Topo-Club 2016

Rapport sur les contributions

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ID de Contribution: **0** Type: **Non spécifié**

Effects of genome position of supercoiling-dependent gene expression

Global transcription regulators are factors that can influence the activity of several bacterial promoters and can thus play a key role during bacterial adaptation to a change in growth environment. These factors include small metabolites, such as ppGpp, abundant nucleoid-associated proteins and DNA topology. The latter two are not equally repartitioned throughout the genome and could thus influence gene expression differently depending on the gene's position along the genome (1,2,3). Our previous work provided evidence for a genome-position dependence of the activity of an H-NS dependent promoter (4). This effect became particularly evident when gene expression was compared as a function of growth rate, growth phase or temperature. Recently we have begun studying the activity of supercoiling-dependent promoters in order to determine whether the effects of DNA topology also depend on genomic position, as suggested by existing data on the binding sites of topoisomerases (5). In this study we have found both local and global effects of genomic position on a supercoiling-dependent promoter, furthermore our results show that the activity of nucleoid proteins can either buffer or amplify these effects depending on the genomic context.

1.Sobetzko, P., Glinkowska, M., Travers, A., and Muskhelishvili, G. (2013). DNA thermodynamic stability and supercoil dynamics determine the gene expression program during the bacterial growth cycle. Mol Biosyst 9, 1643–1651.

2. Scolari, V.F., Bassetti, B., Sclavi, B., Cosentino Lagomarsino, M. (2010) Gene clusters reflecting macrodomain structure respond to nucleoid perturbations. Molecular BioSystems, DOI: 10.1039/C0MB00213E.

3. Zarei, M., Sclavi,B. Cosentino Lagomarsino, M. (2013) Gene silencing and large-scale domain structure of the E. coli genome, Mol. BioSyst., 2013, 9 (4), 758 - 767

4. Brambilla, E. and Sclavi, B. (2015) Gene regulation by H-NS as a function of growth conditions depends on chromosomal position in E. coli. G3, Genes, Genomes, Genomics, 5, 605-614.

5.Jeong, K.S., Ahn, J., and Khodursky, A.B. (2004). Spatial patterns of transcriptional activity in the chromosome of Escherichia coli. Genome Biol. 5, R86.

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

Identification des modifications post-traductionnelles de l'histone H3 impliquées dans la condensation des chromosomes "in embryo"

Epigenetic modifications and nuclear architecture are globally rearranged after fertilization. Unlike somatic cells, mammalian embryos for example present a unique organization of pericentromeric heterochromatin. In mouse embryos, this part of the heterochromatin is not organized in clusters but in spherical structures around nucleolar precursor bodies forming a "cartwheel". This pericentric heterochromatin is characterized by specific epigenetic marks, in particular trimethylation of histone H3 at Lysine 9 (H3K9me3), together with the heterochromatin protein 1 (HP1b). Surprisingly, we recently found out that this heterochromatin also contains histone H3 phosphorylated at serine 10 (H3S10P) from early interphase through mitosis over several embryonic cycles (Mason et al., 2012). In somatic cells, it is known that when histone H3 is phosphorylated at S10 at the end of the cell cycle, HP1b is ejected from the chromatin upon the entry in mitosis. We therefore questioned the "colocalization"of H3S10P and HP1b we observed in early stage embryos, even in G1/S phases.
To better understand the mechanisms behind HP1b association/ejection in early embryos, we performed immunostaining with several antibodies directed against HP1b, H3K9me3, H3S10P as well as with an antibody specific to the double modification H3K9me3S10P.
In order to get a deeper insight into the colocalization between these epigenetic marks/proteins we also developed a new approach based on the in situ PLA technology (Proximity Ligation Assay). This technique allows the fluorescent detection of two targets when they are in close proximity (<40 nm) without any genetic manipulation of the cells in contrast to other techniques such as BRET or FRET.
Altogether our results demonstrate that H3S10P and H3K9me3 can both colocalize with HP1b but that only the double modification H3K9me3S10P is responsible of HP1b ejection from chromatin only upon the first mitosis in mouse embryos.

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

DNA double-strand breaks induced by transcription-blocking topoisomerase I lesions

Topoisomerase I (Top1) relaxes DNA supercoiling generated during transcription by producing transient Top1-DNA cleavage complexes (Top1cc). These Top1cc intermediates can be stabilized under a broad range of physiological conditions including oxidative base damage, alkylation by carcinogenic compounds and nicks, and by ribonucleotide misincorporation. Stabilized Top1cc are potent transcription-blocking lesions and our observations indicate that they can be converted into DNA double-strand breaks (1-4). We will discuss the mechanism of production of these co-transcriptional DNA double-strand breaks and their potential relevance in the pathogenesis of neurodegenerative diseases.

