

Physics of epigenome regulation

Theoretical models of chromatin-based control of gene expression

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Résumé

The general objective of this research project is to build quantitative models of the epigenomic-based mechanisms of gene regulation involved in the short- and long-term cellular response. Using theoretical approaches from statistical and numerical physics and in close collaboration with experimentalists, we propose to study the "1D" assembly of the different eu- and heterochromatin states along the genome (nucleation, spreading, compartmentalization and mitotic/meiotic inheritance), and their "3D" folding and nuclear organization. At the nucleosomal and gene scales, we are currently developing molecular models accounting for the combined action of chromatin regulators and DNA binding proteins to understand the mechanisms by which chromatin states achieve their functions. At larger scale, we use coarse-grained models to derive the structural and dynamical properties of the epigenome : (i) the regulation of epigenomic domains (ii) their folding into spatial compartments and (iii) finally the coupling between 3D organization and 1D assembly. Our main goal is to provide a general framework to understand how the epigenome is regulated and how its affect gene expression : from its establishment during development and differentiation to its deregulation in diseases. Ultimately we propose to understand how the epigenome (via the chromatin regulators) "sense" environmental, metabolic or genomic changes (stresses) and, in some cases, translate transient stimuli into stable,inheritable expression pattern changes while in other cases act as a "buffer" for these perturbations.

The complete version can be downloaded at :
<http://perso.ens-lyon.fr/cedric.vaillant/doku.php?id=divers>, “ProjetRecherche”.

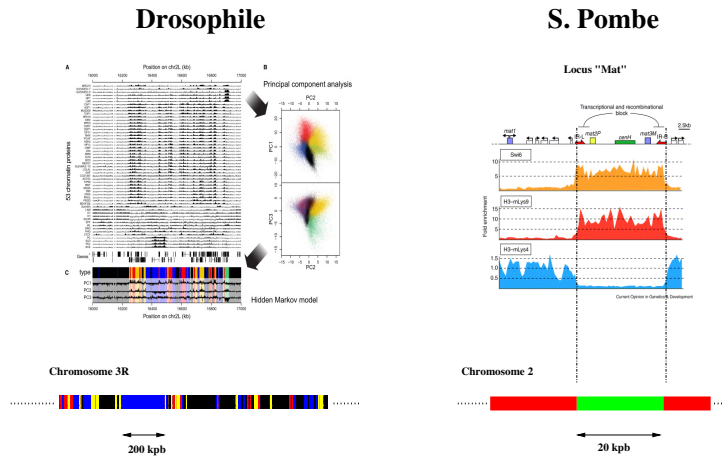


FIGURE 1 – Epigenomic domains in *Drosophila* (1) (Left) and in *S. pombe* (Right).

1 INTRODUCTION

The ability of organisms to precisely regulate gene expression is central to their development. Proper temporal and spatial expressions of genes in higher eukaryotes require activation of transcription during the appropriate developmental stages. In response to environmental and developmental cues, cells can adopt different gene expression patterns to differentiate into a variety of cell types; once established, this pattern is frequently maintained over several cell divisions despite the fact that the initiating signal is no longer present. This ability of translating transient external stimuli into diverse (plasticity) and stable (robustness) phenotypes without alteration of the genomic sequence is at the heart of “epigenetic” regulation of gene expression. Transcription in eukaryotes is regulated by trans-acting factors that associate with the genome in a cell-type and condition-specific manner at specific cis-regulatory elements including proximal promoters, enhancers and repressors. The packaging of eukaryotic DNA into chromatin contribute to this regulation via the modulation of the accessibility and specificity of regulators to their DNA cognate sites. The local chromatin state is characterized by various features like the nucleosome positioning or the covalent modifications of DNA and histones tails. This pattern of chromatin state along the genome, the so-called "epigenome", is itself regulated by the combined action of different specialized chromatin regulators like chromatin remodellers, modifying enzymes, histone chaperones.

1D compartmentalization of the epigenome. The general picture that emerges from the genome-wide high-resolution profiling of structural and functional chromatin markers obtained in various organisms and cell types (1, 2, 3, 4), is that eukaryotic genomes are linearly organized into distinct epigenomic domains (Fig 1). These domains extend over few kb up to few megabases, are characterized by a specific type of chromatin and are isolated from their neighborhood by boundary elements such as insulators. Euchromatin, less condensed, early replicating and containing most active genes, is generally distinguished from heterochromatin, typically highly condensed, late replicating and inhibitory to transcriptional machinery. Heterochromatin is classified into two subtypes : constitutive and facultative heterochromatin. Constitutive heterochromatin contains highly repetitive DNA sequences such as those found at centromeres and telomeres, and serves to stably silence transposable elements as well as facilitates chromosome segregation. In contrast, facultative heterochromatin is typically associated with developmentally regulated genes whose chromatin structure may change in response to cellular differentiation signals. In many higher eukaryotes, from plants to mammals, statistical analysis of hundreds of chromatin markers have identified only a small number of main chromatin types (1, 5, 3), typically 4 or 5, covering the well-known constitutive HP-1-like heterochromatin or the facultative Polycomb-like heterochromatin but also a less-characterized ultra-repressive heterochromatin enriched in genes that are expressed in very few tissues, the so-called black chromatin (1, 6).

3D compartmentalization of the epigenome. Interestingly, within epigenomic domains, regulatory sequences such as enhancers may be located far from the target genes and multiple elements

Compartmentation 1D

Compartmentation 3D

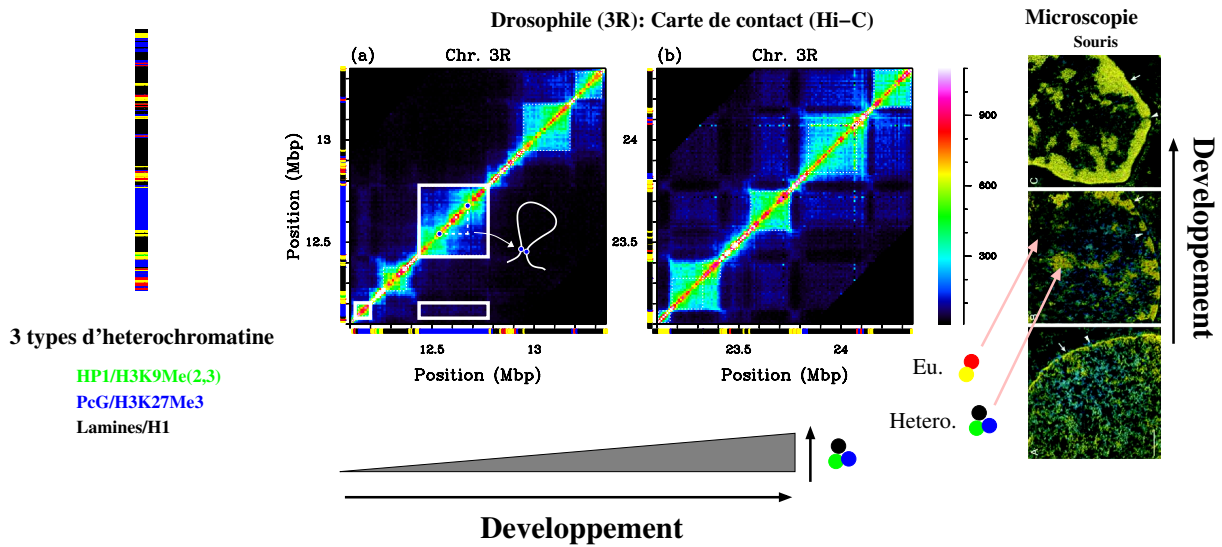


FIGURE 2 – 1D and 3D compartmentalization of the epigenome.

that are arrayed over large regions may collaborate or compete for the regulation of individual genes or gene clusters. This implies the existence of long-range mechanisms where regulatory elements could act over large genomic distances up to hundreds of Kb or more. A first possibility is the linear spreading of a regulatory signal (e.g. repressive chromatin state) from nucleation sites (e.g. silencers) to target-sites (e.g. promoters). Another -but not exclusive- strategy is to take benefit of the polymeric nature of chromatin and to induce spatial co-localization of regulatory sequences with their target. Recently, 3C-based studies have indeed shown that regulatory elements can act over large genomic distances by chromatin looping (7, 8) forming active or repressive chromatin higher-order structure at particular developmental-regulated genes. These pair-wise 3D interactions are mediated by DNA binding proteins such as insulators or cohesins that would cluster in space and bridge distant cis-regulatory sites. At a genomic scale, the contact maps of drosophila (9, 10), mouse (11) and human (11, 12) chromosomes have further revealed a remarkable 3D compartmentalization where epigenomic domains fold into independent “spatial domain”, the so-called topologically-associated domains (TADs), characterized by (i) high intra-domain contact frequencies; (ii) three-dimensional insulation between adjacent domains; (iii) and in many cases, significant contacts between distal domains of the same chromatin type (Fig. 2). This compartmentalization is consistent with the nuclear structure revealed by imaging techniques such as EM and immuno-FISH (13, 14, 15) that clearly shows a phase separation between eu- vs heterochromatin and to some extent between the different heterochromatin types (16).

