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Emergent properties of mitotic chromosomes

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Abstract

As a cell prepares to divide, its genetic material changes dramatically in both form and function. During interphase, a dynamic interplay between DNA compartmentalization and transcription functions to program cell identity. During mitosis, this purpose is put on hold and instead chromosomes function to facilitate their accurate segregation to daughter cells. Chromatin loops are rearranged, stacked, and compressed to form X-shaped chromosomes that are neatly aligned at the center of the mitotic spindle and ready to withstand the forces of anaphase. Many factors that contribute to mitotic chromosome assembly have now been identified, but how the plethora of molecular mechanisms operate in concert to give rise to the distinct form and physical properties of mitotic chromosomes at the cellular scale remains under active investigation. In this review, we discuss recent work that addresses a major challenge for the field: How to connect the molecular-level activities to large-scale changes in whole-chromosome architecture that determine mitotic chromosome size, shape, and function.

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Visualizing the spatial organization of chromosomes: Mapping DNA contacts and imaging chromatin

Understanding how mitotic chromosome properties emerge requires deep knowledge of their structure. Recent advances in genomics and imaging have allowed us to visualize mitotic chromosomes in unprecedented detail and challenged the field to think beyond hierarchical models of mitotic chromosome structure depicted in textbooks.

The development of a chromosome conformation capture technique (Hi-C), which maps the frequency of inter- and intra-chromosomal contacts genome-wide, has revolutionized how we think about chromosome organization [1]. In interphase cells, a major feature that emerges is topologically associated domains (TADs). These megabase-sized regions of the chromosome contain DNA sequences that physically interact with each other more frequently than with regions outside the TAD and are thought to regulate gene expression. A recent landmark study applied Hi-C to highly synchronized cultured cells to closely track any rearrangements of the genome at the onset of mitosis. The authors showed that during prophase, shorter-range interactions and TADs were replaced by a set of longer-range interactions that uniformly spanned the length of each chromosome [2**]. Combining these observations with extensive modeling, these long-range interactions were proposed to represent a 'spiral staircase' of loops that form perpendicular to the central axis of each chromosome and are broadly consistent with the radial loops observed decades ago by electron microscopy of salt-extracted chromosomes [3].

How do the fundamental units of chromatin, nucleosomes that consist of 2 copies each of histones H2A, H2B, H3, and H4, fit into this picture? A long-standing model in the field was based on hierarchical folding, in which nucleosomes are first organized into 30-nm fibers before being further packaged into the final condensed state of the mitotic chromosome. However, evidence for the formation of 30-nm chromatin fibers in vivo as examined by several techniques was lacking [4–6]. Recent studies made possible by advances in electron tomography have thoroughly dispelled the hierarchical folding model by showing that although nucleosomes are packed at a higher density in mitosis compared with interphase in both cultured vertebrate cells and fission yeast, they are irregularly arranged and lack a discernible higher order structure, appearing more like a molten polymer [7,8]. Moreover, depletion of core histones from *Xenopus* egg extracts revealed that nucleosomes are not required for creating the rod-like shape of mitotic chromosomes, although chromosomes lacking histones were fragile, appearing fuzzy and less compacted [9*]. Thus, nucleosome packing and higher order chromosome assembly may be largely independent processes [10].

Taken together, these studies present a new paradox: how do we reconcile the structured, large-scale DNA loop arrangement seen in Hi-C data with the apparent randomness of nucleosome-level organization in mitotic chromosomes?. In this review, we discuss a number of recent mechanistic studies that provide new clues as to how many distinct molecular activities give rise to higher order chromosome morphology and physical properties.

