie de l'Ouest)

Classification de Session: Oral

ID de Contribution: **12** Type: **Non spécifié**

Replicon dynamics during the cell cycle in Escherichia coli

During cell cycle, the bacteria must transmit all the genetic information it contains to its daughter cells. To do this, the chromosome and the plasmids, which form the replicons, must be duplicated through replication, then segregated in each daughter cell. However, replication and segregation events must be finely coordinated with cell division. Many proteins are involved in the regulation of this cell cycle notably the MatP protein, and its DNA binding site matS, which participates in the positioning of the ter regions of sister chromatids at the center of the cell 1; and the recombinases XerC and XerD which separate dimers formed at the end of replication, by site-specific recombination at the dif site 2. Nevertheless, the entire mechanisms that drive the regulation is not fully understood. It is known that a lot of proteins involved in this regulation can interact with the Topoisomerase IV (TopoIV). TopoIV is an heterotetrameric (ParC2ParE2) ATPase-dependent complex, which has a key role in the regulation of the cell cycle through its catenane and pre-catenane resolution activity [3]. Catenanes are interlinked DNA molecules, and these links must be removed to allow their segregation. To better understand the decatenation activity in vivo, I use the model bacterium Escherichia coli with Temperature sensitive mutants of TopoIV. Their activity is investigated by analyzing the accumulation of plasmid catenanes at non-permissive temperature, as well as their resolution by the recovering TopoIV.
Experiments are carried out with pUC18, which is a small (2.7kb) plasmid with a high copy number; and its derivative pMIN33 where a dif site has been added. We know that TopoIV activity is strong at this site [4]. To study the effect of MatP on the activity of TopoIV, a matS site has been added on these plasmids. My results suggest that the presence of matS and dif sites on the plasmid can improve its decatenation, but also that the combination of matS and dif have a synergic effect on TopoIV activity.

1. Mercier, R. et al. The MatP/matS Site-Specific System Organizes the Terminus Region of the E. coli Chromosome into a Macrodomain. Cell 135, 475–485 (2008).
2. Blakely, G. et al. Two Related Recombinases Are Required for Site-Specific Recombination at dif and cer in E, coli K12, Cell 75, 351-361 (1993).
3. Kato, J. et al. New Topoisomerase Essential for Chromosome Segregation in E. coli, Cell 63, 393-404 (1990). 4.El Sayyed, H. et al. Mapping Topoisomerase IV Binding and Activity Sites on the E. coli Genome. PLoS Genet 12, e1006025 (2016).

Auteur principal: VEYRIER, Iris (LMGM CBI)

Orateur: VEYRIER, Iris (LMGM CBI)

Classification de Session: Oral

ID de Contribution: **13** Type: **Non spécifié**

How does the Ter linkage par3cipate in chromosome organiza3on and segrega3on in E. coli

Coordina3ng chromosome segrega3on with cell division is a key-step of the cell cycle for any bacteria. E. coli does not seem to require an ac3ve system to segregate its chromosomes. Instead, entropy and a specific organiza3on of the chromosome into domains seem to explain the observed choreography. MatP is the DNA-binding protein responsible for organizing the ter region by preven3ng the SMC proteins, MukBEF, to compact it. It also helps localizing the Z-ring at midcell, by interac3ng with ZapB, which interact in turn with ZapA and FtsZ, forming the so-called Ter-linkage. MatP also keeps the replicated ter regions at the septum un3l cell division.
First, we studied MatP and its ability to bind ter by following the short-term dynamics (20s) of fluorescent chromosomal loci in strains deleted for matP and/or zapB. We also used Tethered Par3cle Mo3on (TPM), as well as gel shiU assays to beVer characterize how MatP binds to DNA.
Taken together, these results revealed that tetramers of MatP very dynamically bridge the ter region of the chromosomes with the septum (i.e ZapB) before replica3on, and constrain their movements only at this point (Crozat et al, 2020).
We are now studying these mutants by fluorescent microscopy. Varying the amount of ZapB enables us to shed light on a peculiar mechanism ac3ng on chromosome segrega3on, and the precise role played by MatP at this stage of the cell cycle.

Auteur principal: BRENDON, Estelle (LMGM-CBI, Université Toulouse 3)

Co-auteurs: TARDIN, Catherine (IPBS CNRS/UPS); CORNET, francois (CNRS - UPS Toulouse 3)

Orateur: BRENDON, Estelle (LMGM-CBI, Université Toulouse 3)