Chromatin state and folding transitions during development During development, cell differentiation proceeds by global and concomitant rearrangements of epigenomic profile, chromatin organization and transcriptional activity (17, 18, 19, 20). Developmental cell specification is globally accompanied by a progressive chromatin restriction, starting from open (3D) and permissive (1D) chromatin organization in pluripotent ES to increasingly repressive, compact and segregated state in differentiated cells.

2 GENERAL OBJECTIVES

Altogether, these experimental data reveal a clear link between the epigenome, the 3D architecture and the gene transcription pattern. However, it is still unclear what are the mechanisms behind one-dimensional epigenome assembly and its three-dimensional folding, and how these 1D and 3D organizations precisely contribute to epigenetic gene regulation. The general objective of this long term project is, in continuity to my previous and current research activities, by using

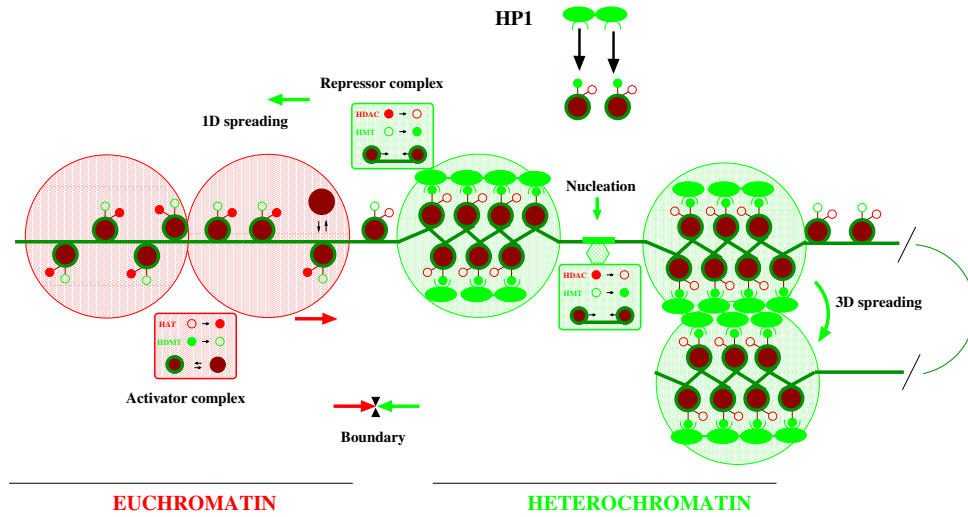


FIGURE 3 – Molecular model of epigenome assembly (and folding)

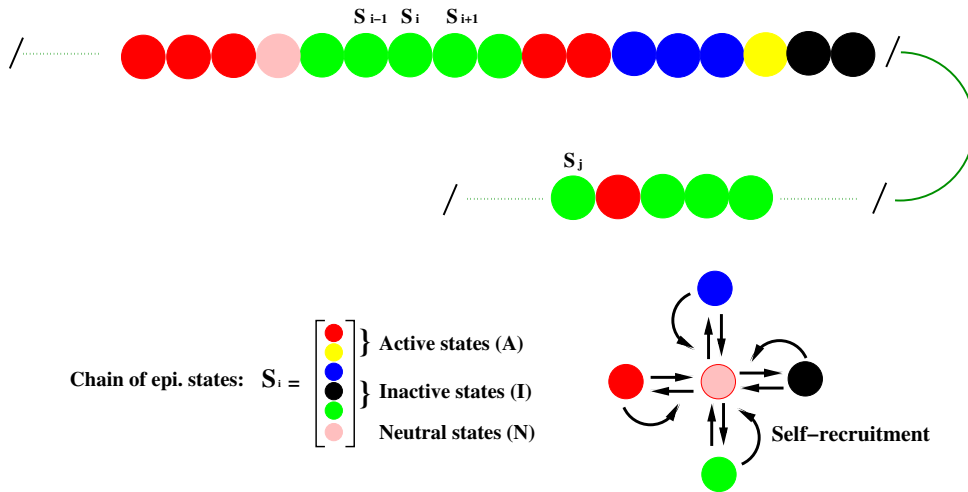


FIGURE 4 – Effective spin-like model of epigenome assembly

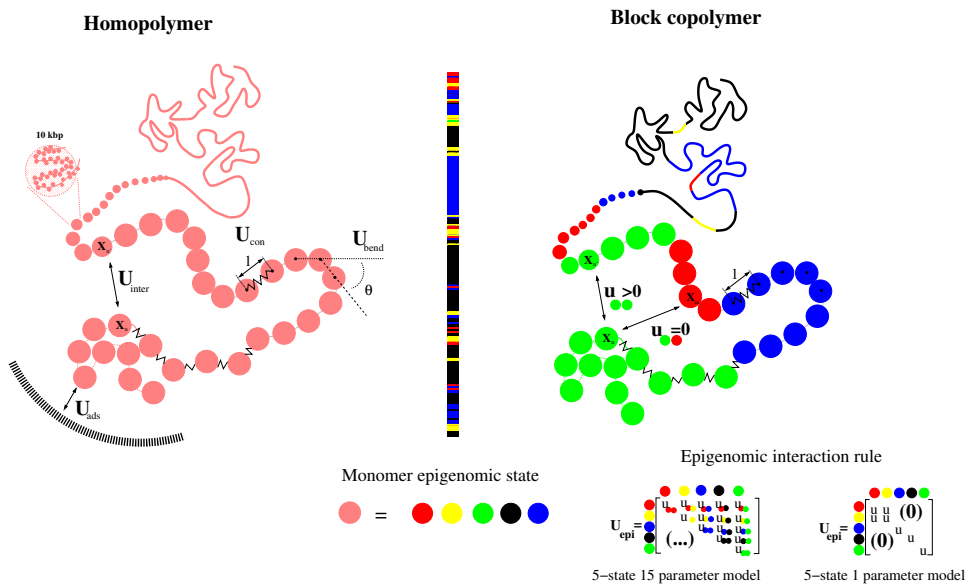


FIGURE 5 – Effective model of chromatin folding.

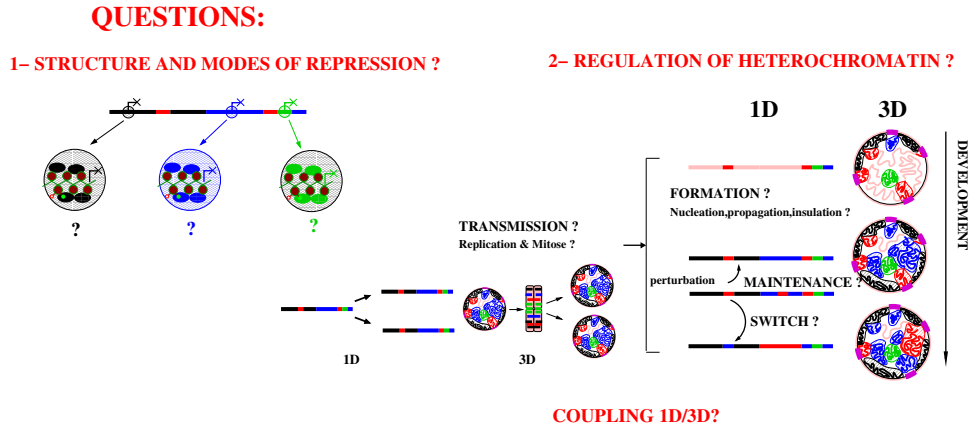


FIGURE 6 – General objectives

theoretical approaches from equilibrium and non equilibrium statistical physics, to identify and model some chromatin-based mechanisms of gene regulation involved in cellular development : from first principles and with a minimum (ie tractable) parametrization, can we account for the different chromatin states, their spatial folding and nuclear organization? For their dynamics during cell cycle , differentiation? Can we understand how these 1D & 3D organizations of the genome contribute to stably activate or repress genome activity? How chromatin regulatory pathway “sense” environmental changes and, in some case, translate transient stimuli into stable, inheritable expression pattern changes while in other cases act as a “buffer” of external as well as genetic/genomic perturbations?

There are two main axes that are in continuity with my previous and current research activity and a third one, more exploratory :

– **Epigenome assembly, folding and function (Fig. 6)**

Question : Can we account for the different epigenomic domains and of their spatial organisation observed in the various cell types and organisms?

Based upon current *in vivo* and *in vitro*-derived experimental knowledges, we will introduce local molecular models (Fig. 3) to investigate the macromolecular structure and dynamics associated to the different types of chromatin. In a thermodynamical framework, as a function of relevant control parameters (i.e. chromatin regulators activity, architectural proteins availability, binding strength, interactions strength, effective temperature) we will compute the equilibrium distribution of chromatin states along genomes. For a given genomic domain, we will explore the parameter space to show conditions at which thermodynamically distinct chromatin states (heterochromatin/euchromatin/intermediate) can occur at equilibrium and thus delineate regions of monostability (i.e. where there is a single stable chromatin state) and regions of multistability (ie where different stable chromatin states can occur).