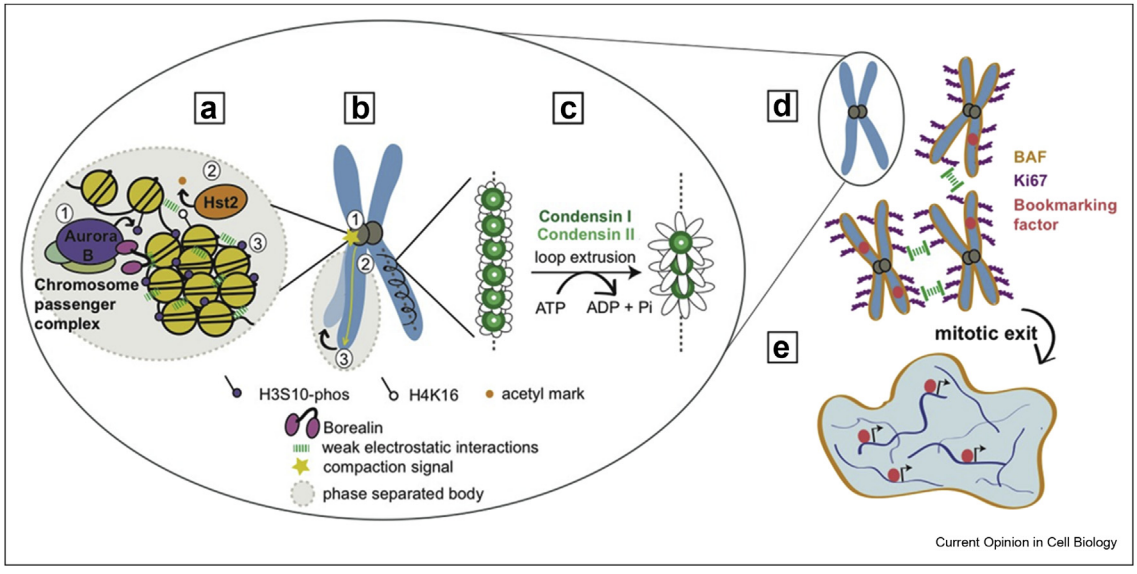
Histone tail modifications and biomolecular condensates drive chromosome compaction

At the nanometer length scale, the current view is that core histones themselves influence higher order chromatin compaction by modulating nucleosome–nucleosome interactions during the cell cycle. Supporting this idea, it was shown that nucleosome arrays reconstituted using histones isolated either from interphase or mitosis resulted in either less or more compact structures, respectively [11*].

Regulation at the level of the nucleosome appears to involve a series of post-translational modifications (PTMs) on histone tails that are either erased or added [12] and contribute to compaction of chromatin. The best understood example begins with Aurora B, the kinase subunit of the chromosomal passenger complex (CPC), which performs numerous roles in mitosis in all organisms [13] (Figure 1a). In budding yeast, it was shown that Aurora B phosphorylates serine 10 of histone H3 (H3S10) at mitotic onset, which then recruits Hst2, an enzyme that removes acetyl groups from lysine 16 of histone H4 (H4K16) and promotes internucleosomal interactions between the positively charged histone H4 tail and an acidic patch on histone H2A [14]. This in vivo study agrees nicely with in vitro data using reconstituted nucleosome arrays [15].

How does this molecular-scale mechanism play out in the context of an entire chromosome? An exciting study in budding yeast suggests that this signaling cascade may originate at the centromere [16**]. The authors observed that removing the centromere from a chromosome prevented centromeric phosphorylation of histone H3S10 and impaired condensation of that specific chromosome. Conversely, inserting a centromere sequence into an artificial minichromosome was

