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Chromosome organization through the cell cycle at a glance

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ABSTRACT

Genome organization and the three-dimensional folding of chromosomes are now seen as major contributors to nearly all nuclear functions including gene regulation, replication and repair. Recent studies have shown that in addition to the dramatic metamorphoses in chromosome conformation associated with entry to, and exit from mitosis, chromosomes undergo continual conformational changes throughout interphase with differential dynamics in loop structure, topological domains, compartments and lamina-associated domains. Understanding and accounting for these cell-cycle-dependent conformational changes is essential for the interpretation of data from a growing array of powerful molecular techniques to investigate genome conformation function, and to identify the molecules and mechanisms that drive chromosome conformational changes. In this Cell Science at a Glance article and the accompanying poster, we review Hi-C and microscopy studies describing cell-cycle-dependent conformational changes in chromosome structure.

KEY WORDS: Cell cycle, Chromatin folding, Nuclear genome organization

Introduction

In the late 19th and early 20th centuries, the concept of chromosomes occupying distinct sub-volumes or territories in interphase nuclei began to take hold (Boveri, 1909; Rabl, 1887). Opinion varied over the course of the 20th century, but the chromosome territory theory is now definitively proven and widely accepted (Cremer and Cremer, 2010). The ubiquitously familiar X-shaped, highly condensed, mitotic chromosome has garnered a lot of attention over the years due to ease of observation under light microscopy in cells of rapidly dividing cultures. However, as cells enter interphase, chromosome structures change dramatically becoming much more decondensed. And although individual chromosomes occupy distinct territories within the interphase mammalian nucleus, it is near impossible to determine where one chromosome territory starts and another ends without advanced chromosome labeling technologies (Cremer et al., 1993; Manders et al., 1999; Manuelidis, 1985; Marsden and Laemmli, 1979; Paulson and Laemmli, 1977; Trask et al., 1993). It is therefore no surprise then that early investigations into chromosome structure were dominated by examination of mitotic chromosomes. Two major models emerged to explain the intricate folding of DNA. The radial-loop model proposed that the condensation of metaphase chromosomes consists of a non-histone protein backbone from which loops emanate radially at regular intervals (Marsden and Laemmli, 1979; Paulson and Laemmli, 1977). The hierarchical helical folding model, which does not require a central proteinaceous backbone, proposed a 10–30 nm chromatin fiber that undergoes coiling and sequential helical winding, to finally form the metaphase chromosome (Belmont et al., 1987; Finch and Klug, 1976; Kireeva et al., 2004; Sedat and Manuelidis, 1978). The introduction of chromosome conformation capture techniques, such as 3C, 4C and Hi-C (see Box 1; Denker and de Laat, 2016), provided molecular approaches complementary to microscopy to assess chromosome conformations, but unlike the microscopy studies, which focused on single cells or individual mitotic chromosomes, 3C techniques averaged chromosome conformations across millions of cells. Early 3C studies using the powerful Hi-C method (Lieberman-Aiden et al., 2009; see Box 1) focused on rapidly dividing cell cultures in which more than 80% of the cells are in interphase. Thus, the first details of the genome organizational principles of the interphase cell began to emerge. The two dimensional Hi-C heat maps, which represent genome-wide matrices of pair-wise contact frequencies between restriction fragments, revealed megabase-sized domains of self-interaction known as topologically associated domains (TADs) that displayed locally reduced contacts to adjacent neighboring regions (Dixon et al., 2012; Nora et al., 2012; Sexton et al., 2012). CCCTC-binding factor (CTCF), which had been previously shown to mediate loop formation by promoting long-range interactions (Splinter et al., 2006), was found to be enriched at TAD boundaries along with cohesin, a ring-shaped protein complex (Dixon et al., 2012; Nora et al., 2012; Sexton et al., 2012). More recently, formation and maintenance of TADs has been shown to be dependent upon CTCF–cohesin interactions (Wutz et al., 2017; Nora et al., 2017; Rao et al., 2017). CTCF–cohesion-mediated promoter–enhancer loops that bridge large genomic distances have generally been associated with controlling gene expression profiles. However, several studies (reviewed by Schoenfelder and Fraser, 2019) indicate that enhancer–promoter interactions can be maintained by alternative mechanisms and that CTCF–cohesin loops play only a minor role in determining gene expression profiles (Thiecke et al., 2020).

At the chromosomal scale a ‘plaid’ pattern of enriched and depleted contact frequencies between multi-megabase regions suggests that there are two nuclear compartments, A and B (Lieberman-Aiden et al., 2009) (see poster). A-compartments are composed of preferential contacts between active domains, while B compartments consist of preferential associations between inactive domains, potentially mirroring the separation of euchromatin and heterochromatin first observed in light microscopy (Heitz, 1928) and later with 4C (Simonis et al., 2006). Unlike TADs, compartment maintenance is independent of cohesin, indicating that TADs and compartments are established and maintained by different mechanisms (reviewed in Schoenfelder and Fraser, 2019). Although population Hi-C experiments revealed the above-mentioned organizational features of chromosomes based on contact frequencies or probabilities, single-cell Hi-C (scHi-C) provided the first molecular characterizations of individual chromosome conformations, revealing considerable variation in