Additionally, we will further investigate the coupling between epigenome assembly and the 3D organisation of chromatin. We will refine and extend the simple block copolymer model (see below) that we already developed ; in particular, the idea is to introduce finer scale epigenomic variability as well as other constraints such as the ability of anchoring at the nuclear membrane and of long-range bridging (site-specific looping) between regulatory sites. Topological constraints (excluded volume) that are likely to control large-scale organisation of chromosomes will be treated more accurately. Using analytical-numerical mean-field approaches and efficient numerical simulations we will compute equilibrium phase diagrams. However this model states that the epigenome is “quenched”. The main task of this project will be to relax this hypothesis and to develop a theoretical framework that couples the dynamics of epigenome assembly and its 3D organisation : the “living polymer” framework. The principle behind this, is that there is a self-reinforcing positive

feedback loop between 1D and 3D organization which mainly relies, for example in the case of heterochromatin, on the oligomerization and self-recruitment ability of repressive complexes (21, 22, 23, 24) : the 3D compartmentalization of chromatin might be a way of coordinating and reinforcing the functional output (repression, activation) of genomic regions by colocalization and mutualization of the same specific regulators (Fig 8).

– **Epigenome dynamics : Epigenetic inheritance (maintenance) and transition (Fig. 6) ?**

Question : Can we account for the regulatory pathways driving chromatin state and folding transitions during development ?

Having identified the “equilibrium” configurations, we will investigate their stability to various perturbations such as molecular noise, genetic polymorphism, or environmental perturbation. In our thermodynamical framework, molecular noise account for the effective temperature : for given control parameters values (chromatin regulators activity), it controls the “intrinsic” fluctuations of the chromatin states ; genetic polymorphisms may account for genome modifications (insertion of parasitic DNA, mutations in regulatory sites) or mutations in the regulators-encoding genes : this might induce modification of the phase diagram. Environmental perturbation would mainly account for chromatin regulators activity modulation and thus for changes in the control parameter space. Formally, this mean that we will study the relaxation dynamics of a given equilibrium state following a change in the control parameter : what changes (of what amplitude and duration ?) are needed to induce a transition between two equilibrium states ? What is the corresponding kinetic and conformational path during the transition ? What is the role of spontaneous fluctuation (noise) in driving transitions ? Of particular interest for differentiation process will be the study of multistability which indeed accounts for plasticity but also for “memory” effects ie irreversible switch as well as for glassy dynamics (see our work on epigenome folding).

We will also investigate the influence of replication on chromatin state stability and maintenance. Provided a molecular model of chromatin state “redistribution”, we will study the non equilibrium dynamic of chromatin states when periodically submitted to replication-induced rearrangement. Depending on the “transmission” model (equi-repartition of chromatin states between the two replicated strands, biased transmission due to “spatial” effects....) and on the duration time of cell cycle, some equilibrium states might be driven through transition or reversely, consolidated.

The objective is to perform this theoretical study in the “Living Chromatin” framework, ie when both 3D and 1D dynamics are coupled.

– **Cellular Signaling**

Question : How epigenome “sense” environment ?

Having studied some principles behind the regulation of the epigenome a final question would be : what regulates epigenome regulation ? How indeed environmental signals such as temperature, mechanical and nutrient availability changes can be translated into stable modification of the epigenome and its functional output. What are the thermosensitive, mechanosensitive and chemosensitive mechanisms that influence the epigenetic processes governing differentiation by modulating chromatin regulators activity ? Mechanical stresses can be transmitted to chromatin (to genes) either indirectly, via a standard cytoplasmic biochemical cascade affecting a regulatory gene network or directly via the connection between cytoskeleton and the nuclear membrane (25) ; the question is, how does it affect chromatin regulators ? By relocation into the interior of the nucleus... ? Chemical cues (nutrient availability) can be transmitted by the metabolism pathways that produce metabolites that are key ingredients of chromatin regulators, connecting epigenetics and metabolisms (26).

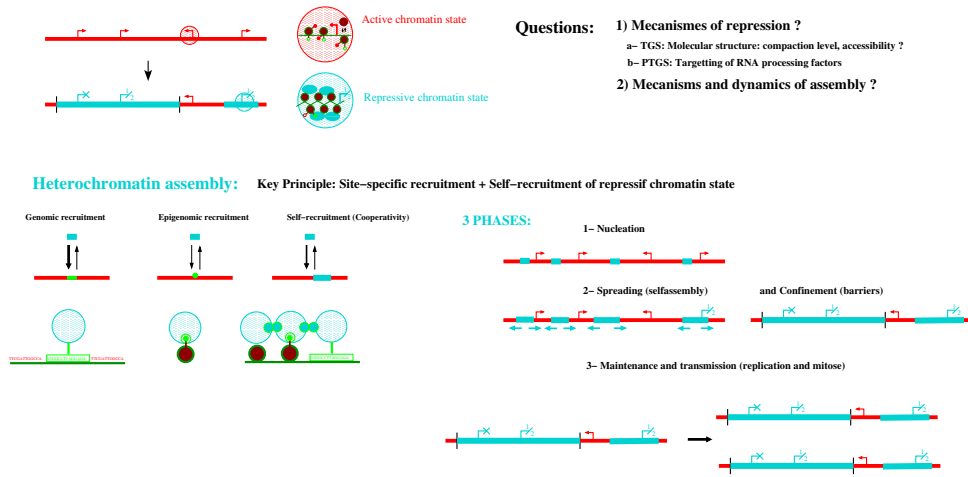


FIGURE 7 – The “1D” problem

How metabolism dynamics translate into epigenetic dynamics? The same question holds for temperature, for example in the vernalization process (27). Simple theoretical models that couple external signal dynamics to epigenome regulation will be developed.

3 MODELS AND THEORETICAL APPROACHES

3.1 “1D” : Epigenome assembly (Fig. 7)

Current model of epigenome assembly mainly consider a spin-like chain models (Fig 4) where nucleosomes can adopt 2 or 3 “internal” (or more) epigenomic states (Active/Repressive/Undetermined) (I.B. Dodd *et al.* (28) and D. Jost (29)). Then dynamics of conversion (from Active to Repressive....) is controlled by the interplay between an effective temperature (random interconversion) and coupling factors (guided conversion) between the states of the nucleosomes; it is assumed that nucleosomes are fixed along a given genomic domain. Large-scale (at the domain scale) equilibrium and dynamical properties are then computed via stochastic simulation or in a mean-field approximation to extract phase and bifurcation diagrams as a function of coupling factors strength. The idea is to refine these approaches by introducing local molecular models of the different chromatin states and of their dynamics of formation : nucleosome positioning and turn-over, binding of proteins such as HP1, modification of the histone-tails (Fig. 3) will be included to define local molecular and epigenetic states. This, of course in order to better account for the complexity of local epigenome regulation. We assume indeed here that a minimal additional layer of complexity can be introduced (in comparison to the spin models) in a still easy tractable way (ie without not so much computational effort while keeping some genericity). Moreover, high-resoluted biochemical mapping allow now to build such molecular models.

3.2 “3D” : The co-polymer framework. *Folding of an homopolymer.*

A generic and minimal model to investigate the large-scale 3D organisation and dynamics of chromosomes is to consider chromatin as a semi-flexible self-avoiding homopolymer. The conformation of such polymer is controlled by the interplay between thermal motion, steric repulsion and effective monomer-monomer interactions that may account for steric confinement, true attractive or repulsive interactions between monomers or interactions mediated by the solvent. For example, in a “good solvent” condition, i.e. when steric repulsion dominates, the chain adopts a swollen coil state with a typical size that scales with the number of monomers as $R_g \sim N^{3/5}$ and the contact frequency is given by $P(s) \propto s^{-9/5}$ with s the linear distance between monomers (30); in a “poor solvent” condition, i.e. when effective attraction prevails, the polymer chain becomes compressed and equilibrates into a collapsed globular phase characterized by a high monomer density, a typical size $R_g \sim N^{1/3}$ and a contact frequency that is almost constant at large separation distance; in between, i.e. at the transition point (the so-called θ temperature) where repulsion and attraction counterbalance, the chain adopts a gaussian coil state with $R_g \sim N^{1/2}$ and $P(s) \sim s^{-3/2}$.

Such homopolymeric model with appropriate geometric constraints provide a fairly good description of large-scale conformational properties from yeast to human and in particular a good fit of different scaling behaviours like the dependence of the contact frequency or of the mean distance on the linear genomic distance. In yeast, static and dynamic studies suggest a brush-like equilibrated organisation (31, 32) of chromosomes with scaling properties compatible with a weakly collapsed state; in fly and human, where chromosomes are much longer, large-scale organisation is characterized by a dense state and a contact probability $P(s) \propto s^{-1}$ which has been primarily associated to a non-equilibrium dense phase composed of spatially segregated long-lived globular domains (33) but which can also be mapped to a semidilute solution of non concatenated rings (34) at equilibrium. A recent paper by Barbieri et al. (35) has shown that this fractal-like state could also be described by introducing effective attraction via binding molecules in an homopolymer model.

