Genome Organization: Tag it, Move it, Place it

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Abstract

Chromosomes are selectively organized within the nuclei of interphase cells reflecting the current fate of each cell and are reorganized in response to various physiological cues to maintain homeostasis. While substantial progress is being made to establish the various patterns of genome architecture, less is understood on how chromosome folding/positioning is achieved. Here we discuss recent insights into the cellular mechanisms dictating chromatin movements including the use of epigenetic modifications and allosterically regulated transcription factors as well as a nucleoskeleton system comprised of actin, myosin, and actin-binding proteins. Together, these nuclear factors help coordinate the positioning of both general and cell-specific genomic architectural features.

Introduction

A eukaryotic cell houses its genetic material within a nucleus. Here, the longest and heaviest biomolecules, chromosomes, are condensed to fit within this central organelle. In humans ~2 m of nucleic acid polymer totaling ~2.15 X 10^{12} Da is fit into nuclei having diameters of ~5–10 μM. This challenge is compounded since DNA is not stored naked but is an amalgam with proteins and RNA to form chromatin, which minimally doubles its mass [1]. Notably, chromatin is not simply compacted into a tangled mass within interphase cells but rather genomes are spatially arranged reflecting both the developmental type and physiological status of each cell [2]. Likely, methodical chromosome folding fosters access to the necessary tracts of DNA to support homeostasis. Despite the conserved three-dimensional (3D) configuration of genomes [3], the mechanisms mediating genome packaging are still unclear. As chromosome reorganization is a common biological event reoccurring after each cell division and also in response to ever fluctuating environmental and internal cues, a better understanding of this process is imperative. Here, we review recent work supporting

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the model that genomes are packaged using a hierarchical architecture within interphase cells and discuss insights into the cellular pathways mediating the organization process.

Chromosome Architectural Features

Classic work argued that chromosomes are arranged into territories within interphase nuclei [4]. Eventually the preferential placement of every chromosome within a nucleus, relative to the periphery and interior, was shown using fluorescent in situ hybridization (FISH) and chromosome paints [5]. The resolution of chromosome arrangements was greatly enhanced by the development of chromatin conformation capture (3C)-based assays (ie, 3C to Hi-C) that identify statistically significant contact frequencies between DNA sites across a genome [2]. A seminal Hi-C study demonstrated that the human genome partitions into two compartments that are composed of open (active; A) and closed (inactive; B) chromatin [6]. Notably, the A/B compartments correspond to the classic euchromatin and heterochromatin areas originally described by cytological staining [7]. The compartments further divide into smaller units such as topologically associated domains (TADs) or lamin associated domains (LADs) [2]. Of note, these domains dissipate during prometaphase and must be recurrently formed after each cell division [8]. In general, the domain boundaries are constant across cell types and species and the DNA within a subdomain displays a higher frequency of internal chromosomal interactions and limited associations with the adjacent domains [2]. Yet, the genetic connections within a TAD (sub-TADs) and interactions between domains (loops) can differ as well as the relative nuclear positions of the domains [9]. Hence, differences in these chromosomal arrangements likely contribute to cell (eg, tissue type) and physiological (eg, aging and disease) fates. Although it is now evident that cells have both common and specific 3D chromosomal features, how either is achieved is unclear. Minimally, building any genomic structure will rely on the same principles that are central to all organizational systems: 1) the ability to selectively recognize the items to be moved; 2) a mechanism to mobilize the marked units; and 3) the instructions on where to place the objects.

Genome Organization Tags (GOTs)

The most apparent sources of information to initiate chromosome architecture are the DNA elements within a genome sequence as well as epigenetic marks on the histone octamers integrated into the chromatin. We refer to these factors as well as any other physical features that mark chromosome regions for select 3D packaging as Genome Organization Tags (GOTs). Notably, DNA elements and histone modifications can serve to assemble both common or cell-specific structures, since the epigenetic landscape as well as the DNA binding factors expressed in any cell can vary. Likely, these GOTs are key guides in building an appropriate 3D genome structure on a cell-to-cell basis.

