5ème Rencontre scientifique des Grands Causses

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Transcription regulates the spatio-temporal dynamics of genes through micro-compartmentalization

Auteurs: Daniel Jost[1]; genevieve fourel[2]; hossein salari[3]

[1] *Laboratoire de Biologie et Modélisation de la Cellule, ENS Lyon*

[2] *INSERM*

[3] *Laboratory of Biology and Modelling of the Cell, Univ Lyon, ENS de Lyon*

Auteurs correspondants: genevieve.fourel@ens-lyon.fr, daniel.jost@ens-lyon.fr, hossein.salari@ens-lyon.fr

Although our understanding of the involvement of heterochromatin architectural factors in shaping nuclear organization is improving, there is still ongoing debate regarding the role of active genes in this process. In this study, we utilize publicly-available Micro-C data from mouse embryonic stem cells to investigate the relationship between gene transcription and 3D gene folding. Our analysis uncovers a nonmonotonic - globally positive - correlation between intragenic contact density and Pol II occupancy, independent of cohesin-based loop extrusion. Through the development of a biophysical model integrating the role of transcription dynamics within a polymer model of chromosome organization, we demonstrate that Pol II-mediated attractive interactions with limited valency between transcribed regions yield quantitative predictions consistent with Hi-C and live-imaging experiments. Our work provides compelling evidence that transcriptional activity shapes the 4D genome through Pol II-mediated micro-compartmentalization.

2

PRC1 nano-structures compact Polycomb-associated chromatin during Drosophila embryogenesis.

Auteur: Thierry Cheutin[1]

Co-auteurs: Giacomo cavalli [1]; Marco Di Stefano [1]; Nazli Akilli [1]

[1] *CNRS*

Auteur correspondant thierry.cheutin@igh.cnrs.fr

Regulation of gene expression during cell differentiation is a complex process involving several levels of chromatin organization. Transcription factors such as promoters and enhancers act on discrete elements whose accessibility can be controlled by nucleosomes. Polycomb grouped proteins (PcG) are key factor required to maintain silenced chromatin state. Although PcG proteins bind discrete elements, their associated histone mark H3K27me3 spreads over chromatin domains large of several tens of kb, which evidences a higher-order organization at the linear genomic scale. In 3D nuclear space, PcG proteins and H3K27me3 accumulate in structures named Pc foci. Though, the relationship between the linear distribution of PcG proteins and their 3D organization in the cell nucleus still remains poorly understood, mainly because optical microscopy lacks resolution. In this work, we use STED, a super-resolution microscope allowing observations of embryos, to study the architecture of the most intense Polycomb foci which corresponds to repressed Hox clusters. In Drosophila embryos, the biggest Polycomb foci are composed of several sub-structures of about 70 nm in diameter. We confirm their existence using Airyscan microscopy and show that they move rapidly compare to each other in living embryos. Immuno-FISH experiments indicate that chromatin associated to repressed Hox clusters also displays substructures in STED microscopy. Although PRC1 and chromatin substructures mostly co-localize, they form distinct assemblies. Noticeably, discrete elements bound by PcG proteins within Hox clusters are more associated to the PRC1 sub-structures than the rest of chromatin marked by H3K27me3. In null mutant for Polycomb, the only subunit of PRC1 which can bind to H3K27me3, both discrete elements bound by PcG proteins and chromatin only covered by H3K27me3 are less associated with PRC1 substructures. Taken together, we evidence new higher-order PRC1 structures organizing Polycomb associated chromatin and explaining why

H3K27me3 is distributed within genomic domains of several tens of kb and the effect of PRC1 on chromatin compaction and long-range interactions.

3

Chromatin structure from high resolution microscopy: scaling laws and micro-phase separation

Auteur: Loucif Remini[1]

Co-auteurs: Andrea Parmeggiani [1]; Enrico Carlon [2]; Jean-Charles Walter [1]; John Palmeri [1]; Midas Segers [2]

[1] *L2C*

[2] *KU Leuven*

Auteur correspondant loucif.remini@umontpellier.fr

Recent advances in experimental fluorescence microscopy allow high accuracy determination (resolution of 50nm) of the 3D physical location of multiple (up to 10^2) tagged regions of the chromosome. We investigate publicly available microscopy data for two loci of the human genome obtained from multiplexed FISH methods for different cell lines and treatments. Inspired by polymer physics models, our analysis centers around distance distributions between different tags, aiming to unravel the chromatin conformational arrangements. We show that for any specific genomic site, there are (at least) two different conformational arrangements of chromatin, implying coexisting distinct topologies which we refer to as phase "α" and phase "β". These two phases show different scaling behaviors: the former is consistent with a crumpled globule while the latter indicates a confined, but more extended conformation, as a looped domain. The identification of these distinct phases sheds light on the coexistence of multiple chromatin topologies and provides insights into the effects of cellular context and/or treatments on chromatin structure.

