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How to rule the nucleus: *divide et impera*

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Genome-wide molecular studies have provided new insights into the organization of nuclear chromatin by revealing the presence of chromatin domains of differing transcriptional activity, frequency of *cis*-interactions, proximity to scaffolding structures and replication timing. These studies have not only brought our understanding of genome function to a new level, but also offered functional insight for many phenomena observed in microscopic studies. In this review, we discuss the major principles of nuclear organization based on the spatial segregation of euchromatin and heterochromatin, as well as the dynamic genome rearrangements occurring during cell differentiation and development. We hope to unite the existing molecular and microscopic data on genome organization to get a holistic view of the nucleus, and propose a model, in which repeat repertoire together with scaffolding structures blueprint the functional nuclear architecture.

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Introduction

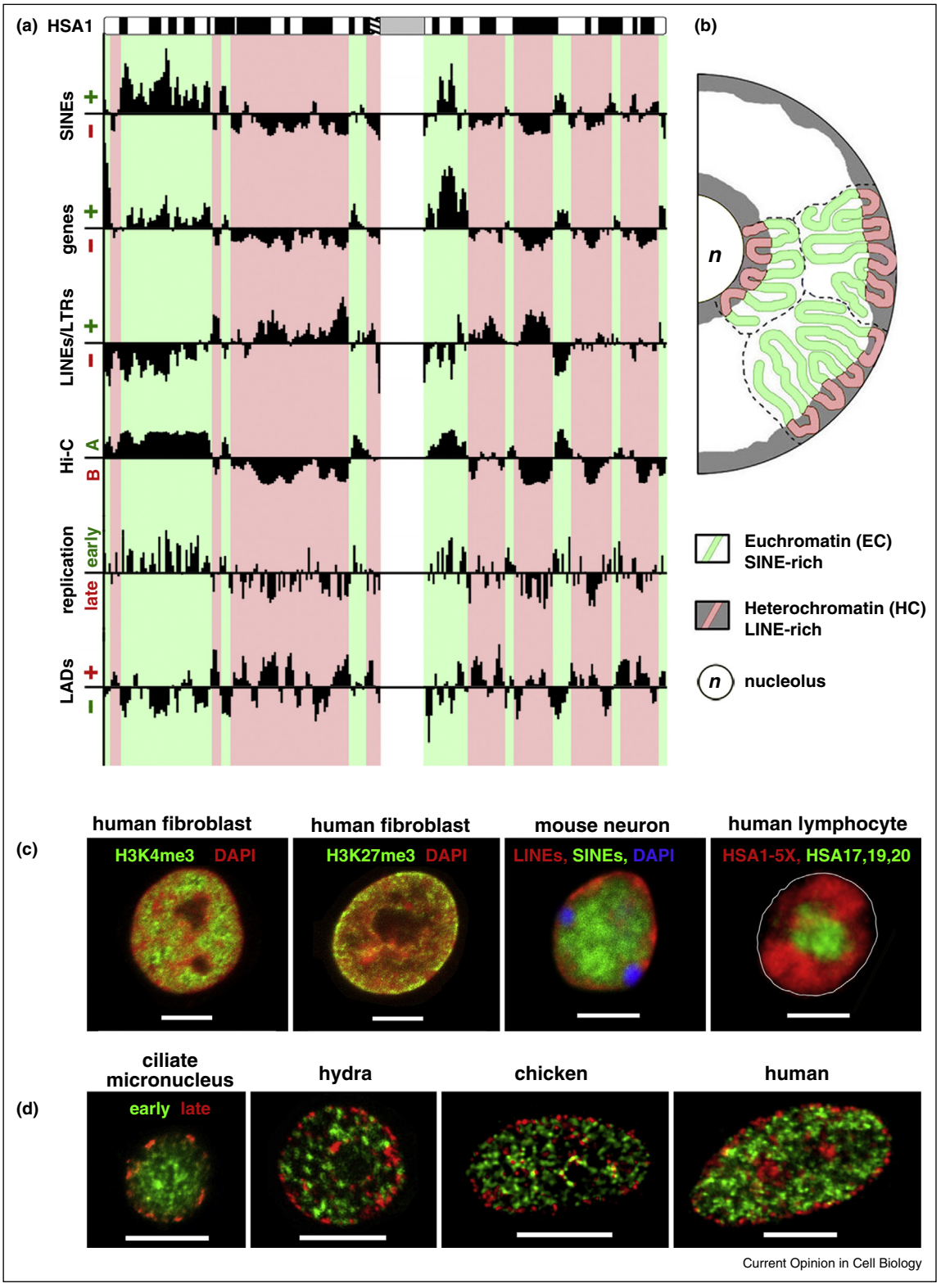
There is growing evidence that spatial organization is the key to genome function. In addition to other epigenetic factors [1], the arrangement of chromatin within the nucleus is important for transcription regulation and for establishing and maintaining cellular identity during differentiation. Recent genome-wide molecular studies have revealed a variety of distinct chromatin units, or domains, that build up the interphase nucleus. Chromatin is subdivided into two compartments, A and B, of juxtaposed transcriptional activity and apparent spatial segregation with loci from the same compartment contacting each other frequently but avoiding contacts with loci from the other compartment [2,3••]. Within compartments, chromatin is self-organized in topologically associated domains (TADs) [4,5], which are structurally conserved and serve as functional platforms for physical interactions between regulatory elements [6–8]. Various other domains, such as lamina-associated domains (LADs) [9,10••,11], nucleolus-associated domains (NADs) [12] or pericentromere-associated domains (PADs) [13•], have been implicated in organizing and anchoring the genome within the nucleus. In cycling cells, genomic regions with time-coordinated replication form replication domains [14–17].

