3D modeling of chromatin structure: is there a way to integrate and reconcile single cell and population experimental data?

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Abstract

The genome is organized in a hierarchical fashion within the nucleus in interphase. This non-random folding of the chromatin fiber is thought to play important roles in the processing of the genetic information. Therefore, a better knowledge of the mechanisms underlying the three-dimensional structure of the genome appears essential to fully understand nuclear processes including transcription and replication. Fluorescent In Situ Hybridization (FISH) and molecular biology methods deriving from the Chromosome Conformation Capture technique are the methods of choice to study genome 3D organization at different levels. Although these single cell and population methods allowed to highlight similar chromatin structures, they also show frequent discrepancies which might be better understood by improving the capacity to generate actual 3D models of organization based on the different types of data available. This review aims at giving an overview of the principles, advantages and limits of microscopy and molecular biology methods of analysis of genome structure and at discussing the different approaches of modeling of chromatin classically used and the improvements that are necessary to reach a better understanding on the links between chromatin structure and its spatial organization.

INTRODUCTION

Processing the genomic information in the nucleus of eukaryotic cells requires regulatory proteins to access DNA assembled into chromatin. Although the chromatin fiber is highly packed within the nuclear space, its density is heterogeneous. For example, looser chromatin regions tend to occupy the center of the nucleus, whereas compact chromatin is enriched close to the nuclear periphery and to the nucleolus. Moreover, it is now evident that the organization of the genome in the nucleus is generally hierarchical and non-random: chromosomes are organized as territories occupying preferential positions depending on their gene-density, with gene-rich small chromosomes frequently located at the center of the nucleus and larger gene-poor chromosomal domains tending to occupy the nuclear periphery (Croft et al. 1999; Bolzer et al. 2005). Within chromosomes, the chromatin fiber is further segmented in mega-base sized domains where loci interact more frequently between them inside the domain than with the neighboring regions. These so called Topologically Associated Domains (TADs) are distributed in different chromatin compartments depending on their epigenetic state (Lieberman-Aiden et al. 2009; Dixon et al. 2012; Nora et al. 2012a). The organization in TADs is thought to favor the establishment of regulatory loops between enhancers and promoters, thus being involved in the regulation of transcription and in the specificity of cell transcriptomes (Lupianez et al. 2015). Although globally organized, this three-dimensional (3D) organization has yet strong dynamic and stochastic components blurring the general patterns and their correlation with nuclear processes (Thomson et al. 2004). Nonetheless, characterizing how the genome is folded in the nucleus is essential to determine how it can constrain or regulate the nuclear processing of DNA including transcription as well as replication and repair (Cavalli and Misteli 2013; Pope et al. 2014).

Two main approaches allow gaining insight on chromatin conformation and organization of chromosomal domains: direct visualization of those structures in the nucleus of cells by microscopy-based methodologies, in particular Fluorescent *In Situ* Hybridization or FISH (Solovei et al. 2002) and molecular biology methods, principally derived from the Chromosome Conformation Capture or 3C (Dekker et al. 2002). Due to the 3D nature of genome organization, it appears that establishing actual 3D models greatly helps in understanding their functional implication and in predicting the consequences of changes in the conformation. Increasing attempts are made to generate such models from the analysis of local region of chromatin (Baù et al. 2011a), chromosomal domains or full chromosomes (Belton et al. 2015), as well as complete genome in the confined nuclear space (Kalhor et al. 2012). These 3D modeling approaches are of several types based on different methodologies and assumptions. However they generally use as input data coming from 3C-derived methods bringing thus limitations due to the population nature of these experiments. Indeed, and in contrast to FISH that allows single cell analysis, 3C-derived results generally consist of a superimposed picture of structures present in a population of cells. And this methodological difference can lead to discrepancies between observations based on FISH and observations based on 3C. Although recovering the natural heterogeneity of genome structure from 3C-based dataset is a challenge, we speculate that accurate modeling could resolve these discrepancies between FISH and 3C. Future implementations of 3D modeling should account for the population aspect of a given experiment and on the structural variability inherent to the chromatin.

In this review, after reminding the principles, advantages, and limitations of FISH and 3C-based approaches, we will discuss the differences between these two approaches. We will further give an overview of the principles of the main 3D modeling methods used to reconstruct genome conformation and we will focus on how, by integrating data from various origins, integrative modeling shall permit to obtain a clearer view of the relation between structure and function of the genome.

I. Single cell and population analysis of the 3D genome architecture

Microscopy-based single cell methods

FISH (Fluorescent *In Situ* Hybridization) allows the visualization of specific DNA loci within the nucleus of fixed cells by the use of complementary probes labeled with modified nucleotides (Markaki et al. 2012). This technique has been widely used to study the organization of the genome at very different scales covering from chromosomes, to specific pairs of loci (Figure 1). FISH notably permitted to validate the existence of chromosome territories (Cremer et al. 2008). Moreover, closer analysis showed that chromatin domains within those territories could further exhibit differences in condensation or in relative localization, which may mainly depend on their gene content (Mahy et al. 2002; Goetze et al. 2007). Additionally, FISH permitted for example to elucidate the reorganization of gene positioning during the process of differentiation (Osborne et al. 2007) and to demonstrate the propensity of related genes to be transcribed in transcription factories (Osborne et al. 2004). The applicability of FISH is however limited by the sensitivity of fluorescently labeled probes or antibodies and by the microscope resolution. This limits the size of the loci to be probed as well as the physical distances that can be resolved. The emerging of high-resolution microscopy methods as well as the development of new labeling strategies are now overcoming these limitations (Hajjoul et al. 2013; Beliveau et al. 2014; Giorgetti et al. 2014; Boettiger et al. 2016). For example, DNA-paint, which uses oligonucleotide-based “Oligopaint” probes, has been recently used to analyze at high resolution the folding of chromatin domains with different epigenetic states (Boettiger et al. 2016). These single cell methods were mainly limited to the analysis of genomic loci in fixed cells, and were not showing the dynamic nature of the chromatin fiber. This limitation has been overcome with the development of genome editing methods and biochemical tools allowing to track specific fluorescently labeled DNA region in living cells (Bystricky 2015).

