CHAPTER THIRTEEN

Relevance and Limitations of Crowding, Fractal, and Polymer Models to Describe Nuclear Architecture

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Abstract

Chromosome architecture plays an essential role for all nuclear functions, and its physical description has attracted considerable interest over the last few years among the biophysics community. These researches at the frontiers of physics and biology have been stimulated by the demand for quantitative analysis of molecular biology experiments, which provide comprehensive data on chromosome folding, or of live cell imaging experiments that enable researchers to visualize selected chromosome loci in living or fixed cells. In this review our goal is to survey several nonmutually exclusive models that have emerged to describe the folding of DNA in the nucleus, the dynamics of proteins in the nucleoplasm, or the movements of chromosome loci. We focus on three classes of models, namely molecular crowding, fractal, and polymer models, draw comparisons, and discuss their merits and limitations in the context of chromosome structure and dynamics, or nuclear protein navigation in the nucleoplasm. Finally, we identify future challenges in the roadmap to a unified model of the nuclear environment.

1. INTRODUCTION

Chromosome architecture plays an essential role for transcription, replication, recombination, and repair, but the molecular mechanisms of these transactions as well as the properties of chromosomes remain major unsolved problems in biology. The best-studied chromosome component is the nucleosome, which is its constitutive element composed of ~150 bp of deoxyribonucleic acid (DNA) wrapped around an octamer of histone proteins, as inferred at atomic precision by X-ray diffraction (Harp et al., 2000; Luger et al., 1997). Beyond the nucleosome level, chromatin structure is far less characterized. Condensed nucleosome arrays that fold into a “30 nm fiber” have been described using electron microscopy of chromatin spread at moderate ionic strength (van Holde, 1989), yet their exact structure still remains strongly debated (van Holde and Zlatanova, 2007), and recent cryo-electron microscopy imaging performed in situ does not even support the existence of the 30-nm fiber (Maeshima et al., 2010; Woodcock and Ghosh, 2010). For genomic distances higher than ~100 kb, long-distance interactions along the chromatin fiber were detected long ago using optical imaging (Mateos-Langerak et al., 2009; Yokota et al., 1995), and more recently by the molecular biology technique called Hi-C, which enables detection of distant chromosome contacts in cis and in trans at the genome wide level (Dekker, 2006).

Topological domains, which are stable across different cell types and highly conserved across species, have recently been identified
(Dixon et al., 2012; Nora et al., 2012; Sexton et al., 2012), allowing for cross talk between distant chromatin loci in three dimensions (Göndör and Ohlsson, 2009) and participating in the regulation of expression (Göndör and Ohlsson, 2009; Krivega and Dean, 2012). The largest level of organization is the chromosome territory, referring to the folding of chromosomes in compact and self-segregating entities (Bolzer et al., 2005). Territories seem to be conserved in daughter cells after mitosis (Gerlich et al., 2003; Walter et al., 2003), following a “space memory” mechanism still under debate (Orlova et al., 2012). Some authors have proposed the existence of a compartment complementary to the chromosome territory, as defined by the interchromatin space (Cremer and Cremer, 2010). This structure, which is mainly devoid of chromatin, forms a porous network between and within chromosome territories. However, the validity of this model is still controversial, in particular due the observation of significant chromatin intermingling at the periphery of the chromatin territories (Branco and Pombo, 2006). Overall, the architecture of chromosomes is defined by a series of structural elements including DNA, the nucleosome, the chromatin fiber, higher-order chromatin loops, coils or folds, chromosome territories and their complement, and the nucleus itself, which are associated with different spatial scales of about 2, 10, 30, 200, 1000, and 20,000 nm, respectively, that is, extending over four orders of magnitude. Whereas adequate space exists in the nucleus such that DNA compaction rate is not so a concern (Carrivain et al., 2012), functional packaging, which consists in providing the genome with the plasticity in the right place and at the right time, remains an open problem that requires proposition and validation of new models.

Chromosomes were viewed as static entities in the early history of the field, but this description has been considerably revisited over the last decade with emerging light microscopy techniques. For instance, fluorescence recovery after photobleaching (FRAP) or any variant of this technique (Bancaud et al., 2011; Hinde and Cardarelli, 2011) to investigate molecular interactions in vivo showed that fluorescence redistribution occurred very rapidly for nearly every chromatin-interacting protein, leading to a dynamic picture for molecular interactions in the chromatin context. Further, the examination of single nucleosome motion by single-particle tracking indicated the local dynamics of these particles in interphase (Hihara et al., 2012). These results are consistent with the spatiotemporal fluctuations of chromosome segments, that is, tandemly repeated bacterial operator sequences inserted in the genome of living cells, which showed random
(Heun et al., 2001; Marshall et al., 1997) and infrequent directional movements (Levi and Gratton, 2008) associated with transcription activation (Chuang et al., 2006). Finally, chromosome reorganization events have been detected during development (Avivi et al., 2004), and they have been correlated to transcription activation (Chambeyron and Bickmore, 2004). The dynamics of chromosomes illustrated in these three examples cover a temporal range of ~5 orders of magnitudes from seconds to days, and models to describe the underlying movements are still severely limited. Thus, the dynamic nature and the complex topology of chromosomes appear as an intractable puzzle.