Although the fine details of genome organization and its functional connections to transcription regulation have been thoroughly discussed in a plethora of recent reviews (e.g., this COCB issue, see also [17–24], it remains a challenge to integrate the genomics-based and microscopy-based views into a cohesive concept. Like stepping back from a pointillist painting, we zoom out to see the nucleus in the pattern of its many domains. First, we define euchromatin (EC) and heterochromatin (HC) domains, discuss how chromatin is spatially organized in the nucleus and propose a model in which the repeat repertoire, together with scaffolding structures, determine chromatin folding. Next, we expand on how EC and HC segregation impacts on chromosome topology and is compatible with the invariant subdivision of chromatin into TADs. Finally, we ask how chromatin folding is regulated during differentiation and development, emphasizing the differences between cycling and postmitotic cells.

Spatial arrangement of EC and HC in the nucleus

To achieve a functional nuclear architecture, the genome is segregated into active EC and inactive HC. EC is gene-rich, includes mostly housekeeping genes, and replicates early in S-phase. By contrast, HC is gene-poor, includes tissue-specific genes, and replicates late in S-phase (Figure 1a; see also [17]). Additionally, EC and HC are differentially marked by interspersed repetitive sequences, which account for up to 45% of the mammalian genome [25,26]. Short interspersed repetitive sequences (SINEs) reside mostly in gene-rich EC, whereas retrotransposon-related long elements (LINEs) and LTRs locate preferably in HC (Figure 1a). Analysis of mammalian genomes by Hi-C revealed that every chromosome can be subdivided into two sets of loci forming A and B compartments, within which loci preferentially interact but avoid interactions with the other compartment [2,3••]. A-compartment and B-compartment closely match EC and HC regarding compaction, gene-richness, expression, replication timing and, consequently, also repeat repertoire (Figure 1a).

Figure 1



Euchromatic and heterochromatic chromosome regions and their spatial separation in the nucleus. **(a)** Euchromatin (EC) and heterochromatin (HC) domains revealed by different approaches. Comparison of human chromosome 1 (HSA1) profiles for genes, SINEs, LINEs/LTRs, A/B-compartments [2], replication timing [119], and LADs [9]. EC is gene-dense, SINE-rich and LINE/LTR-poor, corresponds to A-compartment, replicates in the first half of S-phase, and is depleted in LADs. HC shows inverse genomic characteristics, corresponds to B-compartment, and is