As most single cell approaches, microscopy images are strongly affected by the cell-to-cell variability of the genome. Indeed, despite common rules of organization, the intrinsic dynamic properties of the chromatin fiber as well as the stochastic nature inherent to each of its level of organization are clearly highlighted by the observed variations in most of FISH based studies. Therefore, establishment of models of organization based on FISH data requires the analysis of large number of cells. This need of higher throughput has invariably resulted in the development of methods to facilitate the automation of both the acquisition of images and their analysis (Shachar et al. 2015). However, even allowing the concomitant study of several thousands of cells, such methods remain limited in terms of throughput and resolution in comparison with molecular biology techniques.

Molecular-based population methods

In parallel to the microscopy-based methods described above, the knowledge on genome organization highly benefited from molecular biology techniques. In particular from the derivatives of the original Chromosome Conformation Capture (3C) assay developed 15 years ago by Dekker and colleagues (Dekker et al. 2002). These 3C methods are based on the cross-link of chromatin with formaldehyde, which bridges together DNA loci found in close spatial localization within the cell nucleus (Figure 2A). The frequency of cross-link between two DNA fragments is proportional to the frequency of contact within a population of cells, and is thus assumed to reflect an average physical distance in the nucleus. After chromatin cross-

linking, DNA is further digested with restriction enzymes recognizing target sequences of 4 or 6 bases. For example, digestion of the human genome by these so called 4 or 6 bp-cutters restriction enzymes results in DNA restriction fragments of around 0.4 or 4 kb average length, respectively. The generated DNA ends that have been cross-linked together are further ligated and the ligation events are analyzed (Figure 2B). The original 3C method allowed the analysis of contacts between two given loci using primers designed to amplify regions close to the restriction sites of interest. It therefore limited the analysis to few thousands of bases around a given locus. Further implementations allowed analyzing the structure of larger domains or, more recently, assessing the interactions in a genomic context by coupling the experiment with high-throughput sequencing (Figure 2B). The most used 3C derivative methods are (Figure 2B): (i) 4C, which allows to interrogate the interactions of a locus with the rest of the genome by the use of inverted PCR primers (Zhao et al. 2006); (ii) 5C which uses multiplexed primers covering from hundreds of kilo-bases to several mega-bases, and therefore allows to study interactions within a limited region of a genome (Dostie et al. 2006); and (iii) Hi-C where the digested DNA fragments are biotin-labeled at their restriction ends, allowing their selection after ligation and permitting the use of the selected material to high-throughput sequencing (Lieberman-Aiden et al. 2009). In this last case, the ligation step is performed, either in diluted conditions after partial cell lysis as in the original method (Lieberman-Aiden et al. 2009) or directly in intact nuclei - also called *in situ* or *in nuclei* Hi-C - (Rao et al. 2014). Although each 3C-derived methods have specific advantages depending on the type of analysis required, Hi-C is nowadays becoming the method of choice. The resolution of the Hi-C, which aims at an unbiased detection of all interactions between any pair of restriction fragments in the genome, depends on the sequencing depth. Indeed, identifying all interactions at the fragment-based resolution using Hi-C would require an immense sequencing effort. Hi-C results are thus classically represented as matrices of pairwise contacts resulting from the binning of observed ligation events at a given resolution (Figure 3). Recently, high coverage Hi-C maps have been generated at 1 kb resolution (Rao et al. 2014) but more frequently, they range from 5 to 100 kb (Dixon et al. 2012; Jin et al. 2013).

These molecular methods confirmed several levels of organization already observed by FISH as the organization of chromosomes in territories occupying preferential and distinct positions in the nucleus (Figure 3). But also *de novo* identified the preferential segregation of chromatin domains (Lieberman-Aiden et al. 2009) in compartments (Figure 3) and the existence of a functional segmentation of chromosomes in self-interacting Mb-sized domains called Topologically Associated Domains (TADs – Figure 3) (Dixon et al. 2012; Nora et al. 2012a; Sexton et al. 2012; Jin et al. 2013). In addition, high-resolution analysis demonstrated the existence of specific loops between regulatory sites, enhancer and promoters (Jin et al. 2013; Rao et al. 2014). In summary, 3C derived methods are highly resolutive methods to study the organization of the genome at different scales. Although the resulting frequencies of contacts between loci is assumed to reflect their spatial distance in the nucleus, it is important to keep in mind that this final static picture of contacts actually represents the average of events in the cell population. Attempts have been done to question the heterogeneity and commonality of these contacts by establishing single cell Hi-C methods (Nagano et al. 2013). Such analysis confirmed observations made at the population levels but also highlighted the stochastic and dynamic nature of these contacts (Nagano et al. 2013).

Agreements and discrepancies between FISH and 3C-based results

As mentioned above, Hi-C experiments and FISH are both able to demonstrate the organization of chromosomes into territories as well as their preferential localization within the nuclear space. Additionally, Hi-C and 5C experiments demonstrated that chromosomes are segmented in TADs where the interactions are more frequent between loci within the domain rather than with the neighboring domains (Dixon et al. 2012; Nora et al. 2012a; Sexton et al. 2012; Jin et al. 2013). The use of FISH probes labeling either loci located within a single TAD or within contiguous TADs confirmed their spatial isolation (Nora et al. 2012a). Another result extrapolated from 3C data, and confirmed *in situ*, is the co-localization of regulatory elements with their target promoters located several kilo-bases away. FISH has also confirmed juxtapositions of loci located on different chromosomes (Osborne et al. 2007). However, co-localization of loci was found to occur in a much lower proportion of cells in FISH (Osborne et al. 2007) than it could be speculated from 3C-based results, highlighting the importance of taking precautions in the interpretation of 3C data obtained in a population of cells.