Classification de Session: Posters

ID de Contribution: **14** Type: **Non spécifié**

Lamin A/C deficiency re-wires transcription factor footprints and inhibits EMT

Recent studies suggest an involvement of nuclear lamins in the epithelial-to-mesenchymal transition (EMT) and cancer progression. However, the extent to which nuclear lamins, as genome organizers, are implicated in EMT remains both not consensual and unclear. We have addressed the role of A-type lamins (lamin A/C) in an MCF10A breast epithelial cell model of EMT induction by TGFβ. LMNA transcript levels correlate with expression of EMT markers and with poor prognosis of breast cancer survival. Lamin A/C depletion in MCF10A cells prevents acquisition of an EMT phenotype, establishment of an EMT transcriptome, and of histone modification changes elicited by TGFβ in lamina-associated domains. Lamin A/C depletion also extensively remodels chromatin accessibility detected by ATAC-seq, resulting in a general net opening of promoters of differentially expressed genes, yet attenuating TGFβ-induced increases in promoter accessibility. Absence of lamin A/C also extensively remodels transcription factor (TF) footprints, notably of FOS/JUN pioneer TFs of the AP-1 complex, SMAD2/3 and other factors previously not reported to be associated with EMT. Our footprint changes allude to TF retention, eviction or binding inhibition at/from target DNA binding sites in promoters and enhancers. Changes in TF binding behavior however occur in a manner not necessarily coupled to concurring changes in chromatin accessibility and H3K27 acetylation. We conclude that A-type lamins are essential for TGFβ-induced EMT in the MCF10A model, by enabling a chromatin accessibility landscape and TF binding behaviors at regulatory elements driving EMT gene expression.

Auteur principal: COLLAS, Philippe (University of Oslo)

Orateur: COLLAS, Philippe (University of Oslo)

Classification de Session: Oral

ID de Contribution: **15** Type: **Non spécifié**

The role of intrinsically disordered regions (IDR) of RNA polymerase I machinery in nucleolar organization and chromatin compaction of ribosomal DNA

In eukaryotes, the nucleolus is a specialized nuclear compartment where the early stages of ribosome biogenesis take place. The nucleolar organization and ribosomal DNA compaction reflect ribosome production from yeast to human.
In yeast, ribosomal DNA is a unique 1-2 Mb region of chromosome XII in which 100-200 copies of ribosomal DNA (rDNA) are repeated. Ribosome assembly is initiated by massive transcription of rDNA by RNA polymerase I. In actively growing cells, rDNA is decondensed within the nucleolus, forming a crescent shape compartment occupying about one third of the entire nuclear volume. Upon growth inhibition, rDNA become rapidly condense, associated with global sub-nucleolar region re-organization. The underlying principle allowing such rapid and global re-organization of both rDNA and the nucleolus are poorly understood.
The nucleolus is enriched in protein bearing intrinsically disordered regions (IDRs). IDRs are protein domains exhibiting a high degree of conformational flexibility capable of forming low-energy interaction and thought to promote liquid liquid phase separation and condensate formation.
During my PhD, using yeast as model organism, we will investigate the putative role of IDRs of RNA polymerase I transcription machinery in nucleolar organization and compaction. Using live cells imaging and fluorescent labeled rDNA and nucleolar protein we will study the implication of IDRs deletion on nucleolar organization and rDNA compaction during either exponential growth or growth inhibition.

Auteur principal: DANCHE, Sarah

Orateur: DANCHE, Sarah

Classification de Session: Posters

ID de Contribution: **16** Type: **Non spécifié**

Chromosome elasticity with loop-extrusion activity

The loop-extrusion process has emerged as a pivotal mechanism governing chromosome organization within the nucleus. However, the impact of this process on the effective stiffness of chromosomes remains largely unexplored. In this study, we introduce a novel polymer model to investigate the force-extension behavior of chromosomes with loop-extrusion activity. Our analytical and simulation findings reveal that loop-extrusion activity enhances the effective stiffness of chromosomes in low-force regimes, while demonstrating reduced stiffness at higher force levels, consistent with experimental observations. Moreover, our simulations predict a hysteresis-like behavior in force-extension curves, indicative of memory retention within the loop-extrusion process.

Auteur principal: Dr SALARI, hossein (Laboratory of Biology and Modelling of the Cell, Univ Lyon, ENS de Lyon)

Co-auteur: Dr JOST, Daniel (Laboratoire de Biologie et Modélisation de la Cellule, ENS Lyon)

Orateur: Dr SALARI, hossein (Laboratory of Biology and Modelling of the Cell, Univ Lyon, ENS de Lyon)

Classification de Session: Posters

ID de Contribution: **17** Type: **Non spécifié**

The role of histone acetylation in sub-megabase chromatin folding

Genome 3D organization is highly complex, made of several layers of organization from nucleosome to chromosome territory. At the Megabase scale, the genome is partitioned into Topologically Associating Domains (TADs), that may define functional genomic units. TADs are mostly revealed by cell population-based assays such Hi-C and their organization is defined by the extrusion action of cohesin complexes delimited by CTCF at TAD borders.

To better understand this level of genome organization, we addressed TAD structure at the single cell level using a combination of FISH with Oligopaint and Structured Illumination Microscopy (3D-SIM). Super-resolution microscopy revealed the heterogeneous properties of TADs in mammals as well as the presence of subdomains that we dubbed Chromatin NanoDomains or CNDs. Their formation is independent of Cohesin or CTCF function but appears to depend on the level of histone acetylation. Moreover, their genomic size (in the range of 100-150 kb) is compatible with Enhancer-Promoter communication. Using a combination of genomics approaches and super-resolution microscopy, we are trying to decipher the relationship between acetylation, chromatin folding and genome function, as well as the role of specific histone acetyl transferase (HAT) complexes.