Over the last few years however the efforts of the community to propose physical descriptions of the nuclear environment have intensified to fulfill the demand for quantitative analysis of molecular biology experiments, which provide comprehensive data on chromosome folding, or of live cell imaging experiments that enable researchers to visualize selected chromosome loci in living or fixed cells. Several nonmutually exclusive models have thus emerged to describe the folding of DNA in the nucleus, the dynamics of proteins in the nucleoplasm, or the movements of chromosome loci. These models rely on different assumptions depending on the spatial precision of the experiment: conventional optics, for instance, gathers information at spatial scales larger than ~300 nm so that structural details of chromatin folding can be omitted. On the contrary, the folding principles of chromosomes, as inferred from molecular biology techniques, which now reach 100 kb resolution, can only be analyzed with structural models based on polymer physics. Our goal is to describe three classes of models of chromosomes, namely molecular crowding, fractal, and polymer models (Fig. 13.1), draw comparisons, and discuss their merits and limitations in the context of chromosome structure and dynamics, or nuclear protein navigation in the nucleoplasm. We first focus on molecular crowding, which has proven to be useful to interpret experimental findings on the dynamics of nuclear proteins and the biogenesis of nuclear organelles. We then explain that this model is insufficient to make precise predictions about the architecture of the chromosomes, and we will go beyond this theory, focusing on fractal and polymer models to describe the organization of chromosomes and the nucleoplasm. Finally, we identify future challenges in the roadmap to a unified model of the nuclear environment, in which the spatial organization of chromosomes and reaction–diffusion dynamics of nuclear proteins are accounted for with minimal structural parameters.
Our current knowledge of DNA organization in the nucleus is the result of a century and a half of various experimental breakthroughs to visualize chromatin and analyze its biochemical content. From the first observations of chromosomes (Flemming, 1878) in an organelle (Brown, 1831) to the evidence for chromatin heterogeneous distribution (Baccarini, 1908; Boveri, 1914; Heitz, 1928; Rabl, 1885), and the striking link between gene position and expression (with the discovery of position effect variegation (Muller, 1930), considered for many as the true “birth” of epigenetics), scientists from all kind of backgrounds have struggled to decipher the nature and properties of our genome. Technical progresses in visualization techniques were first reported with electron microscopy of the whole cell (Porter et al., 1945), followed by specific developments for nuclear visualization (Bernhard, 1969). Fluorescence microscopy gained popularity in the 1980s, first in fixed cells (Cremer et al., 2006) and then, in the 1990s, in living cells using fluorescent proteins, which greatly improved our view of the nuclear environment.
complex nuclear architecture, as well as of the dynamics of molecular inter-
actions. Fluorescence microscopy techniques recently received renewed
interest as their spatial resolution increased with super resolution microscopy
methods (Hell, 2003). At the same time, biochemical techniques, which rely
on the cross-linking of DNA protein interactions as initially proposed in
1978 (Jackson, 1978), can now provide genome wide contact maps of chro-
mosome interactions (Dekker, 2006; Dekker et al., 2002; van Steensel and
Dekker, 2010), as well as the genomic distribution of virtually every
chromatin-interacting protein (Mardis, 2007), giving access to an additional
perspective on DNA folding in the nucleus.

3. MOLECULAR CROWDING

3.1. Definition of molecular crowding

The textbook picture of cells, as popularized by illustrations of David
S. Goodsell (Goodsell, 2005), consists of a variety of biomolecules, including
DNA, RNA, and proteins, at high concentrations, sometimes assembled
into large-scale structures. This representation emphasizes the degree of
crowding in the cell, and hints at the fact that biomolecules cannot be con-
sidered as isolated objects flowing in an obstacle-free environment. Con-
versely, conventional molecular biology techniques aim to characterize
interaction properties of purified biomolecules, in order to help the inter-
pretation of in vivo measurements after an extrapolation from in vitro to
in vivo conditions. Although this extrapolation has met considerable success
to characterize molecular reactions, it disregards the impact of crowding,
which is expected to introduce a systematic bias through generic mecha-
nisms, including, among others, molecular crowding or phase separation
(Ellis, 2001; Hyman and Brangwynne, 2011). For example, a crowded
and inhomogeneous environment may induce local onsets in viscosity
and/or repulsions of large complexes, modulate stereo-specific interactions,
and macromolecular complexes may spontaneously segregate due to phase
separation processes (Keating, 2012). Although the impact of these mecha-
nisms remains largely elusive, they are generally thought to have a great
impact on molecular interactions in the nucleus (Iborra, 2007). This argu-
ment has been indirectly supported by the observation that polynucleosomes
extracted from interphase cells self-assemble into compact arrays in vitro in
the presence of crowding agents (Hancock, 2008), suggesting that crowding
is necessary to reproduce in vivo conditions.
The concept of macromolecular crowding applies to continuous media in which a fraction of the volume, typically more than 20% (Ellis, 2001), is occupied by background molecules and, thus, is not accessible to other molecules. The cell nucleus definitely meets the concentration criteria because chromatin alone, which is the main crowding agent in the nucleus, occupies 30–50% of the nuclear volume depending on the cell type, as inferred from contrast analysis of 2D or 3D electron micrographs (Lopez-Velazquez et al., 1996; Rouquette et al., 2009). We propose to overview the consequences of molecular crowding \textit{in vitro} in the following section.

### 3.2. Consequences of molecular crowding \textit{in vitro}

The consequences of molecular crowding have been extensively documented by adding inert co-solutes such as synthetic polymers (dextran, ficoll, polyethylene glycol) or serum proteins (BSA) in test tubes, and by monitoring reaction kinetics as a function of the background molecule concentration. This research has been supported by numerous analytical models (Zimmerman and Minton, 1993) and Brownian dynamics simulations (Schnell and Turner, 2004) which described three main effects associated with molecular crowding (Fig. 13.2).

First, volume exclusion is associated with the inaccessible volume fraction occupied by background species, which is defined by the combination of hard steric repulsion between macromolecules and by soft nonspecific interactions such as electrostatic, hydrophobic or Van der Waal’s interactions (Hall and Minton, 2003) that may reinforce or instead partially cancel steric repulsion (Jiao et al., 2010). In addition, volume exclusion is dependent on the size and shape of probe molecules: small molecules diffuse throughout crowded environments in the interstices between co-solutes, whereas large ones are confined in narrow regions (Minton, 2006). Second, the presence of high concentrations of co-solutes slows down diffusion, because frequent collisions with crowding species impede diffusion-driven motions. The resulting diffusive hindrance, which is defined as the ratio of the diffusion coefficient in a crowded versus constraint-free space, increases exponentially with the concentration of crowding co-solutes, and also increases with the size of the tracer (Muramatsu and Minton, 1988).

Last but not least, volume exclusion alters equilibrium constants and reaction rates of biochemical reactions (Ellis, 2001). For concentrations of co-solutes of $\sim 100$ g/L or more (Hancock, 2004), recent studies have shown nonlinear relationships between the concentration of macromolecules and
equilibrium constants and reaction rates in different contexts (Zhou et al., 2008). The free energy pathway of a reaction involving two reactants, which form an activated state and then a product, can be described in a crowded environment by summing (i) the free energy in dilute conditions, with (ii) the free energy required to place the system in a crowded medium. The latter term depends on the interaction properties between

**Figure 13.2** Consequences of molecular crowding. The presence of high amounts of background (right panel) species reduces the volume accessible to additional macromolecules, inducing volume exclusion (second row). Background species also act as obstacles impeding the diffusion of the molecules (third row), and they alter the thermodynamics of binding reactions, which tend to be shifted toward the bound state (fourth row).
these components (reactant, activated state, and product) with the background species (Minton, 2006), potentially dramatically affecting the reaction properties and dynamics. In the limit of steric repulsion, the inaccessible volume fractions can be used to determine the constants of the reaction (Minton, 1983), showing that molecular crowding tend to stabilize the reaction product, which minimizes the entropic contribution of the free energy. Kinetics are determined by the transition rate to the activated state, as well as the encounter rates between diffusing molecules. Thus, crowding has two antagonistic effects: it stabilizes the activated state and hence accelerates the formation of this activated state, but it lowers the chances that reactants encounter due to diffusion slow down. In fact, it is generally admitted that increasing the degree of crowding ultimately leads to reaction inhibition because reactants can no longer diffuse (Ellis, 2001).