Several examples of more discrepancies between 3C-based data and *in situ* visualizations have been observed. In a recent systematic comparison of data obtained by 5C and FISH for several regions of the genome in different cell types, it was observed that, in many cases, the two approaches agreed (Williamson et al. 2014). However, the work also highlighted discrepancies between the interpretations of the data obtained by each of the two methods. For example, in some cases, regions appearing as highly interacting in 3C-derived data corresponded to highly unfolded regions in the FISH analysis (Williamson et al. 2014). One could hypothesize that these apparent discrepancies are linked to the technical differences in the processing of the samples as for example the thermic treatment required for the hybridization in FISH, even if such process have been shown to mainly preserve the nuclear architecture (Cremer et al. 2007). Or, on the side of 3C-derived methods, fixation process itself could bring artifacts at the time of the interpretation of data. Indeed, although formaldehyde is the most common cross-linking agent used in the study of chromatin, its exact chemical activity in cells remains poorly understood. This agent is known to generate DNA-protein as well as protein-protein cross-links. However, some studies revealed different efficiencies of cross-link depending on the binding dynamics of the proteins involved (Gavrilov et al. 2015). It is therefore possible that sub-nuclear environments with enrichments of specific proteins may have different cross-link propensity. In addition to the uncertain relationship between interaction frequency and spatial distance, the dynamic and stochastic nature of chromatin is also reflected in the single, agglomerative, conformation yielded by the 3C-based approaches. For example, a given interaction frequency between two loci could emerge from various possibilities within the cell population (Figure 4A). Additionally, although some attempts are made to analyze multiple pairs of concomitant contacts in Hi-C data (Darrow et al. 2016), the analysis is generally performed in a pair-wise manner, which does not allow to decipher whether an interaction between 3 or more genomic regions occur at once in a same cell or by pairs in different sub-populations of cells (Figure 4B).

In summary, due to its population nature and the potential biases in the cross-link procedure, the interpretation of 3C results remains challenging. Although the differences observed are probably less relevant at low resolution, it is likely that the discrepancies will increase with the resolution in both imaging and molecular approaches.

II. 3D modelling of 3C-based data

Three-dimensional (3D) spatial representation of the data should help in extracting information not intuitively accessible only from 3C-based experiments. Increasing efforts are made to generate 3D objects that classify, filter and recapitulate the information contained in the single 2D interaction matrices from 3C-based experiments conducted over millions of cells. Ideally, it should allow the generation of models that are not only a comprehensive and accurate representation of the genome structure but also permit to establish new hypothesis on the rules that govern the establishment and maintenance of such structures. Such ideal models would in addition help to understand the differences between single cell observations and population analysis.

Two main global approaches of genome structure modeling have emerged in the last few years. The first one, bottom-up, aim at using the physical and chemical properties of the chromatin fiber to build 3D models. Such approach is particularly useful to test specific biological hypothesis, as for example, the supercoiling of TADs (Benedetti et al. 2014), the loop extrusion model (Fudenberg and Mirny 2012; Rao et al. 2014; Fudenberg et al. 2016), the hierarchical organization of TADs (Fraser et al. 2015), or the spatial co-localization of co-regulated genes (Di Stefano et al. 2013). The second, which is the main subject of this review, correspond to top-down modeling approaches. In this case, the aim is to translate the experimental data into 3D Euclidean distances or spatial contact-maps and to use them to build the models. The projection of an interaction matrix into a list of 3D coordinates is a simple mathematical conversion called multi dimensional scaling. Such methods are already largely used in the field of protein structure determination (Havel et al. 1983). However, this approach is very sensitive to interaction matrices with large structural variability or noise. In this case, a single 3D model cannot explain all the interactions (Trussart et al. 2015). Such problem is precisely one of the limitations of classic 3C-derived experiments where the matrices generated represent a sum over millions of cells and where the number of captured interactions per cell is limited.

Modeling strategies

Top-down modeling strategies consist in either integrating the experimental measures to generate a global solution, *i.e.* one consensus model (Figure 5 [3] and [4]) or in considering that only a heterogeneous population of 3D models account for the variability of the experimental measurements (Figure 5 [5] and [6])(Baù and Marti-Renom 2012; Tjong et al. 2016) Most of the modeling approaches require a distance- or contact-matrix to define the restraints that apply to the 3D models (Figure 5). Since in the case of 3C-derived experiments, the experimental data consists of indirect observations, (*i.e.,* frequency of ligation), the first step usually consists of transforming the observed frequencies into distances. The transformation of interaction data into distances/contacts appears to have reached a global consensus; with the most generally used formula being:

$$D_{ij} \propto \left(\frac{1}{F_{ij}}\right)^{\alpha}$$

where F_{ij} is the frequency of interactions observed between the particles i and j, D_{ij} is the distance between these particles, and α is a correcting factor which reflects the correlation between interaction frequency and 3D distance. Based on polymer models, the α value is usually set to 1.0 in accordance with crumpled or fractal globule models (Grosberg et al. 1988; Lieberman-Aiden et al. 2009; Mirny 2011). This parameter α could however be fixed or optimized depending on the type of data (see (Serra et al. 2015) for a recent review on the

different strategies of optimization). The alternative of transforming interaction matrices into a continuous distance metric is to use directly the observed interactions of the Hi-C experiment. In this case the 3D models are built and optimized by trying to match the observed interactions between chromatin loci with the contacts in the modeled chromatin (Figure 5 [2]).