Early work on TADs and LADs demonstrated that the conserved border regions are marked by CCCTC-binding factor (CTCF) and cohesin binding sites [2]. CTCF and cohesin anchor loops that can connect gene promoters and distal enhancers [10]. The loops are thought to form through an extrusion mechanism in which a cohesin ring translocates along the DNA until it encounters DNA-bound CTCF [11]. Hence, the CTCF DNA elements deliver critical

information to the system, as these are recurrently used to reform TADS following each cell division [12]. Overall, CTCF governs ~80% of all TADs, yet loss of CTCF does not impact partitioning of the A/B compartments [13]. Hence, CTCF serves as a major determinant of TAD boundaries, but additional mechanisms must contribute to genome organization including the formation of some TADs.

The initial discovery of the A/B compartments by Hi-C noted that the partitioned regions correlate with the relative DNase sensitivities of the associated chromatin [6]. A higher resolution Hi-C map further divided the 2 compartments into 6 sub-compartments (A1–2 and B1–4) based on refined DNA interactions and, importantly, select histone modifications (H3K36me3, H3K27me3, H3K4me1, H3K4me2, H3K4me3, H3K9me3, H3K79me2, and H4K20me1) [11] (Figure 1a). As expected the gene-rich A1 and A2 sections favor activating chromatin marks such as H3K36me3, H3K79me2, H3K27ac and H3K4me1 whereas B1–3 correlate with various heterochromatin characteristics such as H3K27me3 (B1; facultative heterochromatin), nuclear lamina/pericentromeric heterochromatin (B2), or lacking an enrichment in any particular mark suggesting common heterochromatin (B3). The B4 region displays a distinctive pattern favoring both activating (H3K36me3) and repressing (H3K9me3 and H4K20me3) modifications [10]. Significantly, using just the epigenetic modification patterns it is possible to predict the coarse 3D structure of a genome de novo leading to the concept that genome architecture self-assembles through a phase separation mechanism that is based upon epigenetic signatures [11,14]. Although the contributions of phase separation to genome folding is yet to be empirically shown, the predictive powers of the epigenetic code suggest that the intrinsic information present along the chromatin polymers is sufficient to guide folding under ideal conditions. In certain respects, this concept is akin to protein folding where the primary amino acid sequence is sufficient to attain a final native protein structure under ideal in vitro conditions yet all polypeptides rely on molecular chaperones for proper folding in vivo [15]. Importantly, the cell interior rarely, if ever, provides ideal conditions and therefore chromosome organization is likely facilitated by extrinsic factors in a manner comparable to molecular chaperones promoting the protein folding quality control process. Despite the correlation between histone code patterns and chromosome compartments, it is still unclear how the epigenetic histone marks functionally contribute to genome organization.

One proposed mechanism to account for an epigenetic-dependent clustering of select genomic regions through a self-assembly mode is the use of histone "reader" proteins that can both selectively recognize histone marks and self-associate [11,16,17]. The prototypical molecule for this concept is Heterochromatin Protein 1 (HP1), which selectively binds H3K9me2/3 and can self-associate [18]. HP1 was originally identified as a heterochromatin-associated protein in *Drosophila melanogaster* and mammalian HP1 maintains a compacted heterochromatic state that is dependent upon HP1 dimerization [18]. It has been suggested that HP1 undergoes a phase separation to form constitutive heterochromatin compartments [16,17]. However, it is unclear whether the phase separation drives heterochromatin formation or if a phase shift happens once the domain is established. We favor the latter since phase separation typically requires high concentrations of the shifting protein, which are unlikely to exist while chromosomes are being actively organized [19]. Minimally, HP1 maintains a heterochromatin state once the chromatin has been clustered by spreading across

adjacent nucleosomes using its distance-limited H3K9me3-induced self-association bridging activity [20,21]. Nevertheless, it is still plausible that HP1 fosters the organization of constitutive heterochromatin compartments independent of its phase separation behavior.