1. A. Cristini et al., DNA-PK triggers histone ubiquitination and signaling in response to DNA double-strand breaks produced during the repair of transcription-blocking topoisomerase I lesions. Nucleic Acids Res In press., (2015).
2. O. Sordet et al., Hyperphosphorylation of RNA polymerase II in response to topoisomerase I cleavage complexes and its association with transcription- and BRCA1-dependent degradation of topoisomerase I. J Mol Biol 381, 540-549 (2008).
3. O. Sordet, A. J. Nakamura, C. E. Redon, Y. Pommier, DNA double-strand breaks and ATM activation by transcription-blocking DNA lesions. Cell cycle (Georgetown, Tex 9, 274-278 (2010).
4. O. Sordet et al., Ataxia telangiectasia mutated activation by transcription- and topoisomerase I-induced DNA double-strand breaks. EMBO Rep 10, 887-893 (2009).

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

DNA minicircles are new tools to study in vitro retroviral integration

Integration is an essential step of retroviral replication and a promising anti-HIV target. It is performed by a viral-encoded enzyme, integrase (IN), and the selectivity of this enzyme for the target cellular DNA is important for retroviral replication. Genetic, biochemical and structural studies have revealed a role of cellular chromatin in this selectivity but the underlying molecular parameters are still under investigation. Indeed, at the level of the nucleosome, both histone modifications specifically recognized by IN and its cofactor LEDGF/p75 and DNA distortions induced by the nucleosome structure are involved in the distribution of integration sites.

To distinguish between these two parameters, we constructed DNA minicircles that reproduce the DNA curvature and torsion present in a nucleosome, but lack the histones. Using various DNA minicircles as integration susbtrates, we observed a large enhancement of integration in these circles with regards of the corresponding linear fragments. This enhancement is observed with both HIV-1 IN and PFV IN but also with the HIV IN-LEDGF/p75 complex. Using high-throughput sequencing of integration products obtained in the DNA minicircles and molecular modelling of these circles, we evaluated the role of DNA structural parameters on the selectivity of integration. With the HIV IN-LEDGF/p75 complex, we observed a periodic distribution of integration sites enriched in outward DNA major grooves, which is consistent with the cryo-EM structure of the intasome obtained with this complex. Surprisingly, this periodicity is less pronounced with HIV IN alone and absent with PFV IN. Using the DNA minicircles, we are now studying the role of the LEDGF/p75 cofactor and IN residues suspected to be involved in the recognition of curved or flexible acceptor DNA substrates. Using under and over-twisted DNA minicircles, we will also evaluate the effect of local DNA superhelicity on integration.

In summary, DNA minicircles allow to study how local structural deformations of the target DNA affect retroviral integration and to determine the separated roles of DNA structure and histone modifications during this enzymatic process.

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

DNA supercoiling activity of DNA gyrases from Francisella strains resistant to quinolones

Quinolones are one of the most commonly prescribed classes of antibacterials agents in the world and are used to treat several bacterial infections in humans. Accordingly, microbiological and clinical data showed that ciprofloxacin, and possibly other fluoroquinolones represent an efficient first-line treatment for oral therapy of tularemia, a disease caused by the Gram negative bacterium Francisella tularensis. These compounds inhibit DNA synthesis through interaction with complexes composed of DNA and either of the two target enzymes, DNA gyrase and topoisomerase IV that belong to type IIA topoisomerases.
A collection of fluoroquinolones resistant clones of Francisella was generated in our lab through an experimental evolution protocol applied on sensitive strains exposed to increasing fluoroquinolone concentrations. Exposure to antibiotic was accompanied by mutations in GyrA and GyrB. While some mutations were restricted to discrete regions of the so-called quinolone-resistance-determining regions (QRDR), amino acid substitutions or deletions never previously reported were also identified.
Here, our aim was to clarify the role of identified GyrA and GyrB mutations in fluoroquinolone resistance. Recombinant WT mutated GyrA and GyrB subunits from Francisella novicida and Francisella philomiragia were expressed in E. coli and purified as soluble proteins. Subsequently, the inhibitory effects of ciprofloxacin and moxifloxacin were evaluated against the functional activity of reconstituted DNA gyrase complexes. The data obtained demonstrated that, as in several bacterial species, the Asp87 residue of GyrA is a mutational hotspot conferring a high degree fluoroquinolone resistance in Francisella. Novel identified GyrA mutations including the conserved Pro43 residue are also implicated.

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

A DNA topoisomerase VI-like complex initiates meiotic recombination

The SPO11 protein catalyzes the formation of meiotic DNA double strand breaks (DSBs) and is homologous to the A subunit of an archaeal topoisomerase (topo VI). Topo VI are heterotetrameric enzymes composed of two A and two B subunits, however no topo VIB involved in meiotic recombination had been identified. Here, we characterized a structural homolog of the archaeal topo VIB subunit (MTOPVIB, for Meiotic TOPoisomerase VIB-like), which is essential for meiotic DSB formation. It forms a complex with the two A. thaliana SPO11 orthologs required for meiotic DSB formation (SPO11-1 and SPO11-2) and is absolutely required for the formation of the SPO11-1/SPO11-2 heterodimer. These findings suggest that the catalytic core complex responsible for meiotic DSB formation in eukaryotes adopts a topo VI-like structure.