Once converted to distances, two alternative strategies are possible. The first consists in using multi-dimensional scaling to directly project the distance matrix into 3D coordinates (Zhang et al. 2013; Lesne et al. 2014)(Figure 5 [3]). The second consists in optimizing the 3D conformation of chromatin models according to a set of restraints defined from the distance matrix (Baù and Marti-Renom 2012) or directly from interaction frequencies (Figure 5 [5] and [6]) (Kalhor et al. 2012; Meluzzi and Arya 2013; Tjong et al. 2016).

Independently of the model strategy, an initial step shared by all is to define a representation of these virtual segments of chromatin. Each bin of the distance or interaction matrix represents a segment of chromatin fiber, and each of these segment is generally represented either as a dot (Figure 6A [1]) or as a sphere (Figure 6A [2]). In the case where segments are represented as tangible objects, other layers of complexity can be added as, for example a soft layer allowing a partial overlap between segments or a heterogeneous representation of segments into particles of different sizes (Figure 6A [3] and [4]). Another aspect of the representation is the type of restraints to be applied between particles (Figure 6B). The physical restraints (U_{PHYS}) that are generally applied can be divided into three types:

$$U_{PHYS} = U_{Excl} + U_{Bond} + U_{Bend}$$

where U_{Excl} are the restraints corresponding to the excluded volume forbidding two particles to occupy the same space, U_{Bond} are the restraints related to the bond between neighboring particles forcing contiguous particle to stay close, and U_{Bend} defines the rigidity of the modeled chromatin fiber (see Figure 6B and 6C, and (Serra et al. 2015) for detailed explanation of the application of restraints). Once defined, different optimization algorithms can be used to apply these restraints on 3D representations of the chromatin and fit their shape with the input interaction matrices (see (Serra et al. 2015) for details). For the optimization, the application of the restraints can be done on a single model (Figure 5 [3] and [4]) or on population of models (Figure 5 [5] and [6]). In the case of population based modeling, several 3D models are optimized together. At the end of the optimization, the sum of all their conformations should reproduce the input distance- or interaction-matrix. In a sense, during the optimization, the models are competing for the input restraints, and this allows that each of the models explains only a portion of the interactions of the Hi-C experiment. With this modeling strategy it is important to consider that, an increase in the number of models, involves an increase in the number of free parameters and in the computation time. An alternative to the optimization of several models together is the resampling strategy (Figure 5 [5]). In this case the number of free parameters is the same as for a single model but the output is a population of models. Methodologically it simply consists in applying the consensus modeling approach repeatedly with different initial states. Resampling approaches are based on the assumption that each local minima of the optimization process corresponds to a subpopulation of cells with similar chromatin folding.

Data quality inference

In all cases, data quality is an important parameter to take into account when choosing for the suitable modeling strategy (Zhang et al. 2013; Lesne et al. 2014; Trussart et al. 2015; Park

and Lin 2016). In particular, a recent work has shown the possibility to assess the accuracy of resulting 3D models by using statistical properties of the input interaction matrices (Trussart et al. 2015). Indeed, a combination of descriptive statistics extracted from the input interaction-matrix can help to understand the quality of the experimental data, and it's suitability for 3D modeling. For example, low structural variability in the folding of the chromatin is usually accompanied with low values of skewness, kurtosis and high number of significant Eigenvalues calculated from the input matrices (Trussart et al. 2015). The results of this work also indicated that structural variability was the principal source of error in 3D modeling.

Resolving structural variability

As mentioned above, the structural variability inherent to the population based 3C-derived methods appear to be an important source of inaccuracy of 3D models (Trussart et al. 2015). Therefore, modeling strategies that aim at identifying population of models within the datasets are in theory more adapted than strategies developing single consensus models. In this case, 3D modeling should allow de-convoluting sub-population of cells with distinct global chromatin folding. With this idea, many population-based modeling strategies have been implemented in the last years (Figure 5 [5]). These techniques were shown to be successful at identifying subpopulation of models within single 3C-derived experiment. However, a particular caution must be taken with the choice of the number of models to optimize as the number of free parameters increases with each of them. Even though the latest modeling approaches are designed specifically to deal with these very low reads per cell ratio (Paulsen et al. 2015; Park and Lin 2016), the main problem of population-based modeling when de-convoluting 3C-based matrices is that the original data consists of the sum very sparse single cell matrices with a very low ratio of observations per cell (Nagano et al. 2013) and precautions should still be taken at the time of the interpretation.

Validation of 3D models

Despite the efforts put in developing realistic modeling strategies, it remains essential to assess the accuracy of the models generated with data obtained independently. Typically, observations from *in situ* by FISH are used to validate inferred 3D models. This methodology can, for example, involve a correlation between the distances of two or more FISH probes and the distances measured on the corresponding positions in a set of 3D models (Baù et al. 2011b; Rousseau et al. 2011; Umbarger et al. 2011; Kalhor et al. 2012; Nora et al. 2012b; Hu et al. 2013b; Peng et al. 2013; Ay et al. 2014; Giorgetti et al. 2014; Le Dily et al. 2014). However, as previously discussed, and beyond the differences in resolution, the main issue with this validation strategy is the low throughput of the FISH experiment. A large number of experimental replicates are usually needed to confirm the predicted folding of the chromatin. It is expected that with the emergence of new *in situ* methods with increased throughput, larger-scale assessment of 3D modeling accuracy will be available.

Integration of distinct types of data in the models

Restraint-based modeling primarily uses 3C-based data. Integrating other kind of data prior to modeling would help in increasing the accuracy of the models generated and in optimizing the modeling parameters as for example the physical scale or degree of compaction of the fiber.