Figure 1



Multiple molecular-level activities cooperate to organize and shape mitotic chromosomes. (**a**) Chromatin compaction at mitotic onset is driven by a cascade of histone post-translational modifications (PTMs) initiated by Aurora B, the kinase subunit of the chromosomal passenger complex (CPC). Aurora B phosphorylates S10 of histone H3 *(1)*, which recruits the deacetylase Hst2 that acts on histone H4 K16 *(2)*. These changes result in increased multivalent interactions among neighboring nucleosomes, driven in part by an unstructured domain of Borealin, another subunit of the CPC *(3)*. Ultimately, these interactions result in increased compaction of chromatin, which could be driven by phase separation. (**b**) In the model proposed by Kruitwagen et al. [16], the compaction signal mediated by Aurora B initiates at the centromere *(1)* and spreads along the chromosome arm *(2)*, resulting in a shortened, more compact chromosome *(3)* (**c**) In the model proposed by Gibcus et al. [2], condensin I and II form loops of DNA perpendicular to the central axis. In this model, loop extrusion by the condensins creates larger loops of DNA, and thus axial shortening of the chromosome arm. (**d-e**) Interchromosomal organization is driven by two DNA-binding molecules: Ki67 and BAF. Ki67 helps individualize chromosomes by acting as a 'surfactant brush', repelling chromosomes from one another. Upon mitotic exit, formation of a complete nucleus is aided by BAF, which cross-links chromosomes at their periphery, preventing chromosome loss. Finally, mitotic bookmarking factors that remain on mitotic chromosomes help reinitiate transcriptional programs upon re-entry into interphase. BAF, barrier to autointegration.

sufficient to drive its compaction. Exactly how the compaction signal is transmitted along chromosome arms is unclear but appears to involve histone deacetylation by Hst2 and regulation by the protein Shugoshin. It also remains to be seen whether or not the centromere is capable of playing a similar role in animal cells, where H3S10 phosphorylation, despite originating at the centromere [17], also occurs along the chromosome arms.

What features of the centromere could allow it to drive compaction of a chromosome? Intriguingly, a recent study indicates that the inner centromere has properties of a biomolecular condensate [18**]. The authors showed that an unstructured domain of the CPC subunit Borealin can drive phase separation of the complex in vitro and provided evidence that the CPC exists in a phase separated state at the inner centromere in vivo. A separate study demonstrated that the unstructured domain of Borealin is also responsible for mediating multivalent interactions between the CPC and nucleosomes, suggesting that chromatin itself also participates in phase separation at the centromere [19]. Taken together, one appealing model is that a cascade of histone PTMs initiated by the CPC drives formation of a biomolecular condensate at the centromere that spreads and compacts chromatin along chromosome arms (Figure 1b). Another factor that could potentially initiate mitotic chromosome compaction in some systems is heterochromatin protein 1 (HP1), which maintains an active role at the centromere during mitosis [20,21] and has been shown to drive phase separation of chromatin in other contexts in vitro and in vivo [22,23].

Beyond the centromere, there is increasing evidence that phase separation, driven by weak multivalent interactions, plays a role in organizing interphase chromatin [24]. A recent study demonstrated that reconstituted nucleosome arrays were capable of phase separating into dense droplets both in vitro and when injected into nuclei of cultured cells. Decreasing their spacing increased the concentration of nucleosomes within the droplets, whereas acetylation of the arrays dissolved the droplets [25]**. These results suggest that tuning multivalent interactions among neighboring nucleosomes can affect the intrinsic compaction of chromatin. It will be of great interest to test these principles in the context of mitotic chromosomes, where it was shown that a transient increase in multivalency in the form of free magnesium during mitotic onset plays a role in chromatin compaction [26].

Taken together, these recent studies suggest that regulation at the nucleosome level can drive a global increase in chromatin compaction through two related pathways: the centromere-mediated initiation and spread of mitotic PTMs along the chromosome and the promotion of multivalent interactions. Both mechanisms involve processes that occur at the nanometer length scale but manifest at the whole–chromosome scale, potentially through a process of phase separation.

ATPases drive whole-chromosome organization and resolution

Although mitotic chromatin itself adopts a compacted conformation, other factors are required to produce structured and individually resolved chromosomes. Key to organization at larger scales is the DNA-based motor condensin, of which there are two types in vertebrates, condensin I and condensin II. When the ATPase subunit shared by both condensins was rapidly depleted from cultured cells, the volume of mitotic chromatin did not change, but chromosomes were misshapen and unresolved [27]. At the molecular level, condensins are ring-shaped protein complexes that change the topology of DNA by creating loops. This activity was directly demonstrated in single-molecule experiments, showing that condensin purified from yeast can create a loop on naked DNA and quickly 'extrude' the DNA to form a progressively larger loop [28**].