Folding of block copolymer. However most of these models consider an homopolymer that cannot obviously account for the regional variability of the chromatin organization and in particular for the spatial compartmentalization of the epigenome. One has to introduce a genomic and/or epigenomic specificity in the folding model. In a recent collaboration with G. Cavalli (50) we posit that chromatin folding is driven by effective epigenomic-dependent interactions between chromatin loci. To test this hypothesis, we propose a generic and new theoretical approach by treating chromatin as a block copolymer, where each block corresponds to an epigenomic domain and where each monomer interacts preferentially with other monomers of the same chromatin type. This is largely motivated by the observations of self-interactions between chromatin type (9) and is also supported by increasing evidence that some architectural proteins might promote physical bridging (24, 23). As already mentioned, we adapt a Gaussian self-consistent approach (??) to derive equilibrium phase diagram and contact maps for any block copolymers, as a function of two control parameters, the specific and non specific monomer-monomer interaction. When considering block copolymers build from the epigenomic landscape of *Drosophila* (1), we have shown that this simple physical model accounts very well for the folding patterns in TADs observed in Hi-C experiments (9). As a main and very original outcome, this model provides a physical basis for the existence of multistability in chromosome organization. We show indeed how some experimental patterns are fully consistent with multistable conformations where topologically-associated domains of the same epigenomic state interact transiently or long-lastly with each other.

Further extension and improvement of the block copolymer model

In our recent work (see “Rapport de Recherche”) , we were interested by identifying some general principles governing chromatin folding. Therefore, for simplicity, we limit our approach to the simplest version of a block copolymer that we can build from the compartmentalization of the epigenome. It assumes that all specific interactions have the same strength whatever the chromatin type and whatever the location along the genome. However such two-parameter model needs to be refined in order to gain in predictability. In particular, the current model considers each domain as an homopolymer with uniform monomer-monomer interactions. This can only provide a coarse-grained understanding of epigenome folding and cannot account for variation in contact frequency and in particular for preferential pairwise (long-range) contact between discrete genomic loci. Recent studies (8, 10) have proposed that these site- and lineage-specific contacts mediated by architectural proteins (insulators, cohesin, mediators) might indeed play a key role in the folding of chromosomes at the sub-Mbp scale. Along the same line, anchoring at the membrane of particular sequence or/and epigenomic domains (via their association with lamina or nuclear pores) has been shown to be crucial for regulating both folding, expression and stability of the genome(36, 37, 15, 38). In addition to the global, non site-specific, interactions investigated by our current model, focal large-scale looping and anchoring might indeed contribute to spatial compartmentalization of domains (9, 10, 39). Therefore, further improvements of the model will require to augment the number of parameters by allowing for variability of interaction at the monomer scale, and to infer specific interaction strengths that predict at best the observed contact maps.

Moreover, we made the assumptions that the polymer chains are equilibrated. Detailed sim-

Coupling 1D/3D: the "living polymer" framework

Key principle: Self-assembly enhanced by long-range contact/Compaction
 Compaction enhanced by ability of bridging
 = Positive feedback loop

How 3D folding and nuclear localization affect 1D assembly ?
 How 1D influence 3D folding and nuclear localization ?

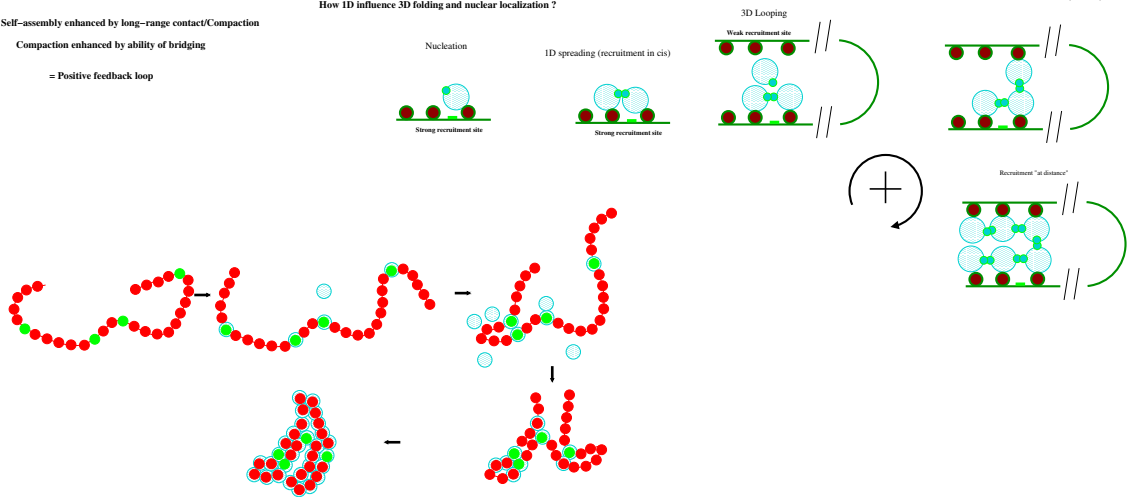


FIGURE 8 – The “1D/3D” problem

ulations of confined self-avoiding homopolymers (34) have suggested that strong topological constraints may slow down the chain dynamics such that equilibration of very long confined chains can be extremely long (34), well above the cell cycle length. However, recent theoretical studies (Rosa & Everaers, personal communications) have estimated that below few Mbps, the typical length scale investigated in our preliminary, topological confinement is negligible and polymer chains may safely be considered as equilibrated. However, it is clear (even at this length scale) that the self-consistent approach can only give an approximate solution of chromatin folding due to the rough treatment of excluded volume. The next step will be to use efficient full numerical simulation such as the one developed by P. Carrivain who is actually post-doc in Cavalli’s lab. Along the same line, A. Rosa and R. Everaers have recently developed a very efficient numerical scheme for computing large-scale conformation statistics of chromosomes and we are currently working on including epigenomic specificity into their modelling.

Inference of structure and potentials from Hi-C maps

The copolymer framework associated with the self-consistent Gaussian approximation may represent an efficient formalism to extract from the available experimental data the effective genomic and epigenomic interactions between chromatin loci. As a promising outcome of such inference process, would be a powerful tool to predict the chromatin organization in various conditions, allowing to investigate *in silico* changes in TAD formations and long-range contacts when altering the epigenome. Hence our model may provide a very interesting framework for understanding how epigenome regulation (resp. dysregulation) during development (resp. disease) could lead to cell phenotypic variations via large-scale chromatin reorganization. This inference (based on bayesian inference) is an ongoing project carried out by Noelle Haddad (our PhD student) ; promising results are already obtained.

3.3 Coupling 1D and 3D : the “Living Chromatin” approach This is the project for a new PhD thesis for September 2015 under the supervision of myself and Daniel Jost and is at the heart of the project with Peter Meister concerning dosage compensation of X chromosomes in *C. elegans*.

Local epigenome assembly relies on the combined action of various enzymes (“chromatin regulators”) that are primarily targeted at given DNA sequences to produce either a permissive (by activators) or a repressive (by repressors) transcriptional state around this “nucleation” sequence. The ability of these regulators to be secondarily targeted at chromatin fragments carrying the epigenomic state that they catalyze increase the cooperativity of their action leading to the 1D spreading (in “cis”) and to an enhanced robustness (stability) of the corresponding epigenomic states (that can be maintained even in the absence of the initial nucleation process). In a sense

this molecular cooperativity balance the small absolute number of regulators that are effectively acting (or even produced) by an increase of the effective local concentration of enzyme. However chromatin is not a 1D object and fragments that are genomically distant can be colocalized spatially leading to a 3D spreading (“in trans”) of the epigenomic states : the enzymes recruited at a given chromatin fragment can catalyze the states of all chromatin fragments that are in the spatial vicinity (and not only genomic vicinity). It would correspond to a 1D system (like spin chains) with long-range interactions leading to an even higher cooperativity (again because of an enhanced local concentration of enzymes). The 1D models presented before (Dodd et al. (28), Jost (29)) include long-range interaction in a mean-field way and our idea is to couple our “structural” polymeric approach (used in our work concerning epigenome folding) (Fig. 5) and their dynamical spin chain model (Fig. 4). Full numerical simulations will be developed by implementing kinetic Monte-Carlo algorithms for the polymer and the chromatin states. Within such model, the “spin coupling” constant will depend on the spatial proximity and as before, specific volume interactions between monomers will depend on their epigenomic state. Thus, the very interesting and original feature that we will be able to study is the “living” feature of chromatin : due to the ability of chromatin fragments of same epigenomic states to cluster in space, the spatial organization will be dynamically coupled to the 1D assembly of the epigenomic states. This correspond to a mechano-chemical coupling that will further increase the cooperativity of the enzymatic action of regulators and thus the robustness of the epigenomic domains.