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

The TopoVIB-Like protein family is required for meiotic DNA double strand break formation

Meiotic recombination is induced by the formation of DNA double strand breaks (DSBs) catalyzed by SPO11, the ortholog of the subunit A of TopoVI DNA topoisomerase (TopoVIA). TopoVI activity requires the interaction between A and B subunits; however whether SPO11 functions alone or through association with another subunit has remained an open question for the last 18 years. Here, we identified a conserved family of plant and animal proteins that we named TopoVIB-Like. They show strong similarity with TopoVIB and have a GHKL domain potentially involved in ATP binding and a transducer domain interacting with SPO11. We further provide evidence that the meiotic recombination proteins Rec102 (S. cerevisiae), Rec6 (S. pombe) and Mei-P22 (D. melanogaster) are TopoVIB homologs, but without GHKL domain. We then demonstrated that mouse TOPOVIBL interacts and forms a complex with SPO11 and is required for meiotic DSB formation. We conclude that the meiotic program has evolved through the combined specialization and differentiation of TopoVI subunit homologs expressed specifically in meiotic cells and with a modified biochemical activity to promote the formation of DSBs.

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

Genome mechanics: let's talk about figures

We wish here to emphasize some physical characteristics of genome organization in order to provide a concrete and quantitative framework in which to interpret DNA metabolism events such as transcription or replication. Indeed, as various molecular motors push, pull and twist DNA, transient forces and torques develop within chromatin, with expected regulatory consequences. How much? How fast? How strong? Various biophysical methods are used to provide answers to these questions and improve our understanding of DNA and chromatin behaviour under physiological physical constraints. We will give a brief overview of our current knowledge in the field and emphasize at the same time the importance of DNA supercoiling as a key parameter in genome mechanics.

References

Cortini, R, Barbi M, Caré B, Lavelle C, Lesne A, Mozziconacci J and Victor JM. (2016). The physics of epigenetics. Rev. Mod. Phys. (in press; available at http://arxiv.org/abs/1509.04145)

Lavelle C. (2014). Pack, unpack, bend, twist, pull, push: the physical side of gene expression. Curr Opin Genet Dev 25:74-84.

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

Phylogenies of type II and type IB DNA topoisomerases

We have updated the phylogenies of type II and type IB DNA topoisomerases (for previous ones, see Forterre et al., Biochimie, 2007, Brochier-Armanet et al., Biology Direct, 2008). Our results suggest that eukaryotic type II could have originated from Large NucleoCytoplasmic DNA viruses (NCLDV). In the case of type IB DNA topoisomerases, we observed that some NCLDV encode the short form typical of bacteria, whereas others encode the long form typical of Archaea and Eukarya. The long form type IB enzymes from NCLDV branch between Thaumarchaea and Eukarya in our phylogenetic tree. This suggests that these TopoIB were recruited by NCLDV from proto-eukaryotes. Our analyses also reveal a strong evolutionary connection between NCLDV and some head and tailed bacterioviruses (phages, Caudovirales).

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

Mapping Topoisomerase IV binding and activity sites on the E. coli genome

Catenation links between sister chromatids are formed progressively during DNA replication and are involved in the establishment of sister chromatid cohesion. Topo IV is a bacterial type II topoisomerase involved in the removal of catenation links both behind replication forks and after replication during the final separation of sister circular chromosomes. We have investigated the global DNA-binding and catalytic activity of Topo IV in E. coli using genomic and molecular biology approaches. ChIP-seq revealed that Topo IV interaction with the E. coli chromosome is controlled by DNA replication. During replication, Topo IV has access to most of the genome but only selects a few hundred specific sites determined by chromatin context for its activity. Strong DNA-binding and catalytic activities are found at the chromosome dimer resolution site, dif, located opposite of the replication origin. We reveal a physical and functional interaction between Topo IV and the XerCD recombinases acting at the dif site, modulated by the MatP protein involved in the organization of the Ter Macrodomain. These results show that Topo IV, XerCD/dif and MatP are parts of a network dedicated to the last step of chromosome management during the cell cycle.

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

Polymer model of supercoiled molecules including multiple structural forms of DNA

DNA supercoiling lies at the core of transcriptional regulation. Except for a few cases, capturing its impact in vivo remains elusive, though. Supercoiling is indeed distributed in a non-trivial way between twist, writhe (plectonemes) and change of structural forms of DNA (including denaturation) and depends, a priori, on genomic sequences. In this talk, we will present a polymer model of DNA that allows studying these properties quantitatively. We will show in particular the possibility to study the behavior of DNA sequences whose length corresponds typically to the topological microdomains that have been experimentally highlighted in *Escherichia coli* and *Salmonella typhimurium*.

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