One could envisage that distinct kind of data can be used as, for example, direct microscopy observations (*e.g.,* FISH), general features of polymer physics models (*e.g.,* rigidity of the fiber), or biological knowledge (*e.g.,* nucleus size or known chromosome tethering). Indeed, several studies of polymer physic based modeling already integrated known biological properties (Tjong et al. 2012), TADs (Barbieri et al. 2012; Fraser et al. 2015) epigenetic state (Jost et al. 2014). An example of integration of FISH data for chromatin modeling is the work from Bolzer and colleagues where chromosome territories where observed by FISH, and used as confinement areas for the polymer models representing chromatin fiber (Bolzer et al. 2005). It is clear that there is still work to be done to translate distinct kind of data into sets of restraints that would be integrated into a single modeling protocol. However, this translation should be the hardest part as restraints-based modeling is already able to integrate data from different sources to define and apply restraints to 3D models (Russel et al. 2012). Beyond the methodological difficulties, the main problem with the integration of distinct types of data in the modeling is the decision of which data should be used. For example, and as mentioned previously, FISH data is usually used to validate the models, and thus cannot also be used to build the models. Therefore, when additional kinds of data are available, a decision has to be made: use the data to validate the model, or use the data to build the model.

Future directions

Recent advances in imaging techniques have increased our capacity to describe the mechanisms of dynamic changes of chromatin (Dion and Gasser 2013). These advances have been complemented with *in silico* models of the chromatin or naked DNA fiber, which have been used to mechanistically explain the physics behind conformational changes of the genome (Amitai et al. 2015; Shinkai et al. 2016). Altogether, both imaging and computational modeling could be used to assess the source of structural variability in 3C-derived experiments, which is likely arising from the polymorphic nature of the DNA flexibility and dynamics. This chromatin heterogeneity, already clear between heterochromatin and euchromatin, is a factor that has not yet been integrated in any of the here discussed modeling strategies although it has been shown to explain an important part of the observed variability in 3C-based interaction matrices (Tokuda et al. 2012). In addition, it may be one the principal sources of discrepancies between direct and indirect observations (Gavrilov et al. 2015; Sexton and Cavalli 2015). From the perspective of 3C-based detection of interactions, more open and thus more dynamic region could result in increased contacts (Williamson et al. 2014). This observation might explain some of the discrepancies between 3C-derived experiment and FISH observations.

From the modeling perspective, this polymorphic nature of the chromatin could be addressed simply by varying the scale parameter (number of nucleotides per nanometer), or the α parameter (see above) and optimizing these modeling parameters independently for different chromatin regions. Different chromatin regions could thus be defined based on prior knowledge obtained from their epigenetic state. Indeed, chromatin structural features are related to the chromatin epigenetic state (Peng et al. 2013; Boettiger et al. 2016; Serra et al. 2016). Being able to predict chromatin structure from epigenetic state is one of the many areas towards which structural biology, and epigenetics are heading. Although tools to do the conversion are already available (Zhu et al. 2016), their application is still limited to interacting pairs of promoters or, and, enhancers within a single TAD. Such predictive tools are still thus in their

early stages, but this kind of analysis could help in validating or even *de novo* reconstructing 3D models of the chromatin.

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Figures:

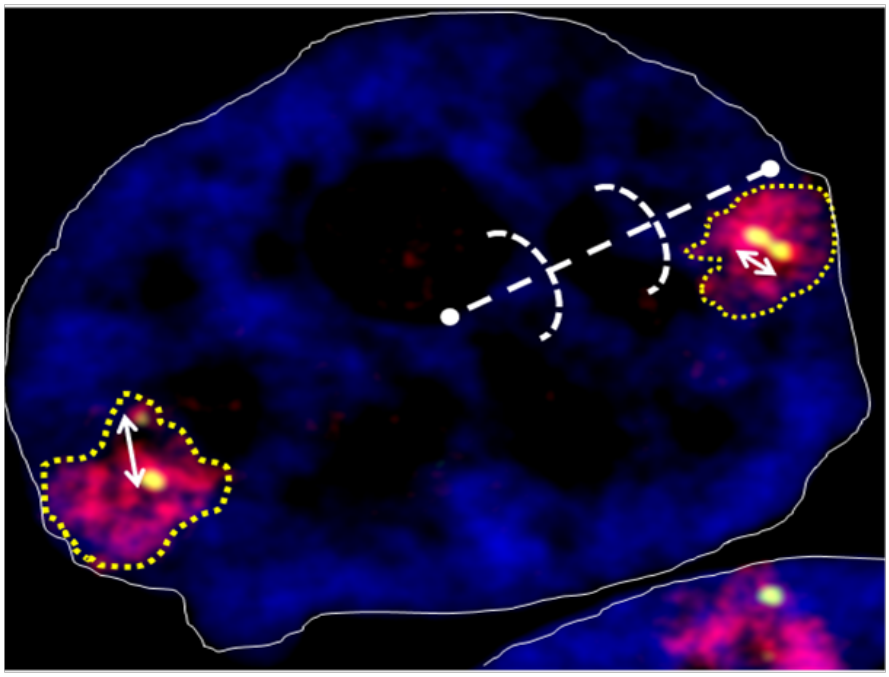

Figure 1: Use of FISH to study the spatial organization of the genome in single cell. Fluorescent In Situ Hybridization (FISH) can be performed on fixed cells to analyze different parameters of the spatial organization of chromosomes or genomic domains. For example, whole chromosome painting can be used to study the position of chromosome territories relative to nuclear structures as well as their radial positioning from nuclear center to periphery (dashed lines - in this example, human chromosome 2 was labeled in red). Multiple specific genomic regions can be labeled in parallel to measure the distances that separate them from each other (double arrows - in this example, 2 different loci of human chromosome 2 were labeled in green).

