Réunion annuelle du GDR ADN & Rencontre scientifique des Grands Causses

Rapport sur les contributions

https://indico.in2p3.fr/e/gdr-adn-2018

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

Cryo - EM evidence of nucleosome conformational changes in concentrated solutions and in interphase nuclei

In Eukaryotes, DNA is wound around the histone core octamer to form the basic chromatin unit, the nucleosome. Atomic resolution structures have been obtained
from crystallography and single particle cryoEM, with identical reconstituted particles. However, native nucleosomes are diverse in DNA sequence and histone content, and the conformational variability of native nucleosomes remains to be
understood,especially in the cellular context. Using cryoelectron microscopy and tomography of vitreous sections (CEMOVIS and CETOVIS) we investigate native nucleosomes, both in vitro, using isolated nucleosomes solubilised at physiologically relevant concentrations (25-50 %), and in situ, within interphase nuclei. We visualise individual nucleosomes at a level of detail that allows us to analyse the conformation of the DNA wrapped at their surface. In particular, we measure the distance between DNA gyres of the superhelix. In concentrated solutions, we evidence a salt -dependant behaviour, with high salt conformations resembling the canonical crystallographic nucleosome, and low salt ones, more open, being closer to the nucleosome conformation in situ. This work shows that CEMOVIS and CETOVIS are powerful tools for chromatin studies, allowing the visualisation and analysis of nucleosomes in their nuclear context. Nucleosomes are known to play a fundamental role not only in genome packaging but also in the regulation of chromatin functions: transcription, replication and repair. Further particle characterisation and cartography are now needed to explore the relationship between the nucleosome conformational variability and chromatin
functional states.

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

Characteristic lengths in bacterial genomes

In condensed matter physics, simplified descriptions are obtained by coarse-graining the features of a system at a certain characteristic length, defined as the typical length beyond which some properties are no longer correlated. From a physics standpoint, *in vitro* DNA has thus a characteristic length of 300 base pairs (bp), the Kuhn length of the molecule beyond which correlations in its orientations are typically lost. From a biology standpoint, *in vivo* DNA has a characteristic length of 1000 bp, the typical length of genes. Since bacteria live in very different physico-chemical conditions and since their genomes lack translational invariance, whether larger, universal characteristic lengths exist is a non-trivial question. In this talk, I will present an analysis of GC content correlations and of the evolutionary conservation of gene contexts (synteny) in hundreds of bacterial chromosomes, which reveals the existence of a fundamental characteristic length around 10-20 kb. This characteristic length reflects elementary structures involved in the coordination of gene expression, which are present all along the genome of nearly all bacteria.

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

Predicting double-strand DNA breaks using epigenome marks or DNA at kilobase resolution

Background

Double-strand breaks (DSBs) result from the attack of both DNA strands by multiple sources, including exposure to ionizing radiation or reactive oxygen species. DSBs can cause abnormal chromosomal rearrangements which are linked to cancer development, and hence represent an important issue. Recent techniques allow the genome-wide mapping of DSBs at high resolution, enabling the comprehensive study of DSB origin. However these techniques are costly and challenging.

Results

We devise a computational approach to predict DSBs using the epigenomic and chromatin context, for which public data are available from the ENCODE project.
Our predictions achieve excellent accuracy (AUC>0.97) at high resolution (<1kb) using available ChIP-seq and DNase-seq data from public databases. DNase, CTCF binding and H3K4me1/2/3 are among the best predictors of DSBs, reflecting the importances of chromatin accessibility, activity and long-range contacts in determining DSB sites and subsequent repairing. We also successfully predict DSB sites using DNA motif occurrences only (AUC=0.839) and identify the CTCF motif as a strong predictor. In addition, DNA shape analysis further reveals the importance of the structure-based readout in determining DSB sites, complementary to the sequence-based readout (motifs).

Conclusion

Double-strand breaks represent a major threat to the cell, and they are associated with cancer development. Here, we show, for the first time, that such DSBs can be computationally predicted using public epigenomic data, even when the availability of data is limited (e.g. DNase I and H3K4me1). By using state-of-the-art computational models, we achieve excellent prediction accuracy, paving the way for a better understanding of DSB formation depending on developmental stage or cell-type specific epigenetic marks. In addition, our work represents the first step toward predicting DSBs using DNA information only, which could guide further locus-specific genome editing.

