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Polymer perspective of genome mobilization

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Abstract

Chromosome motion is an intrinsic feature of all DNA-based metabolic processes and is a particularly well-documented response to both DNA damage and repair. By using both biological and polymer physics approaches, many of the contributing factors of chromatin motility have been elucidated. These include the intrinsic properties of chromatin, such as stiffness, as well as the loop modulators condensin and cohesin. Various biological factors such as external tethering to nuclear domains, ATP-dependent processes, and nucleofilaments further impact chromatin motion. DNA damaging agents that induce double-stranded breaks also cause increased chromatin motion that is modulated by recruitment of repair and checkpoint proteins. Approaches that integrate biological experimentation in conjunction with models from polymer physics provide mechanistic insights into the role of chromatin dynamics in biological function. In this review we discuss the polymer models and the effects of both DNA damage and repair on chromatin motion as well as mechanisms that may underlie these effects.

Keywords

Chromatin motion; Polymer models

1. Introduction

The organization and motion of chromatin underlies nearly all DNA metabolic processes, including repair, transcription, and replication. Study of chromatin motion is therefore crucial in order to better understand these vital cellular processes. Basal motion of chromosomes has been examined with a variety of techniques, from single-particle tracking using Fluorescent Reporter-Operator Systems (FROS) [1–4] in biological systems to utilizing polymer physics to create dynamic models of chromatin. Regardless of the technique used, it has consistently been demonstrated that chromatin can display non-random motion [5–9]. This motion is impacted by several different factors, including tethering of chromatin to external regions such as the nuclear envelope, internal tethering of chromatin by proteins such as cohesin, activity of ATP-dependent remodelers, intrinsic polymer properties of chromatin, and cell-cycle. Burgeoning research also suggests that nuclear organization is impacted by liquid-liquid phase separation of subnuclear

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Declaration of Competing Interest

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compartments [10]. Many studies have confirmed that DNA damage increases chromatin motion [11–13], a process which is thought to underlie repair mechanisms that utilize homology search. Both DNA damage response and checkpoint proteins further influence the motion of sites of damage [12,14].

Here, we discuss the characteristics of chromatin motion, both basally and following DNA damage and repair. We detail biological factors affecting chromatin motion, as well as describe the polymer models that are used to elucidate mechanisms underlying chromatin motion.

2. Chromosomes in motion

It has long been proposed that chromosomes have a distinct organization within the nucleus. Early studies by Carl Rabl revealed that during anaphase, the centromeres are tethered to the nuclear envelope on one side *via* centrosomes, while the chromosome arms and telomeres extend away distally from the centromeres [15]. This Rabl configuration, and the overall concept of chromosome territories, has since been well-studied in organisms ranging from budding yeast to mammals [16]. While chromatin motion is now considered to be an accepted and well-documented phenomena, initial studies investigating movement of DNA in live cells using time-lapsed microscopy suggested that there was only very limited motion in HeLa interphasic centromeres [17]. Consistent with this, FRAP experiments in human cells initially suggested that interphasic chromatin is mostly immobile [18]. However, this concept of stationary chromosomes belies some of the basic biological functions of DNA, and more recent studies have shown the biological importance of chromatin motion.

A prominent example is homologous recombination (HR), which requires that DNA from one sister strand be brought into base pair proximity to the other strand. Even though sister chromatids are proximal to one another from S to G2 in both yeast (<0.7 μm) [19] and mammals (~0.3 μm) [20], they are quite distant from the perspective of base-pairing distances. More dramatic is the high efficiency of ectopic homologous recombination in both mitosis [21] and meiosis [22], suggesting that these genomic territories are less static than previously thought. Using the LacO/LacI-GFP FROS system [2] to label the LEU2 locus near the centromere and analyzing the data using mean-squared displacement, Marshall et al. quantitated chromosome motion within a constrained radius [23]. Marshall et al., together with subsequent studies in a variety of species confirmed that this motion operates in an apparently subdiffusive manner [5,6,24–26]. Chromosome motion behaves as predicted for a long-chain polymer, *i.e*. the motion of every segment of the chain is constrained by adjacent segments, and is confined to a particular radius smaller than nuclear radius. In addition, novel optical methods pioneered by Bonin et al. provide another approach to measure chromatin dynamics, including that of the DNA damage response [27]. Studies utilizing computational polymer dynamics models, based on established polymer physics models like the Rouse chain model (Fig. 1), characterize chromatin motion by placing polymer models into biologically relevant organizations, *i.e* the Rabl configuration [28].

In budding yeast, chromosomes display region-dependent movement, with more constraints on motion at centromeres [24,28] and at telomeres during interphase [24] (Fig. 2). Loci

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present at nucleoli in human cells also display more constrained motion [29]. This activity is consistent with tethering behavior at these regions from a polymer physics perspective. At the centromeres of *S. cerevisiae*, motion is dependent on tethering to spindle pole bodies *via* microtubules, as indicated by inactivation of the centromere using the galactose promoter [28,30]. Telomeres, meanwhile, are constrained to the nuclear envelope *via* the Ku70/80/Sir4/Esc1 complex [24,31–33]. Actively transcribed genes are also tethered to the nuclear envelope, suggesting this dynamic activity as a mechanism for regulating transcription [26,34]. Surprisingly, a course-grained polymer model, with each monomer representing several kilobases of DNA in a Rabl configuration, is capable of replicating the chromatin motion and chromatin territories observed in *S. cerevisiae* [28]. These data therefore confirm the importance of chromatin territories in regulating chromatin motion.

In addition to external tethering, the internal tethering by proteins, *i.e*. looping of a single DNA strand or cross-linking two DNA strands, contributes to the regulation of DNA mobility (Fig. 2). Recently, structural maintenance of chromosome (SMC) protein complexes condensin and cohesin have been shown to extrude loops *in vitro* [35–38], demonstrating that SMC protein complexes are capable of forming DNA loops. Additionally, cohesin has a well-established role in maintaining sister chromatid cohesion and is essential for proper segregation [3,39], demonstrating that cohesin can cross-link two disparate DNA strands. Both types of internal tethering of DNA has proven to have significant ramifications during mitosis. Polymer physics simulations showed that chromatin loops were sufficient to transform a spherical coil of chromatin into an organization mimicking mitotic chromosomes [40]. In *S. cerevisiae*, pericentric cohesin, which links together centromere loops (C loops) [41–43], coordinates the motion of adjacent chromatin strands, as indicated by DNA polymer modeling in conjunction with *in vivo* observations *of S. cerevisiae* [44]. Polymer simulations of the budding centromere, where each monomer represented 10 nm of DNA, demonstrated the condensin-mediated DNA loops, cross-linked by cohesin, displayed the same type of coordinated motion observed *in vivo* and predicted that pericentric DNA loops were sufficient to explain the pericentric chromatin's organization [44]. Cohesin has been proposed to play a role in DNA motility in the repair response [45,46], which will be further explored below.

Given that internal tethering of some polymer models is sufficient to replicate some biological phenomena, we wish to point out a special type of internal tethering that may have a significant impact on chromatin organization. Reconstituted cohesin complexes from *S. pombe* could topologically encircle DNA and slide along a DNA strand [48], suggesting that cohesin can act as a slip ring if two cohesin complexes dimerize or if a single cohesin can slip along two DNA strands. Slip rings can be thought of as mobile cross-links through which the chromatin chain can slide. Moreover, reconstituted condensin from *S. cerevisae* was able to translocate along a taut DNA strand while bound to different DNA strand [35], demonstrating condensin's ability to cross-link disparate DNA molecules while remaining mobile, effectively mimicking a slip ring. Note that the coiled-coil proteins of condensin, SMC2 and SMC4, are extremely flexible (persistence length of 4 nm), allowing them to adopt numerous configurations [49]. A cross-linked polymer chain is a unique type of entangled network that imparts predictable deviations from the behavior of the Rouse chain model (Fig. 3). Most importantly, cross-linked chains can exhibit different stages of

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relaxation at different time scales [50,51], and represent a novel kind of "topological gel." Such a topological distribution of stress was originally proposed in the Edwards–de Gennes reptation tube model [52] and was demonstrated in a study of the chemistry of polyrotaxanes [53]. Topological gels, also known as hydrogels, can reversibly swell upon addition of water. This robust, reversible swelling is possible due to the redistribution of tension by slip-link connections [53]. In 1983, Earnshaw and Laemmli demonstrated that chromosomes could reversibly swell by varying the ionic concentration of the solvent [54]. Given this classic observation, and cohesin and condensin's ability to cross-link DNA strands and form loops in low-tension chromatin regions [35–38], chromosomes could be considered a form of topological gel. Just as chromosome territories and mitotic chromosome organization can be modeled by the tethering of polymers, perhaps other biological phenomena are a consequence by chromatin's organization into a topological heterogenous gel.

