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Principles of chromatin organization in yeast: relevance of polymer models to describe nuclear organization and dynamics

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Nuclear organization can impact on all aspects of the genome life cycle. This organization is thoroughly investigated by advanced imaging and chromosome conformation capture techniques, providing considerable amount of datasets describing the spatial organization of chromosomes. In this review, we will focus on polymer models to describe chromosome statics and dynamics in the yeast *Saccharomyces cerevisiae*. We suggest that the equilibrium configuration of a polymer chain tethered at both ends and placed in a confined volume is consistent with the current literature, implying that local chromatin interactions play a secondary role in yeast nuclear organization. Future challenges are to reach an integrated multi-scale description of yeast chromosome organization, which is crucially needed to improve our understanding of the regulation of genomic transaction.

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Introduction: the necessary jump toward an integrative view of chromatin organization

The driving forces responsible for the establishment and maintenance of high-order chromatin structure remain the subject of intense research. Our understanding of genome organization has always been intimately linked to technical progresses, which fed new insights that confirmed or contradicted working hypotheses [1,2••]. From the seminal use of dyes by Flemmings to identify chromatin, microscopy was, and still is, a central tool to study nuclear organization [3]. Carl Rabl suggested that interphase chromosome organization was guided by the tethering of centromeres and telomeres in opposite directions, a folding latter named 'Rabl-organisation' [4]. Rabl-like configuration of budding yeast chromosomes was established more than 100 years later [5–8]. At smaller length scales, the heterogenous distribution of chromatin in the nucleus was observed in 1928 by Emil Heitz [9] using optical microscopy of Giemsa stained chromosomes. This heterogeneous organization was confirmed by Transmitted Electron Microscopy (TEM) with a considerable gain in resolution [10]. After extraction of the soluble nuclear material, TEM also led to the observation of the 'nuclear matrix' as a nucleo-skeleton onto which chromatin was attached [11]. Live cell imaging of fluorescently labeled nuclear components were later developed, collectively called F-techniques, and showed that a large fraction of nuclear proteins, some of which present in the nuclear matrix fraction, were highly dynamic [12]. Techniques aiming at labeling chromosome loci based on fluorescent operator–repressor system (FROS), which involve LacI-GFP or TetR-GFP binding to array of 256 *lacO* or 112 *tetO*, equivalently ~10 kb of DNA, have then been developed, and time-lapse analysis of chromosome motion revealed the mobility of chromosomal loci *in vivo* [13–15]. Over the last decade we witnessed the advent of genomic methods to sense nuclear architecture, such as Chromatin immunoprecipitation (ChIP), DNA adenine methyltransferase identification (DamID), and the now widely used intra-molecular ligation of cross-linked DNA, named chromosome conformation capture (3C), and its genome wide derivatives including Hi-C [16,17]. This booming field calls for new models to integrate datasets of different nature (microscopic distance measurements, ChIP, DamID, contact frequency map from 3C. Coarse-grained polymer physics models met some success in the recapitulation of heterogeneous data with a single and unified representation [18]. Some improvements are nonetheless still needed to recapitulate the folding principles of DNA, chromatin and chromosomes. Here we wish to discuss the successes of these models in the context of *S. cerevisiae* nuclear architecture, as well as the

clarifications that are needed to reach a better understanding of chromosome organization *in vivo*.

Models of nuclear architecture: direct *versus* indirect modeling

In the last 25 years, essentially two classes of models have been proposed to describe genome organization: direct (or data driven) modeling or inverse (or physics driven) modeling (for review, see [19••]). In direct modeling, experimental datasets are used as inputs, and modeling is built by minimizing the discrepancy of the model to the data. Therefore, such models are tailored to recapitulate input data but by construction, they have little or no predictive value, and new datasets must be obtained before generating a modified model. They can be however very useful since they recapitulate complex data in a frame which is usually amenable to be visually interpreted directly. The other approach consists in building a model with a set of assumptions involving, among others, the mechanics of chromosomes (rigidity and friction) and the geometry of the nucleus. The output of the model can be compared with experiments [20•,21••,22••], and its predictive value can be challenged with novel datasets or whenever the set of microscopic parameters that are used to fit the experiments appears to be inconsistent with the literature. In most cases, however, genome modeling is not sufficiently explored to evaluate the consistency of a model based on its fitting accuracy, because the key molecular parameters to describe nuclear architecture are still debated. At this step we propose to highlight some of the main conclusions inferred from modeling of eukaryotic organization with polymer physics.