While genome partitioning into chromosomes is crucial for proper cell division, it seems to be dispensable for genome function during interphase. Following mitosis, chromosomes decondense and form a seamless chromatin mass, in which individual chromosomes are microscopically indistinguishable, unless visualized with paint probes revealing their territorial organization [27]. EC and HC regions of proximal chromosomes form homotypic intra-chromosomal and inter-chromosomal contacts thereby ensuring spatial segregation. EC occupies the nuclear interior, while HC is predominantly associated with the periphery and the nucleoli (Figure 1b,c). This nuclear organization has been maintained in eukaryotes over more than 500 million years of evolution (Figure 1d) with only a few exceptions (e.g., [28]). Why is EC and HC segregation crucial for nuclear function? Separation of silenced from highly expressed regions in the nucleus could be analogous to separating eating from sleeping by having dedicated rooms. Actively transcribed genes aggregate in foci enriched in transcription and splicing factors [29–33]. Gene silencing occurs in loci with high concentration of HC-establishing enzymes, such as DNMTs, HDACs and HMTs, Polycomb repressive complexes, as well as HC-binding proteins, such as HP1 and MECP2 [20,34–39]. Abrogation of segregation leads to dramatic changes in the developmental program, as was shown recently for *Caenorhabditis elegans* embryos expressing mutant form of CEC-4, a protein anchoring HC to the nuclear envelope [40••].

While segregation is prominent in terminally differentiated cells, it has to be re-established after each division in cycling cells. Mitosis disperses EC and HC, which then gradually separate in two phases during interphase, as oil gradually separates from water in emulsion. In cycling cells, for instance, centromeres are considerably dispersed in G1 phase, but increasingly cluster during transition to S-phase and especially upon exit from the cell cycle [41]. In differentiated cells chromosomal contacts are more stable and the likelihood of chromosomal loci to find each other is increased [28,42].

Chromosome folding and repeat repertoire

EC and HC are unevenly distributed along linear chromosomes. This is reflected, for instance, in the R/G-banding pattern of mammalian chromosomes (Figure 1a). The spatial segregation of EC and HC in the nucleus implies that linear chromosomes with alternating EC/HC distribution are folded and weave between the two compartments (Figure 1b). This view is supported by the polar organization of interphase chromosomes noticed earlier in microscopic studies. Regardless of their linear arrangement on the chromosome, gene-poor late-replicating segments localize closer to the nuclear periphery, whereas gene-rich early-replicating segments position more centrally [43–46] resulting in a clear radial orientation of each chromosome within the nucleus.