Chromosome motion further is impacted by biological factors such as cell cycle and ploidy. In S phase, there is decreased motion that is restored to G1 phase levels upon inhibition of replication with hydroxyurea treatment [24]. This cell-cycle specificity is consistent with regulation of sister chromatid cohesion by cohesin, indicated by yeast studies that show that cleavage of cohesin subunit Scc1 increases chromatin motility in S phase but not G1 [45]. In addition, haploids appear to have greater chromatin mobility compared to diploids [51]. While the exact mechanism is unknown, overall increased motility at certain loci in haploids may facilitate HR following DNA damage, whereas less motility is needed in homologous chromosome-containing diploids.

Processes that rely on ATP, such as ATP-dependent chromatin remodelers, also impact the motility of DNA. ATP-dependent chromatin remodelers play an important role in regulating chromatin structure, and therefore influence nearly all DNA-dependent processes [55]. These remodelers facilitate transcription and repair by shifting nucleosomes and therefore increasing DNA accessibility. One such remodeling complex, Ino80, is recruited to double stranded breaks (DSBs) *via* transcriptional activator VP16 [56] and phosphorylated H2A [57] and requires the actin-related protein Arp8 for its remodeling activity [56,58]. Recruitment of VP16 to various chromatin loci increases DNA motion in these regions, whereas motility is reduced in VP16-targeted cells lacking Arp8; similarly, recruitment of Ino80 also enhances motility at various loci [8]. These data therefore confirm the importance of ATP-dependent processes in influencing motility of chromatin.

2.1. Chromatin modeling and polymer physics

The higher order organization of chromatin is complex, consisting of a series of different structures (loops, fractal globules, etc.). A useful tool in exploring these dynamics is polymer models, in which the genome is assumed to take the form of a number of different chains [44,59–61]. Marko and Siggia were the first to suggest that the DNA strand can be modeled by a worm-like chain [62] (see Fig. 1). Since then, improvements to this model have incorporated subnuclear properties such as excluded volume and hydrodynamic interactions [63]. These models allow for an exploration of the intrinsic polymer-like properties of chromatin that regulates its motion. Furthermore, results from these models suggest that polymer-polymer phase separation underlies the compartmentalization of DNA

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domains, including regions such as the pericentromere and nucleolus [64–67]. Here we will discuss common components of polymer models used to characterize chromatin motion.

Within the nucleus, thermal forces, which underlie Brownian motion are caused by the collision of molecules (water, proteins, etc.) with each other. The collisions occur many times a second and are randomly oriented, so that a monomer would move in a "random walk" of diffusive motion [68]. In terms of thermal energy, each collision transfers an energy of k_BT, where k_B is the Boltzmann constant and T is the absolute temperature. The viscosity of the environment and the size and shape of the monomers will affect the thermal motion of the polymer. The Einstein relation, $D = \frac{k_BT}{\gamma}$, combines the thermal energy, k_BT, with a drag term, γ, to describe diffusive motion. For a spherical monomer, drag is defined as $\gamma = 6\pi\eta R$, where η is the viscosity of the nucleus environment and R is the radius of the sphere. In particular, the nucleus' environment is very viscous, meaning that when the driving force of a molecule is gone, the particles will immediately stop in their course until another driving force acts upon them [68,69]. The composition of the nucleus also allows it to exhibit both viscous and elastic behaviors at different time scales; in other words, the nucleus behaves as viscoelastic material [70]. The magnitude of external force acting on chromatin compared to the magnitude of random noise and drag influences the degree of directed motion. In the nucleus, these magnitudes are similar, resulting in a reduction in directed motion. Excluded volume, on the other hand, is defined as the portion of space occupied by a polymer (a section of a chromosome) that cannot be occupied by another part of that same polymer. This force therefore results in an energy penalty between two overlapping segments of chromatin and can be increased or decreased by monomer-monomer repulsion or attraction. The repulsive/attractive force between monomers can be altered by solvent conditions [71]. In polymer modeling, excluded volume has been used to appropriately model inter- and intra-chromatin chain interactions, or topologically associated domains [65].

Lastly, polymer stiffness will affect polymer motion. Polymer stiffness is frequently defined using persistence length (L_p). Paraphrasing from Rubinstein and Colby [71], persistence length is the length scale at which local correlations between polymer bond vectors decay. Informally, persistence length is the length over which a polymer is stiff. The persistence length of naked DNA is 50 nm [71]. Given that a base pair of DNA is on average 0.34 nm, the motion of the first and last base pairs of a 100 nucleotide oligomer (34-nm contour length) of DNA would be highly correlated, while the motion of the first and last base pairs of a 1 megabase chromosome (340-μm contour length) would not be correlated (on the basis of persistence length). The relationship between chromatin motion and persistence length is not intuitively obvious. A metric commonly used to characterize polymers is the radius of gyration, $R_g2 \overset{\text{def}}{=} \frac{1}{N}\sum_{i=1}^{N}\left(\overrightarrow{R}_i - \overrightarrow{R}_{cm}\right)^2$ where $R_g{}^2$ is the square radius of gyration, N is the number of monomers, $\overrightarrow{R}_i$ is the position vector of monomer i, and $\overrightarrow{R}_{cm}$ is the position vector of the polymer's center of mass [71]. The radius of gyration describes the average radius of the sphere created by a polymer chain at its highest entropic state, roughly describing the volume of the polymer. The radius of gyration is dependent on the stiffness of the polymer (L_p) and contour length of the polymer [71]. The radius of gyration of DNA is usually larger

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than that of the cell nucleus, implying that other factors influence chromosome condensation. For instance, the radius of gyration for the bacterial chromosome, modeled as an ideal random chain, is 13 μm. This is ten times the length of a typical bacterial cell. The largest of budding yeast has a radius of gyration of 2.9 μm, nearly three-fold the radius of the nucleus. The largest human chromosome, chromosome 1, has a radius of gyration of approximately 376 μm. In contrast, a human nucleus is approximately 10 μm.

A measurement that is commonly used to inform polymer models of chromatin is mean squared displacement (MSD). MSD describes the diffusive exploration of a polymer locus, calculated as $MSD(\tau) = \langle (r_{t+\tau} - r_t)^2 \rangle$, where r is the position of the polymer at time t, and τ is the lag time, and the average, denoted by $\langle \ldots \rangle$, is taken over all observed values of time t. The MSD plot describes whether the motion of a particle, *i.e.* a fluorescently labeled subset of a chromosome, is a random walk (*e.g.*, gas law), directed (also called superdiffusive; *e.g.*, motion that is driven by ATP-dependent forces), or subdiffusive (random walk of a chain of beads, where a given bead is constrained through its linkage to the next bead). For a random walk (purely diffusive motion), MSD is linearly related to the time lag (τ) as $MSD(\tau) = 6D\tau$ where D is the diffusion coefficient. In general, the relation can be written as $MSD(\tau) = 6D\tau^{\alpha}$ where α is the scaling exponent. The scaling exponent, α, is found by the initial slope of the MSD curve. If $\alpha > 1$ the motion is superdiffusive, while if $\alpha < 1$ the motion is subdiffusive and, as described above, $\alpha = 1$ indicate regular diffusion.

Both confinement and tethering can lead to subdiffusive motion. While the shape and mechanics of the nucleus are regulated by chromatin polymers, they are also constrained by lamins in the nuclear envelope [72]. In addition to functioning as a location for chromatin tethering, the nuclear envelope acts as a physical boundary for polymer diffusion. This boundary is therefore an important parameter to consider when developing models of nuclear polymer dynamics. Such boundaries can be applied to polymer models of chromatin dynamics in order to account for this constraint, and can be manipulated in order to evaluate effects such as the rigidity of the boundary [59,73].