While molecular crowding was beautifully illustrated by in vitro experiments and convincing evidence based on diffusion hindrance or aggregation reaction kinetics was reported in prokaryotes (de Vries, 2010; Foley et al., 2010; Ignatova and Gierasch, 2004; Konopka et al., 2006), its relevance for eukaryotes remains less investigated. In the following section, we review recent experimental studies indicating that molecular crowding is a consistent ingredient for modeling the nucleus of eukaryotes.

3.3. Confirmations of molecular crowding predictions in eukaryotes

Confocal imaging of living cells showed that the steady-state distribution of inert fluorescent tracers, such as microinjected dextrans or GFP arrays, was heterogeneous in the nucleus, and that these probes were excluded from regions enriched in chromatin, that is, heterochromatin foci (Gorisch et al., 2003; Verschure et al., 2003). This effect appeared to be enhanced as the tracer size increased, as inferred from the simultaneous observation of 25 and 500 kDa dextrans labeled with two different fluorophores in heterochromatin foci (Bancaud et al., 2009). Volume exclusion was also detected in nucleoli, which are dense nuclear compartments containing rDNA sequences, specific transcription machinery, ribosomal subunits, as well as different nucleolus-associated proteins (Handwerger et al., 2005). Furthermore, modifications of chromatin compaction by histone hyperacetylation, which induces chromatin decondensation through electrostatic repulsion (Tóth et al., 2004), or by overexpression of the histone methyltransferase Suv39H1 (Bancaud et al., 2009) that imprints epigenetic marks characteristic of heterochromatin modulated the degree of exclusion of...
fluorescent probes. Chromatin therefore appears to be a crowding component, which induces volume exclusion of physical tracers.

The mobility of fluorescent tracers was also monitored by FRAP, showing that their diffusion throughout the nucleus is rapid so long as their molecular weight is smaller than \( \sim 500 \text{kDa} \) (Görisch et al., 2005; Seksek et al., 1997). Their mobility is moderately slowed down by a factor of 3–5 in comparison to free solution (Beaudouin et al., 2006; Seksek et al., 1997; Wachsmuth et al., 2000), and the diffusive hindrance is constant with the tracer size (Bancaud et al., 2009; Pack et al., 2006; Seksek et al., 1997). Analyzing further the dynamics of the tracers showed an enhanced diffusive hindrance in nucleoli and heterochromatin in comparison to euchromatin (Bancaud et al., 2009; Pack et al., 2006), in agreement with the enhanced degree of volume exclusion and diffusion hindrance in these compartments.

The effect of crowding on reaction equilibria and rate constants was also investigated in heterochromatin versus euchromatin by performing FRAP on three generic chromatin-interacting proteins, namely the guanine nucleotide exchange factor RCC1, the linker histone isoform H1.1, and the C-terminal tail of H1.1 (H1t) (Bancaud et al., 2009). Fluorescence redistribution was slower in heterochromatin for the three proteins, suggesting that their association with chromatin was enhanced in this dense compartment. The same effect was observed by comparing redistribution kinetics before and after chromatin hypercondensation by osmotic shocks (Martin and Cardoso, 2010). These observations suggest a generic enhancement of the binding to chromatin in dense chromatin regions, in line with the theoretical predictions. Moreover, a recent study monitoring the folding kinetics of the phosphoglycerate kinase showed that this protein adopts a more compact configuration in the nucleus of living yeasts than in dilute in vitro media (Dhar et al., 2011).

3.4. Relevance of molecular crowding for nuclear compartmentalization

The enhanced binding reactions in dense environments have been often speculated to play a role for the formation of nuclear compartments, which are dynamic and self-organizing structures defined by specific and transient protein–protein, chromatin epigenetic status, and/or probably protein–RNA interactions (Dundr, 2012; Meldi and Brickner, 2011; Rajapakse and Groudine, 2011; Tripathi and Prasanth, 2011). The molecular mechanism driving their formation remains elusive, and it has been suggested that
molecular crowding plays a role for the formation and maintenance of these macromolecular assemblies (Cho and Kim, 2012; Richter et al., 2007, 2008), in addition to biochemical cues delivered during interphase (Lavau et al., 1995).

Molecular crowding may act as a self-reinforcing mechanism, stabilizing the interaction of proteins in defined nuclear regions and the initial specific nucleation event between pioneering scaffolding molecules (Dundr and Misteli, 2010), as was suggested by Marenduzzo and colleagues for the clustering of RNA and DNA polymerases into factories (Marenduzzo et al., 2006a,b). The segregation mechanism may be spontaneous (Hancock, 2004; Iborra, 2007), facilitating the binding of additional diffusing scaffolding proteins to the nucleator until the compartment reaches a steady-state size (Bancaud et al., 2009). This model has been supported by the evidence that molecular crowding was necessary for the maintenance of nucleoli and nuclear bodies in isolated nuclei (Hancock, 2004). Notably, this research involved changes in osmotic pressure, as was performed in numerous studies showing that reorganizations of chromatin compaction occurred after hyper- or hypo-osmotic treatment (Brasch et al., 1971; Delpire et al., 1985) (Fig. 13.3). However, this method induces adverse changes in ionic conditions (Martin and Cardoso, 2010), and the role of crowding remains a topic of discussion because both effects alter chromatin compaction in vitro (Hancock, 2007; Maeshima et al., 2010). In line with this discussion, our observations suggest that crowding is neither sufficient to induce the formation of heterochromatin nor necessary for its maintenance (Walter et al., 2013). The driving force generating distinct compartments may then arise from chromatin polymeric properties, because polymers with distinct physical properties spontaneously segregate in crowded media (Keating, 2012), a scenario recently proposed to account for the formation of the nucleolus in budding yeast (Wong et al., 2012). Note that this mechanism could be enhanced by changes in crowding and/or ionic strength. Thus, the generic role of molecular crowding in nuclear compartmentalization remains unclear and more studies are still needed to clarify the relative contribution of the crowding as compared to other biophysical processes such as phase separation or colloidal behaviors (Iborra, 2007). We propose that this research could benefit from the possibility of tuning the amount of DNA in the nucleus of fission yeast up to 16-fold with minimal change in nuclear volume (Neumann and Nurse, 2007), hence circumventing the artifacts of osmotic stress.
Despite the oversimplification of molecular crowding, which assumes a random distribution of background co-solutes, this model met some success in accounting for the dynamics and the assembly properties of nuclear proteins and macromolecular assemblies. However, it fails to precisely describe the properties of nuclear diffusion. Several authors have reported, for instance, that the diffusive hindrance is independent of the molecular weight of the probe in the nucleus (Pack et al., 2006; Seksek et al., 1997), in contradiction