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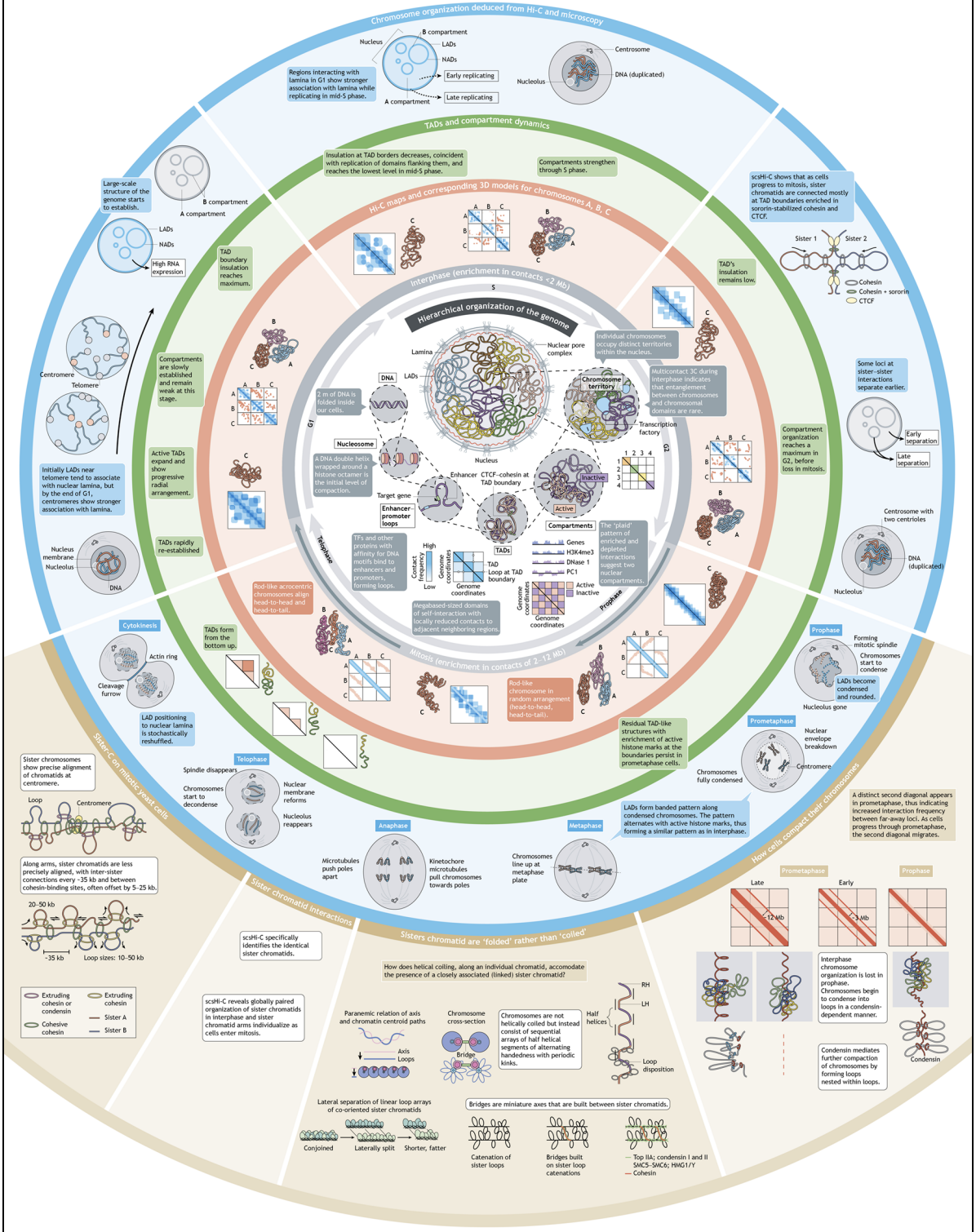

Abbreviations: CTCF, CCCTC-binding factor; H3K4me3, histone 3 lysine 4 trimethylation; HMG1/Y, high mobility group protein 1; LADs, lamina-associated domains; LH, left-handed; NADs, nucleolus-associated chromatin domains; PC1, polycystin 1; RH, right-handed; scsHi-C, sister chromatid sensitive Hi-C; SMC5–SMC6, structural maintenance of chromosomes 5–6 complex; TAD, topologically associating domain; TF, transcription factor; Top IIA, topomerase IIA.

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Box 1. Chromosome conformation capture methodology

Chromosome conformation capture (3C) methods have been developed for over two decades and have provided various insights into the genome topology. The original 3C method (Dekker et al., 2002) measured the frequency of contacts between pairs (a one-to-one approach) of genomic loci. Variants of this method were developed, such as 4C (circularized 3C) to capture contacts with a one-to-all approach (Simonis et al., 2006; Zhao et al., 2006), 5C, which captures contacts with a many-to-many approach (Dostie et al., 2006), and, finally, Hi-C (for 'high-resolution chromosome conformation capture'), which captures all contacts genome wide in an unbiased manner (all-to-all approach) (Lieberman-Aiden et al., 2009). Hi-C involves crosslinking the chromatin material in cells with formaldehyde, restriction enzyme digestion of genomic DNA and biotin incorporation at the fragment ends. This is followed by a ligation step, which joins fragments in close spatial proximity due to chromosome folding in 3D space within the nuclei. After reversal of crosslinks and DNA purification, the ligated DNA is fragmented and the biotinylated ligation junctions are captured on streptavidin beads. Finally, deep paired-end sequencing reveals restriction fragments that were in close spatial proximity in the nucleus at the time of fixation, and are referred to as Hi-C contacts (Lieberman-Aiden et al., 2009). Hi-C data can be represented as a heat map matrix of contact frequencies between pairs of genomic loci. These 2D heatmaps, where each row and column represents a chromosome, and the color intensity represents the contact frequency. The characteristic feature of the map is a strong central diagonal that represents increased contact frequencies between pairs of fragments that are in proximity to each other in the linear genome sequence (see poster).

chromosome conformation from cell to cell (Nagano et al., 2013). Here, we provide an overview of chromosome conformation capture and microscopy studies that have shed light on the 3D organization of chromosomes through the cell cycle.