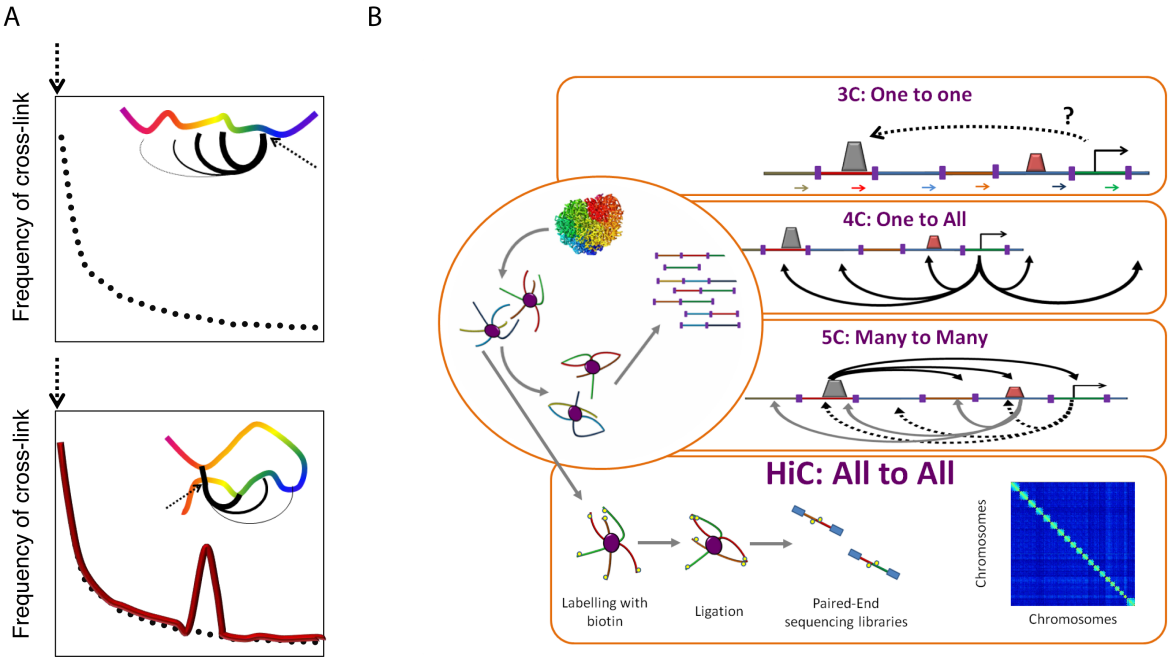

Figure 2: Principle of Chromosome Conformation Capture and derivative methods. (A) 3C-based methods are based on the cross-link of chromatin with formaldehyde. The probability of cross-link between two regions is dependent on the spatial distance that separates them. In the case of a randomly organized molecule (top panel), the frequency of cross-linking will decrease as a function of the genomic distance that separate two loci whereas this relationship is broken in the case two region are establishing preferential contacts (bottom panel). **(B)** 3C-based methods use the frequency of ligation of cross-linked DNA after digestion by restriction enzyme (RE) to recover this information in a quantitative output. If the original 3C methods uses specific primers to detect one-to-one those potential interactions, further derivative allow to analyze the interactions at higher levels, either one-to-many/all (4C), many-to-many (5C) or all-to-all (Hi-C) depending on the detection method used.