MSD of a polymer model will increase with increasing persistence length (L_p). From the perspective of the spring-like characteristics of chromatin ($k_s = k_B T/(L_p)^2$) [69], the spring constant increases with increasing temperature (Boltzmann constant $k_B T$) and decreases with increasing persistence length. This reflects the fewer entropic states that chains with greater persistence lengths can adopt. A weaker spring will exhibit more variance in fluctuations and may therefore exhibit greater motion. Using our chromosome dynamics simulator, we have shown that MSD of a polymer model of a doubly tethered circular molecule of DNA (simulating a dicentric plasmid, Fig. 4C, solid lines) increases over a length scale of L_p from 5 to 500 nm in the absence of histones compaction and condensin. However, internal tethers (Fig. 3), such as cross-linkers and loop extruders, modify this behavior, and the relationship between MSD and L_p is non-monotonic. The MSD increases from L_p 5–50 nm, with L_p = 50 being a suitable fit between simulation and experimental DNA motion in our model of the pericentromere [44]. Upon simulated addition of histones and condensin-mediated loop extrusion, MSD decreases in polymers with L_p in the 200–500 nm range (Fig. 4C, dashed lines). It has been shown in Rouse simulation models that MSD decreases with increasing L_p at short timescales, and increases with increasing L_p at long

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timescales [74]. However, the Faller work [74] only examined chains over a persistence length range of 1–5 monomer diameters. While chromatin changes that increase or decrease L_p will clearly manifest as changes in MSD, the relationship between these parameters cannot be simply deduced from experimental observations in the complex cellular milieu.

Multiple studies have supported roles for subdiffusive and directed motion underlying the motility of DNA [13,14]. While initial studies appeared to point towards solely random subdiffusive motion in chromatin dynamics [23], further studies suggest that this motion may not be entirely random [5–8]. In the budding yeast pericentromere, ATP-inhibitor sodium azide inhibits the radius of confinement by approximately 80 %, pointing towards a strong role of ATP-dependent processes [47]. Despite the dependence on ATP, it is appropriate to think of these energy-consuming machines in aggregate as increasing the temperature of the system, and thus the ATP-dependent motion remains random in trajectory. This dependency on non-thermal ATP-dependent forces is conserved in other species, including bacteria [7]. In contrast, a role has been proposed for microtubule-dependent directed motion of damaged DNA [9]. The oftentimes non-random motion of DNA is consistent with other factors modulating motility, such as the aforementioned tethering of centromeres and telomeres, and intrinsic loop modulators such as cohesin and condensin.

While MSD is a useful and highly utilized method of analyzing chromatin motion, there nevertheless exists several caveats that must be considered when interpreting these results. One such consideration is the size of the labeled array, such the size of the lacO array in biological studies, or the number of labeled beads in a Rouse model. Using such a model, Lawrimore et al. found that the larger the labeled array of chromatin, the less motion is observed with an MSD plot [44]. Smaller fragments of labeled DNA allow a more accurate measure of diffusive motion relative to a larger population of labeled DNA. Imagine trying to assess the average motion of one fan in a large stadium. If you measure the motion of 1000 fans as one “spot,” the centroid of the 1000 fans may not appear to move. If instead you have to ability to detect a single fan, you will be able to track when the individual moves away from the aggregate.

In addition to the size array, the sampling rate also influences the interpretation of results. Several studies have observed differences in chromatin motion when examined at different time scales, which has led to the identification of different types of movement or chromatin characteristics [51,75]. As mentioned above, a network of cross-linked chains exhibits different stages of relaxation at different time scales [50,51,74]. Therefore, the time scale is an important variable in designing experiments/models and interpreting results.

A major challenge in applying concepts of polymer dynamics to chromosomes is predicting dynamics in a regime where the chains are exceedingly long, entangled *via* strand crossing and/or slip link cross links (*e.g.* cohesin and condensin) and heterogeneous in chain properties. Multiple models, such as the freely jointed, worm-like, and Rouse chains, have been devised in an attempt to describe the polymer-like characteristics of chromatin (Fig. 1). The freely jointed chain (FJC) is the simplest polymer model, consisting of a series of rigid, equal-length segments connected together by loose joints. Each segment has a length of b

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(twice the persistence length), and there are N of these segments, resulting in a total polymer length of L = Nb. However, due to entropy a polymer is rarely a straight line in a solvent, the chain must be in a collapsed form. Therefore, the joints between consecutive segments are bent at any angle such that the two segments do not overlap. The radius of gyration, set by the persistence length and the contour length (length of the polymer when it is maximally extended) of the polymer, characterizes these chains [76].

An alternative model, the worm-like chain (WLC), can provide additional information on the polymer properties of DNA. Unlike the FJC model, the WLC lacks sharp joints between segments, and instead is smooth and flexible along its length, more closely resembling that of a DNA strand [62,76]. The radius of gyration of the worm like chain can be determined by its persistence length L_p.

The Rouse model, also known as the simple bead-spring model, incorporates many of the aforementioned characteristics of a polymer chain [77] (Fig. 1). Instead of consisting of straight lines, each segment in this model is assumed to be a sphere or bead. Each sphere connects to the neighboring bead *via* a spring. This spring system simplistically embodies all the attractive and repulsive interactions of a polymer. One caveat, however, is that interactions only occur between neighboring beads. In addition, this model allows for the possibility of beads to overlap, although this can be prevented by assuming the presence of excluded volume forces. ChromoShake, for example, modifies the Rouse model by including an excluded volume metric, and simulating the pericentromere by the addition of cohesin rings and condensin springs [44].

The power of these models is their ability to provide experimentalists with intuition in an environment very foreign to our own inertia-dominated world. It is remarkable how far the simple models go toward helping us understand sub-diffusive motion, effects of tethering, chain softening or stiffening and the contribution of non-thermal ATP-dependent fluctuations. However, as the fields advance, we must remain critical of the limitations inherent in course-grain analyses. For instance, we can find citations for chromatin stiffening [78] or chromatin flexibility [79] that drives increased motion. We appreciate that models predict a change in relation between chain stiffness and motion depending on the timescale of analysis and, depending on the geometry of the system, there can be non-monotonic behavior of chain stiffness vs. motion (Fig. 4).

An additional feature of these models is the simplification of the topology of the chains. In the polymer world this is referred to as phantom chains. The chains are free to pass through one another, a concept that can be rationalized due to the function and abundance of enzymes such as topoisomerase II that can cut one chain and pass another one through. As we discover new function-driving features of the chromatin polymer (*e.g.* loops) and interact more with the polymer models, we realize that considerations such as polymer fluctuations in the tangled regime may be important in particular regions or sub-domains of the nucleus. Entanglements are topological constraints resulting from chains that cannot cross through one another. The lateral displacement of a chain in an entangled regime is prohibited. It can only “snake” or “reptate” through the network in a one-dimensional diffusive motion.

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In the un-entangled state, diffusion is inversely proportional to contour length (as length increases, diffusion coefficient decreases), however for the entangled state, diffusion is inversely proportional to the square of the number of links/chain (as number of cross-links squared increases, diffusion coefficient decreases) [80]. One can consider domains such as the nucleolus and the pericentromere to most likely to be in the entangled regime due to enrichment of slip ring cross-links such as condensin and cohesin. However, it is still possible for polymer systems to be in the entanglement regime without these cross-links.