TAD and compartment dynamics through the cell cycle

Comparison of Hi-C data from enriched interphase and mitotic cell populations found that TADs and compartments were not detectable in mitotic chromosomes, revealing two functionally distinct organizations of chromosomes (see poster) (Naumova et al., 2013). Plots of intra-chromosomal contact frequency as a function of distances showed that mitotic chromosomes were relatively enriched in contacts in the 2–12 Mb range, whereas interphase cells were relatively enriched in contacts under 2 Mb. The relative enrichment of long-range contacts in mitotic cells reflects not only the higher compaction of mitotic chromosomes but suggests a model of a metaphase chromosome as a compressed array of consecutive loops (Gibcus et al., 2018; Liang et al., 2015; Lieberman-Aiden et al., 2009; Naumova et al., 2013).

Using scHi-C on thousands of unsynchronized cells, Nagano et al. (2017) observed stark differences in chromosome conformation between mitotic and interphase cells (see poster). The authors also discovered that plotting the decreasing frequency of mitotic range contacts per cell (which occurs as cells exit mitosis) against the steadily increasing frequency of short-range contacts (<2 Mb) per cell (that occurs as cells progress through interphase), produced a cyclical pattern. Further experiments with cell-cycle-sorted cells confirmed that the cyclical plot of individual cells was in fact mirroring the cell cycle, demonstrating that the cell cycle was coincident with a continuous chromosome conformation cycle. Examination of chromosome organizational features in thousands of individual cells arranged in cell cycle order revealed differential dynamics of TADs and compartments (Nagano et al., 2017). Previous time-lapse microscopy of genomic loci had shown that chromosomes have increased mobility during mitotic exit in the first few hours of G1 as compared to that in later interphase stages (Thomson et al., 2004). During this time, rapid decompaction of active regions and radial positioning of A and B compartments takes place, with inactive B regions repositioning toward the nuclear periphery, and active A regions tending toward the interior of the nucleus (Nagano et al., 2017). Compartments strengthen continually through the cell cycle coincident with refinement of contacts between domains of similar activity and epigenetic states. TADs on the other hand are rapidly reestablished upon mitotic exit (Nagano et al., 2017) (see poster). By using 4C, rapid re-establishment of TADs during time points in G1 phase was shown to be coincident with establishment of the replication timing program (defined by genomic regions undergoing replication in a temporal order in S phase) (Dileep et al., 2015). Analysis of thousands of individual cells representing the entire cell cycle by scHi-C confirmed rapid TAD reestablishment in very early G1 with border insulation reaching a maximum in late G1 (Nagano et al., 2017). As cells entered S phase, insulation at TAD borders decreased, coincident with the replication timing of their adjacent domains, reaching their lowest levels in mid-late S phase, which was maintained through G2 (see poster). A subsequent study proposed a bottom-up model of TAD re-organization during the metaphase to G1 transition (Zhang et al., 2019). Here, synchronized populations of late mitotic cells in anaphase and telophase displayed loops at a sub-TAD scale that appeared to coalesce into TADs in early G1 cells (Zhang et al., 2019). Collectively, these molecular studies added to and confirmed the long-established structural differences between mitotic and interphase chromosomes, and revealed a differential dynamics of TADs and compartments, clearly implicating the cell cycle as a major source of determining dynamic genome organization.

LADs, NADs and NORs

Another feature of the nucleus that aids in controlling functional nuclear organization of the genome are the lamina-associated domains (LADs), which are large genomic regions that closely associate with the peripheral nuclear lamina during interphase (Briand and Collas, 2020). Upon entry into mitosis, chromosomes compact, and the nuclear envelope breaks down, and lamin proteins are re-distributed to the cytoplasm (Kind et al., 2013). DamID labeling (see Box 2) of LADs enabled live-cell tracking as cells progressed through mitosis (Kind et al., 2013). As chromosomes begin to condense in prophase, LADs lose contact with the lamina.

Box 2. Dam identification

Dam Identification (DamID) is a method that identifies chromatin sequences interacting with a nuclear protein of interest (Van Steensel and Henikoff, 2000). A fusion protein consisting of bacterial DNA adenine methyltransferase (DAM) and a protein of interest is expressed at low levels *in vivo*. DAM methylates the adenine in GATC sequences that are in proximity to the protein of interest. The methylated sequences are enriched by digestion with a methylation-sensitive restriction enzyme (DpnI), followed by blunt end ligation of PCR adapters, and then by PCR to obtain a DNA–protein of interest binding profile for the entire genome (Vogel et al., 2007). As shown by Kind et al. (2013), this technology can be modified to track the fate of genomic regions labeled through prior contact with a protein of interest. By using the methyl-binding domain of DpnI devoid of its endonuclease activity, fused to GFP, Kind et al., followed the dynamics of LADs through mitosis and a subsequent interphase.