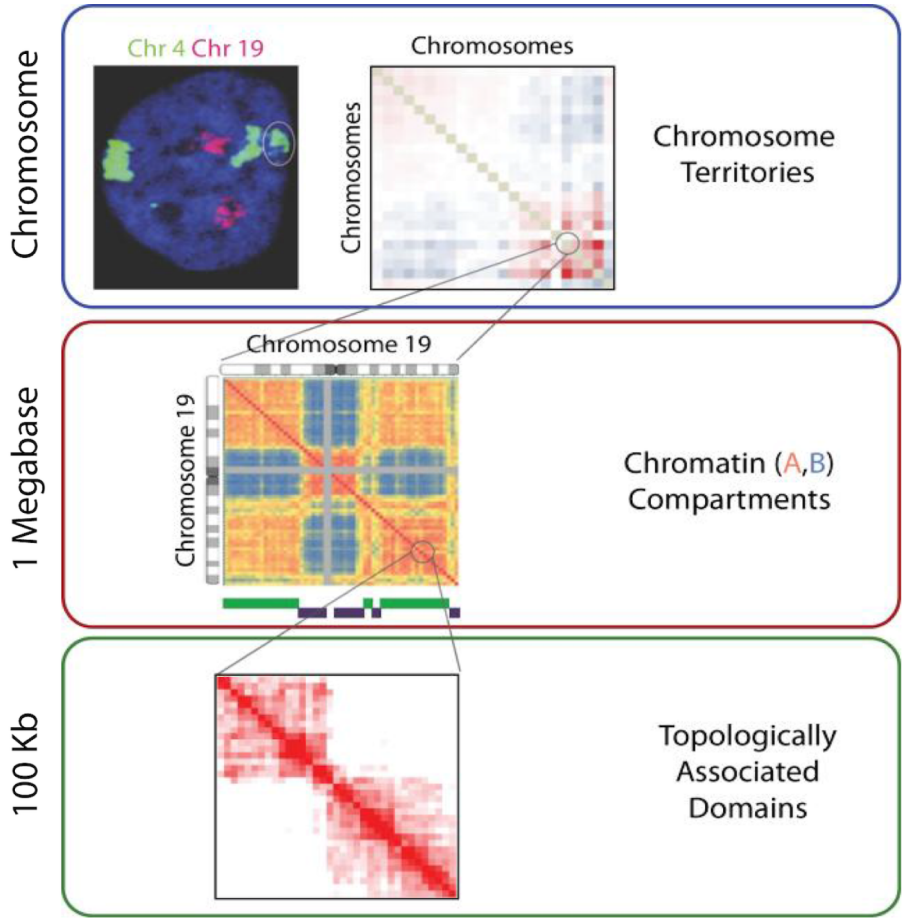

Figure 3: Hi-C allows to study genome conformation at different scales and demonstrate a hierarchical organization. Binning the results of Hi-C experiment at different scale (*e.g.,* whole chromosome, 1 Megabase, 100 kb windows or lower) permits to identify various levels of organization of the genome: chromosome are organized as territories which occupy preferential relative positions. Chromatin is segregated into two compartments depending on its active or inactive state and chromosomes are segmented in Topologically Associating Domains (TADs).

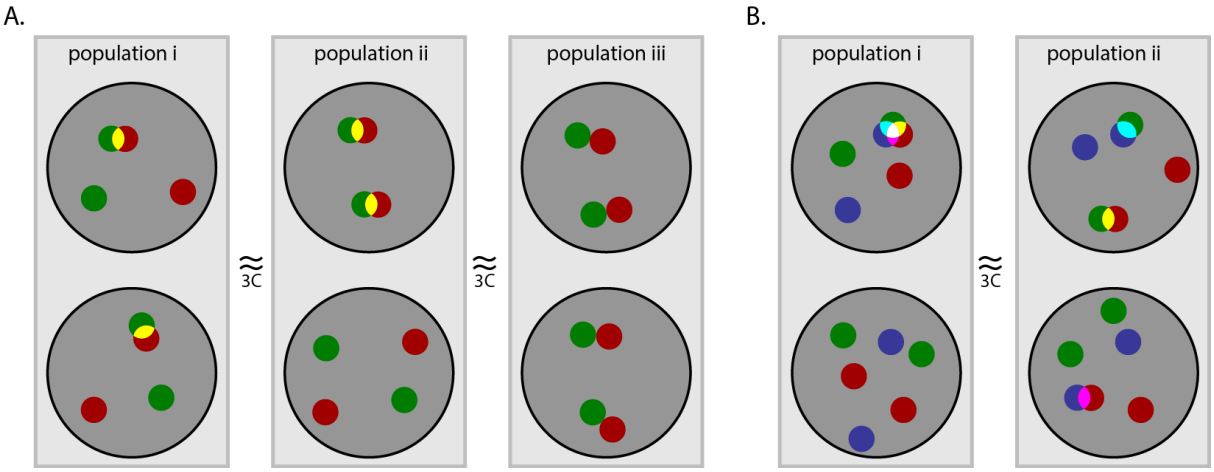

Figure 4: Interpretation of 3C-based results as compared to single cell FISH. Classical 3C-based data do not allow to determine whether co-localization of genomic element occur within a single cell or if they represent the sum of distinct organization co-existing in the population. (A) i, ii, iii correspond to different models of organization of 2 loci (green and red) which would give relatively similar results in 3C-derived datasets: in i, the red and green loci are co-localized in every cell of the population whereas the two other loci are located further away from each other. In ii, two subpopulations of cells are depicted, one presenting the co-localization of the two loci (green and red), the other without any co-localization. In iii, the green and red loci do not co-localize but their movement are restrained in a limited area which favor their random collision and increase the probability of cross-link. (B) Classically 3C-derived data give pair-wise information which do not permit to decipher whether 3 loci (blue, red and green) co-localize in a same cell at the same time (population i) or whether two of them are interacting in different cells or at different time (population ii).

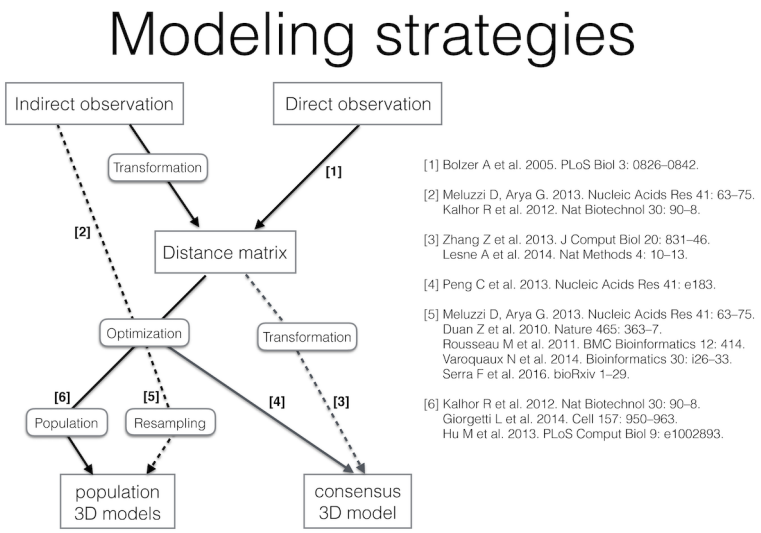

Figure 5: Modeling Strategies. Six main different modeling strategies are currently available for modeling genomes and genomic domains. Those methods diverse in their representation, scoring and sampling of the data. [1] (Bolzer et al. 2005), [2] (Kalhor et al. 2012; Meluzzi and Arya 2013; Giorgetti et al. 2014; Tjong et al. 2016), [3] (Zhang et al. 2013; Lesne et al. 2014; Shavit et al. 2014; Paulsen et al. 2015; Hirata et al. 2016), [4] (Parker 1970; Peng et al. 2013; Park and Lin 2015; Park and Lin 2016; Zou et al. 2016), [5] (Duan et al. 2010; Rousseau et al. 2011; Baù and Marti-Renom 2012; Tokuda et al. 2012; Meluzzi and Arya 2013; Ay et al. 2014; Varoquaux et al. 2014; Nowotny et al. 2015; Trieu and Cheng 2015), and [6] (Kalhor et al. 2012; Hu et al. 2013a; Giorgetti et al. 2014; Wang et al. 2015; Tjong et al. 2016). Refer to the text for detailed explanation of each strategy.