**Figure 13.3** Chromatin architecture and hypotonic shocks. The different panels represent confocal image sequences of living 3T3 cells expressing the core histone H2B fused to EGFP submitted to hypotonic shocks, which are induced by dilution of the imaging medium with distilled water (35:65%, v/v, respectively). To rescue the isotonic chromatin compaction level, cells were microinjected with PBS (upper row), a mixture of PBS and water 40:60 (v/v) supplemented with NaCl at a final concentration of 320 mM (middle row), or with polyethylene glycol (MW = 1500 g/mol) at a dilution of 25% (w/v) (bottom row). While the addition of PBS alone did not induce changes in chromatin compaction, chromatin rich compartments could be detected upon addition of salt or polyethylene glycol. The position of the tip of the microinjection needle is indicated by green arrowheads. The scale bar represents 5 μm.

### 3.5. Limitations of molecular crowding model

Despite the oversimplification of molecular crowding, which assumes a random distribution of background co-solutes, this model met some success in accounting for the dynamics and the assembly properties of nuclear proteins and macromolecular assemblies. However, it fails to precisely describe the properties of nuclear diffusion. Several authors have reported, for instance, that the diffusive hindrance is independent of the molecular weight of the probe in the nucleus (Pack et al., 2006; Seksek et al., 1997), in contradiction
with several *in vitro* studies that indicated an increase of the hindrance with the size of the probe in random crowded media (Han and Herzfeld, 1993; Muramatsu and Minton, 1988). A second discrepancy is related to the anomaly of diffusion in the nuclear environment. Monte-Carlo simulations as well as *in vitro* studies indicate that, except at very short timescales, tracers moving within a crowded medium composed of mobile co-solutes usually display pure Brownian diffusion (Dauty and Verkman, 2004; Saxton, 1994). This result is essentially explained by the fact that the crowding co-solutes define an unstructured medium after a travel displacement larger than the average distance between obstacles, so that the crowding medium appears as homogeneous and normal diffusion occurs. Anomalous diffusion persists in the case of a structured pool of obstacles, that is, a polymer melt obtained with polymer solutions at high concentrations (Banks and Fradin, 2005). This structuration of co-solutes is consonant with the observation of an anomalous diffusion for GFP multimers containing one, two, five, and ten GFPs at length scales smaller than ~300 nm in the nucleus of living cells, as inferred from fluorescence correlation spectroscopy, which consists in measuring fluorescence intensity fluctuations in the confocal volume of an epifluorescence microscope, and from single-particle tracking (Bancaud et al., 2009; Grünwald et al., 2008; Pack et al., 2006; Wachsmuth et al., 2000). Anomalous diffusion is characterized by the anomaly parameter $\alpha$, which is defined using the power-law scaling of the temporal evolution of the mean square displacement (MSD) with the time $t$:

$$
\text{MSD}(\Delta t) \propto \Delta t^\alpha
$$

(13.1)

$\alpha$ is equal to 1 for normal diffusion in free space, but its value somewhat varied between 0.5 and 0.8 in the nucleus. Although the molecular mechanism accounting for anomalous diffusion remains unclear (Saxton, 2012; Szymanski and Weiss, 2009), these results do not support a conventional molecular crowding picture. Furthermore, the analysis of the spatial redistribution of the three chromatin-interacting proteins RCC1, H1.1, and H1t (Bancaud et al., 2009), the navigation of which is determined by diffusion and interaction dynamics in the nucleus, showed fractal-like dynamics meaning that the association constant of these proteins to chromatin appeared to be time-dependent (Kopelman, 1988). This result could not be reproduced assuming a homogeneous repartition of chromatin obstacles in the nucleus, and rather hinted to the fractal organization of the nucleoplasm, as will be discussed in the following section.
Overall, the assumption that the nuclear environment can be modeled with a homogeneous network of obstacles appears questionable, supporting the modern view of chromatin as a highly ordered structure (van Steensel, 2011). In the meantime the fractal model has gained popularity to describe chromatin architecture (Mazza and McNally, 2010; Mirny, 2011; Sanyal et al., 2011), and we recently surveyed some of the main experimental evidence supporting its validity (Bancaud et al., 2012). We will now briefly overview the physical description and consequences of the fractal model.

### 4. FRACTAL MODELS

#### 4.1. The fractal hypothesis for nuclear diffusion

Fractal objects were initially imagined to describe geometrical objects with irregular appearances (Mandelbrot, 1982), which cannot be mapped with a finite number of geometrical elements. The evaluation of the contour of a coastline is, for instance, characterized by an infinite length $l$ because the measured length increases as the resolution to sample the contour increases. The morphology of this object can nevertheless be characterized by the fractal dimension $f$, which is determined by assessing the mass distribution (i.e., the number of structural elements $N$) at a given zoom factor $R$ according to the following relationship:

$$f = \frac{\ln (N)}{\ln (R)} \quad (13.2)$$

The fractal dimension is a noninteger number, which varies somewhat between 1 and 2 for a line in 2D: its value is 1.25 for the British coastline (Mandelbrot, 1967). Mathematical fractals are self-similar over an unlimited range of scales, but natural fractals are self-similar only within a spatial domain with upper and lower scaling limits of $\sim 20$ μm and $\sim 2$ nm in the nuclear context, corresponding to the lateral dimension of the nucleus and the DNA diameter, respectively.

Let us note that the assumption of a fractal repartition of obstacles has been investigated in the seminal contributions of M.J. Saxton on molecular diffusion in the presence of obstacles, which were randomly placed on a lattice (Saxton, 1994) or in percolation or diffusion-limited clusters (Saxton, 1993), which exhibit self-similar properties characterized by fractal dimensions of 1.9 and 1.7 in 2D, respectively. These molecular dynamics simulations showed that diffusion was anomalous over a broad spatial
domain, and this result suggested that the anomalous diffusive response observed in living cells originated from the fractality of the nucleoplasm (Münk et al., 1999). The fractal dimension of the accessible nucleoplasm has then been derived by modeling interaction kinetics of chromatin-interacting proteins, revealing that the fractal architecture of the nucleoplasm of euchromatin and heterochromatin were markedly distinct and associated with fractal dimensions of \( f \sim 2.6 \) and 2.2, respectively, in the \( \sim 2–100 \) nm space domain (Bancaud et al., 2009). These values appear to be consistent with the idea that heterochromatin is more condensed than euchromatin because the topography of the complement of heterochromatin is smooth and leaves a smaller part of chromatin surface for transcription factors to scan for target sites. By contrast, nucleoplasm in euchromatin is defined by a larger fractal dimension, occupying more of the total volume, and therefore giving access to a larger part of the rough chromatin surface for scanning chromatin-interacting proteins.

