Sixième réunion du GdR Architecture et Dynamique Nucléaire (ADN)

Report of Contributions

https://indico.in2p3.fr/e/gdradn2017

Contribution ID: **16** Type: **not specified**

Polymer model for reconstructing chromatin and generating SPTs

Chromatin organization is probed by chromosomal capture data,from which the encounter probability (EP) between genomic sites is represented in a large matrix. However, this matrix is obtained by averaging the EP over cell population, where diagonal blocks called TADs, contains hidden information about sub-chromatin organization. Our aim here is to elucidate the relationship between TADs structure and gene regulation. For this end, we reconstruct the chromatin dynamics from the EP matrix using polymer model and explore the transient properties, constrained by the statistics of the data. To construct the polymer, we use the EP decay in two steps: first, to account for TADs, we introduce random connectors inside a restricted region defining the TADs. Second, we account for long-range frequent specific genomic interactions in the polymer architecture. Finally, stochastic simulations show that only a small number of randomly placed connectors are required to reproduce the EP of TADs, and allow us to compute the mean first time and the conditional encounter probability of three key genomic sites to meet. These encounter times reveal how chromatin can self-regulate. The present polymer construction is generic and can be used to study steady-state and transient properties of chromatin constrained on 5C data.

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Contribution ID: **18** Type: **not specified**

CTCF mediates allele-specific sub-TAD organization at paternally imprinted gene loci

Mammalian genes can be mono-allelicly expressed depending on their parental origin, a process called imprinting. Though only a relatively small number of genes are imprinted, this mechanism is of great importance for correct embryonic development.

Imprinting is governed by allele-specific DNA methylation at defined Imprinting Control Regions (ICRs), which often influences the binding of the architectural CTCF protein. In collaboration with the group of Robert Feil (IGM-Montpellier, France) we have used a mix of allele-specific ChIP-seq, high-resolution 4C and microscopy to study how 3D chromatin organization is involved at the paternally imprinted Dlk1-Dio3 and Igf2-H19 loci.

At both loci, the large majority of 3D interactions are containing within Topologically Associating Domain (TAD). Within these TADs dramatic allele-specific differences can be observed though. The maternal alleles form an invariant and more localized 3D structure that is demarcated by a mix of constitutive and multiple allele-specific CTCF binding sites. As such, these maternal specific sub-TADs encapsulate the ncRNA genes that are active on the maternal allele.

In contrast, the paternal alleles adopt a more locus specific 3D organization. The imprinted genes at the Igf2-H19 locus are contained within a sub-TAD with little specific internal structure and dynamics. In contrast, the imprinted protein-coding genes at the Dlk1-Dio3 locus co-occupy a large sub-TAD. Upon activation of these genes, in neuronal cells, interactions globally increase, indicative of a more compacted 3D configuration.

In conclusion, our study reveals that paternally imprinted gene loci in mammals are organized into allele-specific sub-TADs. Maternal alleles adopt the most structured and stable 3D organization, despite the imprint being present on the paternal allele. We hypothesize that imprinted gene expression at these loci relies most on the maternal 3D architecture, as supported by previous knock-out studies.

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Contribution ID: **19** Type: **not specified**

How pinching a DNA plectoneme facilitates the interaction between distant genes

Normally separated by a distance on the order of 10 nm, the two opposite double-strands of a DNA plectoneme must be brought closer if a protein implicated in genetic regulation is to be bound simultaneously to both strands. We propose an analytic calculation of the energetic barrier, of elastic nature, required to bring closer two loci situated on the two double-strands. We also examine how this energy barrier depends on the DNA supercoiling.

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Contribution ID: **21** Type: **not specified**

TALEs fused to chromatin modifiers for epigenetic engineering at satellite DNA repeats in mouse and human centromeric regions

Centromeric regions of eukaryotic chromosomes contain large numbers of tandemly repeated DNA sequences, also called satellite DNA sequences. These sequences represent the main component of constitutive heterochromatin, a compact type of chromatin. They also support the attachment of the kinetochore, thereby contributing to the stability of the genome. Although centromeric repeats are left out from most chromatin studies, several investigations have pointed to the presence of specific epigenetics marks, such as DNA methylation, the presence of specific histone variants, hypoacetylation and histone modifications. If there is no doubt about the involvement of various chromatin structures in the functional role of centromeres, the real contribution of each specific epigenetic mark and the interplay between chromatin structure and genome stability, remain poorly known.

We developed an epigenetic engineering technique that will aim to induce chromatin modifications specifically at satellite repeats at centromeric loci in human and mouse cells. The production of transcriptional activator-like effectors (TALEs) fused to fluorescent proteins, targeting a 18 bp sequence highly represented in human alpha-satellites repeats or targeting major satellite repeats in mouse cells, permitted us to validate, by immunofluorescence experiments, the correct recruitment of TALEs fusion proteins in these compact heterochromatin regions. The fusion of chromatin modifiers (JMJD2B and JMJD2D histone demethylases) at the C-terminal region of the TALEs, induces a decrease of the immuno-staining of the H3K9me3 histone marks at the targeted regions in TALE-positive cells. In mouse fibroblast cells, we can observe that the decrease of the trimethyl mark of H3K9 is associated with the appearance of the di- and mono-methyl marks of H3K9. Furthermore, this lost of H3K9me3 marks is also associated with modifications of chromocenter structures, in a TALE concentration dependent manner. These results comfort us for the use of TALEs fusion proteins for epigenetic engineering at tandem repeats in human and mouse centromeric regions. This will permit us to decipher the role of this histone mark in centromere integrity and chromosome stability.