4 EXPERIMENTAL SYSTEMS AND COLLABORATIONS

4.1 Heterochromatin assembly in Fission yeast : transcribing for repressing! This is a common project with Andre Verdel (Institut Albert Bonniot) and with Daniel Jost (TIMC-IMAG) : We received a financial support (5000 euros) associated to this project by the IXXI institute : the full description of the project can be found at : <http://perso.ens-lyon.fr/cedric.vaillant/doku.php?id=divers>.

In collaboration with the experimentalists the objective is to develop molecular models of heterochromatin assembly (Fig. 3). *S. pombe* is a very well suited system since heterochromatin of type “HP1” is a very well conserved mode of repression found in all multicellular organisms. Repression by DNA-based and RNA-based nucleation pathways (Fig. 9) are also two very-well conserved modes of repressions. I propose to particularly focus on the RNA based mechanisms of gene regulation that have, to date, received very few theoretical investigations. Diverse classes of RNA, ranging from small to long non-coding RNAs, have emerged as key regulators of gene expression, genome stability and defence against foreign or endogenous genetic mobile elements (40). Small RNAs modify chromatin structure and silence transcription by guiding Argonaute-containing complexes to complementary nascent RNA scaffolds and then mediating the recruitment of histone and DNA methyltransferases. In addition, recent advances suggest that chromatin-associated long non-coding RNA scaffolds also recruit chromatin-modifying complexes independently of small RNAs. These co-transcriptional silencing mechanisms form powerful RNA surveillance systems that detect and silence inappropriate transcription events, and provide a memory of these events via self-reinforcing epigenetic loops.

The objective is to formalize by quantitative modeling the current biological models (Figs 9, 10) of heterochromatin assembly at the DNA and nucleosome scales. Starting from the current experimental knowledge of the various biochemical reactions and their corresponding kinetic rates (eg nucleosome deacetylation, remodelling, methylation, ncRNA transcription rate...) involved in both nucleation and the further assembly of heterochromatin, we will perform Gillespie kinetic Monte-Carlo simulations for different genomic loci : the Mat locus, the meiotic genes, the pericentromeres and telomeres and the retrotransposons.

4.2 Epigenome reprogramming during spermatogenesis *Collaboration with Saadi Khochbin, Institut A. Bonniot, Genoble*

An essential feature of spermatogenesis is the generation of a transportable genome placed in the nucleus of highly specialized cells, spermatozoa, capable of leaving and surviving the parent organism. After meiosis, young spermatids (known as round spermatids) inherit a chromatin-

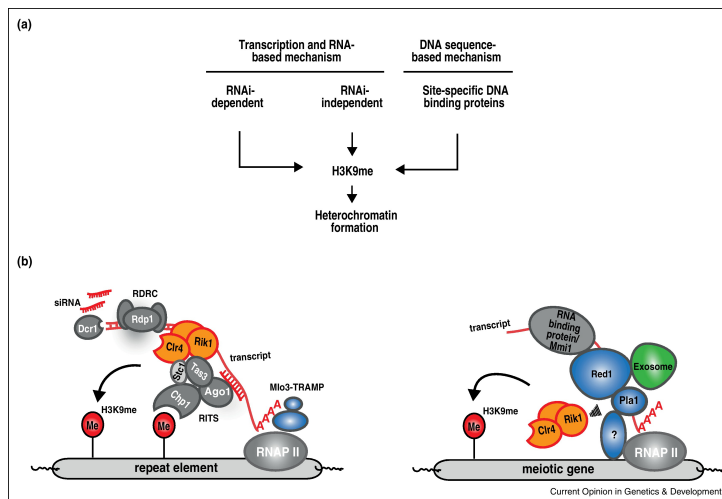


FIGURE 9 – (a) Heterochromatin nucleation is mediated by RNA-based and DNA sequence-based mechanisms. RNA-based mechanisms to establish heterochromatin include both RNAi-dependent and RNAi-independent pathways. DNA-sequence based mechanisms rely on sequence-specific DNA binding factors to recruit chromatin-modifying activities. (b) RNA-based heterochromatin nucleation pathways. The RNAi-dependent pathway (left) involves RITS, RDRC and Dicer. Guided by siRNAs, the RITS complex localizes to nascent transcripts where it interacts with *Stc1*, a scaffold that bridges RITS to *Clr4*. *Clr4* methylates H3K9 to assemble heterochromatin. Repeat transcripts are polyadenylated by Mlo3-associated TRAMP, a factor that mediates processing of RNAs by the exosome and the RNAi machinery. The RNAi-independent pathway (right) requires the RNA elimination machinery to assemble facultative heterochromatin at meiotic genes. The RNA binding protein *Mmi1*, along with factors involved in pre-mRNA 3' end processing and the RNA elimination machinery, recognize specific meiotic RNAs. *Red1*, a protein that interacts with the exosome, may form a specialized complex that recruits *Clr4* required for the assembly of heterochromatin at specific meiotic genes. From (41)

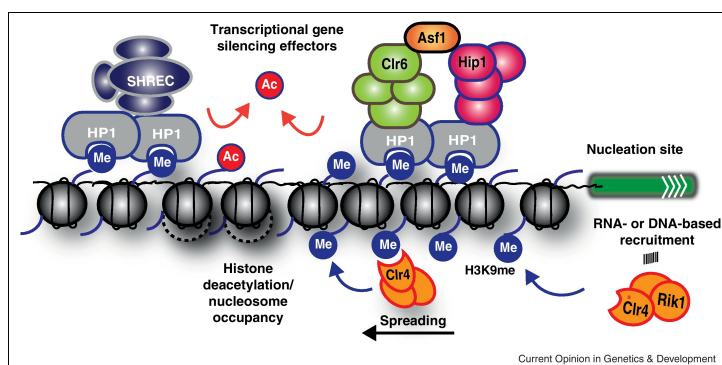


FIGURE 10 – Heterochromatin promotes transcriptional silencing through histone deacetylation and changes in nucleosome occupancy. H3K9me that is initially targeted to nucleation sites by DNA- or RNA-based mechanisms can be spread to surrounding sequences via a process that involves *Clr4* binding to methylated H3K9. HP1 proteins (*Chp2* and *Swi6*) bound to H3K9me provide a recruiting platform for loading of the histone deacetylase complexes SHREC and *Clr6*-*Hip1* complex, and the histone chaperones *Asf1*-*HIRA*. *Clr6* and SHREC have overlapping functions in limiting RNAPII occupancy at heterochromatic loci. *Asf1*-*HIRA* facilitates deacetylation of histones by *Clr6* HDAC. *Asf1*-*HIRA* and SHREC also promote nucleosome occupancy and eliminate nucleosome-free regions that are thought to prevent access to the transcriptional machinery and enforce transcriptional gene silencing. From (41)

containing transcriptionally active genome that, during subsequent stages in elongating spermatids, undergoes a genome-wide histone hyperacetylation followed by histone removal and the assembly of transition proteins (TPs) and protamines in condensing spermatids. These chromatin transitions constitute a unique feature among eukaryotes, since the universal nucleosome-based organization of the genome undergoes a metamorphosis into new and unique genome-packaging structures based on nonhistone proteins. This transition (nucleosome-TP-protamines) has been shown to occur through the complete replacement of conventional histones H2B by one of its variant TH2B (42). Although essential to the life cycle, the molecular basis of these dramatic changes remains one of the most obscure issues in modern biology.

The project here is to provide a quantitative modelling of the different stages of these global transition. The analysis of all the epigenomic datas (nucleosome positioning, histone variant replacement, histone acetylation...) obtained, in particular in the group of S. Khochbin at the different stages of spermatogenesis will allow us to build dynamical models of this global epigenome reprogramming. The main objective of this project is to provide fundamental insights concerning transgenerational epigenetic inheritance via the epigenome regulation of male gametes.

4.3 Fly development *Proposal of ANR project ("EpiDevoMath" 2015) with G. Cavalli :*

The Cavalli lab has adapted the original Hi-C method to map chromosome contacts in order to improve its resolution and simplify the protocol. This allowed obtaining high-resolution contact maps of the genome at late *Drosophila* embryogenesis, which showed the existence of domains of 100 *Kb* average size. Many contacts are formed within each domain, whereas much fewer contacts are formed between domains, such that one may define these as physical or topological domains. The analysis showed that physical domains are strongly correlated with the chromatin states of underlying genes, i.e. most genes within the same physical domain have similar chromatin states. They showed the existence of four different types of physical domains, with predominant i) active chromatin, ii) heterochromatin, iii) Polycomb chromatin and iv) silent chromatin without notable marks. Furthermore, we identified interdomain contacts that associate preferentially physical domains of the same kind. In order to improve this analysis we plan to increase the sequencing depth by over one order of magnitude (from 300 million to approximately 5 billion reads). Moreover, we will produce a developmental time course in order to deduce when physical domains are formed and compare this to epigenomic domains. We will focus on early stages of embryogenesis, starting from stages preceding the onset of transcription (syncytial nuclear cleavage 8), then stages between cleavage 8 and cellularization (nuclear cleavage 14), and finally cellular blastoderm embryos. We can obtain at least 106 cells for each of the stages and this is sufficient to perform Hi-C. If we find that the earliest point still resembles late embryogenesis, we will establish Hi-C maps of earlier stages, up to the oocyte. We have already schemes that allow to obtain thousands of unfertilized eggs, and may establish single-cell Hi-C to analyze hundreds of different eggs if necessary. In order to complement the developmental maps, we will also analyze cultured cells derived from embryos in G1, S, G2 and M states, obtained by FACS sorting. This will allow deriving the stability of chromatin interactions during the cell cycle. For comparison, we will also produce Hi-C maps for different *Drosophila* species, such as *Drosophila simulans* and *Drosophila pseudoobscura*.