Moreover, it was recently shown that the comparison of the fractal dimension \( f \) to the anomaly parameter \( \alpha \) provides general predictions regarding the target-search mechanism in fractal environments (Bénichou et al., 2010; Condamin et al., 2007, 2008). Exploration by diffusion is compact when \( \alpha > f \), meaning that diffusing molecules systematically visit their neighboring sites, and oversample their surrounding environment. On the contrary, the time to reach a target locus is independent of the distance from the initial position for \( f \geq \alpha \), so diffusing molecules rapidly travel over large distances while overlooking nearby sites. This second regime is referred to as non-compact exploration. Because the fractal dimension of the heterochromatin nucleoplasm is lower than in euchromatin but the anomaly parameter is similar (Bancaud et al., 2009), exploration is more compact in heterochromatin, and chromatin-interacting proteins should systematically bind to all of their available binding sites in heterochromatin, in agreement with the long residence of generic chromatin binding proteins observed in heterochromatin (Bancaud et al., 2009) and with the higher frequency of transient protein trapping in heterochromatin (Grußwald et al., 2008). On the contrary, the target-search strategy in euchromatin likely favors a faster exploration, which is presumably adapted to scan for comparatively rare regulatory elements in the genome. Notably, it was recently shown that the facilitated diffusion model, which describes the search for a target site based on alternating phases of free diffusion in the bulk and sliding diffusion of a bound complex, was a robust mechanism for a fractal template such as chromatin (Bénichou et al., 2011). In turn, this study indicated that the
exploration strategy could be finely tuned by adjusting the molecular interactions on chromatin. This hypothesis could be tested by tracking the motion of, for example, transcription factors at the single-molecule level (Normanno et al., 2012), potentially shedding new light on the regulation of transcription activation; chromatin might be able to switch the expression of different loci by altering its fractal structure, as was suggested by theoretical models (Meyer et al., 2012).

4.2. Fractal hypothesis for chromosome architecture

The fractal model has also been applied to describe the structure of chromatin by image analysis of fluorescently labeled DNA, showing a fractal topography characterized by a fractal dimension of \( f \approx 2.5 \) in the 0.15–2.7 \( \mu \text{m} \) spatial range (Einstein et al., 1998; Huisman et al., 2005; Tóth et al., 2004). Furthermore, textural analysis of transmission electron micrographs of cell nuclei stained with uranyl acetate as a contrast agent indicated that chromatin organization was fractal (Castelli and Rosa, 2001). Neutron scattering, which is presumably the optimal method to probe fractal architecture over a broad spatial range of 15 nm to 10 \( \mu \text{m} \) (Lebedev et al., 2005, 2008), has been applied to isolated chicken erythrocyte nuclei, showing a power-law scaling relationship between the scattering intensity and the scattering vector which is characteristic of a fractal organization. A biphasic response with a fractal dimension of \( f \approx 2.4 \) in the length spectrum 15–400 nm and \( f \approx 2.9 \) for larger length scales was detected. The response could then be studied in finer details by assaying the contribution of DNA architecture that also exhibited two different regimes of fractality with a fractal dimension of \( f \approx 2.2 \) in the 15–400 nm space domain, and a \( f \approx 3.2 \) exponent for larger length scales ((Lebedev et al., 2005), see table in (Bancaud et al., 2012) for a classification of measured fractal dimensions). Last but not least, the fractal model gained additional support from Hi-C (Lieberman-Aiden et al., 2009), which unraveled a power-law scaling associated with a slope of \( -1.08 \) in the range 0.5–7 Mb, which corresponds to a spatial range of \( \sim 0.5–2 \mu \text{m} \). This structural property appeared to be consistent with a fractal organization of DNA characterized by a fractal dimension of \( f \approx 3.0 \). This folding can be assigned to the folding of DNA into a crumpled globule conformation, as defined by Grosberg and coworkers (Grosberg et al., 1993). The crumpled globule is not entangled, and large-scale loops should be reorganized at a low energetic cost with no need to break physical contacts to liberate genomic sequences.
Consequently, several experimental lines of evidence support the fractal organization of the nucleus, but the available experimental data that refer to DNA, chromatin, the nucleoplasm, or the whole nucleus and the estimates of the fractal dimension are not always consistent (Bancaud et al., 2012). In addition, the estimate of the fractal dimension cannot be assigned to a unique structure because a variety of fractal objects can be imagined to match a given fractal dimension. Notably, the link between the polymer folding properties and the fractal dimension obtained by Hi-C highlights the need for a polymer model to derive a molecular description of chromatin organization in the nucleus (Heermann et al., 2012). In the following section we therefore offer an overview of some of the main conclusions obtained by describing chromosomes with a polymer model, and we will then attempt to bridge the conclusions of the fractal and the polymer models.

5. POLYMER MODELS FOR CHROMOSOMES

5.1. Polymer models for chromosome architecture

The conformation of a polymer chain can be described by measuring the physical distance \( L \) between genomic loci as a function of their genomic separation \( G \):

\[
L \propto G^{1/e}
\]

with \( e \) the fractal dimension of the line of polymer in space. \( e \) is \textit{a priori} unrelated to the fractal dimension \( f \), as shown by the thought experiment of A. Grosberg in which we consider a chromosome and freeze it instantly. We then imagine that linker DNAs are cut systematically, while the whole structure remains frozen. For the resulting disconnected system, the concepts of \( e \) or any Hi-C type information are fully lost, because the structure is genetically disconnected, but \( f \) remains perfectly measurable. This thought experiment is particularly relevant in the case of the fractal dimension of the protein component of chromosomes. This quantity can be estimated by computing the distribution of fluorescently labeled histones in living cells, but it is not related to DNA polymer properties.