4

Unraveling the principles regulating chromosome spatial organization during differentiation

Auteurs: Marco Di Stefano[1]; Ivana Jerkovic[1]; Giacomo Cavalli[1]

[1] *Institut de Génétique Humaine Montpellier*

Auteurs correspondants: marco.di-stefano@igh.cnrs.fr, giacomo.cavalli@igh.cnrs.fr, ivana.jerkovic@igh.cnrs.fr

Chromosomal structural organization contributes to fundamental processes in the cell nucleus, including DNA transcription, replication, and repair. Experimental and theoretical works unveiled that chromosome organization consists of a complex aggregate of layers: entire chromosomes occupy distinct regions of the nucleus, called territories; below, at the tens of Mega-bases (Mb) scale, active and repressed regions form (A/B) compartments; and at the intermediate Mb scale, local domains (TADs) and loops may bring in contacts gene promoters with enhancers. However, the forces regulating this organization and its interplay with transcription activity are still elusive. Here, I present a project where we study these organizing principles at the *Zfp608* locus in mouse embryonic stem cells (ESC), where *Zfp608* is transcriptionally inactive, and neural progenitor cells (NPC), where the gene is active. By applying biophysical structural 3D modeling, we focus on epigenomic-driven interactions between chromatin of the same type (e.g., active chromatin attracting other regions with the same chromatin marks), loop-extrusion dynamics, and the effect of promoter-enhancer interactions. Furthermore, we perform extensive quantitative analysis and comparison with Capture Hi-C experimental data to drive model parameterization. This project shows 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.

5

The RD-HRS Method : New insights on the relationship between phase separation, DNA and RNA

Auteur: Nathan Lecouvreur[1]

Co-auteurs: Annick Lesne [2]; Cosette Rebouissou [1]; Thierry Forné [1]

[1] *IGMM*

[2] *CNRS*

Auteurs correspondants: nathan.lecouvreur@igmm.cnrs.fr, cosette.rebouissou@igmm.cnrs.fr, thierry.forne@igmm.cnrs.fr, lesne@lptmc.jussieu.fr

The RD-HRS method: new insights on the relationship between phase separation, DNA and RNA

Nathan Lecouvreur1, Cosette Rebouissou1, Annick Lesne1,2, Thierry Forné1

1 Institut de Génétique Moléculaire de Montpellier, UMR5535, CNRS-Université de Montpellier, 1919 Route de Mende, 34293 Montpellier Cedex 5, FRANCE.
2 Laboratoire de Physique Théorique de la Matière Condensée, CNRS UMR 7600, Sorbonne Université, 4 place Jussieu, 75252 Paris Cedex 5, France.

- email: nathan.lecouvreur@igmm.cnrs.fr or thierry.forne@igmm.cnrs.fr

Recent experimental advances suggest that nuclear bodies dynamically self-assemble and disassemble through condensation and phase separation mechanisms. However, the links between the formation of these nuclear bodies and their association with genomic sequences, and the functional consequences of this association, remain almost entirely unexplored. The difficulty of isolating such semi-liquid structures is a technological barrier that has long prevented in-depth study of their implications for genome architecture and functions.

Our group developed the RD-HRS method (RNA/DNA High-salt Recovered Sequences), a novel experimental approach that allows to purify nuclear bodies by making them insoluble thanks to high-salt treatments of transcriptionally-active cell nuclei. After quantifications of nucleic acids in the insoluble and soluble fractions of HRS assays by quantitative PCR or high-throughput sequencing, we are able to identify not only DNA genomic sequences (Baudement et al., 2018), but also RNAs (Lecouvreur et al., in preparation) associated with them. Using mouse embryonic stem cells, we previously showed that, as expected, the DNA sequences enriched in HRS assays essentially correspond to sequences associated with nuclear bodies (Histone genes/Histone Locus Body; snRNA genes/Cajal bodies; Malat1 and Neat1 genes/speckles and paraspeckles⋯).

Here, using human fetal lung fibroblasts (IMR-90 cells), we performed RT-qPCR experiments on total RNA purified from HRS assays and we show that many, but not all, long non-coding RNAs (lncRNAs) expressed in these cells, like NEAT1, are highly enriched in such RNA-HRS assays compared to messenger RNAs of housekeeping genes. This result emphasizes the important architectural role that lncRNAs play as integral nuclear body components. Our results also indicate that unspliced pre-messenger RNAs of some highly expressed cell-type specific genes, like IGFBP5, are also strongly enriched in the HRS insoluble fraction. Interestingly, both NEAT1 gene and super-enhancers at the IGFBP5 locus are highly enriched in DNA-HRS assays suggesting that not only paraspeckles but also transcriptional condensates are retained in HRS insoluble fraction.