In the course of this project, we will use additional experimental data to refine the estimates of interaction forces. In particular, we will test if the mathematical models make quantitative predictions in the form of virtual Hi-C maps for the effects of erasing topological insulation effects, for the consequence of chromosomal inversion, or for the folding of chromosomes in different *Drosophila* species. To this end, we will produce Hi-C maps in the In(3LR)sep inversion as well as for mutants of border binding proteins such as CTCF and Cp190. We will also test predicted distance distributions between selected loci, as predicted in our molecular dynamics simulations of chromosome folding, by Fluorescent in situ DNA hybridization (DNA FISH), using both confocal and superresolution microscopy with an OMX apparatus . Furthermore, we will attempt to improve the quality of the biological Hi-C maps by deeper sequencing (depending on cost reduction in the coming years). These gradual refinements of the predictions followed by experimental testing will lead to improvements that should ultimately lead to a very good match between predictions and data for the fly genome and represent the first step of a revolution in our understanding the relation between chromatin components and their role in setting up

chromosome architecture.

Extending predictive 3D epigenomics to mouse and human cells. Recent work has shown that topological domains exist in mouse and human cells, with the notable difference in size, which approaches 1Mb in these organisms . However, the existing maps are restricted to very low sequence coverage when compared to those of the *Drosophila* genome. We calculated that at least 100 fold increase in sequencing depth would be required to match the resolution achieved in flies, and the Cavalli lab secured funds to perform Hi-C of 1011 paired-end reads in total, that will be used to map mouse ES cells as well as their differentiated counterparts that give rise to neuronal progenitors or terminally differentiated neurons. We will thus produce predictive Hi-C maps for these cell types and match them to the experimental maps that we will obtain by the end of 2014. In the context of the present project, we will then predict the effect of reducing Polycomb proteins and insulators and produce knockdown and knockout cells for these chromatin components and compare predictions with Hi-C data in a similar way as in flies. As a second mammalian model for nuclear organization change, we will tackle cellular senescence. Senescence is characterized by an irreversible cell cycle arrest in response to various forms of stress, including activation of oncogenes, shortened telomeres (replicative senescence), DNA damage, oxidative stress and mitochondrial dysfunction. Senescent cells acquire an alteration of cell morphology and metabolism, epigenetic changes, increase in senescence-associated β -galactosidase activity and a major change in nuclear architecture with the formation of senescence-associated heterochromatic foci, so called SAHFs. A recent analysis suggests that SAHFs do not arise as a consequence of major changes in the epigenome marks along the chromosome, but rather on a change in their 3D organization . We will revisit this notion by investigating a series of epigenome marks as well as by performing Hi-C experiments in normal versus senescent cells. For this, we dispose of a model where we can induce either replicative senescence or oncogene-dependent senescence. The two systems will be compared and virtual Hi-C maps will also be produced for these cells in order to compare them with experimental data. Together, these two paradigms should lead to an extension of the predictive power of the physical models from the small fly genome to the larger and more complex mammalian genomes

In the frame of this collaboration and project, I propose to investigate the fascinating phenomenon of Hox genes regulation in both *Drosophila* and vertebrate systems (43). During embryogenesis, Hox genes control the specification of the anterior to posterior axis; in vertebrate they also organize structures along other body axis such as the appendicular (44, 17). All these genes are organized into genomic clusters and, remarkably, the relative order of Hox genes within this cluster corresponds to the relative position of the structures they instruct along the anterior-to-posterior body axis (Fig. 11 A,B). Recent studies have shown that this “colinear” activation is associated with a “wave” of concomittent spatial and epigenomic reorganisation of chromatin along the clusters (Fig. 11A,B). As proposed by D. Duboule (43), the emerging picture is that such “collinearity” is based on five main principles . Hox gene regulation and the question of colinearity might thus be a great modelling task to investigate within our “living polymer” framework.

4.4 Acetylcholine receptor gene coregulation by long distance interactions *ANR project “Young Investigator” Alexandre Mejat (LBMC, ENSL), with D. Jost*

The muscle fibre is an ideal model to probe how cellular function is linked to nuclear architecture. Muscle differentiation is a well-characterized model of cellular differentiation with mononucleated myoblasts irreversibly withdrawing from the cell cycle and differentiating into myocytes that fuse to form multinucleated terminally differentiated myotubes in an ordered series of events. In vivo, each multinucleated fibre is contacted by the axon of a motor neuron initiating the formation of a highly specialized structure dedicated to muscle / nerve communication, called the neuromuscular junction (NMJ). Motor innervation leads to the restriction of expression of synapse-specific genes to a few nuclei located directly under the synapse. Thus, the resulting mature muscle fibre is unique in mammals as it is the only multinucleated cell in which different genetic programs are expressed in different nuclei of the same cell. A further incentive to study nuclear architecture in muscle is the fact that mutations in nuclear envelope components have been linked to several human muscular dystrophies, suggesting that nuclear organization is critical for muscle differentiation/function. However, little is known about how

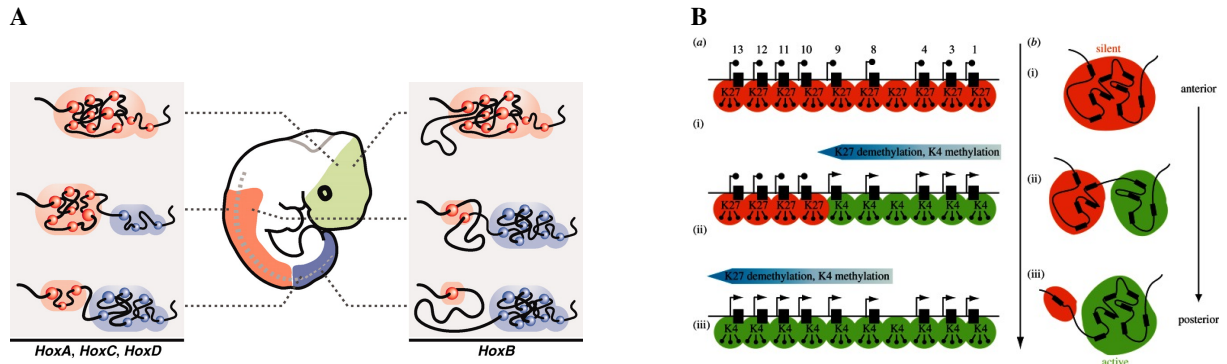


FIGURE 11 – A) Model of the 3D organization of Hox gene clusters, at various stages of colinear gene activation. Transcriptionally inactive genes are depicted in red and active genes in blue. Gene activation is paralleled by a transition from one 3D domain, matching the presence of H3K27me₃, to another domain of active transcription (marked with H3K4me₃). Although the same dynamics are observed for the HoxA, HoxC, and HoxD clusters (left), the HoxB cluster (right) shows a slight variation with a large piece of intergenic DNA looping out from these two domains. B) Collinearity during trunk extension and chromatin dynamics at Hox clusters. Expression of Hox gene along the anterior-to-posterior (AP) embryonic axis is collinear with gene order within the cluster. (a) During axial extension, the sequential onset of Hox gene transcriptional activation is accompanied by a transition in histone modifications over the gene cluster. In ES cells (i), the whole cluster is labelled with H3K27me₃ (orange), a mark associated with Polycomb-mediated silencing. In the developing embryo, this mark is progressively erased and replaced by H3K4me₃ (green), concomitantly with gene activation. (b) Active and silent Hox loci segregate into distinct spatial compartments along the AP axis. In embryonic tissues where the whole cluster is repressed, such as the forebrain (i), Hox clusters form a compact three-dimensional structure. In regions where subsets of Hox genes are expressed (anterior trunk, ii), active and silent genes segregate in distinct compartments, labelled with either H3K27me₃ (silent compartment) or H3K4me₃ (active compartment). In posterior embryonic regions (iii), most genes are transcribed and participate in the active compartment.