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After nuclear envelope breakdown, LADs form a banded pattern along condensed chromosomes that alternates with regions containing active histone marks (Kind et al., 2013). Interestingly, as the nuclear lamina reassembles in anaphase and telophase, LADs labeled in the previous interphase varied in their interaction with the newly formed lamina. Even by several hours post-mitosis, only a subset of the parental LADs localize near the newly formed lamina. Furthermore, the subset of LADs that associate with the lamina is different between daughter cells. Collectively, these results demonstrate that lamina interaction and peripheral positioning of LADs is not inherited from parent to daughter cells but is stochastically re-shuffled after every mitosis (Kind et al., 2013; Thomson et al., 2004). Time-lapse imaging of telomeres and DamID technology provide further insight into LAD dynamics in interphase. As cells enter G1, telomeric LADs tend to associate with the lamina more strongly, but as G1 progresses, LADs near the centromere show stronger association with the nuclear lamina, whereas telomeric LADs detach, suggesting gradual reorientation of chromosomes early in interphase (van Schaik et al., 2020) (see poster).

The nucleolus is a major subnuclear organelle, and is the hub of ribosomal RNA transcription and ribosome biogenesis. It is known to cluster and form around ribosomal (r)DNA repeat sequences, known as nucleoli-organizing regions (NORs). As cells enter mitosis, RNA polymerase (Pol) I transcription is shut down and nucleoli disassemble, but as cells approach G1, nucleoli begin forming around NORs (reviewed in detail in McStay, 2016). Similar to LADs are nucleolar-associated domains (NADs), which are heterochromatic, late-replicating regions that associate closely with the nucleolus during interphase (Dillinger et al., 2017). It has been shown that LADs and NADs are interchangeable and can switch positions after mitotic events (Kind et al., 2013). The molecular basis behind LAD and NAD association with the lamina and the nucleolus, respectively, is not completely understood, but their dynamics and non-random positioning within the nucleus suggest they play an important role in the organization of interphase chromosomes.

Chromosome organization in mitosis

Hi-C analyses of cell populations synchronously released into mitosis have been used to detail chromosome conformation changes in mitosis (Gibcus et al., 2018). Within minutes, insulation at TAD boundaries declines sharply, as does the plaid pattern characteristic of interphase compartment organization. Contact maps from cells in inferred early prometaphase revealed a second diagonal running parallel to the central diagonal (see Box 1), indicating increased contact frequency between loci separated by ∼3 Mb (see poster). This was followed by progressive migration of the second diagonal away from the central diagonal, indicating increased contact frequency between loci further apart on the linear genome as cells progressed through prometaphase. Polymer modeling suggested a possible pathway of chromosome folding as interphase organization is lost during entry to mitosis. TAD and compartment organization is lost in prophase in favor of condensin-dependent formation of 60 kb loops emanating from a central condensin scaffold, progressing to ∼80 kb inner loops that are nested within ∼400 kb outer loops in prometaphase (see poster). The loop arrays, which emanate from a central condensin spiral staircase like a scaffold, were proposed to acquire a helical arrangement creating the second diagonal, which progressively widens to ∼12 Mb during prometaphase (Gibcus et al., 2018).

However, other studies challenge the hypothesis of helical coiling of individualized sister chromatids in prometaphase. For instance, live imaging of GFP-labeled topoisomerase IIα, a major component of the chromatid axis, reveals inter-axes bridges at the sister–sister interface once sister chromatids have individualized in prophase (Chu et al., 2020). These bridges appear to be evenly spaced along the entire length of the sister chromatids (∼400 nm between adjacent bridges). As chromosomes shorten in prometaphase the bridges decrease in number, whereas the spacing between them is maintained. The bridges persist through to metaphase then disappear in anaphase as chromosomes separate (see poster). The authors suggested the helical coiling proposed in Gibcus et al., (2018) would cause sister chromatids to convolute unless the connections were able to undergo dynamic adaptations during helical coiling (Chu et al., 2020). However, no evidence of sister chromatids intertwining, or dynamic adaptability at the interface was observed. In contrast, examination of chromatid slices along their axis indicated that the axes are not helically coiled, but instead have evenly spaced half-helical segments with alternating left and right handedness, resulting in a ‘net zero’ for the overall axes helicity. Furthermore, loop density did not change, but their size increased as chromosomes shortened and widened (Chu et al., 2020).

Two other recent studies further developed Hi-C to specifically identify inter-sister chromatid interactions (Mitter et al., 2020; Oomen et al., 2020), which is not possible with standard Hi-C because the DNA sequences of newly replicated sister chromatids are identical. Mitter et al. (2020) replication-labeled human chromatids with a nucleoside analog and then induced point mutations by nucleoside conversion to identify the chromatids containing the newly replicated Watson- and Crick-strand by Hi-C. They observed inter-sister chromatid contacts in G2 cells with high rates of contact along entire domains of TADs enriched in H3K27me3 (histone 3 trimethylated at K27; a facultative heterochromatin mark), and globally between most TAD boundaries. Indeed, inter-sister contacts were enriched at most CTCF sites that mark TAD boundaries, and sororin-stabilized cohesin was required to maintain contacts and alignment between sister TADs. Once cells entered mitosis, sister chromatids separated and individualized (Mitter et al., 2020). If sister chromatids are completely resolved bodies in mitosis, then the inter-sister bridges described in Chu et al. (2020) must not contain DNA.