How are the smaller scale DNA loops formed by condensins organized into higher order chromosome architecture? Based on Hi-C data from condensin-depleted vertebrate cells, it was proposed that condensin II forms large DNA loops during prophase, which are subsequently partitioned into smaller, nested loops by condensin I in prometaphase [2**]. The interplay between these two condensin-driven activities result in a series of loops arranged into a twisted helical structure perpendicular the central axis of a chromosome (Figure 1c). In the model proposed by the authors, axial shortening of the chromosome occurs as a result of loop extrusion by the two condensins.

Another open question is how the large-scale organization of DNA in chromosomes is directed by the spatial arrangement and temporal activity of condensins. One study addressed this question using quantitative, high resolution imaging and fluorescence correlation microscopy in single cells to measure absolute numbers, spacing, and dynamics of condensin molecules during mitotic progression [29**]. The authors then used direct measurements of chromosome length and published loop extrusion models [30] to derive DNA loop sizes as a function of mitotic progression, generating a comprehensive, quantitative model for condensin-driven mitotic chromosome compaction. Although many of the qualitative findings of this model are not new, its quantitative and predictive power is unprecedented and will undoubtedly aid future experimental design. Findings in other systems add further complexity. For example, in budding yeast, the binding kinetics of condensins on mitotic chromatin is not only dynamic but also requires

regulation by nonchromatin enzymes [31,32]. In addition, a study in human cells showed that the two ATPases in each condensin ring have different activities, with one promoting loop formation and the other stabilizing higher order connections between loops [33]. In the future, it will be interesting to integrate these recently discovered parameters into models of loop extrusion-based mitotic chromosome formation.

In addition to condensin, the ATP-dependent DNA strand-passing enzyme topoisomerase II (topo2) is also required to resolve mitotic chromosomes from each other as they condense, which was beautifully demonstrated in a reconstitution experiment that identified core histones and their chaperones, condensin I, and topo2 as sufficient to remodel sperm chromatin into individualized chromatids [34]. Moreover, in a molecular dynamics simulation, loop extrusion by condensins and strand-passing mediated by topo2 was sufficient to convert initially globular interphase chromosomes into elongated structures that resembled prophase chromosomes [35]. In vivo, however, interactions between topo2 and condensin activities appear to be more complex [36]. Acute inactivation of condensin I in *Drosophila* embryos in mitosis caused topo2-dependent chromosome concatenation and hypercompaction, thereby blocking the resolution and anaphase segregation of chromosomes [37*].This result suggests that condensin is required continuously to drive decatenation rather than catenation of DNA molecules by topo2.

Taken together, these data suggest an exciting model in which the molecular-level ATP-dependent loop extrusion by condensin I and II, in cooperation with the DNA strand-passing activity by topo2, is the driving force that organizes mitotic chromosomes at a whole-chromosome scale.

Barrier to autointegration and Ki67 organize chromosomes at their periphery

In addition to being individually condensed and structured at a single—chromosome scale, mitotic chromosomes must also be organized relative to one another to facilitate their proper segregation. This level of organization is mediated by a set of proteins that act at the chromosome periphery. Promoting mitotic chromosome individualization is Ki67, a protein that acts as a brush-like surfactant on each mitotic chromosome, preventing coalescence of the genome into a single large mass [38]. At the end of mitosis, dimers of the barrier to autointegration (BAF) protein bind to chromatin. Containing two high affinity DNA-binding sites, BAF functions as a cross-linker to connect chromosomes and promote the formation of a single nucleus [39*]. Thus, Ki67 and BAF use the simple molecular-scale activities such as DNA-binding and electrostatics to regulate the degree of whole-chromosome resolution and clustering. This feature of whole-chromosome organization is thought to be particularly important at the exit from mitosis, when the genome is recompartmentalized inside the nuclear envelope and must exclude large cytoplasmic components that could not be exported through nuclear pores.