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

Numerical model of checkpoint dependent replication origin activation in the Xenopus in vitro system

The initiation of DNA replication in multicellular organisms begins at thousands of genomic positions known as replication origins, which are activated at different times during the S phase in a regulated manner. Furthermore, few origins are grouped into so-called replication clusters that fire more or less synchronously. Previous studies point out that in the *Xenopus in vitro* system the ATR-Chk1 dependent checkpoint pathway is necessary to globally inhibit origin activation in the presence and absence of exogenous replication stress. Using DNA combing we showed that checkpoint inhibition did not lead to the inhibition of origins in already activated replication clusters, close to stalled forks.

The stochastic nature of the initiation process together with limitations of the experimental techniques, require the use of numerical models to obtain more information about the spatial and temporal activation of replication origins.
To this purpose, I tested different models by comparing Monte Carlo simulations and data from DNA combing experiments in the presence and absence of Chk1 inhibition. I used a genetic algorithm which allows to optimize the fitting of the multitude of different replication parameters. The best accordance with experimental data was obtained with a model that combines three notions: 1) a random initiation by an increasing limiting factor, 2) a strong global inhibition of origins firing by Chk1 protein and 3) an enhanced initiation probability near active replication forks together with a local repression of the Chk1 action. The model is consistent with the fact that replication origins are grouped into different temporal clusters. Combining numerical simulations with new models and experimental data will allow us to develop a new global model of the replication program in eukaryotes.

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

Polycomb proteins and 3D genome folding in the epigenetic regulation of development

The eukaryotic genome folds in 3D in a hierarchy of structures, including nucleosomes, chromatin fibers, loops, chromosomal domains (also called TADs), compartments and chromosome territories that are highly organized in order to allow for stable memory as well as for regulatory plasticity, depending on intrinsic and environmental cues. Polycomb Group (PcG) and trithorax group (trxG) proteins form multimeric protein complexes that regulate chromatin via histone modifications, modulation of nucleosome remodeling activities and regulation of 3D chromosome architecture. These proteins can dynamically bind to some of their target genes genes and affect cell proliferation and differentiation in a wide variety of biological processes. Polycomb group proteins form two main complexes, PRC2 and PRC1, which coregulate a subset of their target genes, whereas others are regulated only by one of the complexes. We have recently described the 3D architecture of the genome and identified the Polycomb system as one of the fundamental folding and regulatory principles. Our progress in these fields will be discussed

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

Stochasticity in drosophila chromosome organization

At the kilo- to megabase pair scales, eukaryotic genomes are partitioned into self-interacting modules or topologically associated domains (TADs) that associate to form nuclear compartments. Here, we combine high-content super-resolution microscopies with state-of-the-art DNA-labeling methods to reveal the variability in the multiscale organization of the Drosophila genome. We find that association frequencies within TADs and between TAD borders are below ~10%, independently of TAD size, epigenetic state, or cell type. Critically, despite this large heterogeneity, we are able to visualize nanometer-sized epigenetic domains at the single-cell level. In addition, absolute contact frequencies within and between TADs are to a large extent defined by genomic distance, higher-order chromosome architecture, and epigenetic identity. We propose that TADs and compartments are organized by multiple, small-frequency, yet specific interactions that are regulated by epigenetics and transcriptional state.

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

Dynamic interplay between enhancer-promoter topology and gene activity

A long-standing question in gene regulation is how remote enhancers communicate with their target promoters, and in particular how chromatin topology dynamically relates to gene activation. Here, we combine genome editing and multi-color live imaging to simultaneously visualize physical enhancer–promoter interaction and transcription at the single cell level in Drosophila embryos. Examining transcriptional activation of a reporter by the endogenous even-skipped enhancers 150 kb away, we identify three distinct topological conformation states and measure their transition kinetics. We find that sustained proximity of the enhancer to its target is required for activation, yet is not sufficient. We show that transcription affects the 3D topology, as it is associated with a temporal stabilization of the proximal conformation and a further spatial compaction of the locus. Moreover, the facilitated long-range activation results in transcriptional competition at the locus, causing corresponding developmental defects. Our approach thus offers means to quantitatively study the spatial and temporal determinants of long-range gene regulation and their implications on cellular fates.