The second study investigated sister chromatid contacts in yeast (Oomen et al., 2020). To that end, the authors developed a similar method called Sister-C, which employs BrdU incorporation into new replicated chromatids followed by strand specific degradation to distinguish inter- and intra-sister chromatid interactions based on read orientation. In contrast to the findings presented in Mitter et al. (2020), these authors observed that interactions between yeast sister chromatids are maintained at 35 kb intervals into mitosis, indicating that yeast sister chromatids are not completely individualized in mitosis (Oomen et al., 2020) (see poster).

Obviously, the differences in sister chromatid organization might be due to differences between the relatively small chromosomes of yeast and the enormous chromosomes of higher eukaryotes; however, the seemingly conflicting results on chromosome organization in mitosis suggest that mitotic chromosome structure is still not completely understood.

Conclusions

With the advent of chromosome conformation capture methods, key features of genome organization, such as TADs and compartments, have been identified. Several lines of evidence suggest that these features of chromosome folding and organization, and their ability

to dynamically change during the cell cycle, are important for proper nuclear function. However, the precise molecular conformations, mechanisms and full biological roles of these features remain an active area of research. For example, TADs appear to be regulatory domains that limit promoter–enhancer interactions to elements within the TAD (Symmons et al., 2014). What then is the molecular and biological significance of TAD insulation that peaks during G1 and decreases during S phase? How are compartments formed and maintained in the absence of CTCF and cohesin (Nora et al., 2017; Rao et al., 2017), and what role does refinement of compartment contacts through the cell cycle play in setting up cell-type-specific genome organization in cycling cells versus post-mitotic cells? Both TADs and compartments are lost during mitosis, and in interphase evidence suggests that TADs are formed by CTCF–cohesin-mediated loop extrusion, whereas compartments are thought to be formed by phase separation (reviewed by Hildebrand and Dekker, 2020). Perhaps an 'epigenetic memory' or a transcription factor 'bookmark' carried through mitosis is responsible for reestablishing the transcriptional pattern and higher-order organizational features in interphase, as has been suggested previously (Michelotti et al., 1997; reviewed by Miura and Hiratani, 2022)? Depletion of Rap1-interacting factor-1 (RIF1, a critical factor in determining the replication timing program) in cells disrupts the replication timing program in S phase, and consequently disrupts genome compartmentalization and the epigenetic landscape (Klein et al., 2021). These effects are magnified in ensuing cell cycles (Klein et al., 2021), suggesting that the replication timing program might be important in re-establishing the epigenetic landscape and 3D genome structure in interphase. Recent evidence also shows that mitotic bookmarking by histone marks such as H3K27ac (H3 acetylated at K27) is involved in rapid reestablishment of transcriptional activity (Pelham-Webb et al., 2021), and H3K9me3 is important in maintaining binding of certain other bookmarking proteins to mitotic chromosomes (Djeghloul et al., 2022 preprint). Nevertheless, our understanding of the molecular mechanisms that drive the formation and dynamics of TADs and compartments, and how the two higher-order organizations may be functionally related to each other remains limited.

Upon entry into mitosis, replicated interphase chromosomes undergo dramatic changes in morphology to shorten and compact so as to enable error-free segregation into daughter cells. The compact nature of mitotic chromosomes makes them difficult candidates for structural studies by microscopy, and the identical sequence of sister chromatids further complicates determining the structure of individual chromatids by chromosome conformation capture studies. Because of this, there is some disagreement in the field regarding how the sister chromatids are positioned with respect to each other through mitosis, including the question of how mitotic chromosomes compact – either by loop growth and progressive helical winding (Gibcus et al., 2018), or through a more linear compaction with an increase in loop size and no helical winding (Chu et al., 2020). Both the Gibcus et al. and Chu et al. studies implicate loop extrusion as a mechanism by which chromosomes widen and consequently shorten during mitosis. More recently, simulations based on Brownian dynamics in fission yeast introduces 'diffusion capture' as a potential contributor to mitotic chromosome compaction, in addition and possibly in cooperation with loop extrusion (Gerguri et al., 2021). Diffusion capture is posited as the ability of condensin already bound to DNA to capture and stabilize an interaction with another condensin-binding site that comes in proximity by Brownian motion (Gerguri et al., 2021). Our understanding of the molecular mechanisms behind mitotic chromosome formation and the precise details of the molecular conformation are thus continuously evolving.

Among the finer features of chromosomes, the 30 nm fiber has long been thought to be integral to chromosome compaction. Observing chromatin structure using a combination of electron tomography and DNA labeling, Ou et al. (2017) were among the first to provide conclusive evidence that 30 nm fibers are not present as an intermediate chromatin hierarchical folding state *in situ*, but possibly are artefacts introduced during DNA extraction or nuclei isolation in the presence of detergents and salts or other *in vitro* conditions (Rattner and Lin, 1985; Sedat and Manuelidis, 1978; Song et al., 2014). Instead, chromosomes are long, densely packed and disordered chains of varying diameters (Ou et al., 2017). More recently, tracing of linker DNA paths from nucleosome to nucleosome has revealed the trajectory of chromatin fibers with no evidence of hierarchical folding states (Beel et al., 2021), thus supporting the findings in Ou et al. (2017). Clearly there is much work to be done, but one thing is clear, studying 3D genome organization dynamics and function remains an exciting field that has much to reveal in the unfolding story of how the genome controls information flow from genes to cells to organisms.