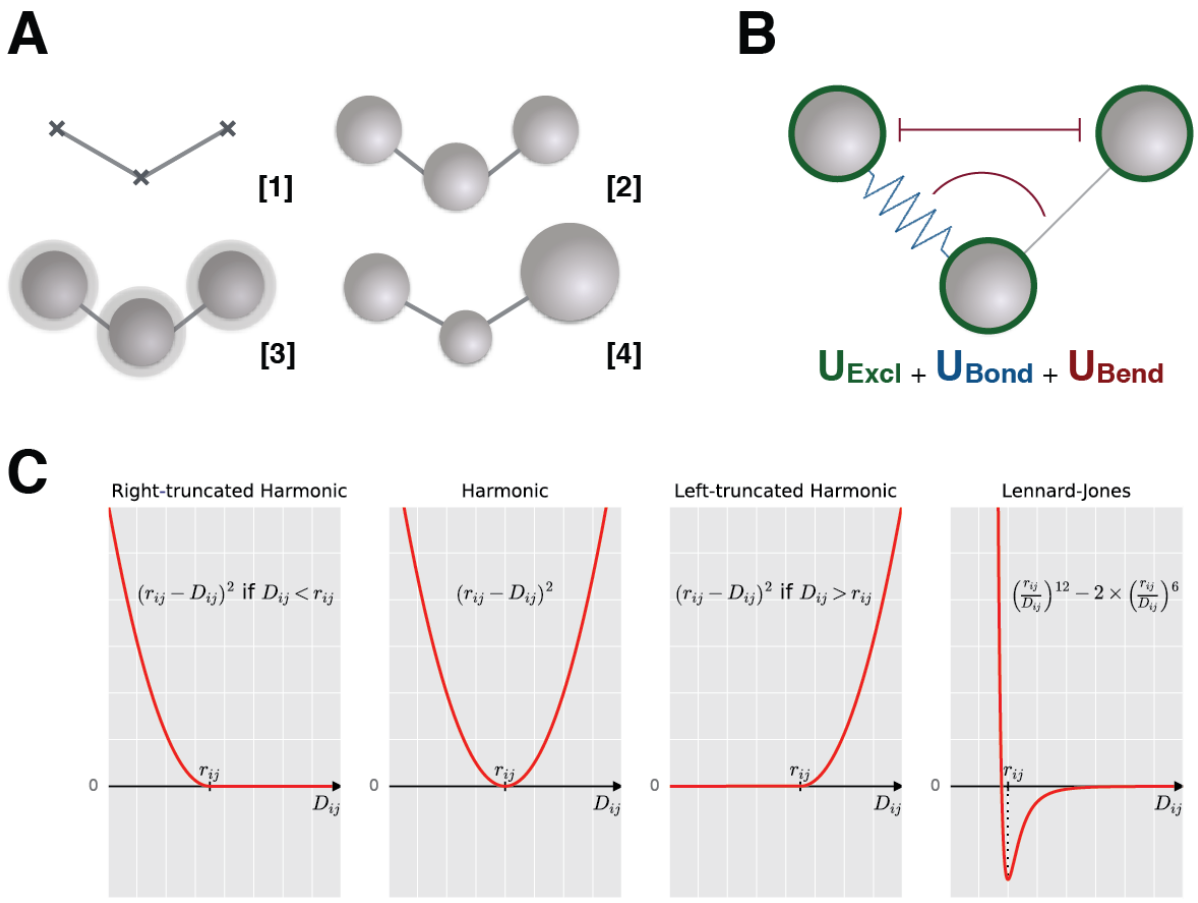

Figure 6: Chromatin model representation. (A) Different ways of representing modeled chromatin loci: [1] as dots, with no physical consistency (Rousseau et al. 2011; Hu et al. 2013a; Zhang et al. 2013; Lesne et al. 2014; Shavit et al. 2014; Varoquaux et al. 2014; Nowotny et al. 2015; Park and Lin 2015; Paulsen et al. 2015; Hirata et al. 2016; Park and Lin 2016; Zou et al. 2016). [2] as spheres (Cremer et al. 2008; Duan et al. 2010; Baù and Marti-Renom 2012; Kalhor et al. 2012; Tokuda et al. 2012; Meluzzi and Arya 2013; Peng et al. 2013; Ay et al. 2014; Giorgetti et al. 2014; Trieu and Cheng 2015; Wang et al. 2015; Tjong et al. 2016). [3] as spheres with complex physical properties, for example a soft external surface and a hard core (Kalhor et al. 2012; Giorgetti et al. 2014). [4] as sphere of different sizes (either representing different content in nucleotides, or different levels of compaction) (Baù and Marti-Renom 2012; Kalhor et al. 2012; Tjong et al. 2016). (B) Schematic representation of the application of the three main physical restraints, the excluded volume (U_{Excl}), the bond restraint (U_{Bond}) here represented either as a spring that could be modeled by a Lennard-jones potential or as a harmonic restraint, the bending (U_{Bend}) that could in this example be modeled explicitly imposing an angle between 3 particles or by limiting the distance between two non-consequtive particles (i, i+2) using a right-truncated harmonic. **(C)** Different kind of potentials to be applied between particles *i* and *j*. D_{ij} being the Euclidian distance between them, r_{ij} the optimal distance inferred from, for example, the experimental data.