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

Multiscale Structuring of the E. coli Chromosome by Nucleoid-Associated and Condensin Proteins

As in Eukaryotes, bacterial genomes must be accurately folded to ensure their proper function. Bacterial genetic information is generally carried on a single circular chromosome with a unique origin of replication from which two replication forks proceed bidirectionally to the opposite terminus region. Here we investigated the higher-order genome architecture of the model bacteria Escherichia coli. We show that the chromosome is partitioned into two structurally distinct entities through a complex and intertwined network of contacts: the replication terminus (ter) region and the rest of the chromosome. Outside ter, the condensin MukBEF and the ubiquitous nucleoid-associated protein (NAP) HU promote DNA contacts in the megabase range. Within ter, the MatP protein prevents MukBEF activity and contacts are restricted to ~280 kb creating a domain with unique structural properties. We also show how other NAPs contribute to nucleoid organization, such as H-NS that constrains and insulates short-range interactions. Combined, these results reveal the contributions of major, evolutionary conserved proteins in a bacterial chromosome organization.

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

Imaging nanoscale chromatin compaction in vivo

How metazoan genomes are structured at the nanoscale in living cells and tissues remains largely unknown. In addition, it still remains difficult to explore chromatin in vivo, particularly at both nucleosomal array level and single cell definition. Here, we applied a quantitative FRET (Forster resonance energy transfer)-based fluorescence lifetime imaging microscopy (FLIM) approach to measure nanoscale chromatin compaction in living primary cells and also at the scale of an organism, Caenorhabditis elegans. By measuring FRET between fluorescently-tagged core H2B histones, we spatially visualized distinct chromosomal regions and quantified the different levels of chromatin structuration.
In C.elegans, we combined RNAi approach to specifically define the heterochromatin state and showed that its architecture presents a nanoscale-compacted organization controlled by HP1 and SETDB1 H3-lysine-9 methyl-transferase homologs in vivo. Furthermore, we found that condensin I and condensin II regulate differentially the heterochromatin compaction state.
Altogether, our experimental system offers the exciting prospect to explore the effects of genetic and environmental factors on nanoscale chromatin compaction in living cells and in whole organisms.

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

Looping and Clustering model for the organization of protein-DNA complexes on the bacterial genome

The bacterial genome is organized by a variety of associated proteins inside a structure called the nucleoid. These proteins can form complexes on DNA that play a central role in various biological processes, including chromosome segregation. A prominent example is the large ParB-DNA complex, which forms an essential component of the segregation machinery in many bacteria. ChIP-Seq experiments show that ParB proteins localize around centromere-like parS sites on the DNA to which ParB binds specifically, and spreads from there over large sections of the chromosome. Recent theoretical and experimental studies suggest that DNA-bound ParB proteins can interact with each other to condense into a coherent 3D complex on the DNA. However, the structural organization of this protein-DNA complex remains unclear, and a predictive quantitative theory for the distribution of ParB proteins on DNA is lacking.

Here, we propose the Looping and Clustering (LC) model, which employs a statistical physics approach to describe protein-DNA complexes. The LC model accounts for the extrusion of DNA loops from a cluster of interacting DNA-bound proteins that is organized around a single high-affinity binding site. Conceptually, the structure of the protein-DNA complex is determined by a competition between attractive protein interactions and the configurational and loop entropy of this protein-DNA cluster. Indeed, we show that the protein interaction strength determines the "tightness" of the loopy protein-DNA complex. Thus, our model provides a theoretical framework to quantitatively compute the binding profiles of ParB-like proteins around a cognate parS binding site.

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

Modélisation Multi-échelle de l'Architecture Nucléaire

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

Effect of gene position in the genome and in the cell on transcription regulation

Orateur: SCLAVI, Bianca (LBPA, UMR 8113)

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

Transcription et dynamique de la fibre de chromatine

Orateur: GADAL, Olivier (CNRS)

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

Conférence grand public: Le Projet Lutétium

Le Projet Lutétium est un projet vidéo à la rencontre de la recherche expérimentale, de la médiation scientifique, et des arts musical et graphiques. Via sa chaîne YouTube, il vise à faire découvrir par des expériences visuelles des sujets de recherche actuels et peu communs en communication grand public. Pour l'événement, une présentation du Projet accompagné de projections de vidéos commentées sera réalisée.

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

Effect of epigenetics and extrusion on chromatin organization and dynamics

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