We are now targeting NEAT1 lncRNA using an RNA interference approach in order to inactivate specifically paraspeckles in both proliferative and senescent IMR-90 cells. We will then perform DNA/HRS-seq and HRS/RNA-seq to obtain the first global profiling of DNA and RNA associated with these nuclear bodies. This should bring new insights about the role of paraspeckles in the alteration of genomic organization during senescence.

6

Order in the disordered - Molecular determinants of phase separation and its physiological role in plant temperature sensing

Auteur: Stephanie Hutin[1]

Co-auteurs: Janet R Kumita ; Philip Wigge ; Luca Costa ; Mark Tully ; Chloe Zubieta

[1] *LPCV/CNRS*

Auteur correspondant stephanie.hutin@cea.fr

Order in the disordered - Molecular determinants of phase separation and its physiological role in plant temperature sensing

S. Hutin, JR. Kumita, P.Wigge, L. Costa, M. Tully, C. Zubieta

As sessile organisms, plants must live with constant environmental fluctuations and stress constraints. The increased average temperature and prolonged periods of extreme weather due to climate alter plant phenology, presenting a critical challenge for our food security. While direct sensing of their environment is critical for plant survival, the mechanisms plants use to monitor their environment have remained elusive. Our recent studies suggest that one important mechanism for a direct environmental sensing is via protein-mediated liquid–liquid phase separation (LLPS). LLPS is an important mechanism enabling the dynamic compartmentalization of biological macromolecules, such as proteins and nucleic acids, as a function of the cellular environment. In vitro, phase separation is sensitive to pH, ionic strength and, perhaps most notably, temperature [1]–[3]. This suggests that, in vivo, LLPS may act as a wide-ranging sensing mechanism, allowing a fine-tuned response to the changing physicochemical environment. Recent studies established that protein-mediated LLPS serves as an environmental sensing mechanism in response to external stimuli including temperature, water and nutrient availability, pathogen challenges and stress conditions, demonstrating that it is likely an important mechanism for directly sensing biotic and abiotic variables [4]–[11]. However, the underlying molecular mechanisms, the different physicochemical variables that trigger LLPS in vitro and in vivo, and its physiological role are the subject of ongoing debate due to the challenges in studying this complex biophysical phenomenon. We have identified an environmental sensing proteins involved in temperature sensing and response via LLPS; EARLY FLOWERING 3 (ELF3) [4]. The protein contains a largely unstructured prion-like domain (PrLD) that act as a driver of LLPS in vivo and in vitro. The PrLD contains poly-glutamine (polyQ) tracts, whose length varies across natural Arabidopsis accessions.
We use a combination of in vivo, biochemical, biophysical and structural techniques to investigate the dilute and condensed phases of the ELF3 PrLD with varying polyQ lengths. We demonstrate that the dilute phase of the ELF3 PrLD forms a monodisperse higher order oligomer that does not depend on the presence of the polyQ sequence. This species undergoes LLPS in a pH and temperature-sensitive manner and the polyQ region of the protein tunes the initial stages of phase separation [12]. Furthermore, the condensed phase rapidly undergoes aging and forms a hydrogel as shown by fluorescence and atomic force microscopies. We demonstrate that the ELF3 PrLD hydrogel assumes a semi-ordered structure using small angle X-ray scattering, electron microscopy and X-ray diffraction [12]. These experiments demonstrate a rich structural landscape for a PrLD protein and provide a framework to analyse and tune the structural and biophysical properties of biomolecular condensates.

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7

The bacterial DNA segregation complexes ParBS display a twofold phase separation

Auteur: Jean-Charles Walter[1]

[1] *L2C, CNRS & Montpellier Université*

Auteur correspondant jean-charles.walter@umontpellier.fr

The bacterial DNA segregation is prevalently performed through the ParABS system for most chromosome and low-copy number plasmids. It consists in two proteins: (i) ParB a DNA binding protein with a specific binding site at parS. It has been recently found to be a CTP-ase; (ii) ParA, an ATP-ase whose ATP hydrolysis is catalyzed by ParB and (iii) parS, a centromere-like sequence where ParB can bind specifically.

We show that the bacterial DNA segregation consists in a two-fold liquid-liquid phase separation (LLPS) (i) an equilibrium LLPS due to the formation of a droplet of ParB proteins centered on parS. The parS sequence acts as a kinetic catalyzer optimizing both the specificity and the speed of nucleation of the droplet and (ii) an out-of-equilibrium LLPS due to the action of ParA leading to the separation and the positioning of the two replicated droplets of ParB. The energy consumption of PArA is needed to counterbalance the increase of the surface tension.

8

The cell cycle as a phase separation cycle controlled by protein phosphorylation

Auteur: Liliana Krasinska[1]

Co-auteur: Daniel Fisher [2]

[1] *IGMM CNRS*

[2] *IGMM, CNRS, Montpellier*

Auteur correspondant liliana.krasinska@igmm.cnrs.fr