Neuvième réunion annuelle du GdR Architecture et Dynamique Nucléaires (ADN)

Rapport sur les contributions

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ID de Contribution: **43** Type: **Présentation orale**

Global and ancestral regulation by DNA supercoiling is tuned by promoters' structure

Usual models of transcriptional regulation are centered on transcription factors, which recognize specific sequences in genes' promoters. However, at a more global scale, the physical state of the double helix, embedded in the chromatin fibre, plays a crucial role in genes' expression in vivo. In particular, torsional stress (or DNA supercoiling) is an ubiquitous feature in DNA transactions, which has a strong influence on the initiation step of transcription in bacteria as well as eukaryotes, notably by strongly modulating the energetic cost of opening the double helix for RNA synthesis. DNA supercoiling may thus constitute an ancestral and global transcription factor, as suggested by many recent transcriptomics studies, but for which no quantitative or even qualitative model is available. I will present a thermodynamic model of supercoiling-assisted promoter opening which shows how promoters can be globally and selectively activated by supercoiling variations depending on their structure. Although it might be valid for both bacterial and eukaryotic promoters due to their common structural features, the quantitative analysis is carried more specifically on bacterial promoters, where DNA supercoiling plays a predominant role in the chromatin organization and more data have been accumulated. In vitro and in vivo transcription data are analyzed, which quantitatively confirm the predictions of the models. The universality of the mechanism, which is based on the basal interaction between RNA Polymerase and the promoter elements independently of any additional transcriptional regulator, is then demonstrated by analyzing transcriptomes of several distant bacterial species under conditions of supercoiling variations by antibiotics. We finally reproduce model predictions in vivo on individual promoters in Escherichia coli, by monitoring their expression during time and their response to opposite supercoiling variations. All together, our results demonstrate that DNA supercoiling could act as an important ancestral mode of regulation currently underestimated.

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ID de Contribution: **44** Type: **Présentation orale**

NUCLEOSOME CONFORMATIONAL VARIABILITY IN VITRO & IN SITU EXPLORED BY CRYO-ELECTRON MICROSCOPY AND TOMOGRAPHY OF VITREOUS SECTIONS

In most eukaryotic cells, DNA is packed into the chromatin « bead on string » filament of nucleosomes, formed by 145–147 bp DNA wrapped into 1.65 turns of a left-handed superhelix around a histone octamer. Atomic resolution structures have been obtained from X-ray crystallography, and more recently from cryo-EM of identical, symmetric and highly stable engineered particles, which has led, together with a highly conserved structure, to a canonical static view of the particle. However, nucleosomes are now being recognised as pleiomorphic and dynamical, which is so far documented in vitro and in silico, but unknown in the cellular context. Using cryo-electron microscopy and tomography of vitreous sections, we analyse the structure and local organisation of nucleosomes within interphase nuclei of different cell types (human cell lines, Drosophila embryo). We visualise individual nucleosomes at a level of detail that allows us to analyse the conformation of the DNA gyres wrapped at their surface. We measure the distance between gyres of the superhelix, and show that nucleosomes are polymorphic, with variable conformations, more open in situ than in canonical crystallographic structures, with an increase of the distance between DNA gyres. These observations are compatible with a "gaping"conformation, proposed by theoretical approaches and detected experimentally in vitro, and/or an increase of the pitch of the DNA superhelix. To characterise this conformational variability, we combine analyses of individual nucleosomes, sub-tomogram averaging and 3D classification based on normal mode analysis. To decipher the mechanisms at work in this conformational variability, we use isolated native nucleosomes solubilised at physiologically relevant concentrations (25-50%) and in various ionic environments. We evidence a salt–dependant behaviour, with high salt conformations resembling the canonical crystallographic nucleosome, and low salt open ones, closer to the nucleosomes in situ. This highlights the role of ionic effects, already known to play a central role at many levels of chromatin organisation, demonstrating that, at the nucleosome level, DNA gyres open or close. Nucleosomes are known to play a fundamental role not only in genome packaging but also in the regulation of major cellular processes. Further particle characterisation and cartography are now needed to understanding the relationship between nucleosome conformational variability and chromatin functional states.

