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Principles of Chromatin Organization in yeast:

Relevance of polymer model to describe nuclear organization and dynamics

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Abstract

Nuclear organization can impact on all aspects of 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 *S. 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 regulation of genomic transaction.

Running head: Chromatin Organization in yeast

Keywords: Nuclear structure; chromatin dynamics; chromatin organization; *Saccharomyces cerevisiae*

Introduction: the necessary jump toward 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 chromosome was established more than 100 year 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 organization was confirmed by Transmitted Electron Microscopy (TEM) with considerable gain in resolution [10]. Further after extraction of the soluble material, TEM 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 developed, collectively called F-techniques, and showed that a large fraction of nuclear proteins, some of which present in nuclear matrix fraction, were highly dynamic [12]. Techniques to label 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 also been set up, and time-lapse analysis of chromosome motion revealed the mobility of DNA *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 most 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), and coarse-grained polymer physics models met some success in the recapitulation of heterogeneous data with a single and unified representation [18]. Necessary improvements are still needed to enhance the spatial resolution of polymer models so as to recapitulate the folding principle 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 to

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

Models of nuclear architecture: Direct vs 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 to build 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 [20-22], and its predictive value can be challenged with novel datasets or whenever the set of microscopic parameters that fit experiments appear to be inconsistent with the literature. In fact the consistency of the model with experiments does not imply its validity, especially because the essential parameters to describe large-scale nuclear architecture remain elusive. 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 the self contacts resulting from the DNA molecules being folded in chromosomes within the live nucleus and is therefore reflecting this architecture (See on figure 1a this 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 is organized as an equilibrium globule in agreement with physical models (see figure 1d) [21,22]. This simple view have been however challenged as additional Hi-C data obtained with standardized protocols became available [27], because 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 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 [30-33]. It has been suggested that one way around these technical limitations was to combine 3C methods with microscopy observations, which are now combined [17,25]. We thus conclude that 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 locus is release 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, *i.e.* 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 proteins, as well as RNA, which are expected to screen out hydrodynamic interactions. As was described for the bacterial chromosome [39], the dynamics of chromosomes in yeast was proposed to follow the Rouse model, which assumes that chromatin fiber behaves as a homogeneous series of beads connected by elastic springs, with the notable exception of ribosomal DNA (rDNA) in the nucleolus [40,41]. For an isolated chain and disregarding volume exclusion, the motion of a locus in chain composed of N monomers, of known stiffness characterized by the Kuhn length (Lk) is described by 3 consecutive regimes (Figure 2). For very short time intervals, elastic interactions between neighboring monomers do not restrain motion. This is only valid for small displacements ($MSD \ll Lk$; ~ 30 nm for chromatin), which are difficult to access experimentally (see below). For long displacements larger than the polymer diameter ($MSD \gg Lk \cdot \sqrt{N}$), the entire polymer chain diffuses freely in solvent. In the yeast nucleus, this behavior is not relevant because chromosomes are confined in the nucleus and tethered at their centromere [7,42,43] and telomere [44,45]. Notably this tethering also induces topological constraints that do not allow for reptation, *i.e.* longitudinal diffusion of a monomer along the contour path of the chain. In between those two regimes (for $Lk \ll MSD \ll Lk \cdot \sqrt{N}$), Rouse regime is characterized by $MSD(\tau) = \Gamma \tau^\alpha$, with scaling exponent α of 0.5, which increases to 0.54 whenever volume exclusion is considered. Although this simplistic model overlooks local variations in chromatin structure, it appeared to be consistent with the motion of loci located on chromosome XII, XIV and IV in *S. cerevisiae* [40,41]. These results are further supported by molecular simulations using a polymer model of the entire yeast genome [40,41]. A recent publication however challenged the relevance of the Rouse model by monitoring the motion in 3D of *GAL* genes and control loci in the 0.5-5 s time domain [46]. These authors indeed showed that the exponent of subdiffusion was $\alpha \sim 0.7$, which is apparently not compatible with Rouse regime. The authors suggested that fractional Brownian motion (fBm) could account for this scaling exponent,

though the underlying physics accounting for this behavior remains unclear. Another possibility is that the Rouse model remains valid despite the unexpected exponent. High precision 3D microscopy is achieved with strains in which chromosome loci are characterized by high signal-to-noise ratio. These imaging conditions are obtained by increasing the number of bacterial operator binding sites from 56 to 224 *tetO* sequences, equivalently 2.5 to 11 kb of DNA, in order to enhance brightness of the locus. However, due to the increased length of the labeled DNA, the initial free diffusive motion is expected to be slower than for a single monomer (Fig. 2). The transition to the Rouse regime should hence be delayed, leading to an “intermediate” exponent lower than 1 but greater than 0.54 in the short time regime, as was for instance discussed for particle migration in a dense meshwork [47]. In fact we recently observed the same behavior in clones with long FROS labels (Fig. 2). The dynamics of a locus on chromosome XII showed a smooth transition to the Rouse regime after ~5 s. Note that this two-phase response was not detected in our previous report using clones with shorter FROS labels [40,45]. Consequently, the Rouse polymer model does not seem to be invalidated by analyzing the MSD over a limited temporal domain comprised between 0.5-5 s. Nevertheless we suggest that further validations of the Rouse model to describe chromosome motion require additional analyses, including among others step distribution functions, velocity autocorrelation function of locus trajectories [48], or probability of backward motion.