The emergence of whole-chromosome mechanics and dynamics

As a result of the many mechanisms that generate higher order mitotic chromosome structure, distinct physical features emerge. One such property is the resistance to stretching forces, a parameter that can be directly measured by micromanipulating chromosomes isolated from human cells [40]. Interestingly, depleting condensin from chromosomes decreased their stiffness 10-fold, whereas increasing condensin levels had the opposite effect [41]. At the level of the nucleosome, increasing histone methylation, but not H3K9 acetylation, stiffened mitotic chromosomes [42]. Thus, factors that modulate organization over both shorter and longer length scales can affect physical properties of whole chromosomes.

Organization also occurs at an even higher level that determines how mitotic chromosomes are arranged within the cell. For example, a toroidal distribution of chromosomes at nuclear envelope breakdown facilitates their interaction with spindle microtubules [43]. Recently, it was observed that the two haploid sets of chromosomes are spatially segregated from one another throughout mitosis [44]. This 'antipairing' behavior was observed in normal human and mouse somatic cells but lost in a carcinoma cell line. The function of this organization is thought to prevent recombination between homologs, which would result in genetic instability. It will be of great interest to discover the set of molecules that mediate this type of interchromosomal organization.

Emergent functions of mitotic chromosomes

The ultimate properties of condensed chromosomes including their size, shape, and stiffness facilitate their segregation by the mitotic spindle. These properties are dynamic and can be precisely tuned during a single cell division. For example, introducing an extra-long chromosome in budding yeast results in hypercondensation of that chromosome during anaphase, a response that requires Aurora B phosphorylation of H3S10 [45]. Chromosome properties are also tuned across multiple cell divisions, for example to allow chromosome size to scale with cell size during early development, when many rapid cell divisions occurring in the absence of cell growth lead to progressively smaller cells [46]. The mechanisms tuning overall chromosome morphology, the downstream effects on the physical properties of chromosomes and the mechanics of cell division, remain poorly understood.

Other, more subtle functions are also programmed into mitotic chromosome structure and organization. A fascinating example is mitotic 'bookmarking' in which regions of the genome are primed for transcription in the ensuing interphase [47,48]. Bookmarking factors can either reside within the chromatin itself, in the form of histone PTMs, or be mediated by transcription factors (TFs). A recent study in mouse embryonic stem cells showed that 100 of 500 TFs tested were enriched on the mitotic chromosome relative to the cytoplasm [49]. These bookmarking TFs interact dynamically with mitotic chromatin, and in some cases, are able to preserve the local nucleosome architecture at the promoter [50]. Whether or not these bookmarking factors affect large-scale chromosome morphology, mechanics or dynamics remains to be seen.

Conclusions and future directions

In conclusion, multiple biochemical activities at the molecular level come together to mediate the large-scale chromosome organization and dynamics observed during mitosis. Despite recent advances in the field, two major challenges remain: (1) integrating the many sources of structural information (imaging, sequencing, biochemistry) into a comprehensive, multiscale model for mitotic chromosome architecture, and (2) relating the structure and mechanics of mitotic chromosomes to their functions in cell division and epigenetic inheritance. To tackle these challenges, there is a great need in the field for reconstituted systems to study mitotic chromosome structure and function at the whole-chromosome level. So far, *Xenopus* egg extracts is one of the few systems in which this is possible. A second approach that will undoubtedly push the field forward is to test our models of chromosome structure and function against the extremes that biology has to offer. For example, species with large genome sizes [51] or small chromosome numbers [52] could help us stretch our imaginations.

Conflict of interest statement

Nothing declared.

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