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Competing interests
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References
Beel, A. J., Azubel, M., Matteï, P.-J. and Kornberg, R. D. (2021). Structure of mitotic chromosomes. *Mol. Cell* **81**, 4369-4376.e3. doi:10.1016/j.molcel.2021.08.020

Belmont, A. S., Sedat, J. W. and Agard, D. A. (1987). A three-dimensional approach to mitotic chromosome structure: evidence for a complex hierarchical organization. *J. Cell Biol.* **105**, 77-92. doi:10.1083/jcb.105.1.77

Boveri, T. (1909). Die Blastomerenkerne von Ascaris Megalocephala und die Theorie der Chromosomenindividualität. *Arch. Zellforsch.* **3**, 181-268

Briand, N. and Collas, P. (2020). Lamina-associated domains: peripheral matters and internal affairs. *Genome Biol.* **21**, 1-25. doi:10.1186/s13059-020-02003-5

Chu, L., Liang, Z., Mukhina, M., Fisher, J., Vincenten, N., Zhang, Z., Hutchinson, J., Zickler, D. and Kleckner, N. (2020). The 3D topography of mitotic chromosomes. *Mol. Cell* **79**, 902-916.e6. doi:10.1016/j.molcel.2020.07.002

Cremer, T. and Cremer, M. (2010). Chromosome territories. *Cold Spring Harbor Perspect. Biol.* **2**, a003889. doi:10.1101/cshperspect.a003889

Cremer, T., Kurz, A., Zirbel, R., Dietzel, S., Rinke, B., Schrock, E., Speicher, M. R., Mathieu, U., Jauch, A., Emmerich, P. et al. (1993). Role of chromosome territories in the functional compartmentalization of the cell nucleus. *Cold Spring Harbor Symp. Quant. Biol.* **58**, 777-792. http://symposium.cshlp.org/content/58/777.short. doi:10.1101/SQB.1993.058.01.085

Dekker, J., Rippe, K., Dekker, M. and Kleckner, N. (2002). Capturing chromosome conformation. *Science* **295**, 1306-1311. doi:10.1126/science.1067799

Denker, A. and de Laat, W. (2016). The second decade of 3C technologies: detailed insights into nuclear organization. *Genes Dev.* **30**, 1357-1382. doi:10.1101/gad.281964.116

Dileep, V., Ay, F., Sima, J., Vera, D. L., Noble, W. S. and Gilbert, D. M. (2015). Topologically associating domains and their long-range contacts are established during early G1 coincident with the establishment of the replication-timing program. *Genome Res.* **25**, 1104-1113. doi:10.1101/gr.183699.114

Dillinger, S., Straub, T. and Németh, A. (2017). Nucleolus association of chromosomal domains is largely maintained in cellular senescence despite massive nuclear reorganisation. *PLoS ONE* **12**, e0178821. doi:10.1371/journal.pone.0178821

Dixon, J. R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J. S. and Ren, B. (2012). Topological domains in mammalian genomes identified by

analysis of chromatin interactions. *Nature* **485**, 376-380. doi:10.1038/nature11082

Djeghloul, D., Dimond, A., Kramer, H., Brown, K., Patel, B., Wang, Y.-F., Futschik, M. E., Whilding, C., Montoya, A., Veland, N. et al. (2022). Loss of H3K9 tri-methylation alters chromosome compaction and transcription factor retention during mitosis. *BioRxiv*, 2022.02.01.478684. doi:10.1101/2022.02.01.478684

Dostie, J., Richmond, T. A., Arnaout, R. A., Selzer, R. R., Lee, W. L., Honan, T. A., Rubio, E. D., Krumm, A., Lamb, J., Nusbaum, C. et al. (2006). Chromosome conformation capture carbon copy (5C): a massively parallel solution for mapping interactions between genomic elements. *Genome Res.* **16**, 1299-1309. doi:10.1101/gr.5571506

Finch, J. T. and Klug, A. (1976). Solenoidal model for superstructure in chromatin. *Proc. Natl. Acad. Sci. USA* **73**, 1897-1901. doi:10.1073/pnas.73.6.1897

Gerguri, T., Fu, X., Kakui, Y., Khatri, B. S., Barrington, C., Bates, P. A. and Uhlmann, F. (2021). Comparison of loop extrusion and diffusion capture as mitotic chromosome formation pathways in fission yeast. *Nucleic Acids Res.* **49**, 1294-1312. doi:10.1093/nar/gkaa1270

Gibcus, J. H., Samejima, K., Goloborodko, A., Samejima, I., Naumova, N., Nuebler, J., Kanemaki, M. T., Xie, L., Paulson, J. R., Earnshaw, W. C. et al. (2018). A pathway for mitotic chromosome formation. *Science* **359**, eaao6135. doi:10.1126/science.aao6135

Heitz, E. (1928). *Das heterochromatin der moose*. Bornträger.