What do we learn from chromosome conformation capture?

The genome wide implementation of the 3C technique (Hi-C) enables the mapping of the self contacts resulting from the DNA molecules being folded in chromosomes within the live nucleus and is therefore reflecting this architecture (see Figure 1a a contact map for the yeast genome, [23]). Direct 3D modeling [24] applied on this contact map leads to a 3D structure which recapitulates known features of yeast chromosomes organization such as strong centromere clustering, weaker telomere co-localization and the spatial segregation of long and short chromosomal arms (Figure 1b). A pending question is whether or not this organization is quantitatively compatible with polymer physics. In the seminal Hi-C paper, the authors compared their data with two polymer models describing chromosomes as crumple or equilibrium globules [25]. These models differ in their predictions on the decrease of the contact probability P between two loci on the same chromosomes as a function of their genomic distance s (see Figure 1c). The finding that $P(s)$ followed a power law decrease with s characterized by an exponent close to -1 ($P(s) \sim s^{-1.08}$) appeared to be in agreement with the crumple globule model. Other results were later published on different organisms, including the yeast *S. cerevisiae* [26]. They seemed to indicate that metazoan genomes shared common folding principles with a similar exponent of -1 whereas the yeast genome, which has shorter chromosomes are organized as an equilibrium globule in agreement with physical models (see Figure 1d) [21••,22••]. This simple view has been challenged as additional Hi-C data obtained with standardized protocols became available [27•]. It was for instance found that the exponent of $P(s)$ somewhat varied in the range of -1.5 to -1 for different human cell lines [28]. The general relevance of the crumpled globule model has therefore been called into question, because $P(s) \sim s^{-1.5}$ is expected to be detected in equilibrium globules. Concerning the yeast *S. cerevisiae*, only two genome-wide datasets are available [23,29], and more data and analysis are needed to confirm or invalidate the actual folding scheme. Notably, GC content biais, possible fixation artifacts (some of which can be normalized) in 3C techniques, and the difficulties to convert contact frequency to physical distances should not be ignored [24,30••,31,32,33•]. One way around these technical limitations is to combine 3C methods with microscopy observations [17,25]. In conclusion, the folding principles of chromosomes at the *entire* genome level remain controversial, but the number of contributions in this booming field should rapidly clarify these central questions. Conversely the motion of a chromosome locus is associated to the *local* properties of chromatin, and the main results obtained by physical modeling of spatial fluctuations will be described in the following paragraph.

What do we learn from chromosome motion analysis?

Chromatin loci are in constant random motion within some finite volume of confinement detectable with long time-lapse acquisitions [13,14,34,35]. When the locus is released from chromosome (i.e. through inducible excision of tagged chromatin rings), chromatin is diffusing in the nucleoplasm, and boundaries are defined by the nuclear envelope [34,36]. Chromosomal loci instead seem to be confined in a 'gene territory', as defined by the region of preferential steady-state localization [37]. For shorter time scales, the displacement of chromosome loci was mainly analyzed based on the mean square displacement (MSD). The MSD was adjusted with models of diffusion or sub-diffusion, meaning that power-law scaling describing its temporal dependence was characterized by an exponent of 1 or lower than 1, respectively. Notably normal diffusion is expected to occur for isolated objects, that is, influenced by thermal fluctuations and viscous friction only. In the case of polymer loci, elastic interactions between neighboring monomers and long-range hydrodynamic interactions associated to solvent flux have to be considered [38]. The nucleus is a concentrated environment composed of DNA, diffusing and bound