The ability of HP1 to specifically bind H3K9me2/3 satisfies the first step of an organization system—selective recognition of the item to be moved. Missing is how the binding of HP1 to these marks would allow sizable regions of chromatinized chromosomes to be coerced together into an organized unit—what nuclear system might drive the motion of these large chromatin polymers? We suspect that other protein partners connect HP1 to such a system. In particular, we believe that the Actin related protein 6 (Arp6), which associates with HP1 from flies to humans [22,23], links chromatin-bound HP1 to an actin-based system in the nucleus (Figure 1b), which is described below. While a direct role for HP1-Arp6 in this process is yet to be shown, it is notable that Arp6 colocalizes with HP1 at pericentric heterochromatin [23,24]. Regardless of the exact role(s) of HP1 with heterochromatin, additional information beyond broadly used epigenetic marks will be required to properly organize genomes.

The most apparent source of detailed information to coordinate chromosome architecture is the genome sequence itself. Yet, few examples of DNA elements capable of directing chromatin organization in interphase cells, besides CTCF elements, have been reported. A notable exception is the pioneering work exploiting the *INO1* gene in budding yeast as a model to understand directed chromosome motion [25]. In brief, the *INO1* locus is inactive and localized predominantly to the inner nucleoplasm in the presence of inositol but colocalizes with the nuclear membrane following inositol starvation and gene activation [25]. Significantly, movement of *INO1* to the nuclear periphery is dependent upon a Gene Recruitment Sequence I (GRS I) upstream of the transcription start site along with the Put3 transcription factor that binds to GRS I [26]. Loss of Put3 or ablation of GRS I reveals a second site, GRS II, capable of mobilizing *INO1* to the nuclear membrane in conjunction with the Cbf1 transcription factor [26]. Intriguingly, the DNA sequences of either GRS do not resemble the known consensus elements of the associated transcription factors. This divergence in sequence of the element dictating nuclear positioning might help explain why it has been generally difficult to identify DNA elements associated with genome architecture. Significantly, the *INO1* system was instrumental in discovering a potential nuclear pathway that satisifies the second step of an organization system—a mechanism to mobilize a marked unit.

Actin-based Transport in the Nucleus

Cargo transport in a cell is often associated with a cytoskeleton system where actin, actin-binding proteins (ABPs), and myosin motors move large biomolecules precisely from one locale to another [27]. Although actin was discovered in the nucleus over half a century ago by subcellular fractionation [28], a role for actin-based transport in the nucleus had been met with skepticism based, in part, on a limited capacity to detect filamentous actin in the nucleus using the common actin-stain phalloidin [29]. Fortunately, the development of new detection reagents (eg, ABPs or actin-nanobodies fused to GFP) have yielded significant new insights in the existence and relevance of a nucleoskeletal system incorporating various

actin species including monomers, polymers, and rods [30]. Perhaps one of the more exciting concepts to emerge is a seemingly general use of the nuclear actin system in genome organization events.

In normal eukaryotic cells from yeast to flies to mammals visualization of nuclear actin with an actin-nanobody reveals a punctate pattern [31–34]. At least in yeast, the puncta are lost following treatment with an actin polymerization inhibitor [34]. Further interrogation of the actin-nanobody signal in yeast using an Airyscan confocal microscope revealed a dynamic polymerization behavior for nuclear actin [34]. Intriguingly, various cell perturbations including cell division, environmental cues, or DNA damage readily stabilize actin filaments in the nucleus.

The functional relevance of nuclear f-actin appears broad including for the expansion of the nuclear volume and decondensing chromosomes following mitosis or during embryonic development [35]. In the course of development nuclear actin shifts from puncta near the DNA to a filamentous cage surrounding the chromosomes within oocytes [36]. More subtle physiological events also correlate with f-actin formation such as activation of G protein-coupled receptors that trigger polymerization of f-actin starting at the nuclear membrane and moving into the nucleoplasm to alter chromatin organization [37]. Conversely, DNA damage events produce actin filaments to promote the repair process by minimally transporting double-stranded DNA breaks (DSB) from the inner nucleoplasm to the nuclear periphery [32,33]. Our group has shown that actin polymerization is required to transport activated genes to the nuclear membrane although stable actin polymers were not part of this process [34]. In several cases it has been noted that the association between actin and chromatin requires different histone marks including H3K27me3 and H3K9me3 during oogenesis [38] or decreased H3S10p and increased H4K16ac during the mitotic exit [37], which might explain site-specific nucleation of actin at certain chromatin sites.