To understand how chain stiffness is related to motion in an entangled regime we have simulated the motion of bead-spring chains with and without cross-linking proteins that introduce loops (Fig. 2). This is a simplification of an entangled network, but that nonetheless serves as a simple geometry to assess the consequences of topological constraints. For chains without cross-links, one observes a monotonic relationship between L_p and MSD. As we increase L_p, the spring constant decreases and MSD increases (Fig. 4 and see above). In contrast, for chains with cross-links, we see a non-monotonic relationship between L_p and MSD. Interestingly, for flexible chains (L_p 5, 50) MSD increases, while for stiffer chains (L_p 200, 500) MSD decreases. There are also major differences in the structural organization of the chains as a function of L_p in the presence of cross-links. For flexible chains, the loops are dynamic and more or less distributed evenly over a range of distance. In contrast, for stiffer chains the loops are less dynamic and cluster into discrete size classes (Fig. 4D). Recently, reconstituted budding yeast condensin was found to form a particular type of loop within loop structure, dubbed a Z-loop, that allows for two condensin complexes to partially traverse each other resulting in bi-directional loop extrusion [81]. Our model, which allows for the presence of loops within loops, also exhibits these Z-loops. These simple polymer simulations reveal critical insights into chromosome organization and motion. The results indicate that chromatin stiffness (L_p) and cross-linkers may function as tuning forks for higher order chromosome organization. Secondly, we need to appreciate that chain properties will exhibit non-monotonic behavior as soon as we invoke the simplest of topological constraints.

3. DNA damage and its effects on DNA motility

3.1. Double stranded breaks as a model of DNA damage

Double-stranded breaks (DSBs) occur endogenously in organisms as a byproduct of DNA replication, collapsed replication forks, transcription, and as a consequence of repair intermediates [82]. These breaks are associated with an increase rate of mutations and genomic instability that are fundamental to the development of cancer. As a model for DNA damage, DSBs can be generated by exposing cells to gamma ionizing radiation (IR), DNA damaging drugs from the bleomycin family (phleomycin, zeocin), or more targeted approaches such as induction of the homothallic (HO) switching or I-SceI endonucleases [83]. The method of DSB-induction (*e.g.*, IR vs. endonucleases) differentially affects the process of repairing a break, including resection and recruitment of repair proteins [84], making this an important consideration in interpretation of DNA damage studies. While endonucleases generate a product with 3′OH and 5′-phosphate ends, IR-based approaches tend to yield a more heterogenous population of intermediate products, and bleomycin

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creates a 3′-phosphoglycolate end [84–86]. These disparities likely explain differences in the cellular responses to the break. In addition, higher concentrations of zeocin are required in yeast to instigate damage-induced motility [87], another possible confounding factor when interpreting DNA damage studies.

3.2. Effects of DNA damage on chromatin motion

Damaged DNA often is followed by a homology search within the genome in order to facilitate HR. Repair of DSBs commonly occurs through either non-homologous end-joining (NHEJ) or HR [88]. NHEJ is the favored repair pathway in mammalian cells, while yeast rely more heavily on HR [89]. While NHEJ ligates the ends of the broken strands together, often resulting in small mutations around the break point, HR requires a homologous template located elsewhere in the genome in order for repair to begin. Which repair pathway is favored is partially influenced by the cell-cycle. As sister chromatids are the preferred template for repair, HR is suppressed in G1 phase, whereas NHEJ can be utilized throughout the cell cycle [90,91]. Homology search is associated with changes in DNA dynamics and higher order structures. Lisby et al. first demonstrated in 2003 that DSBs on two different chromosomes in yeast (III and IV) both co-localized with the same Rad52 repair foci, implying that one or both chromosomes must migrate [92]. Furthermore, in a yeast strain where a single DSB was induced without a homologous donor (*e.g.*, a DSB that cannot be easily repaired), the DSB moves towards the nuclear periphery [93], suggesting a mechanism for constraining the location of certain breaks. Induction of a DSB in G1 in homologous-donorless cells followed by 3C analysis revealed no change in interaction frequency, suggesting that homology search precipitates damage-induced motion [93]. However, direct analysis of motion of a DSB in G1 cells reveals a global increase in chromatin motility [94]. The single cell analysis is a more direct measure of motion, while 3C is a population-average of interaction frequency. One way of reconciling these results is to consider DNA damage as an increase in temperature. From this perspective, the increase in motion will not result in a change in 3C since it is the interaction frequency and not necessarily the absolute distance that is perturbed. In a diploid yeast strain, a DSB in S/G2 phase increases movement of both the broken chromosome and its unbroken homologue, as measured by MSD analysis [95]. This is consistent with a DSB increasing activity overall in the genome in the subsequent homology search.

Prior to homology search, motion of the damaged DNA is also regulated during the resection phase. Using the ParB-INT FROS system to fluorescently label resected DNA near a DSB, Saad et al. found that there is a transient confinement of the DNA early in resection [96]. This activity is consistent with the need for more stationary DNA during resection, allowing for the proper protein machinery to bind.

The motion of chromatin, as mentioned previously, is constrained at centromeres and telomeres, an activity which regulates the response to DNA damage. While it was initially reported that release of centromeres from microtubules drives DNA motility [30], this contradicts further studies which suggest centromeres remain tethered to spindle pole bodies *via* microtubules following formation of nearby DSBs [4]. An alternative interpretation is that rather than centromere release, the mechanism that clusters centromeres is relaxed,

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which increases their configurational degrees of freedom [97]. This result may be due to either binding and unbinding processes, or relaxation of DNA tethering. However, detachment of telomeres, as shown by using yKu70/Ku80 mutants, increases the mobility (radius of confinement) in a manner similar to that of cells damaged with phleomycin [4]. It has further been suggested that telomere tethering is essential for the proper repair of DSBs [98]. These data therefore support the hypothesis that telomere tethering regulates DSB-induced chromatin motility.

In addition to extrinsic tethering of centromeres/telomeres, it has also been hypothesized that the intrinsic properties of DNA regulate its motion as a polymer and contribute to DNA damage-induced motility. In particular, the rigidity of DNA (measured by the aforementioned persistence length) has been proposed to underlie DSB-induced chromatin motility. Analysis of super-resolution images of LacI-GFP labeled foci suggests that zeocin treatment induces a stiffening of the chromatin, rather than decondensation [99]. Furthermore, while damaged chromatin has increased motility when measured at longer time scales, I-SceI-induced DSBs in budding yeast causes the chromatin to have reduced motility at short time scales (10 and 100 ms) [51]. This result is again consistent with an increased persistence length (chromatin rigidity) due to chromatin modifications. In addition to rigidity, internal tethering by proteins such as cohesin has been suggested to play a role in damaged DNA motility. Expansion of the cohesin barrel (visualized by Smc3-GFP) was observed in yeast treated with phleomycin or induced with a single DSB with HO endonuclease [4]. This suggests that DSB cause changes in the internal chromatin tethering regulated by cohesin, which may underlie corresponding changes in motility.

As an early and well-characterized response to DNA damage, phosphorylation of histone H2A variant X (γH2AX, or γH2A in yeast) has also been implicated in the dynamics of the DNA damage response [100]. This modification takes place rapidly (within several minutes) following IR in yeast and mammalian cells [101,102], and covers a roughly 60 kb region on either side of an HO-induced DSB in yeast [103]. Interestingly, in a mutant yeast strain lacking phosphorylatable H2A, zeocin treatment resulted in decreased intra-chromosomal distances in the mutant compared to the wild-type [99]. The modest increase in motion in the mutants indicates that histone modification is integral to the physics that underlies the magnitude of DNA-damage induced motion. However, as γH2AX is an early event in the DNA damage response it is likely that additional downstream events contribute to the increase in motility as well.

An additional component to the DNA damage response is the INO80 ATP-dependent chromatin remodeling complex, which binds to phosphorylated H2A [57] and, as previously mentioned, influences DNA motility [8]. In zeocin treated yeast, mutants of Ino80 subunits Arp5 and Arp8 have a decreased radius of confinement compared to wild-type cells [87], suggesting that this remodeling complex influences motility at damaged loci.