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ID de Contribution: **45** Type: **Présentation orale**

Cohesin-mediated chromatin folding constrains homology search during DNA repair

DNA break repair by homologous recombination (HR) entails a search for homology carried out by a specialized nucleoprotein filament (NPF). The search mechanism remains elusive, notably its interplay with the folding of chromatin in the nucleus. We show in the tractable model system *S. cerevisiae* that it takes place in the reorganized context of individualized metaphase-like chromosomes structured by cohesins into arrays of chromatin loops. Functionally, cohesin-mediated chromatin folding primarily constrains homology search intra-chromosomally, which provides a kinetic advantage for DNA strand invasion at intra- over inter-chromosomal donors. The sister chromatid plays a secondary inhibitory function. Using a pds5 mutant to artificially enlarge cohesin loops, we further show that the DSB region acts as a cohesin (and thus directional loop expansion) barrier, which promotes donor identification in cis. Cohesin thus constrains homology search intra-chromosomally in multiple ways, inhibiting and orienting genome-wide homology search.

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ID de Contribution: **46** Type: **Présentation orale**

The development of Nano-C allows the characterization of TAD boundaries with unprecedented resolution

Chromosome conformation capture (3C) methods are a powerful tool for uncovering 3D genome organization. Short-read Illumina sequencing restricts 3C to the description of average conformations within large cell populations. Significant variation between individual cells within the same population may be present though. For instance, if different contacts happen simultaneously, exclusively, or in subsets, cannot be discerned by methods based on population-level pairwise contacts.

In my project, I aim to determine the degree of cellular heterogeneity of TAD (Topologically Associating Domain) boundaries. TADs are separated by boundaries that are often bound by the CTCF insulator protein. Several recent studies have reported that CTCF often binds at multiple sites around TAD boundaries (e.g. Madani Tonekaboni et al, 2019). We have recently reported that many TAD boundaries provide a more gradual insulation, suggesting they act like 'transition zones'(Chang et al, 2020). Considering the dynamic nature of CTCF binding to the DNA (Hansen et al, 2017), clustering of CTCF binding at boundaries may thus stabilize the insulation of neighboring TADs.

To measure the variable nature of TAD boundaries, I have developed "Nano-C", a PCR-free multi-contact assay that combines 3C with Nanopore sequencing, to identify multiple interactions from defined genomic loci in single cells. Using Nano-C, I have confirmed that individual CTCF binding sites contribute additively to TAD boundary function in individual cells. Moreover, I have found that this cell-to-cell variation contributes to the insulation-strength of different TAD boundaries. These results support the notion that clustering of CTCF binding at boundaries provides redundancy to stabilize TAD structure and gene regulatory function.

References:

Chang, L.-H., Ghosh, S. & Noordermeer, D. TADs and Their Borders: Free Movement or Building a Wall? *J Mol Biol* **432**, 643-652 (2020).

Hansen, A. S., Pustova, I., Cattoglio, C., Tjian, R. & Darzacq, X. CTCF and cohesin regulate chromatin loop stability with distinct dynamics. *Elife* **6** (2017).

Madani Tonekaboni, S. A., Mazrooei, P., Kofia, V., Haibe-Kains, B. & Lupien, M. Identifying clusters of cis-regulatory elements underpinning TAD structures and lineage-specific regulatory networks. *Genome Res* **29**, 1733-1743, (2019).

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ID de Contribution: **47** Type: **Présentation orale**

Folding and persistence times of intramolecular G-quadruplexes transiently embeded in a DNA duplex

G-quadruplex (G4) DNA structures have emerged as important regulatory elements during DNA metabolic transactions. Most *in vitro* studies have focused so far on the G4 kinetics within single-stranded DNA. However, G4 structures potentially form in genome regions where their stability is challenged by a complementary strand. Since the energy of hybridization of Watson-Crick structures dominates the energy of G4 folding, this competition should play a critical role on the persistence of G4 structures *in vivo*. Here, we addressed the kinetics of G4 folding and unfolding in presence of a complementary strand. We designed a single molecule assay allowing measuring G4 folding and unfolding while the structure is periodically challenged by the complementary strand. By repeating cycles of opening and closing of the DNA duplex, we quantified the folding rate and the persistence time of biologically relevant structures from three different genomic origin, namely human oncogene promoters, human telomeres and an avian replication origin. We show that the dynamics of G4 formation varies largely in ways not fully predictable by current knowledge. In addition, we show that folding and persistence time increase upon treatment with a G4 binding ligand or an anti-G4 antibody. Our assay opens new perspectives for the measurement of G4 dynamics in vitro in biologically inspired substrates.