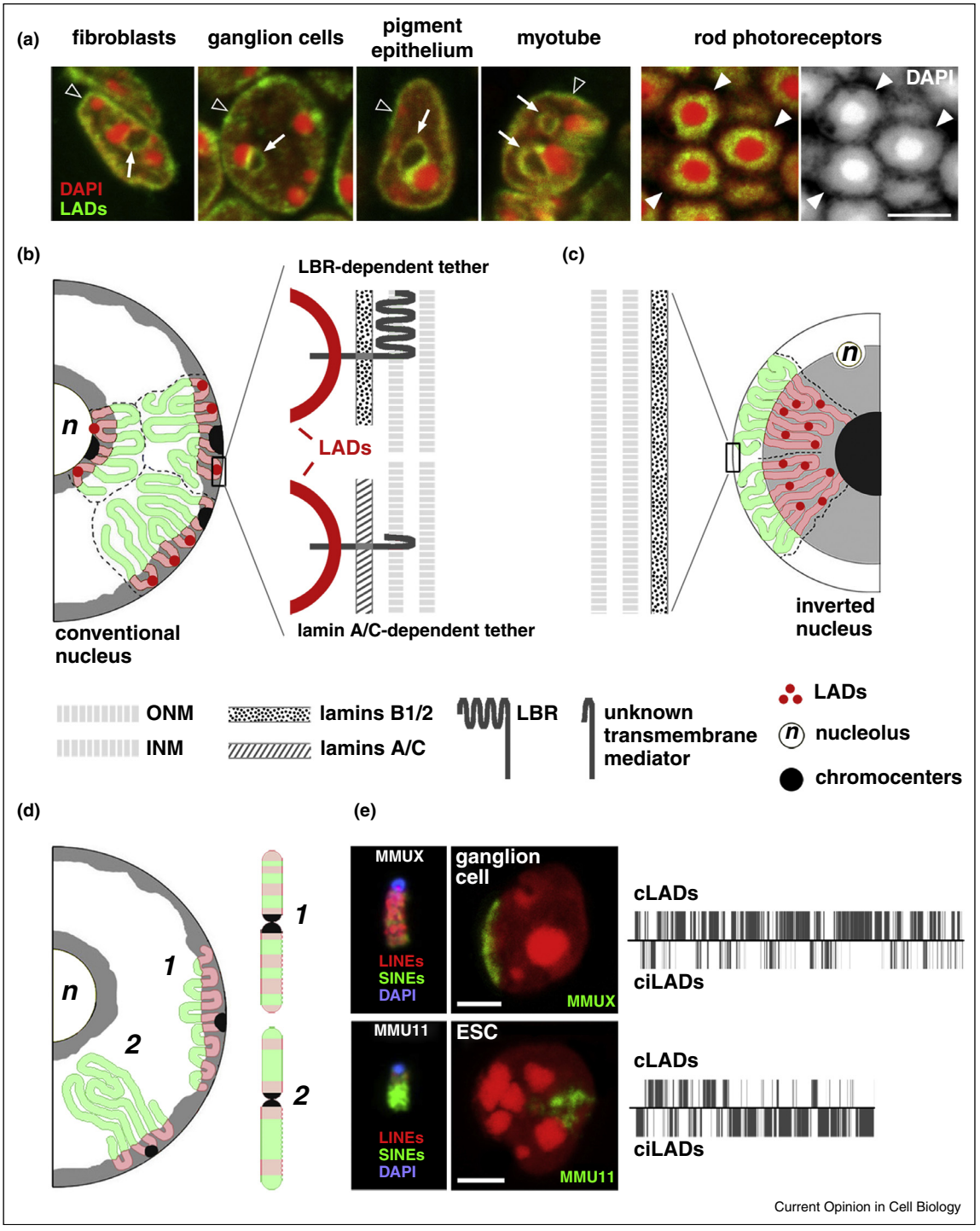
Which mechanisms could keep EC and HC apart? We and others [36,47,48] propose that similarly typed sequences exhibit a high affinity to each other thereby driving the separation of the two opposing compartments. For example, satellite sequences of centromeric and subcentromeric regions fuse into chromocenters during interphase [13•,41,48]. Likewise, attraction between dispersed repetitive sequences selectively enriched in EC and HC, that is, SINEs and LINEs/LTRs, could drive the gathering of homotypic chromatin. The abundance and scattering of the repeats along the chromosome axis enhances the formation of stable contacts between homonymous repetitive sequences, which serve as nucleation points for compartment formation, aptly described by the proverb 'birds of a feather flock together' [49]. Thus, mutual attraction of chromatin marked by the same repetitive sequences, enforced by binding of architectural and epigenetic factors, might serve as a minimal model for the separation of EC from HC.

Segregation by scaffolding

Chromatin segregation is facilitated by binding of HC to scaffolding structures, the nuclear lamina and the nucleolus. 35–40% of the mammalian genome is bound to the lamina in LADs with a median size of 0.5 Mb [9,50]. LADs are distributed along all chromosomes and alternate with inter-LAD regions (iLADs) that rarely contact the lamina [10••,51••,52•]. While LADs are enriched in LINE elements and are thus more abundant in the HC compartment, iLADs feature high gene and SINE content. Nevertheless, there is no exact match between LADs/iLADs and the linear HC/EC chromosomal distribution (Figure 1a see also [53•]). LADs and iLADs can be either cell-type specific (facultative, fLADs) or invariant between cell types and species (constitutive, cLADs and ciLADs) [10••,11,52•]. Although initially discovered as regions contacting the lamina, LADs substantially overlap with NADs [12]. As a result of stochastic reshuffling following mitosis, some LADs reposition towards the nuclear interior where

(Figure 1 Legend Continued) enriched in LADs. EC and HC regions have an alternating distribution along the chromosome, but in the nucleus they form two continuous compartments with distinct spatial segregation (b,c). **(b)** EC resides in the nuclear interior, whereas HC localizes to the nuclear and nucleolar peripheries. Therefore, to contribute to both compartments, chromosomes are folded, weaving between interior and periphery or interior and nucleolus. **(c)** Examples of EC and HC localization in mammalian nuclei revealed by active (H3K4me3) and repressed (H3K27me3) chromatin immunostaining, hybridization with probes for LINEs and SINEs, and visualization of gene-rich (HSA17, 19, 20) and less gene-rich (HSA1-5, X) chromosomes. **(d)** Spatial segregation of chromatin domains is reflected in the replication pattern with early replicating EC (green) and late replicating HC (red) located to the nuclear interior and periphery, respectively; this pattern is remarkably conserved in the evolution from single cellular organisms to mammalian cells. Scale bars = 5 μm.