Conclusion: toward an integrated view of nuclear organization and dynamics

This overview suggests that the implementation of a physics model providing an integrated picture of yeast nuclear organization is under rapid and constant improvement. Based on the current literature, we may propose that each chromosome arm extends in the nucleus from its centromere, behaving as a space-filling polymer in a preferential path dictated by the centromere to nucleolus axis (Fig. 3). Volume exclusion in the nuclear volume defines a characteristic tube in which chromosome can be described as a series of polymer beads, called blobs, in which the chain behaves as an ideal constraint-free polymer. In this description, the Rabl-like organization of yeast chromosomes is marginally dictated by polymer-polymer interactions, rather chromosomes behave as extended polymer chains organized by volume exclusion. This description covers the length spectrum from the Kuhn length to the nucleus size, and disregards the folding of chromatin fiber. We argue that the next challenge is to build multi-scale models with improved description at every spatial dimension to explore

gene specific properties: Gene relocalization documented in yeast during gene expression such as tRNA gene preferential interaction with NPC or nucleolus [49,50], peripheral recruitment of SAGA-regulated genes [51-53], the formation of replication factory [54] or increased motility toward DNA damage [55-57]. The benefits of this effort are expected to help clarify how the cells organize its genome for gene expression regulation or efficient repair. Finally, the recent organization of chromosome arm in fission yeast in globule by local cohesin association at specific sites demonstrate that combination of biophysical properties of chromatin and mapping chromatin bound factors will allow tremendous progress in our understanding of chromatin architecture *in vivo* [58].

Figures captions

Figure 1 : 3C insights on the structure of the *S. cerevisiae* chromosomes.

(A) Contact map of the 16 chromosomes as obtained by genome-wide 3C ([23], individual chromosomes are labeled with roman numbering). The map has been normalized so that the sum over each line and column is equal to one [32]. Note that the colorscale is in log 10. (B) Direct 3D modeling from this map [24]. The colorcode for each chromosome on the structure is indicated in (A). Telomeres and centromeres are labeled with respectively purple and black beads. (C) Zoom on the intra chromosomal contact map of chromosome XV, corresponding to the blue box in (A). The value $p(s)$ is obtained for each genomic distance s with averaging the signal in the red box. (D) Plot of the mean number of contacts obtained in the experiment with varying distances (s). Note that these number of contacts were not normalized. Picture adapted from [22].

Figure 2: Rouse model of polymer dynamics.

(A) This figure describes the difference in segmental dynamics of one monomer or a fragment with n monomers (color-coded in green or blue, respectively). In the short time limit, the motion of the locus is not restricted by elastic interactions with its neighboring polymer segments, leading to a regime of free diffusion. The larger dimension of a fragment with n monomers is associated to a slow down in diffusion (the blue curve is below the green one). This second regime corresponds to Rouse model of polymer dynamics, in which the MSD increases with time with a power-law scaling of 0.54. The red rectangle represents the observation window in a real experiment with finite temporal and spatial resolutions. (B) The blue dataset represents the average MSD over 45 trajectories for a selected bright locus located at position 380 kb on chromosome XII (room temperature). Note that standard errors are indicated as vertical caps. Two regimes can be distinguished with a sharp increase of the MSD at short time scales (scaling exponent of 0.75) followed by a behavior consistent with the Rouse regime (scaling exponent of 0.54). Respective trend lines are shown in black and red.

Figure 3 : Two schematic representation of yeast nucleus.

(A) Schematic representation of yeast nucleus for biology point of view. Chromosome arms are depicted in color lines. Centromeres (CEN; yellow circle) are attached to spindle pole body (SPB; black circle) by microtubules (red lines). Telomeres (TEL) (green circle) are distributed near nuclear envelope (NE) (double-black-lines circle). Nucleolus (red crescent abutting NE) contains rDNA (bold purple line). Blue and brown crosses depicts respectively nuclear and nucleolar center (B) Schematic representation of yeast nucleus for polymer physics point of view. The color circles represent polymer blobs, containing Rouse chain (lines within blob). Each chains represent one chromosome arms anchored at both end (yellow circle and green circle). Blobs are fulfilling nucleoplasm (gray circle). Nuclear and nucleolar center are forming a central axis around which chromosome are organized. Note that rDNA in the nucleolus has property distinct from nucleoplasm.

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