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ID de Contribution: **48** Type: **Présentation orale**

The histone variant H3.3 and its chaperone DAXX preserve heterochromatin integrity of embryonic pluripotent cells.

Within the nucleus, heterochromatin domains segregate in particular compartments such as the chromocenters that contain pericentromeric heterochromatin (PCH) regions, or the lamina-associated domains (LADs) that localize at the periphery of the nucleus. In most cell types, DNA methylation is essential for heterochromatin formation, directly contributing to the transcriptional repression of DNA repeats and the maintenance of genome stability. Active DNA demethylation during early embryogenesis is a critical step for development but requires alternative pathways to maintain heterochromatin. Yet, the functional importance of heterochromatin and the molecular factors involved remain elusive.

Here, we address the role of DAXX, the H3.3-specific chaperone for heterochromatin deposition, in heterochromatin maintenance in Embryonic Stem Cells (ESCs). We observe that DAXX is essential for ESCs survival when grown in low DNA methylation conditions. Upon active DNA demethylation-mediated damages, DAXX relocalizes to PCH, and recruits H3.3, PML and SETDB1 to promote heterochromatin formation. In DAXX knock-out ESCs, the 3D-architecture and the physical properties of pericentric heterochromatin are impaired, resulting in overexpression of major satellite RNA. By using epigenome editing tools, we demonstrate that H3.3 and its modification on the lysine 9 directly contribute to PCH spatial conformation.

Altogether, our results demonstrate that DAXX and H3.3 are essential for the maintenance of heterochromatin in response to DNA damages in pluripotent stem cells.

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ID de Contribution: **49** Type: **Présentation orale**

Visualisation 3D des séquences répétées dispersées du génome humain.

L'architecture tridimensionnelle des génomes eucaryotes joue un rôle important dans leur régulation fonctionnelle. Le développement de méthodes de capture de la conformation chromosomique, telles que le Hi-C, a mis en évidence l'existence de deux compartiments appelés A et B, séparés dans l'espace, qui correspondent respectivement à l'euchromatine (gènes exprimés) et à l'hétérochromatine (gènes non exprimés). Les mécanismes qui sous-tendent la formation de ces compartiments demeurent mal compris.
Dans des cellules de souris, il est possible de visualiser les compartiments A et B par des expériences d'hybridation de fluorescence in situ (FISH) (Solovei et al 2009). Les sondes utilisées prennent pour cible des séquences répétées dites dispersées connues pour être enrichies dans l'euchromatine et dans l'hétérochromatine. Les séquences répétées dispersées comprennent de très nombreuses familles qui diffèrent entre les espèces. Il a été suggéré qu'elles pourraient jouer un rôle dans le repliement tridimensionnel des génomes, en interagissant les unes avec les autres au sein d'une même famille (Cournac et al 2016).
Afin de pouvoir étudier les mécanismes conduisant à la mise en place des compartiments A et B dans des cellules humaines, nous avons recherché les familles de séquences répétées les plus enrichies dans l'un ou l'autre des compartiments dans un modèle cellulaire humain, les cellules DLD1. Focalisant sur la famille AluJb pour le compartiment A, et sur la famille L1PA7 pour le compartiment B, nous avons recherché les séquences de sondes oligonucléotidiques les plus aptes à détecter l'un ou l'autre des compartiments, préalablement obtenus en analysant des données de Hi-C. Ces sondes ont été utilisées dans des expériences de FISH qui ont permis d'observer les compartiments chromatiniens au microscope à fluorescence. Des analyses statistiques des images obtenues permettent de comparer les propriétés des différentes sondes.
Les résultats obtenus montrent qu'il est possible pour la première fois de distinguer les compartiments A et B dans des cellules humaines et ouvrent la voie à de nouvelles expériences visant à comprendre les mécanismes conduisant à la formation de ces deux compartiments.