Hildebrand, E. M. and Dekker, J. (2020). Mechanisms and functions of chromosome compartmentalization. *Trends Biochem. Sci.* **45**, 385-396. doi:10.1016/j.tibs.2020.01.002

Kind, J., Pagie, L., Ortabozkoyun, H., Boyle, S., de Vries, S. S., Janssen, H., Amendola, M., Nolen, L. D., Bickmore, W. A. and Van Steensel, B. (2013). Single-cell dynamics of genome-nuclear lamina interactions. *Cell* **153**, 178-192. doi:10.1016/j.cell.2013.02.028

Kireeva, N., Lakonishok, M., Kireev, I., Hirano, T. and Belmont, A. S. (2004). Visualization of early chromosome condensation: a hierarchical folding, axial glue model of chromosome structure. *J. Cell Biol.* **166**, 775-785. doi:10.1083/jcb.200406049

Klein, K. N., Zhao, P. A., Lyu, X., Sasaki, T., Bartlett, D. A., Singh, A. M., Tasan, I., Zhang, M., Watts, L. P., Hiraga, S. et al. (2021). Replication timing maintains the global epigenetic state in human cells. *Science* **372**, 371-378. doi:10.1126/science.aba5545

Liang, Z., Zickler, D., Prentiss, M., Chang, F. S., Witz, G., Maeshima, K. and Kleckner, N. (2015). Chromosomes progress to metaphase in multiple discrete steps via global compaction/expansion cycles. *Cell* **161**, 1124-1137. doi:10.1016/j.cell.2015.04.030

Lieberman-Aiden, E., Berkum, N. L. V., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B. R., Sabo, P. J., Dorschner, M. O. et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289-293. doi:10.1126/science.1181369

Manders, E. M. M., Kimura, H. and Cook, P. R. (1999). Direct imaging of DNA in living cells reveals the dynamics of chromosome formation. *J. Cell Biol.* **144**, 813-822. doi:10.1083/jcb.144.5.813

Manuelidis, L. (1985). Individual interphase chromosome domains revealed by in situ hybridization. *Hum. Genet.* **71**, 288-293. doi:10.1007/BF00388453

Marsden, M. P. F. and Laemmli, U. K. (1979). Metaphase chromosome structure: evidence for a radial loop model. *Cell* **17**, 849-858. doi:10.1016/0092-8674(79)90325-8

McStay, B. (2016). Nucleolar organizer regions: genomic 'dark matter' requiring illumination. *Genes Dev.* **30**, 1598-1610. doi:10.1101/gad.283838.116

Michelotti, E. F., Sanford, S. and Levens, D. (1997). Marking of active genes on mitotic chromosomes. *Nature* **388**, 895-899. doi:10.1038/42282

Mitter, M., Gasser, C., Takacs, Z., Langer, C. C. H., Tang, W., Jessberger, G., Beales, C. T., Neuner, E., Ameres, S. L., Peters, J. M. et al. (2020). Conformation of sister chromatids in the replicated human genome. *Nature* **586**, 139-144. doi:10.1038/s41586-020-2744-4

Miura, H. and Hiratani, I. (2022). Cell cycle dynamics and developmental dynamics of the 3D genome: toward linking the two timescales. *Curr. Opin. Genet. Dev.* **73**, 101898. doi:10.1016/j.gde.2021.101898

Nagano, T., Lubling, Y., Stevens, T. J., Schoenfelder, S., Yaffe, E., Dean, W., Laue, E. D., Tanay, A. and Fraser, P. (2013). Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. *Nature* **502**, 59-64. doi:10.1038/nature12593

Nagano, T., Lubling, Y., Várnai, C., Dudley, C., Leung, W., Baran, Y., Mendelson Cohen, N., Wingett, S., Fraser, P. and Tanay, A. (2017). Cell-cycle dynamics of chromosomal organization at single-cell resolution. *Nature* **547**, 61-67. doi:10.1038/nature23001

Naumova, N., Imakaev, M., Fudenberg, G., Zhan, Y., Lajoie, B. R., Mirny, L. A. and Dekker, J. (2013). Organization of the mitotic chromosome. *Science* **342**, 948-953. doi:10.1126/science.1236083

Nora, E. P., Lajoie, B. R., Schulz, E. G., Giorgetti, L., Okamoto, I., Servant, N., Piolot, T., Van Berkum, N. L., Meisig, J., Sedat, J. et al. (2012). Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* **485**, 381-385. doi:10.1038/nature11049

Nora, E. P., Goloborodko, A., Valton, A. L., Gibcus, J. H., Uebersohn, A., Abdennur, N., Dekker, J., Mirny, L. A. and Bruneau, B. G. (2017). Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization. *Cell* **169**, 930-944.e22. doi:10.1016/j.cell.2017.05.004

Oomen, M. E., Hedger, A. K., Watts, J. K. and Dekker, J. (2020). Detecting chromatin interactions between and along sister chromatids with SisterC. *Nat. Methods* **17**, 1002-1009. doi:10.1038/s41592-020-0930-9

Ou, H. D., Phan, S., Deerinck, T. J., Thor, A., Ellisman, M. H. and O'Shea, C. C. (2017). ChromEMT: visualizing 3D chromatin structure and compaction in interphase and mitotic cells. *Science* **357**, eaag0025. doi:10.1126/science.aag0025

Paulson, J. R. and Laemmli, U. K. (1977). The structure of histone-depleted metaphase chromosomes. *Cell* **12**, 817-828. doi:10.1016/0092-8674(77)90280-X

Pelham-Webb, B., Polyzos, A., Wojenski, L., Kloetgen, A., Li, J., Di Giammartino, D. C., Sakellaropoulos, T., Tsirigos, A., Core, L. and Apostolou, E. (2021). H3K27ac bookmarking promotes rapid post-mitotic activation of the pluripotent stem cell program without impacting 3D chromatin reorganization. *Mol. Cell* **81**, 1732-1748.e8. doi:10.1016/j.molcel.2021.02.032

Rabl, C. (1887). *Uber Zelltheilung*. W. Engelmann. https://books.google.com/books?id=8pSCHAAACAAJ.