At heterochromatic DSBs actin is selectively recruited to the damaged site by the combined efforts of Mre11, which is a subunit of the MRN complex that recognizes the damaged DNA site, and HP1 [32,33]. In brief, the Mre11 and HP1 nucleate the Arp2/3 complex, Arp2/3 fosters f-actin polymerization towards the nuclear periphery, and the Hsp90 co-chaperone Unc45 activates the nuclear myosins (Myo1A, Myo1B, and MyoV) to transport the DSBs to the nuclear membrane (Figure 2a). In some respects, the activated *INO1* gene uses a parallel mechanism to move to the nuclear periphery.

At the *INO1* locus inositol starvation triggers the recruitment of several transcription factors including Ino2, Ino4, and Put3 [26, 34]. As mentioned, Put3 binds to the DNA element, GRS I, required to move *INO1* to the nuclear periphery whereas Ino2 and Ino4 bind the upstream activation sequences (UASs) of the *INO1* promoter to recruit the factors driving gene transcription including the INO80 and SWR-C chromatin remodelers that mediate the exchange of H2A/H2A.Z. In addition to gene promoters, the non-canonical H2A.Z histone is enriched at most TAD boundaries [12,39]. Both of these remodelers contain an actin monomer along with several Arp subunits, which facilitate an interaction with f-actin [40]. At *INO1* this function stabilizes a Put3-mediated nucleation of the unconvention myosin Myo3 to the chromatin that allows the single-headed myosin to transport *INO1* to the

nuclear periphery [34]. As with DSBs, motion of this chromatin site requires an Unc45 homolog along with the Hsp90 chaperone itself (Figure 2b). In contrast to DSBs, Arp2/3 is not used for actin polymerization nor are stable actin filaments apparent during the motion but rather Formin homologs are required to drive *INO1* transport [34]. Besides *INO1*, Formins have proven important for the transcriptional response to serum stimulation as well as mammalian cell spreading [31,41,42].

A common thread in many of the actin-dependent genome organization events is the inclusion of one or more ABP. ABPs were first reported in the nucleus in 1987 when cofilin was found to interact with nuclear actin rods [43]. Of note, cofilin1 is vital for f-actin assembly during mitotic exit to increase the nuclear volume of daughter cells [35]. In general, ABPs serve to control the behavior of actin [27]. A critical class of ABPs are the Actin-related proteins (ARPs), which are conserved from yeast to human. ARPs share structural features with conventional actin despite varied primary sequences [44]. Initial characterization of the ARPs in budding yeast identified 10 homologs with 4 primarily found in the cytoplasm (Arp1, 2, 3, and 10) and 6 enriched in the nucleus (Arp4, 5, 6, 7, 8, and 9) [45]. ARP nomenclature is based on an increasing diversity relative to actin with Arp1 sharing 52% identity and Arp10 17% [44]. Overall, actin and the ARPs belong to a larger protein family that includes the 70 kDa heat shock proteins (Hsp70s), hexokinases, and glycerol kinases where the actin-fold is minimally used as a conformational switch point [46].

As the best studied ARPs, Arp2 and Arp3 form a complex enriched in the cytoplasm that controls actin polymerization through actin nucleation and branching [27], it was anticipated that most ARPs would govern actin dynamics. Yet, nuclear ARPs are primarily known as components of large, multi-subunit chromatin modifying complexes including nucleosome remodelers (INO80, SWR-C, SWI/SNF, RSC, p400) and histone acetylases (NuA4) where an ARP effectively caps the barbed end of a monomeric actin co-subunit [40]. However, chromatin modifiers often contain additional ARP-subunits that are not in contact with the actin subunit. Moreover, the majority of the nuclear ARPs are not part of large protein structures (i.e. >150 kDa), which would be comparable to most chromatin modifying complexes [46]. Hence, the functional relevance of most nuclear ARPs is not clear. Yet, the capacity of the ARPs to contribute to f-actin binding, even when part of a large chromatin modifier, suggests that mediating actin interactions is a major ARP contribution within the nucleus. Might the interaction between HP1 and Arp6 serve a similar role to coordinate an initial clustering of chromosome domains after cell division in conjunction with a dynamic nuclear actin network (Figure 1b)? Intriguingly, in *Arabidopsis* the different nuclear Arps display distinct nuclear localization patterns [44], which might be useful in either establishing or maintaining different chromosome domains.