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4. Repair of damaged DNA and its effects on DNA motility

4.1. Homologous recombination repair pathway

Homologous recombination involves a number of steps in the repair process. Fig. 5 overviews this pathway in both a traditional linear model, as well as what is observed in ell biology and polymer models. Following the formation of the DSB, the MRX complex (Mre11, Rad50, Xrs2 in yeast; MRE11, RAD50, NBS1 in mammalian cells) is recruited to the site of the break [104]. The MRX complex, together with the Sae2 protein in yeast (CtIP in mammals) resects the 5′ end of both strands [105]. The nucleases Exo1 and Dna2, as well as the RecQ helicase Sgs1 in yeast (BLM in humans) provide further resection of the DNA [106,107]. The resulting single-stranded DNA (ssDNA) is then bound by heterotrimeric replication protein A (RPA), which protects the ssDNA from excessive nuclease activity [108]. In addition to its role in resection, the MRX complex triggers the activation of DNA damage checkpoint pathways, which includes the phosphoinositide 3-kinase related protein kinases (PI3Ks) Tel1 (ATM in mammals) and Mec1 (ATR in mammals) [109]. The MRX subunit Xrs2/NBS1 recruits Tel1/ATM to the site of DSBs [110], while RPA-coated ssDNA overhangs are necessary to recruit Mec1/ATR and its associated protein Ddc1 [111,112]. The heterotrimeric complex 9-1-1 (Ddc1-Rad17-Mec3 in budding yeast; RAD9-RAD1-HUS1 in humans), which is loaded at ssDNA/dsDNA junctions in a Rad24-RFC-dependent manner [113], and stimulates Mec1 activation [114,115]. Mec1-Ddc1 further phosphorylates Rad53, a major checkpoint kinase involved in many downstream processes, in a Rad9 (53BP1 in mammals) dependent manner [116].

In order to allow recombination repair to proceed, Rad52 binds to DNA-bound RPA, overcoming the inhibitory effects of RPA and allowing Rad51 to bind [117,118]. Rad51, a mitotic recombinase, oligomerizes onto the ssDNA to form a nucleoprotein filament, also referred to as a presynaptic filament. Presynaptic filaments are responsible for searching for homology and promoting strand invasion [119]. Rad54 then interacts with Rad51, and promotes the formation of displacement loops (D-loops) [120,121]. Following DNA synthesis on the invading strand, HR can proceed by pathways such as synthesis-dependent strand annealing (SDSA) or double-stranded break repair (DSBR) that generates double Holliday junctions [119].

4.2. Role of cohesin in DNA repair

The SMC protein cohesin is also an important component of the DNA damage and repair response [123]. Cohesin is composed of a Smc1/Smc3 heterodimer, as well as the Scc1 (Mcd1/Rad21) and Scc3 subunits [124]. Loading of cohesin onto DNA is regulated by the Scc2-Scc4 complex (NIPBL-A or NIPBL-B-MAU2 in humans) [125]. Cohesin regulates sister chromatid cohesion by tethering the sisters together, promoting proper segregation during mitosis. Due to the close proximity of sister chromatids, mitotic cells therefore preferentially use sister chromatids as a template for homologous recombination [126,127]. Studies in budding yeast further confirmed that cohesin is recruited to DSB sites in late S and G2, with a dependency on Scc2/Scc4 for cohesin localization to the site of the break as well as for proper sister chromatid cohesion [103,128,129]. In human cells, cohesin localizes to sites of DNA damage [130], although it is restricted to a smaller range around the site of

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damage compared to yeast [131]. Human cells depleted of cohesin display enhanced levels of damage measured by γH2AX staining [131,132]. Interestingly, timely removal of cohesin may also be important for continuation of proper repair. In budding yeast, a separase-resistant allele of Scc1 displays reduced resection of DSBs, and reduces the efficiency of resulting repair product formation [133].

Beyond DNA repair, cohesin may also have a role in regulating transcription through its ability to modulate DNA domains. Cohesin associates with regions of actively transcribed genes [134,135]. The chromatin structure remodeling (RSC) complex, which is a conserved ATP-dependent remodeler essential for transcription of numerous genes, recruits the cohesin loader Scc2/Scc4 at promoter regions [136–138]. It has been proposed that cohesin enables interaction between promoters and enhancers by facilitating long-range interactions in *cis* and looping distal DNA domains closer together [139,140]. However, further work is needed to elucidate this mechanism.

4.3. Role of cohesin in facilitating motility-induced repair and chromatin motility

Cohesin, as previously mentioned, facilitates sister chromatid cohesion and therefore also repair pathways that utilize recombination between sister chromatids [141]. In budding yeast, cohesin plays a role in regulating the mobility of chromatin during repair. Cleavage of the cohesin subunit Scc1 using a Scc1-TEV protease construct results in increased motion (radius of confinement) of the Rad52 complex in zeocin-damaged cells [45], suggesting that cohesin-mediated cohesion limits chromatin motility. Since cohesin maintains cohesion between sister chromatids, it has further been hypothesized that cohesin prevents recombination between distant DNA sequences by limiting its motion. Consistent with this, cohesin represses end-joining events between distant DSB sites in human cells, whereas those in close proximity remain capable of end-joining [46]. This result suggests that cohesin-mediated DNA motility may affect repair mechanisms.

4.4. Impact of DNA repair proteins on DNA damage-induced motion

As they are recruited to sites of DNA damage, proteins involved in repair have also been implicated in influencing the dynamics of damaged DNA. In particular, various studies have found that Rad51, which binds to the ssDNA exposed following a DSB, influences the motility of damaged chromatin. A diploid yeast strain with mutant Rad51 displays no corresponding increase in radius of confinement following DSB induction with I-SceI, indicating that Rad51 is integral for DSB-induced chromatin motion [95]. Similar results were seen in haploid strains of mutant Rad51 [87,142]. Localization of repair foci are also regulated by Rad51, as non-functional Rad51 localizes to the nuclear periphery whereas functional Rad51 remains internal [45]. Furthermore, the reduced motility of damaged chromatin observed at short time scales is dependent on Rad51 [51]. While the exact mechanism for Rad51 has yet to be determined, polymer physics suggests that an increased persistence length of chromatin (*e.g.*, stiffer chromatin) predicts this pattern of higher motility at larger time scales, and lower motility at shorter time scales [74]. Therefore, Rad51 may be responsible for stiffening chromatin surrounding a DSB. Accordingly, this activity is specific solely to the damaged chromatin – there is no suppression of motility at an undamaged loci in a Rad51 mutant [87].

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In addition to Rad51, various other proteins involved in the repair response such have all been shown to regulate the motility of damaged DNA [4,95,142]. Rad54, Rad9 (53BP1 in mammals), and Mec1 (related to mammalian ATR) mutants in budding yeast all display a decreased radius confinement upon DNA damage [142], as does a Sae2 mutant (CtIP in mammals) [95]. These results suggest that following a DSB, the recruitment of proteins to the exposed ssDNA as an important contribution in HR-mediated motility [11]. In addition to these measures of chromatin motility, repair proteins also affect the intrinsic properties of chromatin. At the yeast pericentromere, phleomycin-induced expansion of the cohesin barrel is dependent on both Rad9 and H2A phosphorylation [4]. These data suggest that repair proteins may also influence intrinsic tethering of damaged DNA. Furthermore, as we have demonstrated that certain proteins affect chromatin motion in a manner dependent on L_p (Fig. 4C), recruitment of repair proteins may also affect L_p and therefore the motility of damaged DNA. However, future studies are needed to explore this perspective.

4.5. Role of liquid-liquid phase separation in DNA repair and chromatin motility

While as previously mentioned, polymer physics elucidates many of the mechanisms underlying the motility of chromatin, recent studies suggest an exciting role for liquid-liquid phase separation (LLPS) in regulating genome organization. The protein HP1 α, for example, is involved in LLPS of heterochromatic regions [143,144], and LLPS underlies the compartmentalization of nucleolar domains [145]. Using a novel CRISPR-Cas9 system in human cells, Shin et al. determined that intrinsically disordered proteins (IDPs) cause phase separation preferentially in low-density chromatin regions [10]. The authors further find that a minimal physical model is consistent with these results, suggesting that there is a lower mechanical energy required for liquid droplet formation in lower-density chromatin regions.