Auteur principal: BANTIGNIES, Frédéric (CNRS)

Co-auteurs: CAVALLI, Giacomo (CNRS); REBOUL, Henri (LUPM UMR 5299)

Orateur: BANTIGNIES, Frédéric (CNRS)

Classification de Session: Oral

ID de Contribution: **18** Type: **Non spécifié**

Single-molecule tracking reveals the functional allocation, in vivo interactions, and spatial organization of universal transcription factor NusG

During transcription elongation, NusG aids RNA polymerase by inhibiting pausing, promoting anti-termination on rRNA operons, coupling transcription with translation on mRNA genes, and facilitating Rho-dependent termination. Despite extensive work, the in vivo functional allocation and spatial distribution of NusG remain unknown. Using single-molecule tracking and super-resolution imaging in live E. coli cells, we found NusG predominantly in a chromosome-associated population (binding to RNA polymerase in elongation complexes) and a slowly diffusing population complexed with the 30S ribosomal subunit; the latter provides a "30S-guided" path for NusG into transcription elongation. Only ~10% of NusG is fast diffusing, with its mobility suggesting non-specific interactions with DNA for >50% of the time. Antibiotic treatments and deletion mutants revealed that chromosome-associated NusG participates mainly in rrn anti-termination within phase-separated transcriptional condensates and in transcription-translation coupling. This study illuminates the multiple roles of NusG and offers a guide on dissecting multi-functional machines via in vivo imaging.

Auteur principal: EL SAYYED, Hafez (University of Oxford)

Orateur: EL SAYYED, Hafez (University of Oxford)

Classification de Session: Oral

ID de Contribution: **19** Type: **Non spécifié**

Biophysical models of the Zfp608 locus in mouse cells

Marco Di Stefano, Ivana Jerkovic, Giacomo Cavalli
Institute of Human Genetics, Université de Montpellier, CNRS, Laboratoire de Chromatine et Biologie Cellulaire, 34094 Montpellier, France

Chromosome structural organization contributes to fundamental processes in the cell nucleus, including DNA transcription, replication, and repair. Experimental and theoretical works unveiled that chromosome spatial organization is a complex aggregate of layers: entire chromosomes occupy distinct volumes of the nucleus, called territories; regions of tens of Mega-bases (Mb) tend to organize in active and repressed (A/B) compartments; regions up to one Mb organize in domains (TADs); and loops may bring in contacts gene promoters with enhancers. However, the forces regulating these layers and their interplay with transcription activity are still elusive. Here, I will present an approach to studying these organizing principles in the genomic region around the Zfp608 gene in mouse Embryonic stem cells, where the gene is transcriptionally inactive, and in neural progenitor cells (NPC), where it is active. By applying biophysical structural modeling, we focus on epigenomic-driven interactions between chromatin of the same type (e.g., active chromatin attracts other regions with the same chromatin marks), loop-extrusion dynamics, and the effect of promoter-enhancer interactions. Extensive quantitative analysis and comparison with Capture Hi-C data drives the models'parameterization. This project aims to show that biophysical models can help explain how experimentally observed structures are formed and unravel potential factors and molecular mechanisms regulating chromosome organization in different cell types.

Auteur principal: DI STEFANO, Marco (IGH)

Orateur: DI STEFANO, Marco (IGH)

Classification de Session: Posters

ID de Contribution: **21** Type: **Non spécifié**

New insights into resistance to the metabolic inhibitor 2- deoxyglucose from studies in fission yeast

The rewiring of glucose metabolism from mitochondrial respiration to fermentation is a hallmark of cancer. This metabolic adaptation has been exploited in ongoing therapeutic and diagnostic strategies. Notably, treating cancer cells with the glucose analog 2-deoxy-D-glucose (2DG) reduces their proliferation presumably by dampening the rate of glycolysis. However, this treatment leads to frequent resistance, through mechanisms that remain incompletely characterized. This issue is important to improve the efficacy of this compound while preventing resistance, and to rationalize its use in the clinics, either alone or in combinatorial approaches.
We are using fission yeast as a new model to understand the cellular effects of 2DG and how cells evade its toxicity. Indeed, we accumulated evidence that new resistance mechanisms exist in this species, which were not identified in studies previously performed in budding yeast and other model systems. Two epigenetic regulators, SAGA and SWI/SNF, appear to have critical roles in 2DG resistance in fission yeast, suggesting a new chromatin-based mechanism. In particular, our preliminary data suggest a differential degree of action within the SAGA complex, revealing a leading role for the histone acetyl transferase subunit in 2DG resistance whose molecular mechanisms have yet to be determined.

Auteur principal: BIROT, Adrien (Université de Bordeaux et de Montpellier - CNRS)

Orateur: BIROT, Adrien (Université de Bordeaux et de Montpellier - CNRS)

Classification de Session: Posters