Once a chromosome site/domain has been targeted for transport, a force-generating activity, such as a myosin motor, will be engaged at least for physiological events that require immediate action such as the response to nutrient deprivation (*INO1*) or DNA damage (DSBs). For example, homology-dependent pathways directing either DNA repair or allele-paired transcription rely on nuclear myosin I (NMI) or myosin VI, respectively [47,48]. In general, it seems that most rapid long distance chromatin movements depend on nuclear

myosin and actin [49,50]. A recent report from Christof Gebhardt's group following single molecules of nuclear myosin VI found that transcription-dependent long-range chromatin rearrangements occurred along nuclear actin filaments [A Große-Berkenbusch et al., unpublished]. Whether all chromosome movements will require a myosin motor or if other avenues are utilized is an open question. For instance, it is possible that large-scale partitioning events such as the formation of the A/B compartments might just rely on the rapid actin polymerization/depolymerization found within interphase cells [34]. In this case, the Arp-dependent connections between histone code readers and the dynamic actin network would facilitate local concentration gradients formed by the weak interactions between the same reader proteins (eg, HP1 dimers) thereby clustering the chromosome regions with the same epigenetic signatures (Figure 1b). As each large-scale domain coalesces, the finer details of cell-specific chromosome architecture would be further guided by the DNA elements along the genome in conjunction with the expressed cognate transcription factors. Once a suitable concentration of the reader is achieved, a phase transition could then occur to enforce the maintenance of the newly formed domain. Persistence of such a dynamic actin-based system throughout interphase also provides a plausible explanation for variances in genome organization that have been noted in single cell experiments [51].

Where does it end?

While a variety of reports are providing exciting insights into the first two steps governing an effective genome organizing system (ie, marking items to be moved and a mechanism to move the items), less is understood about the parameters determining the final step—instructions on where to place the objects. What signals and/or mechanisms are in place to guide chromosome domains and/or chromatin sites to select areas of the nuclear space. For instance, placement of a GRS I sequence within an unrelated gene is sufficient to guide that site to same spot along the nuclear periphery as the inositol-dependent reorganized *INO1* locus [26]—what cellular signaling network provides the necessary long-distance information to the nuclear transport system to guide it from the inner nucleoplasm to a select site along the nuclear membrane? Recent super-resolution microscopy work as well as proximity techniques (i.e. DamID and TSA-Seq) might be revealing these details by showing how various nuclear bodies contribute to genome organization [52,53] as well as how the packaging fluctuates in response to transcription activation including interactions with the nuclear envelope [54] and splicing speckles [55]. Clearly, there is much left to be discovered on how cells make critical decision on where and when to position their genomic information.