Rao, S. S. P., Huang, S.-C., Hilaire, B. G. S., Engreitz, B., Perez, J. M., Kieffer-Kwon, E. M., Sanborn, K. R., Johnstone, A. L., Bascom, S. E., Bochkov, G. D. et al. (2017). Cohesin loss eliminates all loop domains. *Cell* **171**, 305-320.e24. doi:10.1016/j.cell.2017.09.026

Rattner, J. B. and Lin, C. C. (1985). Radial loops and helical coils coexist in metaphase chromosomes. *Cell* **42**, 291-296. doi:10.1016/S0092-8674(85)80124-0

Schoenfelder, S. and Fraser, P. (2019). Long-range enhancer–promoter contacts in gene expression control. *Nat. Rev. Genet.* **20**, 437-455. doi:10.1038/s41576-019-0128-0

Sedat, J. and Manuelidis, L. (1978). A direct approach to the structure of eukaryotic chromosomes. *Cold Spring Harbor Symp. Quant. Biol.* **42**, 331-350. doi:10.1101/SQB.1978.042.01.035

Sexton, T., Yaffe, E., Kenigsberg, E., Bantignies, F., Leblanc, B., Hoichman, M., Parrinello, H., Tanay, A. and Cavalli, G. (2012). Three-dimensional folding and functional organization principles of the drosophila genome. *Cell* **148**, 458-472. doi:10.1016/j.cell.2012.01.010

Simonis, M., Klous, P., Splinter, E., Moshkin, Y., Willemsen, R., de Wit, E., Van Steensel, B. and De Laat, W. (2006). Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). *Nat. Genet.* **38**, 1348-1354. doi:10.1038/ng1896

Song, F., Chen, P., Sun, D., Wang, M., Dong, L., Liang, D., Xu, R. M., Zhu, P. and Li, G. (2014). Cryo-EM study of the chromatin fiber reveals a double helix twisted by tetranucleosomal units. *Science* **344**, 376-380. doi:10.1126/science.1251413

Splinter, E., Heath, H., Kooren, J., Palstra, R.-J., Klous, P., Grosveld, F., Galjart, N. and de Laat, W. (2006). CTCF mediates long-range chromatin looping and local histone modification in the β-globin locus. *Genes Dev.* **20**, 2349-2354. doi:10.1101/gad.399506

Symmons, O., Uslu, V. V., Tsujimura, T., Ruf, S., Nassari, S., Schwarzer, W., Ettwiller, L. and Spitz, F. (2014). Functional and topological characteristics of mammalian regulatory domains. *Genome Res.* **24**, 390-400. doi:10.1101/gr.163519.113

Thiecke, M. J., Wutz, G., Muhar, M., Tang, W., Bevan, S., Malysheva, V., Stocsits, R., Neumann, T., Zuber, J., Fraser, P. et al. (2020). Cohesin-dependent and -independent mechanisms mediate chromosomal contacts between promoters and enhancers. *Cell Rep.* **32**, 107929. doi:10.1016/j.celrep.2020.107929

Thomson, I., Gilchrist, S., Bickmore, W. A. and Chubb, J. R. (2004). The radial positioning of chromatin is not inherited through mitosis but is established De Novo in early G1. *Curr. Biol.* **14**, 166-172. doi:10.1016/j.cub.2003.12.024

Trask, B. J., Allen, S., Massa, H., Fertitta, A., Sachs, R., van den Engh, G. and Wu, M. (1993). Studies of metaphase and interphase chromosomes using fluorescence in situ hybridization. *Cold Spring Harbor Symp. Quant. Biol.* **58**, 767-775. http://symposium.cshlp.org/content/58/767.short. doi:10.1101/SQB.1993.058.01.084

van Schaik, T., Vos, M., Peric-Hupkes, D., HN Celie, P. and van Steensel, B. (2020). Cell cycle dynamics of lamina–associated DNA. *EMBO Rep.* **21**, 1-17. doi:10.15252/embr.202050636

Van Steensel, B. and Henikoff, S. (2000). Identification of in vivo DNA targets of chromatin proteins using tethered Dam methyltransferase. *Nat. Biotechnol.* **18**, 424-428. doi:10.1038/74487

Vogel, M. J., Peric-Hupkes, D. and van Steensel, B. (2007). Detection of in vivo protein–DNA interactions using DamID in mammalian cells. *Nat. Protoc.* **2**, 1467-1478. doi:10.1038/nprot.2007.148

Wutz, G., Várnai, C., Nagasaka, K., Cisneros, D. A., Stocsits, R. R., Tang, W., Schoenfelder, S., Jessberger, G., Muhar, M., Hossain, M. J. et al. (2017). Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins. *EMBO J.* **36**, 3573-3599. doi:10.15252/embj.201798004

Zhang, H., Emerson, D. J., Gilgenast, T. G., Titus, K. R., Lan, Y., Huang, P., Zhang, D., Wang, H., Keller, C. A., Giardine, B. et al. (2019). Chromatin structure dynamics during the mitosis-to-G1 phase transition. *Nature* **576**, 158-162. doi:10.1038/s41586-019-1778-y

Zhao, Z., Tavoosidana, G., Sjölinder, M., Göndör, A., Mariano, P., Wang, S., Kanduri, C., Lezcano, M., Sandhu, K. S., Singh, U. et al. (2006). Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. *Nat. Genet.* **38**, 1341-1347. doi:10.1038/ng1891