Interestingly, there has also been novel research regarding the potential role of LLPS at sites of DNA damage. In human cells damaged with laser microirradiation, IDPs localize to DSB sites, and quantitative imaging suggests that these proteins form liquid droplets [146]. FUS, a mammalian protein with intrinsically disordered domains that is associated with neurodegenerative diseases, also forms liquid domains at sites of DNA damage [147]. Furthermore, 53BP1, a protein involved in the DNA damage response and checkpoint activation, shows liquid droplet-like behavior at sites of DSBs [148] as does the yeast protein Rad52 [149]. As the yeast homolog of 53BP1, Rad9 is required for damage-induced expansion of the cohesin barrel at the pericentromere [4]; a potential role for cohesin in separation of these domains exists as well. These data therefore suggest that LLPS may play a role in the DNA damage response. One possible theory for the mechanism of action of LLPS could be that the change in viscoelasticity due to phase separation which influences MSD through drag and the scaling parameter α, and therefore also the ability of certain proteins (such as loop-extruding factors) to function. However, the importance of phase separation at these loci, as well as the exact mechanism of how these may influence chromatin motility, remains to be explained.

4.6. Role of nuclear filaments in DNA repair dynamics

Nuclear filaments such as actin and microtubules play important roles in nuclear organization, dynamics, and DNA damage responses [150–152]. While filamentous actin (F-

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actin) is more prevalent in the cytoplasm, these filaments have also been detected in the nucleus of mammalian cells [153] and are regulated in a cell cycle-dependent manner, with polymerization occurring during G1 phase [154,155]. DNA damage further instigates actin polymerization. In mammalian cells, various DNA damaging elements results in an increase of Utr230-labeled nuclear actin filaments [156,157]. Blocking nuclear import of actin using an Ipo9 mutant results in increased numbers of pH2AX foci, implying that actin plays a role in the DNA damage response [156]. Furthermore, actin polymerization inhibitor latrunculin A suppresses recruitment of the DNA damage checkpoint protein ATR, suggesting that actin polymers recruit repair proteins [157]. Consistent with this role, a variety of actin-binding proteins, such as actin related protein (Arp) 2/3, 5, and 9 function in DNA repair (for review, see [152]). The Arp2/3 complex, which associates with actin filaments, localize to DSB sites in human cells, and also promotes the mobility of DSB sites [158]. These findings not only implicate actin and its associated proteins in repair, but also suggest that it may mediate these effects *via* control of chromatin motion.

Further evidence in yeast suggests that actin polymerization specifically influences chromosome motion. In yeast treated with latrunculin A, subtelomeric DNA regions have decreased diffusive motion [159]. Similarly, the efficiency of homologous recombination-mediated repair of HO-induced DSBs is inhibited in latrunculin-treated cells [159]. In addition, chromatin remodeling complex Ino80, which contains several Arps and as previously mentioned influences chromatin dynamics, relies on actin polymers to influence chromatin motility [159]. Nuclear actin filaments may also regulate the motion of damaged heterochromatin. In *Drosophila*, nuclear actin filaments along with myosin facilitate the directed motion of heterochromatic DSBs to the nuclear periphery [160]. Furthermore, as the cytoskeleton is linked to the nuclear envelope *via* linker of nucleoskeleton and cytoskeleton (LINC) complex proteins [161], cytoskeletal actin also has the potential to influence chromatin dynamics. Consistent with this hypothesis, latrunculin treatment decreases mobility of nuclear pore complex (NPC) protein Nup49, as indicated with FRAP experiments, and tethering of actin to Nup49 increases the mobility of subtelomeric DNA regions [159]. These data therefore suggest that *via* the NPC, cytoskeleton actin may also influence chromatin dynamics.

Microtubules influence the motility of damaged DNA [4,162], and may further play a role in DNA repair. Fission yeast (*S. pombe*) is characterized by horsetail oscillatory motion of the nucleus, driven by microtubule motor protein dynein [163–165]. Cytoplasmic dynein has been shown to further influence meiotic recombination *via* its influence on nucleus oscillations [164,166]. In budding yeast, multiple DNA damage agents induce *de novo* formation of nuclear microtubules (damage-inducible intranuclear microtubule filaments, DIMs), while repair proteins Rad52 and Rad51 suppress endogenous DIM levels and are responsible for further damage-induced DIM formation [9]. Furthermore, the catalytic component of the microtubule motor protein Kinesin-14, Kar3, is responsible for directed motion of damaged chromatin [9].

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5. Conclusions

Chromatin motion is controlled by intrinsic properties (*e.g.*, polymer stiffness, entanglements, crowding), extrinsic factors such as cross-linkers and loop extruders (*e.g* condensin/cohesin), tethering by microtubules, ATP-dependent processes, and recruitment of repair proteins to sites of damage, among other possible factors. Polymer models of chromatin have been invaluable to the study of chromatin motion and going forward will continue to provide useful insights into the complex world of chromatin. However, even the simplest of polymer models reveal the non-monotonic relationship between key parameters. It will be important in future studies to keep in mind the limitations of such models, especially in regard to extrapolating results to chromatin motion. Lessons from biology continue to provide a rich experimental space to explore with polymer models, including the contribution of loop-extruding factors to chromatin motion, the impact of repair protein recruitment, and liquid-liquid phase separation.

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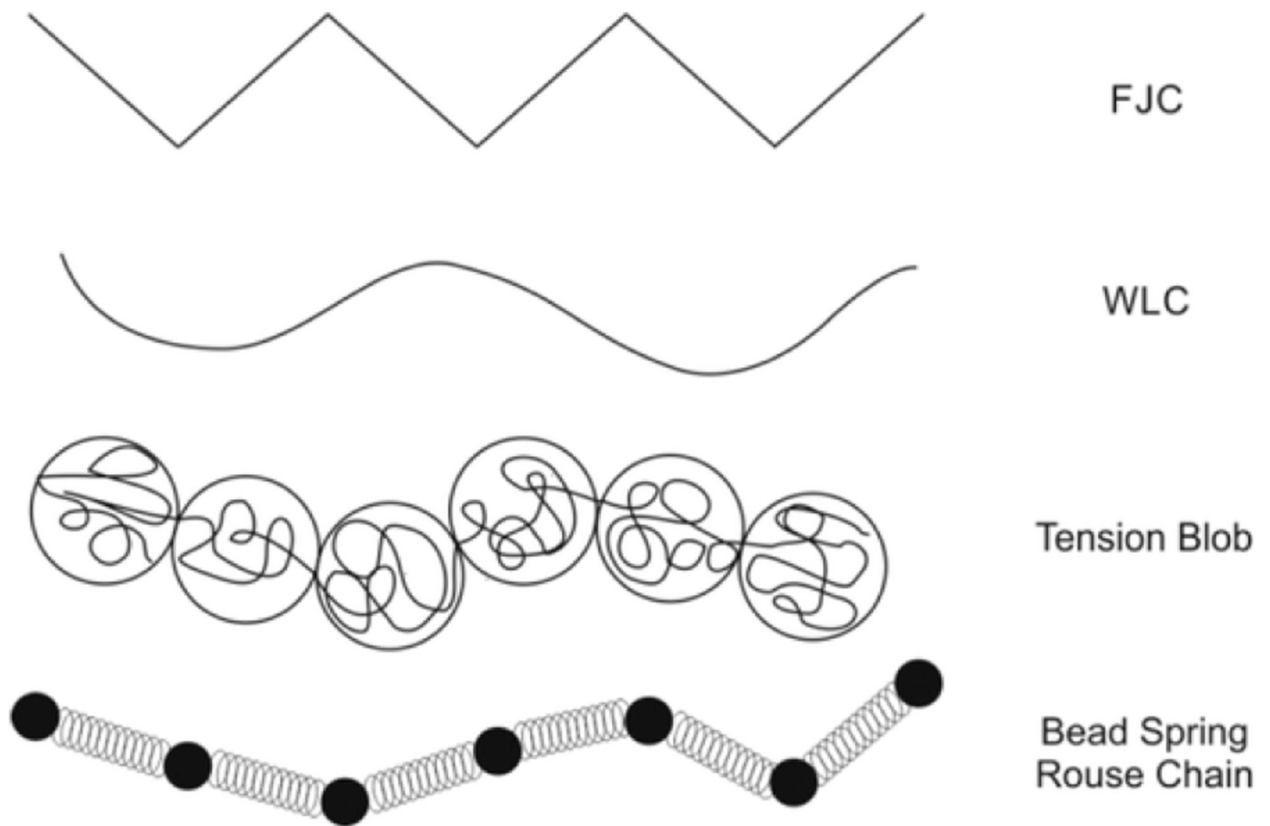

Fig. 1.