Acknowledgements

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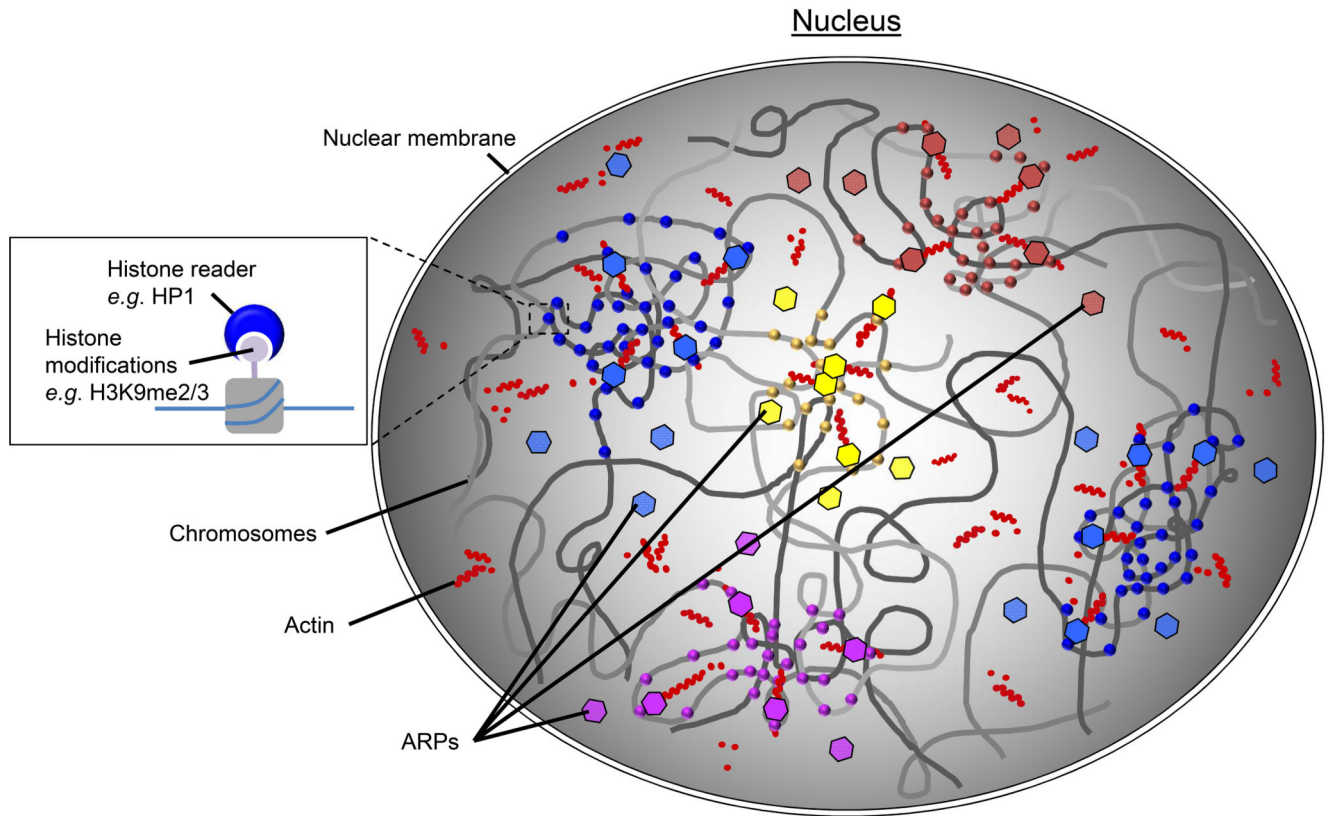
a