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Contribution ID: **22** Type: **not specified**

Surfing on protein waves: proteophoresis as a mechanism for bacterial genome partitioning

Efficient bacterial chromosome segregation typically requires the coordinated action of a three-component, ATP-fueled machinery called the partition complex. We present a phenomenological model accounting for the dynamic activity of this system. The model is obtained by coupling simple linear reaction-diffusion equations with a *proteophoresis*, or "volumetric" chemophoresis, force field that arises from protein-protein interactions and provides a physically viable mechanism for complex translocation. This minimal description captures most known experimental observations: dynamic oscillations of complex components, complex separation and subsequent symmetrical positioning. The predictions of our model are in phenomenological agreement with and provide substantial insight into recent experiments. From a non-linear physics view point, this system explores the active separation of matter at micrometric scales with a dynamical instability between static positioning and travelling wave regime.

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Contribution ID: **23** Type: **not specified**

Imaging DNA repair at the single molecule level

Our genome is constantly damaged by a variety of exogenous and endogenous agents. Among the various forms of DNA damage, double-strand breaks (DSBs) are the most cytotoxic and genotoxic for the cell. Failure to repair such lesions leads to genomic instability or cell death. In higher eukaryotes, mutations in DNA repair genes lead to cancer predisposition. Eukaryotic organisms use several mechanisms to repair
DSBs: non-homologous end-joining (NHEJ), alternative non-homologous end-joining (Alt-NHEJ) and homologous recombination (HR).

Here, we investigate the molecular mechanisms of HR proteins inside cells at the single molecule level in living Saccharomyces cerevisiae yeast. In response to DSB, repair proteins colocalize from diffuse distribution to repair foci located at the damaged DNA site. An enduring question in the DNA damage field is how do repair proteins find their correct target and accumulate within repair foci: how do they diffuse before DNA damage, during focus formation when they have to reach the site of damage, and inside such a small sub-nuclear region formed by a repair focus?

To answer these questions, we use single particle tracking and PALM approaches allowing us to assess the physical properties underlying repair foci formation and decipher the internal structure of these foci.

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Contribution ID: **24** Type: **not specified**

The cohesin complex is required to maintain chromosome integrity at DSB sites

A single DSB can be lethal if unrepaired, particularly in a haploid organism like yeast, and may lead to loss of genetic information and chromosome rearrangements if repaired improperly. In this context, maintaining DSB extremities in close proximity until repair is likely a crucial step to avoid illegitimate joining or initiation of independent recombination events from unrelated chromosome loci that would give rise to deleterious translocations.
To overcome this dramatic event, the DNA damage response (DDR) implements a protein bridge that holds the two DSB ends together and so preserve its integrity. In S. cerevisiae, at least two pathways have been described. One involves a physical bridging by the MRX, the other requires formation of ssDNA, notably by the Exo1 exonuclease.
We show here that the cohesin and SMC5/6 complexes, that share a common 3D structure with MRX and are recruited to DSB, play major roles in maintaining DSB end bridging. Our genetic analysis shows that DSB end tethering requires cohesin loading and that cohesin and SMC5/6 act in the same pathway in part independently of Mre11. We also show that cohesin and SMC5/6 work with Exo1 defining a pathway that act in parallel to MRX.
Our data support a model in which cohesin recruitment to DSB mediated both by MRX and by ssDNA formation allows to entrap DSB proximal DNA in a loop that avoid DSB end separation.

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Contribution ID: **25** Type: **not specified**

Out of equilibrium dynamics of a cross-linking polymer: chromatin as a case study

The structural investigation of large macro-molecular assemblies is an essential step towards understanding the molecular mechanisms at work in cells. Due to the very unstable and labile nature of these assemblies most assays rely on a fixation step, typically achieved using formaldehyde cross-linking, to freeze and capture contacts made by proteins and nucleic acids. Propelled by rapid technological advances such as ChIP-seq and Hi-C chromatin structure is today field of intense activity. Here we use Hi-C which maps contacts between genomic regions both within and between chromosomes in order to quantify the chromatin distortion induced by irreversible cross-link. The analysis of the polymeric structure emerging from the contact maps shows the presence of two different organizations at short and long distances. The large distance behavior reflects the in-vivo structure of the chromosomes and can be interpreted in terms of classical equilibrium polymer model. On the other hand, the short distance behavior depends on the concentration of the cross-linking agent and on exposure time and cannot be interpreted by an equilibrium dynamics. By modeling the cross-linking effect as a polymer irreversible collapse we were able to quantitatively describe this short distance polymeric structure.

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Contribution ID: **26** Type: **not specified**

Making efficient coarse-grain models for long polymers: application to chromatin folding

The physical organization of a chromosome is very crucial for cell functions. Chromosomes are, in general, long and dense-packed polymers. Emphasizing on these characteristics, we developed a generic strategy to develop efficient coarse-grain homopolymer models for chromosomes. From a proper time mapping using the monomer mean-squared displacement, we show that our simulation scheme not only captures the equilibrium properties of the system but also track the same dynamics at different coarse-graining. Our results strongly suggest that accounting properly for the initial configuration, the polymer entanglement length and the base pair density are crucial for quantitatively describing the sequence average behavior of chromosome folding. Finally, we use our formalism to study more precisely the 3D organization of chromosome 3R in drosophila. We show that a combination of our coarse-graining strategy with a one-parameter block copolymer model integrating epigenomic-driven interactions can quantitatively reproduce experimental HiC data at the chromosome-scale and make interesting predictions on the dynamics of chromosome folding.

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