This relationship of Eq. (13.3) can be inferred by \textit{in situ} hybridization of fluorescent oligonucleotides targeted to specific genomic sequences in fixed cells (FISH). \( e \) was \( \sim 2 \) in the 150 nm to 1 \( \mu \)m spatial range, increased to \( \sim 3 \) above 1 \( \mu \)m (Sachs et al., 1995; Yokota et al., 1995), and a confinement (\( \sim 0 \)) was detected for distances larger than 2–3 \( \mu \)m (Mateos-Langerak et al., 2009).
The latter regime is consonant with the existence of chromosome territories in interphase (Cremer et al., 2006), while $\varepsilon \sim 2$ corresponds to a random walk polymer. Different models were suggested to reproduce the changes in $\varepsilon$ with the sampling dimension, assuming that chromatin formed loops of $\sim 1$ Mb in length (Sachs et al., 1995) or of $\sim 200$ kb and bundled in groups of $\sim 5$ (Münkèl et al., 1999). These models have since been challenged by a dynamic loop model, which postulates that the formation of chromosome loops is a random diffusion-driven process and that loops occur transiently (Bohn and Heermann, 2009, 2010). An appropriate set of parameters for the loop formation probability and the loop lifetime enabled reproduction of FISH and Hi-C experiments, as well as the general topography of chromosome territories (Bohn and Heermann, 2010). However, the recent identification of topological domains of $\sim 1$ Mb by Hi-C, which were shown to be an inherent property of mammalian genomes (Dixon et al., 2012; Nora et al., 2012; Sexton et al., 2012), seems to support the pioneering model of Sachs (Sachs et al., 1995), and in turn shows that the folding of chromosomes has not yet been fully clarified. Furthermore, the consistency of these models has recently been questioned because confined polymer models appeared to reproduce FISH data equally well (Emanuel et al., 2009).

Last but not least, the different loop models should be compared to the crumpled globule architecture derived from Hi-C (Lieberman-Aiden et al., 2009). The crumpling was originally imagined to explain relaxation kinetics of polymers rapidly brought into poor solvent conditions (Grosberg et al., 1988). The crumpled conformation is transient, and ultimately collapses into an equilibrium globule, which is a compact conformation favoring polymer–polymer over polymer–solvent interactions. The crumpled globule is not entangled, and is characterized by long-range intrachromosomal interactions as shown by the power-law dependence of $-1$ for contact probabilities in comparison to $-1.5$ for equilibrium globules (Lieberman-Aiden et al., 2009). While the dynamic loop model is stable thermodynamically, the crumpled globule is transient and can only be stabilized kinetically, in agreement with the analysis which showed that the organization of chromosomes in discrete territories is maintained during interphase kinetically (Rosa and Everaers, 2008). Additionally, the crumpled globule may be stabilized over long time periods by DNA sequences nonrandomly repeated along chromosomes, as suggested by the spatial correlation between CTCF binding sites and contacts between DNA fragments derived from Hi-C (Botta et al., 2010).
In another direction, FISH has been applied to characterize the folding principle of chromosomes in the yeast *Saccharomyces cerevisiae*. The physical versus genomic distance data have been analyzed with a worm-like chain (Prorod-Kratky) model which allows for derivation of the fiber persistence length $l_p$ and its packing ratio $P$. The persistence length represents the length over which the fiber behaves as a rigid polymer with thermal fluctuations, and the packing ratio is the number of nucleosomes per 10 nm. Chromatin appeared to be a stiff polymer in yeast, characterized by a persistence length of 200 nm and a nucleosome density of 7–10 per 10 nm (Bystricky et al., 2004). These quantities were also assessed using 3C (Dekker, 2008), suggesting that chromatin persistence length was 66–134 nm and its packing ratio 1.1–3.3 nucleosome/10 nm. So far these results did not clarify whether chromatin is stiff or flexible and whether the density of nucleosomes is high or low. This debate is consonant with the recent questions raised about the existence of a dense and compact 30 nm fiber in vivo. Electron spectroscopic imaging, which distinguishes phosphorus atoms and provides a trace of the path of the DNA in a chromatin fiber (Fussner et al., 2011) and cryo-electron microscopy of frozen nuclei (Maeshima et al., 2010) have indicated that chromatin in cultured mouse embryonic fibroblasts and native mouse tissues is nearly exclusively organized in a 10 nm fiber. Altogether, despite years of research on chromatin structure, the local and global characteristics of chromosomes, which are assessed by the packing ratio, the persistence length, and the looping probability, remain the subjects of intense controversies.

5.2. Polymer models for chromosome dynamics

The physical parameters governing chromosome movements remain poorly understood, though it was established 15 years ago that chromosome loci explore a broad volume of the nucleus in yeast and in metazoans (Heun et al., 2001; Marshall et al., 1997). Nuclear constraints such as the nuclear envelope (Bystricky et al., 2004; Heun et al., 2001) or the position along the chromosome, for example, telomeres (Bystricky et al., 2005) were shown to modulate the motion of loci, but the physical parameters governing these movements were never assessed quantitatively. For instance, it has been proposed that chromatin segments undergo normal Brownian fluctuations at small time scales, and that their motion is confined in volumes of $R \sim 0.3 \mu m$ (Marshall et al., 1997), or that the movement of the GAL1 locus on chromosome II follows an anomalous diffusive behavior.
characterized by an anomalous parameter of $\sim 0.4$ (Cabal et al., 2006). Detailed analysis of chromosome motion in bacteria also showed an anomalous diffusive response in the temporal regime comprised between 1 and 100 s, which has been interpreted with the Rouse model (Weber et al., 2010, 2012). The Rouse model assumes that the motion of chromosome loci is dominated by elastic interactions between nearest-neighbor chromatin segments and the viscous friction of each monomer (Doi and Edwards, 1988). Note that this model disregards hydrodynamic interactions, which are screened out in the nuclear context due to the crowding level in this compartment (Fig. 13.4A).

We have recently developed an imaging platform for 3D high-throughput fluorescence microscopy of living yeast (Hajjoul et al., 2009) that was used to track the motion of 15 loci inserted in chromosome III, IV, VI, XII, and XIV over an extended temporal range of more than four orders of magnitude ($10^{-2}$ to $10^3$ s; Fig. 13.4B). The MSD of chromosome loci, as well as the statistical properties of the trajectories, followed an anomalous diffusive response that was characteristic of the Rouse model (Hajjoul et al., 2013). This behavior was consistently observed for seven loci along the contour of chromosome XII (Albert et al., 2013), and it appeared to be specific to the bulk of chromatin because the motion of loci in the nucleolus was reduced (Fig. 13.4C). In addition, the analysis of the extent of spatial fluctuations suggested that chromatin persistence length is highly flexible in yeast and less than 30 nm. This result is consistent with the recent indications that chromatin is folded in a 10 nm fiber in vivo (see above), and shows that motion analysis provides an efficient solution to assay chromosome structural properties and hence complements conventional techniques such as FISH. In the following section, we illustrate how different techniques can be combined to derive chromosome structural properties.