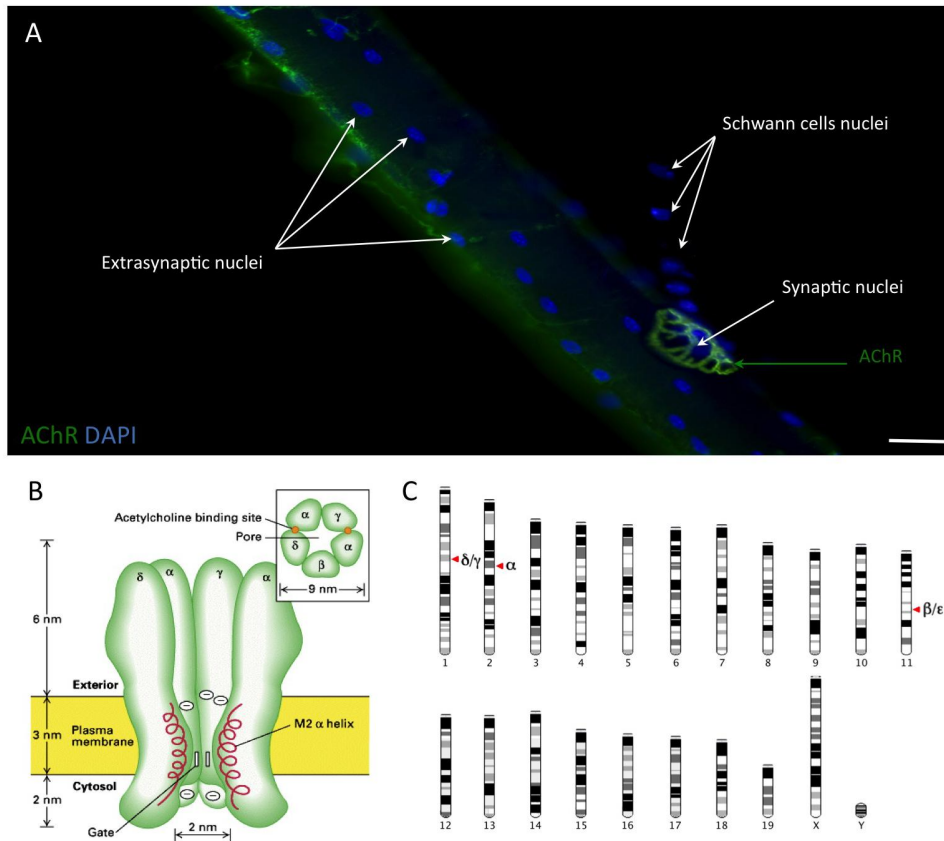


FIGURE 12 – (A) Portion of an isolated muscle fibre showing the acetylcholine receptor network (green) at the neuromuscular junction and the different types of nuclei (blue) in a multinucleated muscle fibre. Bar = $80\mu m$. (B) Structure of the adult acetylcholine receptor showing the different subunits : α , β , δ and ϵ . (C) Genetic locations of the different AChR gene in the mouse genome.

nuclear architecture and dynamics change during muscle differentiation and how nuclear architecture contributes to muscle function. We previously showed how electrical activity elicited by the motor nerve regulates muscle gene expression and identified the histone deacetylase 9 (HDAC9) as a key regulator of muscle chromatin organization and as a potential molecular target to block muscle atrophy. We also addressed the role of a major nuclear lamina component, lamin A/C, in the mature muscle fibre and showed that lamin A/C plays a specific role in muscle synaptic nuclei. Our results demonstrated that lamin A/C mediated neuromuscular junction defects are largely responsible for the disease phenotype in Emery-Dreifuss muscular dystrophy. Our proposal will extend these studies and address the functional role of long-distance interactions in the regulation of muscle gene expression.

We propose to study nicotinic acetylcholine receptor (AChR) subunit genes as a model system to investigate how long-distance interactions between coregulated genes are established and, in a more general way, to elucidate the mechanism(s) controlling gene positioning in muscle nuclei. AChR is a heteropentameric cationic ion gated channel highly enriched at the muscle / nerve synapse, also called neuromuscular junction (NMJ), where it binds the acetylcholine released by the nerve terminal and initiates the muscle contraction (Figure 4.4 B).

In mammals, five AChR subunits, α , β , δ , ϵ and γ , are expressed in muscle and exhibit both coordinated and differential regulation during muscle development. During embryonic development, fetal AChR is composed of the four subunits $\alpha 2\beta\delta\gamma$ and is expressed by all muscle nuclei. AChR subunit gene expression is then under the control of the Muscle Regulatory Factors (MRF) MyoD and myogenin which bind the E box element (CANNTG) present in the AChR gene promoter sequences. In vivo, each multinucleated fiber is contacted by the axon of a motoneuron at 14.5 day of embryonic development initiating the formation of the neuromuscular junction. As nerve contacts the muscle fibre, the electrical activity, triggered by the binding of the acetylcholine to the AChR accumulated at the NMJ, represses myoD and myogenin expression and AChR transcription in extrasynaptic nuclei. In parallel, neurotrophic molecules secreted by the nerve terminal stimulate the expression of synapse specific genes, such as the acetylcholine receptor (AChR) genes, in subsynaptic nuclei via the local activation of the Ets transcription factor GABP. These two independent regulation pathways result in a strict compartmentalization of AChR gene expression and the restriction of the synapse-specific genes expression to a few nuclei located directly under the synapse. Finally, after birth, the γ subunit is replaced by the ϵ subunit in order to modify the electrophysiological properties of the receptor. In Human, AChR subunit α , β , δ , ϵ and γ are encoded by five independant genes, CHRNA1, CHRNB1, CHRND, CHRNG and CHRNE respectively located on different chromosomes (Chromosome 2 for α , δ and γ ; and Chromosome 17 for β and ϵ). The same way, mouse Chrna1 is located on mouse chromosome 2, Chrn1 and Chrne on chromosome 11 and Chrnd and Chrng on chromosome 1 (Figure 4.4C). Although AChR subunit genes are located on different chromosomes, the correct expression of AChR requires a concerted regulation of their expression. We hypothesized that AChR gene expression could be regulated via long-distance gene interactions.

Our long-term goal is to elucidate how the spatial organization of the genome changes during muscle differentiation and how these changes affect genome function. To reach this goal, our proposal has three scientific aims : Aim 1. Measure the radial and relative positioning of AChR subunit genes and the kinetics of their movements during muscle differentiation using Fluorescent In Situ Hybridization (FISH). Aim 2. Define the intra- and inter-chromosomal interactions of AChR subunit genes at the different steps of muscle differentiation using Chromosome Conformation Capture (3C/4C). Aim 3. Modelize the three dimensional organization and transition dynamics of AChR clusters and their chromosomal environment combining biological data (FISH, 3C/4C, ChIP Seq, RNA Seq) and the physical models of 1D/3D epigenome organization : again in the “living polymer” framework the objective will be to understand what drives spatial relocation of some AChR genes and how it is coupled to epigenome reprogramming that occurs during muscle differentiation (45). Due to its importance for this project but also for the following one (*C. elegans*) we will first working on modelling the effect of anchoring at the nuclear lamina.

Taken together, the proposed experiments and collaborations represent a unique set of complementary techniques and expertises to elucidate and modelize the three-dimensional organization and dynamics of the genome in a physiological and well-defined cellular differentiation system. Understanding such mechanism(s) would not only explain how a high order genome or-

ganization is established, it could also provide a pathological mechanism for Congenital Myasthenic Syndromes in which mutations have been identified not in the coding but in the promoter sequences of AChR genes.

4.5 Functional Folding of chromosomes : Dosage Compensation in *C. elegans* as a model to understand the formation of functional subnuclear domains *Collaboration with Peter Meister's group in Bern (<http://www.izb.unibe.ch/content/groups/meister/>) and with D. Jost*

Proposal of ANR project with P. Meister :