Figure 2



Binding of heterochromatin to the nuclear lamina and nucleolus. **(a)** Examples of the spatial distribution of LADs in various mouse cells. LADs are located at the nuclear (empty arrowheads) and nucleolar (arrows) borders in conventional nuclei; in inverted nuclei of rod photoreceptors, LADs dissociate from the nuclear envelope (solid arrowheads) and accumulate around the central chromocenter. FISH with a LAD probe on mouse tissue sections. The probe was generated as described in [11]. Scale bar = 5 μm and applies to all images. **(b)** At least two major tethers of peripheral HC binding are identified, the LBR-dependent and LA/C-dependent tethers. **(c)** Lack of both tethers causes dissociation of HC from the nuclear envelope and its clustering in the nuclear interior, which results in inversion of EC and HC positions, as found in rod photoreceptor cells of nocturnal mammals. **(d)** Quantity and arrangement of HC stretches and LADs determine the radial distribution and shape of chromosomes. Chromosomes enriched in HC and LADs (*cartoon chromosome 1*) obtain a peripheral location adjacent to the nuclear envelope. By contrast, chromosomes depleted in HC/LADs (*cartoon chromosome 2*) reside more internally and have a voluminous shape. **(e)** Example of different

they associate with nucleoli and thus become NADs [51••] (Figure 2a).

Several DNA motifs, histone modifications and proteins have been implicated in directing LADs to the periphery [52•,54–57]. Recently, we identified the inner nuclear membrane protein Lamin B-receptor (LBR) and the lamina-constituent Lamin A/C as major components of two peripheral LAD tethers (Figure 2b) [58••]. Both tethers are used sequentially during differentiation with LBR preceding Lamin A/C. Notably, absence of both tethers results in dissociation of HC from the nuclear envelope, its clustering in the nuclear interior and thus in inversion of EC and HC positions (Figure 2a,c). Although natural inversion is unique to rod photoreceptor cells of nocturnal mammals, where it improves night vision [28], it can be induced in other cell types by deletion of the corresponding tether(s) [58••].

Tethering to the periphery affects chromosome topology, that is, radial positioning and shape. Non-random radial distribution of chromosomes, where high gene density correlates with internal positioning [59–62], can be attributed to the amount of HC stretches enriched in LADs (Figure 2d). Comparison of MMUX and MMU11, which have opposing gene and repeat content, reveals a striking difference in both amount and linear distribution of cLADs [11,52•]. While MMUX is highly enriched in almost homogeneously scattered cLADs, MMU11 contains few cLADs, which are gathered in islands and separated by long iLAD stretches (Figure 2e). Tight binding to the lamina results in a flat shape of MMUX, whereas MMU11 protrudes into the nuclear interior and acquires a more voluminous shape (Figure 2e). Accordingly, radial distribution and shape of a given chromosome can be inferred from the chromosome-specific ratio of EC and HC stretches and, in particular, LAD density and distribution.