6. EVALUATING CHROMOSOME STRUCTURAL PROPERTIES WITH COMBINED TECHNIQUES

The haploid genome of *S. cerevisiae* is composed of 12 million base pairs arranged in 16 chromosomes. The architecture of this small genome has recently been modeled using simulations (Gehlen et al., 2012; Tjong et al., 2012; Wong et al., 2012), reproducing experimental results of Hi-C, telomere repartition, or nucleolus formation with a minimal number of physical principles, including volume exclusion and physical tethering of polymers. Therefore, the yeast nucleus constitutes an excellent model
Figure 13.4 Motion analysis. (A) The nuclear volume is densely filled with chromosomes and nuclear proteins, and volume exclusion between chromosomes has been shown to (Continued)
system to validate physical concepts, and we demonstrate here how two different assays can be recapitulated to extract chromatin persistence length and packing ratio.

Distance measurements between genomic loci on the same chromosome have been analyzed with the WLC model (Bystricky et al., 2004). The validity of this model is unclear, however, because chromosomes are assumed to be ideal chains and monomer–monomer interactions are disregarded, whereas recent simulations showed that volume exclusion was essential to model chromosome conformation (Tjong et al., 2012). These simulations enabled to reproduce the results of gene maps (Wong et al., 2012) (upper panel in Fig. 13.5A) which represent the position of one chromosome loci averaged over a cell population comprising ~2000 individuals, hinting at the relevance of polymer models to model the yeast genome. We thus propose to revisit distance measurements carried out over short distances of less than 400 nm (equivalently to 100 kb) with the blob picture (de Gennes, 1979), which describes the steady-state conformation of a confined polymer as a series of self-avoiding chains (dashed red circles in the left and middle panels of Fig. 13.5B). Note that we showed that this model was consistent with our MSD data using Langevin dynamics simulations (Hajjoul et al., 2013), which also hinted to the structural organization of chromosomes in blob-like geometries (Fig. 13.5B, right panel). According to the blob model, the power-law exponent relating the physical $L$ versus the genomic distance $G$ is 0.6 (Ostashevsky and Lange, 1994), and the prefactor is dependent on $l_p$ and $P$ (de Gennes, 1979):

$$L = \left( \frac{\alpha}{2l_p^2} \right)^{1/5} \times 2l_p \times \left( \frac{G}{K} \right)^{3/5}$$  \hspace{1cm} (13.4)
Figure 13.5 Recapitulation of experimental results with the blob picture. (A) The gene maps on the left represent the statistical distribution in 2D of chromosome loci located at 90 and 170 kb from the centromere on right arm of chromosome XII in living yeast. The plot on the right shows the scale bar, the color scale, and the reference position of the nucleolus, which are used to construct gene maps. (B) The left panel represents the blob picture, in which chromosomes are segmented in consecutive self-avoiding chains (red dashed circles). The diameter of each blob is determined by the crowding level in the nucleus, as suggested by the middle panel. The right panel is the superposition of the conformation of chromosome III in the time course of a Langevin dynamics simulation. The repulsion between chromosomes guides their folding in an elongated

(Continued)
with \( \alpha \), the fiber diameter in nm and \( K \) the number of bp in one Kuhn segment of length \( 2l_p \). Note that Eq. (13.4) is relating the distance to the number of monomers in one Kuhn segment of length \( 2l_p \), and we checked the relevance of this scaling prediction with Langevin dynamics simulations (not shown). \( K \) is related to the physical distance by the packing ratio:

\[
K = P \times 2l_p. \tag{13.5}
\]

We thus conclude that

\[
L = \left( \frac{\alpha}{2l_p} \right)^{1/5} \times (2l_p)^{2/5} \times P^{-3/5} \times G^{3/5}. \tag{13.6}
\]

The left panel of Fig. 13.5C shows that this power-law scaling relationship between the physical and genomic distances is consistent with FISH data (Bystricky et al., 2004). In addition, our recent data on the distance between genomic loci separated by more than \( \sim 100 \) kb on chromosome XII showed a linear regime (green dataset in the right panel of Fig. 13.5C) (Albert et al., 2013), indicating a different folding mechanism consistent with the formation of series of blobs in a tube-like configuration, as shown in the simulation of Fig. 13.5B. The transition between the linear and nonlinear regimes occurs for genomic and physical distances of \( \sim 100 \) kb and \( \sim 400 \) nm, respectively, indicating that the typical dimension of blobs is \( \sim 400 \) nm. Overall, the responses for short and long length scales of the physical versus genomic distance data tend to support the blob picture model.

Moreover, assuming that the contribution of the fiber diameter is negligible, as is conventionally done in FISH analysis (Bystricky et al., 2004;
Ostashevsky and Lange, 1994), we neglect the first term of Eq. (13.6), and deduce from the fit that \( l_p^{2/5} \times P^{-3/5} = 0.31 \text{ nm} \). Given that we obtained with another method a persistence length of less than 30 nm, we propose that the structural properties of chromatin are defined in the region of the parameter space shown in Fig. 13.5D. Obviously, more work is needed to provide the community with reliable estimates, which will involve multiple assays because one technique is generally insufficient to evaluate multiple quantities with precision.

7. UNIFORMITY OF CHROMATIN MECHANICAL PARAMETERS

The bending persistence length of chromatin has been estimated to \( \sim 30 \text{ nm} \) based on single-molecule stretching (Bancaud et al., 2006; Bennink et al., 2001; Cui and Bustamante, 2000), 28 nm by monitoring the recombination frequency of chromatin circles \textit{in vivo} (Ringrose et al., 1999), 50–100 nm by evaluating the circularization frequency in Hi-C (Dekker, 2008), to \( \sim 200 \text{ nm} \) using \textit{in situ} hybridization experiments (Bystricky et al., 2004) (Table 13.1). We first estimated the torsional persistence length on reconstituted chromatin fibers manipulated by magnetic tweezers (Bancaud et al., 2006; Celedon et al., 2009) and showed a surprisingly low value of 5 nm, which is almost 20 times lower than that of naked DNA (Strick et al., 1999). For the stretching modulus, a rough value of \( \sim 5–8 \text{ pN} \) has been derived from the same kind of experiments (Bancaud et al., 2006; Cui and Bustamante, 2000), close to the theoretical estimate (Ben-Haı̆m et al., 2001) and probably reflecting the weak internucleosomal interactions disrupted during the mechanical extension of the fiber, as well as the partial unwrapping of DNA (Victor et al., 2012). Although a pure physical polymer-based model is obviously useful to get general laws of chromosome organization, the discrepancies between different evaluations of the persistence length may be associated with the intrinsic variability caused by the geometrical polymorphism of this complex nucleoprotein assembly (Ben–Haı̆m et al., 2001; Wedemann and Langowski, 2002). For instance, how biochemical modifications of its components affect chromatin mechanical properties is still debated (Lavelle, 2009). In this regard, recent evidence that DNA methylation affects DNA mechanical properties (Severin et al., 2011) and nucleosome structure and dynamics (Choy et al., 2010; Jimenez–Useche and Yuan, 2012) suggests that methylation may regulate gene expression not only through biochemical mechanisms (e.g., serving
as molecular “handles” recognized by various regulatory proteins), but also
through physical mechanisms (changing DNA elastic and dynamical
properties).