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ID de Contribution: **50** Type: **Présentation orale**

Euryarchaeal genomes are organised into domains and chromatin loops but lack transcription-mediated compartmentalization

Chromosome conformation capture (3C/Hi-C)-based methods have been used extensively to probe the average 3D organization of genomes, revealing fundamental aspects of chromosome dynamics. However, when applied to prokaryotic and archaeal species the current protocols are expensive, inefficient, and limited in their resolution. We developed a cost-effective approach that allows the exploration of bacterial and archaeal chromosome conformations at the gene or operon level. We applied it to the Euryarchaea *H. volcanii*, *Hbt. salinarum* and *T. kodakaraensis*. We generated genome-wide contact maps at a resolution of up to 1kb, allowing us to further explore the diversity of chromosome folding in this kingdom. We show that, in contrast to Crenarchaea, these Euryarchaea lack (active and inactive) compartment-like structures, and instead resemble the architecture of bacterial chromosomes. The genomes also display sub-Mb domains and DNA loops. In *H. volcanii*, these structures are regulated by both transcription and the archaeal SMC protein, further supporting the ubiquitous role of these mechanisms in shaping the higher-order organization of genomes in all kingdoms.

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ID de Contribution: **51** Type: **Présentation orale**

3D genome organization and the genetic risk to (some) diseases

Genome-wide association studies have established statistical associations between various diseases and a large number of single-nucleotide polymorphisms (SNPs). However, they provided no simple explanation of the mechanisms underlying this association.
Following the idea that 3D genome organization and its variations may have a functional role in gene regulation, we investigated the distribution of these SNPs with respect to topologically-associating domains (TADs) and their borders. Our computational analyses show that for some specific diseases, including many cancers, disease-associated SNPs are over-represented in TAD borders.
To analyze further this enrichment, its determinants and its consequences, we have selected candidate loci and started an experimental study using two techniques (HRS-seq and 3C-qPCR) developed in the group at IGMM.

Work in collaboration with Thierry Forné (IGMM, Montpellier, France), Julien Mozziconacci (MNHN & LPTMC , Paris, France), Marc-Torsten Hütt (Jacobs University, Bremen, Germany) and their students Marina Villaverde, Coralie Compare, Leopold Carron (now at LCQB, Paris) and Kim Philipp Jablonski (now at ETH, Zürich).

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ID de Contribution: **52** Type: **Présentation orale**

Chromosome structure due to phospho-mimetic H2A modulates DDR through increased chromatin mobility

In budding yeast and mammals, double strand breaks trigger global chromatin mobility together with the rapid phosphorylation of the histone H2A over an extensive region of the chromatin. To assess the role of H2A phosphorylation in this response to DNA damage, we have constructed strains where H2A has been mutated to the phospho-mimetic H2A-S129E. We show that H2A-S129E mutant increases global motion of chromosomes even in the absence of DNA damage. The intrinsic chromatin mobility of H2A-S129E is not due to checkpoint activation, histone degradation or kinetochore anchoring. Rather, the increased intra-chromosomal distances observed in H2A-S129E mutant are consistent with chromatin structural changes. In this context, the Rad953BP1-dependent-checkpoint becomes dispensable. The increase in chromatin dynamics is favorable to NHEJ of a single double-strand break but is accompanied by a sharp decrease in inter-chromosomal translocation rates. We propose that changes in chromosomal conformation due to H2A phosphorylation are sufficient to modulate the DDR and maintain genome integrity.

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ID de Contribution: **53** Type: **Présentation orale**

Genomic contacts reveal the control of sister chromosome decatenation in E. coli

In *E. coli*, Topoisomerase IV is responsible for the untangling of catenanes that are formed during the replication of the chromosome and has been shown to play an essential role in nucleoid segregation. Previous studies have shown that alterations in Topo IV result in a prolonged interaction between sister chromosomes leading to poor chromosome segregation and a loss in cell viability. Using chromosome conformation capture (Hi-C) and fluorescence microscopy, we have shown that the alteration of Topo IV affects the organization of the entire chromosome. As this phenotype only occurs in circular chromosome replicative cells, we hypothesized that this is due to an accumulation of precatenanes throughout the chromosome, allowing loci on different sister chromosomes to interact (inter-chromosomal contacts). We further showed that the butterfly wing positions are dependent on both *matS* and MatP, and that MukB defines the length and density of the butterfly wings. We thereby hypothesized that the *matS*-MatP complex and these Topo IV sites define a decatenation hub where unresolved precatenanes would be "pulled"toward this hub, to be decatenated prior to cell.