Nucleus

A Sub-Compartments

B Sub-Compartments

Chromosomes

Nuclear membrane
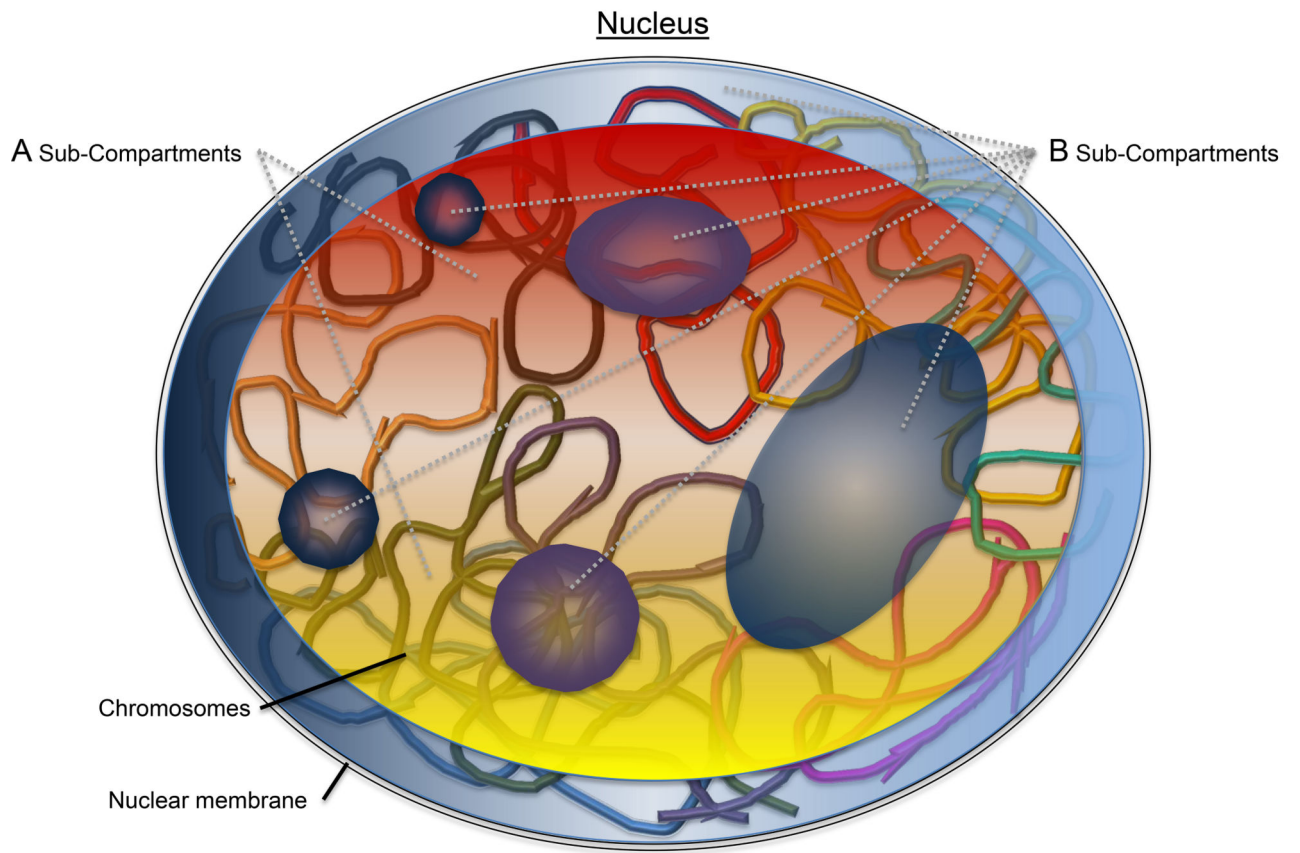
Figure 1. Chromosome regions are clustered based upon epigenetic signatures.
(a) In a nucleus, eukaryotic genomes are partitioned into two main compartments (A/B), as detected by Hi-C [6], that correspond to the classic heterochromatin and euchromatin regions [7]. The main compartments are further sub-divided (compartments A1-A2 and B1-B4) based upon varying epigenetic signatures. Here, the general locale of A1 and A2 sub-compartments are represented by the red and yellow regions primarily found in the nuclear interior whereas the B1-B4 sub-compartments are shown by the blue and purple areas that are minimally enriched at the nuclear periphery and around the nucleolus. While it is clear that the various regions correlate with select epigenetic modifications [11,16], how this information is used to physically cluster the chromatin is yet to be revealed. **(b)** A plausible model to account for the nuclear organization of large-scale genomic features such as the A/B compartments and sub-compartments (A1-B4) is the coordinated use of a dynamic nuclear actin network to effectively move the large chromosome regions, epigenetic readers (eg, HP1) to selectively distinguish the various histone marks (circles), and ARPs (hexagons) to bridge the readers to