Polymer models of flexible chains. A chain can be mathematically defined as a series of rods that are flexible at discrete sites (Freely jointed chain, FJC). A model with a continuously flexible chain (Worm-like chain, WLC) better matches experimentally derived force-extension curves of DNA. Models that capture the dynamics of flexible polymers include tension blobs and thermal blobs. Blobs are small sections of the chain that have defined statistical and mechanical properties. Representing the blob as a spring and modeling the polymer as a bead-spring chain (Rouse chain) has proven invaluable to understanding chromosome behavior.

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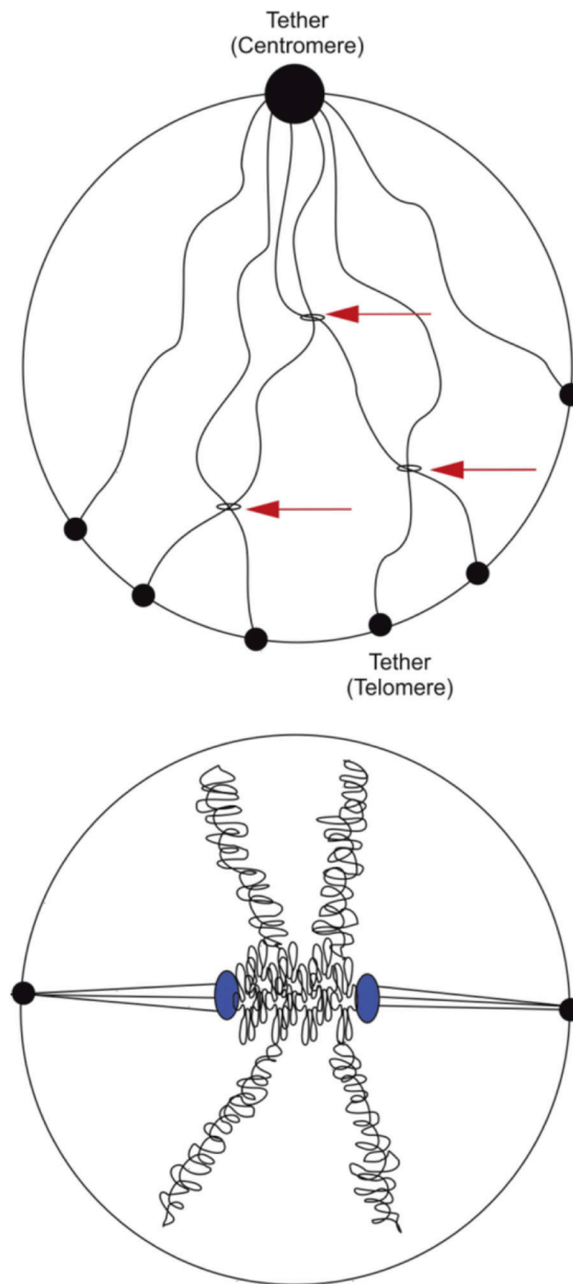

Fig. 2.

Chromosome configuration in budding yeast in interphase (top) and mitosis (bottom).The interphase configuration is known as the Rabl orientation in which centromeres from all 16 chromosomes are tethered to the spindle pole and the chromosome arms extend away from the pole. The telomeres from the 32 chromosome arms are clustered at the nuclear periphery in 6–7 foci. In mitosis, the replicated sister centromeres are clustered between the duplicated spindle poles. The distance between the clusters of sister centromeres is represented as a bottle brush polymer [44,47]. Red arrow- Cohesin rings are one source of internal tether points.

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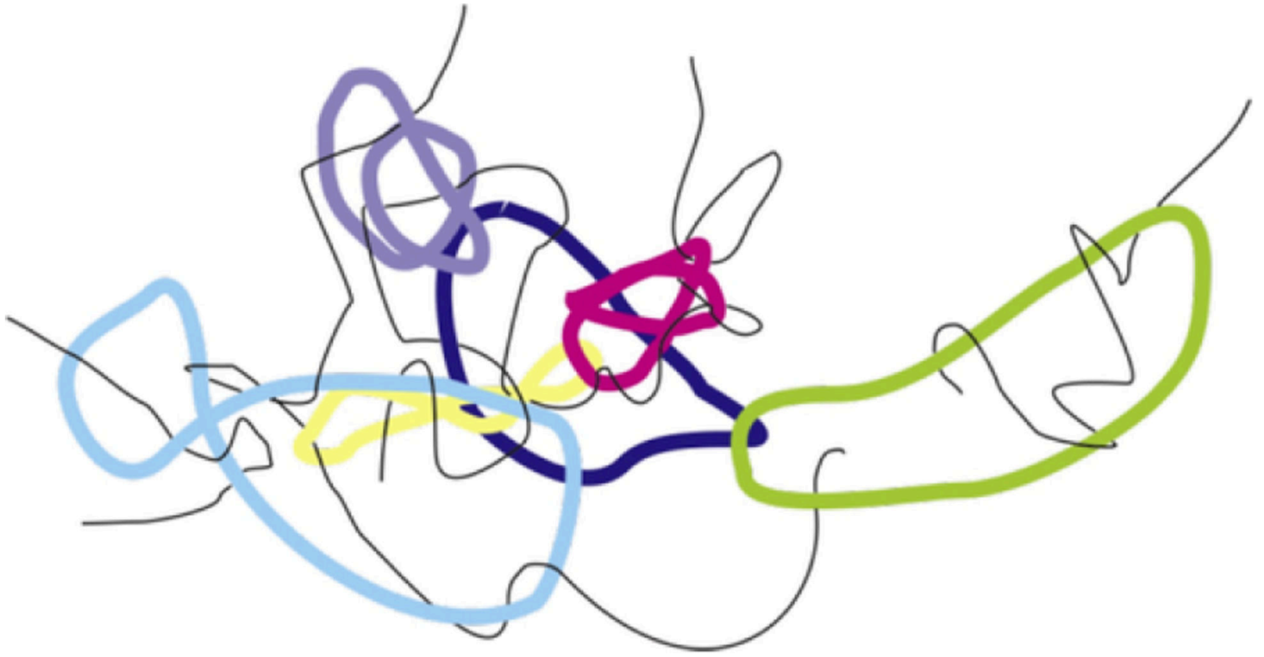

Fig. 3.
Entanglements in polymer melts. The DNA polymer chain (black lines) is linked through various ring polymers (such as cohesin and condensin, indicated by colored rings) and through topological constraints *via* strand crossing (indicated by intertwining between black strands).