Although very different in their shape, physiology and developmental history, all cells of multicellular organisms share the same genetic material. Cell fate is therefore not a function of genome sequence but of genome expression. Gene expression is regulated at many levels, from local DNA packaging and transcription factor binding to higher order 3D chromatin functional domains. There is increasing evidence that the formation and maintenance of these subnuclear domains are strongly correlated with cell fate, suggesting a role in the epigenetic determination of gene expression (46, 47). Therefore, models of subnuclear domains are important to uncover the general principles of chromatin 3D folding and their influence on gene regulation. Dosage compensation (DC) is a promising system to understand how a nuclear domain is formed and maintained over the entire life of an organism. This essential function widely observed in metazoans, ensures adjusted expression of X-linked genes to the X to autosome ratio. The X chromosome forms a specific subnuclear domain, restricted to a single chromosome. In *C. elegans* hermaphrodites, DC is performed by downregulation of transcription from the two X chromosomes (48). Transcription is not completely shut down but only half downregulated, a situation much closer to general gene regulation than the complete heterochromatinization seen in mammalian female cells. Transcriptional downregulation is achieved via association of the Dosage Compensation Complex (DCC, a condensin-like complex). DCC loading and spreading along the X chromosome leads to the restriction of the methylation levels of a specific histone tail residue, a decrease in active chromatin marks and partial eviction of RNA polymerase II from the X chromosome [Ferrari]. The functional link between DCC loading and the downregulation of transcription remains elusive, as there is no direct correlation between DCC presence along the chromosome and transcriptional silencing. Together with the structure of the DCC ; this suggested a role for tridimensional nuclear organization in the creation of a specific "half-repressive" subnuclear domain containing the X chromosome. In previous work of this consortium, we showed that the X chromosome undergoes major structural rearrangements inside the nuclear space in relation with DC (49). In males, the X chromosome is located at the nuclear periphery and interacts with nuclear pore components, which had previously been shown to increase transcriptional output in a variety of biological systems. In hermaphrodite, the X chromosome is located in the middle of the nucleus and its localization depends on DCC loading, which may impair pore interaction and transcriptional activation. Together, this strongly suggests a model of DC in which the X chromosome is creating two different subnuclear domains in males (non compensated) and hermaphrodites (compensated) and these domains impinge on X-linked gene transcription. The dosage compensated X is a perfect model to understand the function of nuclear domains in relation to physical properties of the chromatin fiber : DC occurs in all cells of the organism, the loading sites of the DCC and interaction sites at the periphery are known, interactions with nuclear landmarks have been characterized, the X domain is different between sexes and the transcriptional output is clear and phenotypically screenable. With this project, we aim to understand how the physical properties of the chromatin folding coupled to the loading and spreading of DCC in one sex impact on the embryonic creation and life-long maintenance of functional X chromosome domains. In particular, we want to elucidate the physical and molecular mechanisms of domain wide gene regulation. To this aim, our approach is cross-disciplinary, combining computation modeling of chromatin behavior and a highly sensitive genome-wide mapping technique (DamID-seq). The iterative process of model implementation, confrontation with the data, formulation of predictions and experimental verification will provide new insights into the mechanism of DC.

The project is subdivided into 4 specific aims :

1. Detailed experimental description of the establishment of the compensated X domain
2. Development of a modular computational model of functional subnuclear domains :

In parallel to the characterization of X chromosome conformation in vivo, we will develop an original physical model (“the Living Chromatin” model) that couples the dynamics of the chromatin folding - modeled as a semi-flexible heterogeneous polymer chain - to the stochastic loading and spreading of architectural proteins and epigenetic marks along chromosomal domains. While most of the published computational chromatin models consider chromosomes as homogenous polymers, our recent work led to the development of a heterogeneous polymer model which accounts for the local variability of epigenomic properties (50). This allowed interrogating the role of chromatin landscape along the genome in the formation of subnuclear domains and their impact on gene transcription. In particular, we showed that formation and dynamics of topologically-associated domains may be associated with the folding of chromatin domains due to protein-mediated interactions. To describe the establishment and maintenance of DC, we aim to generalize this approach by modeling the dynamics of chromatin regulators and its crosstalk with 3D chromatin folding and gene expression. To date, models that explicitly assess for this dynamic coupling remains to be developed. Here, we propose to complete the polymer modeling developed in (50) by a stochastic description of the local chromatin state (29). In particular, X chromosomes will be modeled as a confined long semi-flexible polymer where each monomer represents a portion of DNA. The chromatin state of each monomer would dynamically fluctuate between several flavors depicting the presence or not of DCC or other factors. DCC-mediated interactions between monomers will impact on the local and higher-order chromatin organization, while spatial localization will affect DCC spreading and recruitment. Ultimately, we aim to understand the functional impact of chromatin state, folding and nuclear localization in the non-compensated vs compensated X chromosome : for that purpose and as first attempt, local gene activity will be modeled as depending on local compaction/chromatin state and on proximity to nuclear pores. Outcomes of the model will be generated using advanced numerical kinetic Monte-Carlo methods. For a given set of parameters and molecular mechanisms, the living chromatin model would be able to make quantitative predictions on the nuclear 3D chromatin organization of the X chromosomes as well as on X gene expression during the life span of the animal. It will allow relating experimental observations to the critical underlying physical and molecular mechanisms involved in DC establishment and maintenance. Moreover, it is likely that the model will formulate new predictions that will strengthen the description of DC processes and that can be tested in vivo.

3. Role and function of DCC in hermaphrodite X domain maintenance
4. Interpretation of experimental results by the model and experimental tests of the model predictions.

4.6 Approche multi-échelle de la dynamique de régulation des gènes mécano-sensibles chez les cellules souches végétales *Collaboration with A. Boudaoud (Laboratoire RDP, PI of the project), O. Hamant (RDP) and B. Audit (Lab. Physique).*

Here we aim to decipher how chromatin is regulated by physical forces during organismal development, using the model plant *Arabidopsis thaliana*. Indeed it has been recently shown that mechanical pathways are important in gene regulation. However, virtually nothing is known about mechanotransduction when it comes to the interior of the nucleus. As development involves the progressive restriction of cell fates into stable identities, the involvement of mechanosensitive genes in development requires the transduction of mechanical signals into stable outputs. We propose that chromatin remodelling could play an important role in this framework, notably by locking the state of chromatin to memorize these signals and allow the mechano-regulation of gene expression. Starting from original sets of mechanosensitive genes that we will obtain from comparative genome-wide expression profile experiments, we will characterise the chromatin context associated with these sets of genes by combining statistical analyses of epigenetic and functional profiles with signal processing tools and modelling of the nucleosomal array. This will allow us to gather the preliminary data needed to initiate the study of the mechanical regulation of the chromatin state during development.

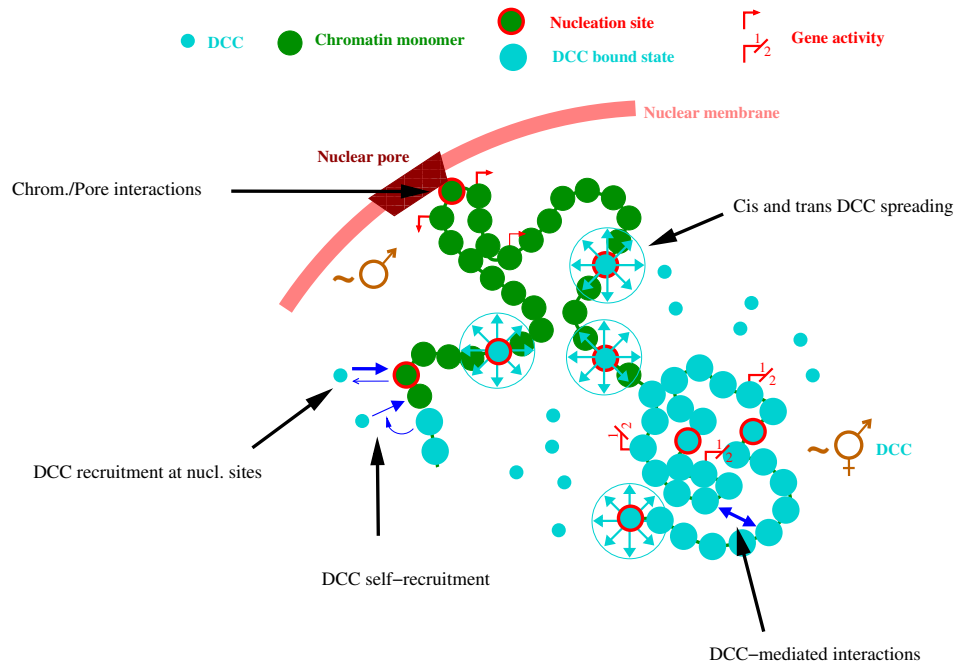


FIGURE 13 – The “Living Chromatin model” applied to DC : Chromatin is modeled as a polymer where monomers interact with each other or with nuclear landmarks, depending on their chromatin state. In males, interactions with nuclear pores induce segregation of the X chromosomes at the nuclear periphery. In hermaphrodite, DCC is recruited at specific sites and spread in cis and in trans by subsequent self-recruitment that is enhanced by 3D long-range interactions.

5 CONCLUSION

This project rely on the strong collaboration with different groups of excellent biologists G. Cavalli, P. Meister, A. Verdel, S. Khochbin..., international leaders in their field ; my objective is, in a joint work with Daniel Jost (TIMC-IMAG Grenoble), Ralf Everaers (Lab. Physique ENSL) and our curent (Noeël Haddad) and future students at the ENS de Lyon, with A. Rosa in Trieste (<https://sites.google.com/site/angelosissa/>), P. Carrivain in Montpellier to combine our theoretical and numerical skills in order to provide original multiscale theoretical modelling of the regulation of epigenome assembly and folding. I propose to carry out this project in the Laboratory of Physics of “ENS de Lyon”. My objective is to work on developing our computational biology group with D. Jost (“Physics of epigenome regulation”) and progressively to explore topics related to cellular adaptation of micro-organisms : how epigenome participate to these strategies of short and long-term (evolution) adaptation in microbial communities in their natural fluctuating environment ?

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