the actin (red dots) network. The various colors of the circles and hexagons, represent the various readers and ARPs found with eukaryotic nuclei. Together, this system would provide an effective means to reorganize a genome after each cell division within a biologically useful timescale (<hour).
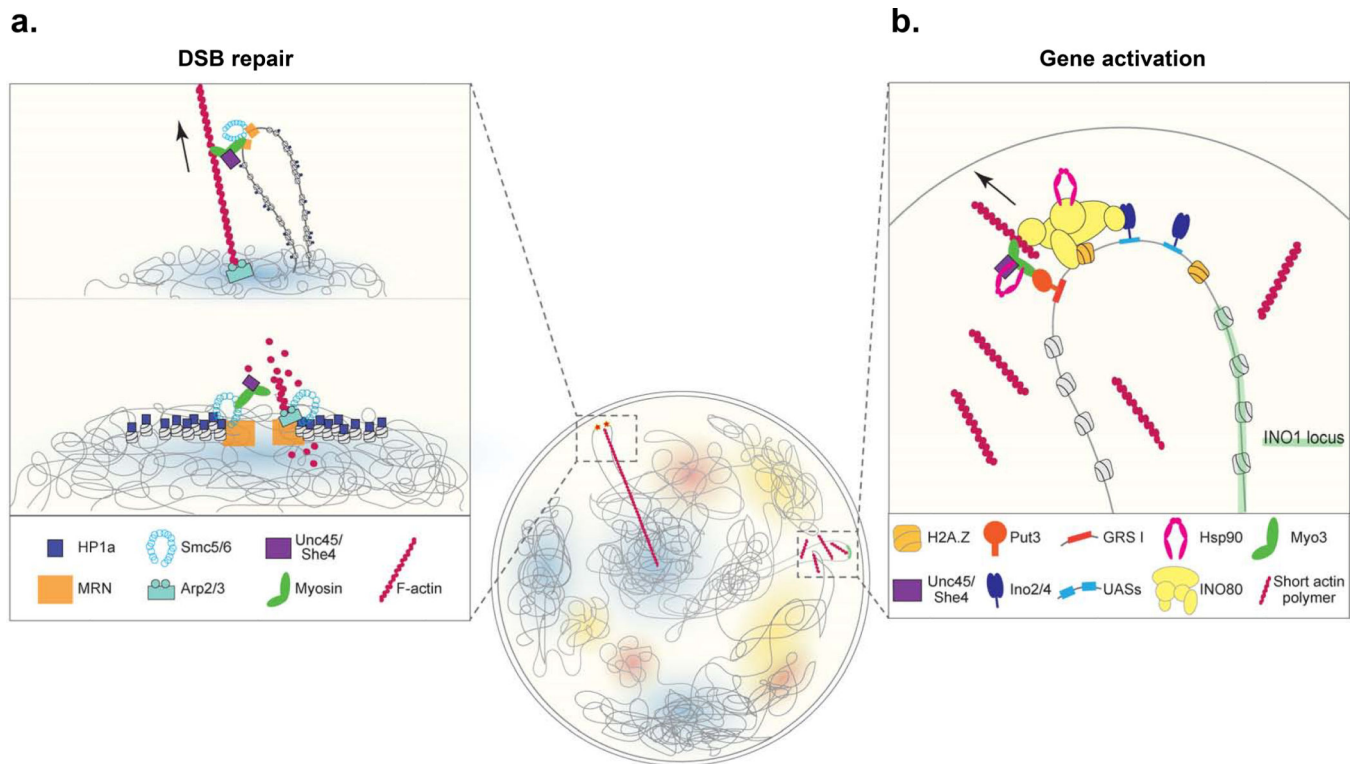
Figure 2. Chromatin sites are reorganized using a myosin motor-driven nucleoskeleton system selectively targeted to a genomic locus.

(a) Double stranded DNA breaks (DSBs) within heterochromatin regions are recognized by the Mre11/Rad50/Nbs1 (MRN) complex, in conjunction with HP1, Arp2/3 is recruited to site to polymerize F-actin towards the nuclear periphery (lower panel). Nuclear myosins along with the Hsp90 co-chaperone Unc45 actively transport the break sites to the nuclear periphery along the actin tracks where the DNA will be effectively repaired (upper panel) [32,33]. **(b)** Upon inositol starvation in budding yeast the *INO1* locus moves from the inner nucleoplasm to the nuclear periphery [25]. The promoter bound transcription factors Ino2/4 at the UASs and Put3 at the GRS I DNA elements nucleate the proteins mediating the transport including the INO80 nucleosome remodeler and Myo3 myosin motor. Together with Unc45 and the Hsp90 chaperone, *INO1* is mobilized to the nuclear periphery utilizing dynamic actin polymers [34].