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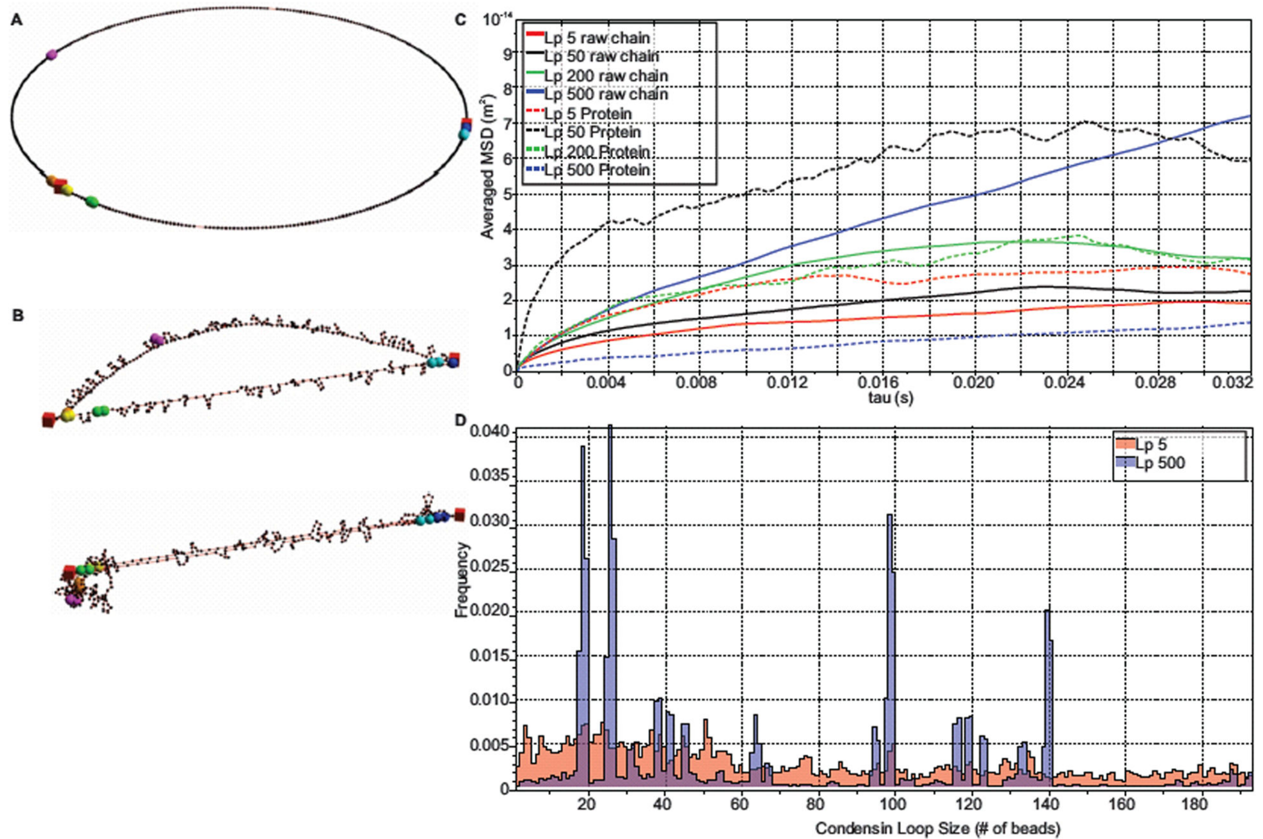

Fig. 4.

Non-monotonic behavior of MSD as a function of L_p in presence of loops. Simulation of Bead-Spring polymer chain. (**A**) Starting configuration of the polymer chain model. The bead spring chain is depicted as black beads along the red line. Two beads representing the centromeres in metaphase of the cell cycle are pinned (depicted as red squares). Histone octamers are modeled as 7-bead loops along the chain. Histone binding is not implemented in the starting configuration. Condensin is depicted as large colored spheres (orange, green, yellow, purple, dk and lt. blue). (**B**) Initializing the code (0.05 ms, near the start of the simulation) results in collapse of the polymer due to condensin compaction and the tendency for the chain to adopt a random coil. The bottom panel (10 ms, at equilibrium state) shows an example of an extruded loop. (**C**) Simulated mean-squared displacement (MSD) as a function of increasing persistence length (L_p) on chains with no histone or condensin (solid lines, Raw chain). Note the monotonic relationship between MSD and L_p. Upon analysis of chains with histone dynamics and condensin loop extrusion (dashed lines, protein chain), the relationship between MSD and L_p is non-monotonic. (**D**) Distributions in the sizes of loops for the protein chains as a function of L_p. The loop size is measured based on the positions of its two attachment sites. For highly flexible chains (L_p =5 nm), the loops are highly dynamic and exhibit a broad distribution. For stiffer chains (L_p =500 nm) the loops cluster into discrete size classes (number of beads/loop). Average bead-bead separation is 10 nm.

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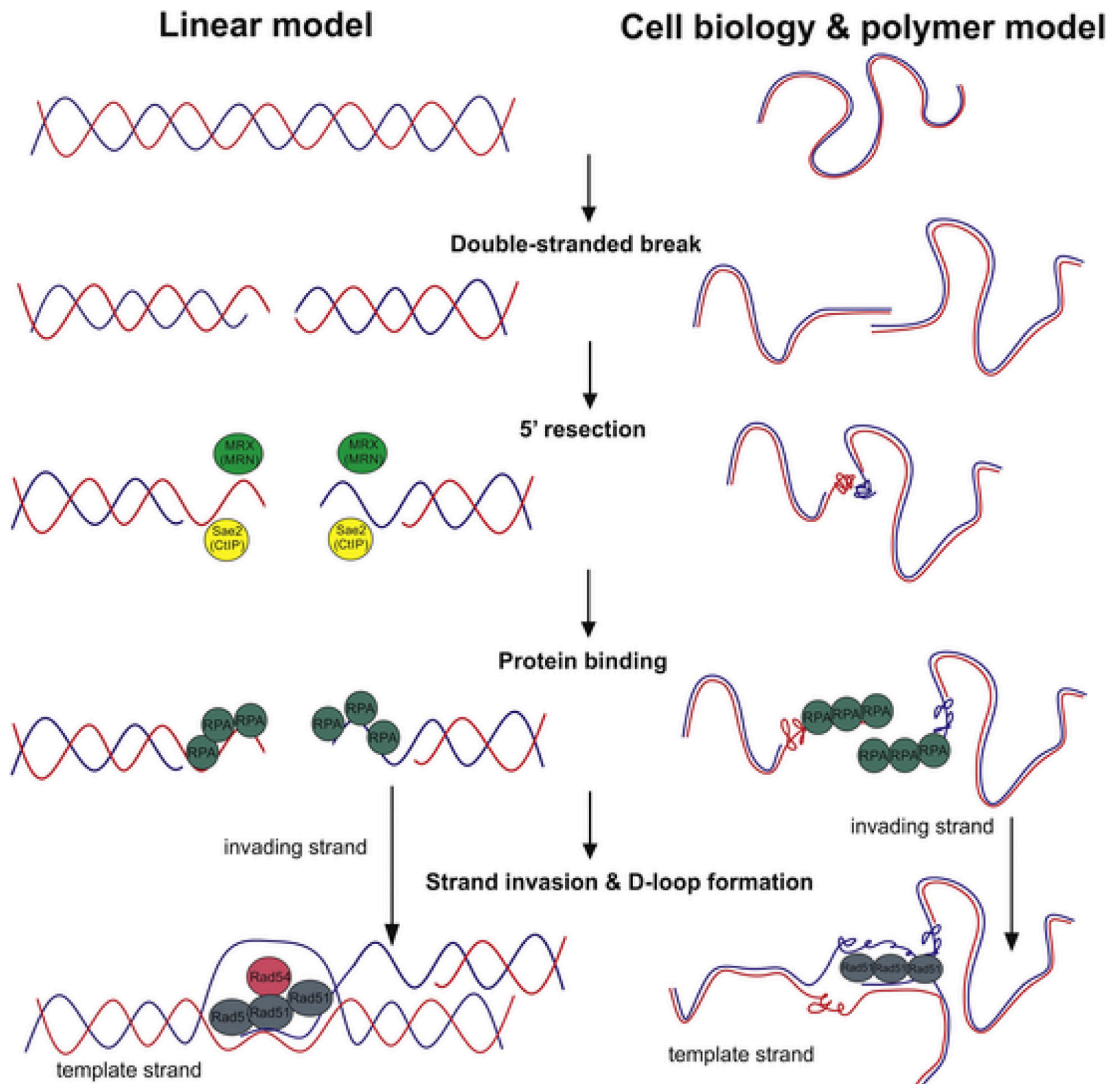

Fig. 5.

Homologous repair pathway of double-stranded breaks in linear and cell biology/polymer models. Proteins names are listed for yeast, and human homologs are given in parenthesis when different from their yeast counterparts. Following a double-stranded break (DSB), the MRX complex (MRN in human) and Sae2 (CtIP in human) binds and resects the 5′ ends. As demonstrated in polymer models, the resulting single-stranded DNA (ssDNA) is more flexible than its double-stranded counterpart, allowing for smaller coils than dsDNA. The ssDNA is then bound by RPA, protecting the DNA from excessive nuclease activity and stiffening the ssDNA [122]. RPA is replaced by Rad51, forming a presynaptic filament, and

allowing strand-invasion and D-loop formation. HR then continues *via* pathways such as synthesis-dependent strand annealing (SDSA) or double-stranded break repair (DSBR).

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