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ID de Contribution: **54** Type: **Présentation orale**

Semi-analytical modeling of chromatin loop-extrusion

At the scale of 10^5-10^6 base pairs, human chromosomes are structured in topologically associated domains (TADs). These are regions of the chromosome within which contacts are more frequent that with adjacent regions, as measured by 'chromosome conformation capture' experiments such as Hi-C. TADs have been shown to result from the action of cohesin, a molecular motor. Cohesin binds to chromatin, reel it in, and extrude it as a loop. This process is called "loop extrusion"(Fudenberg et al. 2016). In this realm, theoretical modelling is able to answer the following questions: How much energy should the cell spend to maintain these structures? with how many concurrent motors? how fast? and how frequently? The current approach to simulate loop extrusion uses explicit molecular dynamics simulations. This approach, while very flexible, limits our possibility (i) to explore the parameter space in an efficient manner and (ii) to dissect the observed effects under the lenses of a coherent analytical theory. I will present our original approach to simulate loop extrusion, that exploits an analytical solution of the probability distribution over a conformational space for the polymer model, and the action of extruders simulated in 1D. I will show how this approach allows highlighting the hallmarks of the out-of-equilibrium processes on chromatin conformation observed in experiments. Finally, this approach permits the definition of the Gibbs entropy of chromosome conformation. I will show how the application of this concept to simplified toy-models increases our analytical understanding of the loop extrusion process.

Fudenberg G, Imakaev M, Lu C, Goloborodko A, Abdennur N, Mirny LA. 2016. Formation of Chromosomal Domains by Loop Extrusion. Cell Reports 15: 2038–2049.

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ID de Contribution: **55** Type: **Présentation orale**

Live imaging and biophysical modelling support a button-based mechanism of somatic homolog pairing

The spatial configuration of the eukaryotic genome is organized and dynamic,
providing the structural basis for regulated gene expression in living cells. In Drosophila melanogaster , 3D genome organization is characterized by the phenomenon of somatic homolog pairing, where homologous chromosomes are intimately paired from end to end. While this organizational principle has been recognized for over 100 years, the process by which homologs identify one another and pair has remained mysterious. Recently, a model was proposed wherein specifically-interacting "buttons"encoded along the lengths of homologous chromosomes drive somatic homolog pairing. Here, we turn this hypothesis into a precise biophysical model to demonstrate that a button-based mechanism can lead to chromosome-wide pairing. We test our model and constrain its free parameters using live-imaging measurements of chromosomal loci tagged with the MS2 and PP7 nascent RNA labeling systems. Our analysis shows strong agreement between model predictions and experiments in the separation dynamics of tagged homologous loci as they transition from unpaired to paired states, and in the percentage of nuclei that become paired as embryonic development proceeds. In sum, our data strongly support a button-based mechanism of somatic homolog pairing in Drosophila and provide a theoretical framework for revealing the molecular identity and regulation of buttons.

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ID de Contribution: **56** Type: **Présentation orale**

Smc3 acetylation anchors cohesin dependent chromatin loops

Past decade has shown that the cohesin is essential for the regulation of mammalian's genomes 3D organization. Cohesin is a ring-shaped complex that interacts with three related hook-shaped proteins composed of HEAT repeats namely Pds5, Scc3/STAG and Scc2/Nipbl that regulate its functions. Cohesin shapes mammalian genomes by establishing chromatin loops along chromosomes, most likely through an extrusion mechanism. Recent single molecules experiments suggest that loops expansion is mediating by cohesins'ATPase activity stimulated by Scc2/Nipbl.