8. CONCLUDING REMARKS

We conclude from this survey of experiments and models of chromo-
some structure and dynamics that the field is particularly active, although
there is still a lack of consensus on the analytical approaches describing chro-
mosome properties. For a long time, FISH has remained the only technique
to probe nuclear architecture \textit{in vivo}, but there has been much progress
recently with the development of high resolution optical microscopy and
other nonoptical imaging techniques for the study of 3D nuclear

\begin{table}[h]
\centering
\caption{Methods used to measure chromatin persistence length \textit{in vitro} and \textit{in vivo} (transparent and gray backgrounds, respectively)}
\begin{tabular}{|l|c|p{10cm}|p{5cm}|}
\hline
\textbf{Method} & \textbf{Persistence length (nm)} & \textbf{Experimental conditions} & \textbf{References} \\
\hline
Force spectroscopy & 30 & Purified chromatin from chicken erythrocytes & Cui and Bustamante (2000) \\
\hline
Force spectroscopy & 28 & Reconstituted chromatin on repeated 5S sequences & Bancaud et al. (2006) \\
\hline
Force spectroscopy & 30 & \textit{X. laevis} egg extract reconstitution & Bennink et al. (2001) \\
\hline
Distance measurements & 196–272 & Chromosome 4 in G1 human fibroblasts (fixed cells) & Ostashevsky and Lange (1994) and van den Engh et al. (1992) \\
\hline
Distance measurements & 170–220 & Chromosomes 5, 6, 14 in \textit{S. cerevisiae} & Bystricky et al. (2004) \\
\hline
Looping probability & 28 & Recombination in human cells & Ringrose et al. (1999) \\
\hline
Looping probability & 66–134 & 3C on chromosome 3 in \textit{S. cerevisiae} & Dekker (2008) \\
\hline
Spatial fluctuations & <30 & Motion analysis on chromosomes 3, 4, 12, 14 & Hajjoul et al. (2013) \\
\hline
\end{tabular}
\end{table}
organization (Markaki et al., 2012; Rapkin et al., 2012), as well as with the advent of high-throughput cell biology technologies. We now have the tools in hand to get a unified picture of nuclear architecture and dynamics. This objective requires the integration of experimental data coming from different approaches, for example, Hi-C and FISH as discussed in Gibcus and Dekker (2013), or FISH and motion analysis as proposed in this report. Models are mandatory to support these integrated researches and the rapid development of multi-scale simulations, which can now reproduce the behavior of the whole nucleus of S. cerevisiae with a minimal number of adjustable parameters (Tjong et al., 2012; Wong et al., 2012), clearly offers a great opportunity. Notably, several problems should be addressed to improve our description of the nuclear environment. For instance, it is important to link nuclear protein dynamics and chromosome structural properties, as was recently initiated by Langowski and collaborators in Fritsch and Langowski (2011). In another direction, the development of a combined approach based on polymer physics including biochemistry to take into account the heterogeneity of the polymer caused by sequence-specific DNA-binding proteins interacting at discrete loci is needed to reach a functional model of chromatin organization in the nucleus. These new efforts and models may be confronted by experiments carried out in living cells, in fixed cells, or in isolated nuclei, provided that the consequences of molecular crowding can be directly investigated with nuclei (Hancock, 2004), and that technical progress to handle and analyze these structures was recently reported (Rowat, 2009).

Several studies have shown that some chromatin “outlooping” can occur upon transcription (Volpi et al., 2000; Williams, 2003) to drive targeted gene into functional nuclear compartments such as transcription factories (Bartlett et al., 2006; Jackson et al., 1993; Osborne et al., 2004). From a mechanistic point of view, outlooping might rely on changes in chromatin conformation and topological state (Mozziconacci et al., 2006), potentially assisted by some ATP-dependent motors (Havas et al., 2000). Some evidence for an actin/myosin system in the nucleus renewed our vision of mechanisms potentially tasked with long-range chromatin movements (Chuang et al., 2006), occurring at velocities ranging from 0.1 to 1 μm/min and over distances of 1–5 μm. The role for an actin/myosin complex in the active translocation of chromatin domains has been suggested by several studies (Kumaran et al., 2008). Motors have to be anchored to generate force, so it has been suggested that nuclear myosin I (Nowak et al., 1997), which binds to RNA polymerases (Fomproix and Percipalle, 2004), interacts with
certain actin polymers, although these nuclear polymers are different from cytoplasmic filaments (McDonald et al., 2006). So far, the literature on chromosome reorganization events has been mostly focused on evidence of directed movements, but the driving force of these reorganizations, the questions on how chromosomes accommodate these mechanical constraints, the distance over which mechanical constraints propagate, and the kinetics of reorganization events mostly remain elusive.

More challenging than transcription-induced chromosome reorganization events are the dynamics of the genome during DNA repair (Dion and Gasser, 2013), cell differentiation and development (Chalut et al., 2012; Fisher and Fisher, 2011; Krijger and de Laat, 2013), or cancer (Göndör, 2013). The link between genome topology and its functions (Cavalli and Misteli, 2013) is increasingly observed experimentally, but these observations have not yet been tested in quantitative models although preliminary contributions investigated the interplay between molecular crowding and DNA repair in prokaryotes (Delmas et al., 2013) or eukaryotes (Amouroux et al., 2010). The regulatory function of the genome 3D organization and its reorganization propensity cause variegated gene expression levels (Krijger and de Laat, 2013) and define cell-specific genomes, which can be understood quantitatively only with concerted efforts of physicists and biologists. This tour-de-force is not only appealing, but also mandatory to clarify our understanding of genome transactions.

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