We have recently showed that yeast mitotic chromosomes are also organized into cohesin-dependent loops. Length of these loops is regulated by two pathways: the Wapl-mediated releasing activity and a mechanism dependent of the acetyltransferase Eco1. Our data suggested that Eco1 inhibits the translocase activity that powers loop formation through a mechanism that is distinguishable from its role in cohesion establishment. However, how Eco1 inhibits this translocase activity is still unknown.
Our latest data show that Eco1 restricts the length of the loops throughout acetylation of the two conserved lysine 112 and 113 located at Smc3's ATPase head. Smc3 acetylation allows anchoring of chromatin loops at specific positions by inhibiting both releasing and translocase activities. Moreover, our data suggest that Smc3 acetylation inhibits translocase activity in a Pds5 dependent manner.

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ID de Contribution: **57** Type: **Présentation orale**

SETDB1 dependent H3K9me3/H3K36me3 dual chromatin regulate cell differentiation by restricting enhancers

Cellular identity is conferred by specific gene expression profiles that are regulated by transcription factors and chromatin. Combination of specific histone modifications characterize gene activity. Active genes typically display an enrichment in histone H3 lysine 4 trimethylation (H3K4me3) at their promoters, whereas SET Domain 2 (SETD2) trimethylates histone H3 lysine 36 (H3K36me3) in their gene bodies. Chromatin signatures of gene silencing are more complex. A large subset of developmentally silenced genes is targeted by proteins of the Polycomb group and usually harbor histone H3 lysine 27 trimethylation (H3K27me3). Other silent genes harbor no typical histone modification, or an enrichment in histone H3 lysine 9 trimethylation (H3K9me3). SET Domain Bifurcated 1 (SETDB1) is responsible for H3K9 trimethylation on genes and endogenous retroviruses. Knocking-out Setdb1 results in early embryonic lethality. Moreover, SETDB1 loss in differentiated cells leads to aberrant lineage specific gene reactivation, showing that SETDB1 is important for development through the maintenance of appropriate cellular identity. Recently, an atypical SETDB1 chromatin has been identified at mouse ES telomeres or on the 3'end of a subset of zinc finger genes in human cancer cells. Enriched in apparently opposing histone marks (H3K9me3 and H3K36me3) the function of this dual chromatin, if any, has not yet been elucidated.

We identified almost 5000 loci harboring H3K9me3 and H3K36me3 on the same nucleosome in mouse ESCs. These dual domains are SETDB1-dependent and NSD dependent, but only partially SETD2 dependent, for H3K9me3 and H3K36me3 respectively. H3K9me3/H3K36me3 dual domains are located on future enhancers to restrict their gene activating functions. Distinct subsets of H3K9me3/H3K36me3 dual domains in ESC become enhancers in distinct tissues. Based on our data, we propose that dual H3K9me3/H3K36me3 helps to maintain stemness.

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ID de Contribution: **58** Type: **Poster**

Transcriptomic response of phytopathogen Dickeya dadantii to an increase of DNA supercoiling by novel antibiotic seconeolitsin

DNA supercoiling, i.e., the presence of torsional stress in the DNA double helix, is an essential mechanism of bacterial chromosome compaction and affects gene expression. Supercoiling level is mainly regulated by topoisomerase I and DNA gyrase. Inhibiting one of these enzymes with an antibiotic leads to a global supercoiling modification and global gene expression changes. Novobiocin, an antibiotic inhibiting DNA Gyrase, thus inducing relaxation, has already been studied. Genes responding to this relaxation where categorized as "supercoiling sensitive". To study the opposite variation of supercoiling and redefine the notion of "supercoiling sensitive"gene, we have measured the transcriptomic effect of seconeolitsin, a non-marketed antibiotic inhibiting topoisomerase I. Topoisomerase I inhibition has never been tested on Gram-negative bacteria. We have analyzed these RNA sequencing data, studied the chromosomal distribution of responsive genes and characterized them at the functional level. All together, our studies show that conformational changes of the chromosome induce a global transcriptomic response, with little functional enrichment but a non-uniform spatial organization. Moreover, the supercoiling sensitivity is not an intrinsic property of a promoter but depends on physiological state of the cell and the direction of supercoiling variations.

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