TADs as basic units of EC/HC segregation

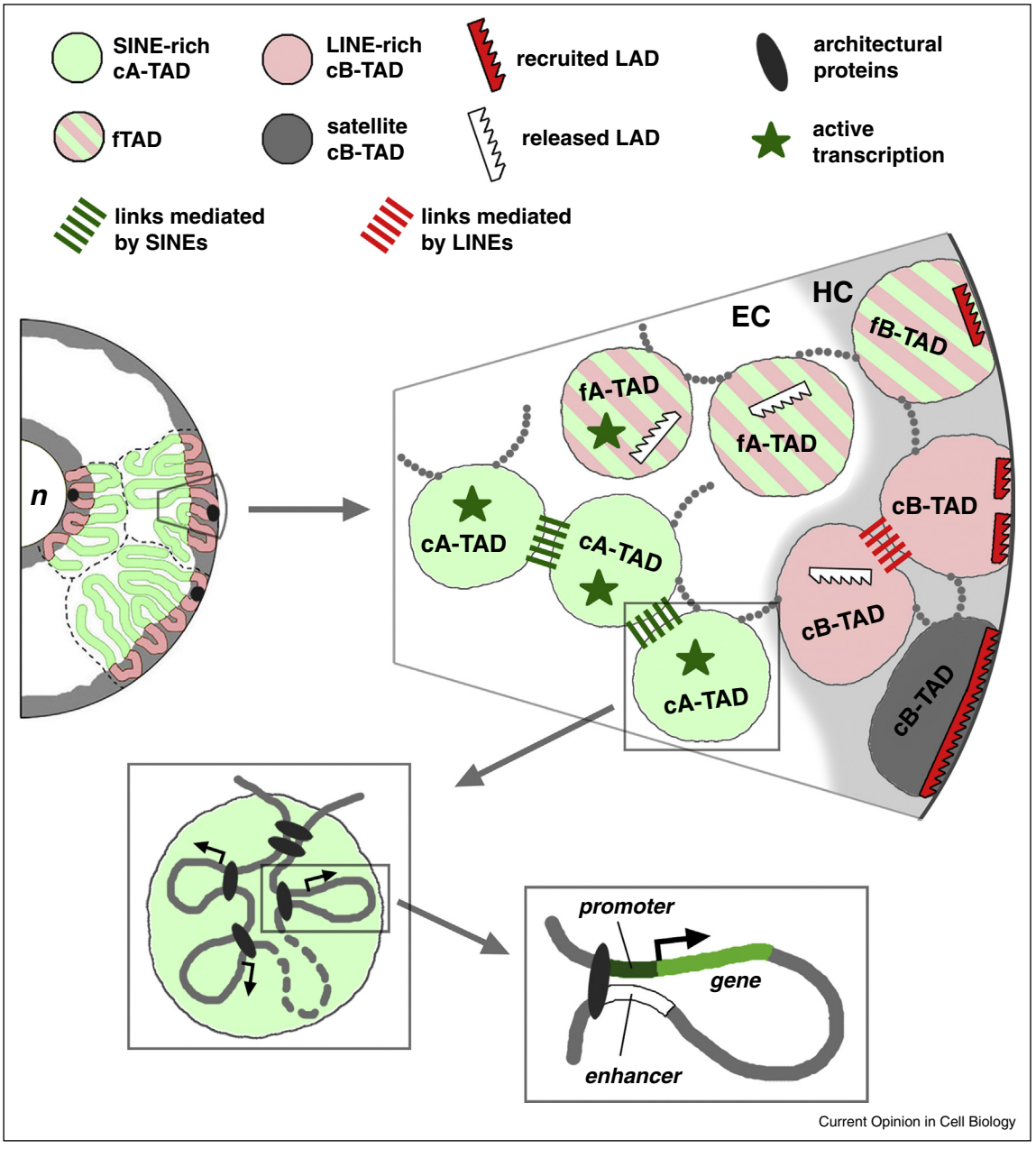
TADs are megabase-sized, self-associating globules demarcated by interaction-depleted boundaries (Figure 3) [4,5,63]. TADs serve as structural platforms for dynamic *cis*-regulatory contacts, in particular between promoters and enhancers [6–8], which govern the establishment of cell-type specific expression [64–66]. Within a TAD, chromatin has a uniform epigenetic signature [4,63] and replication timing [67•,68]. Absence of TADs from silent structures such as metaphase chromosomes [69••] or the inactive X [5,70,71] implies that the organized microenvironment of a TAD is important for transcription regulation. Architectural proteins, such as CTCF, cohesin and Mediator [4,72], assist chromatin folding and participate in the formation of loops between boundaries and within TADs [3••,73,74•,75,76•] but are not required for TAD maintenance *per se* [77–79]. Nevertheless, their deletion changes both inter-TAD and intra-TAD interactions causing gene deregulation [5,8,76•,80••]. Although TADs have been proposed to be relatively invariant between cell-types and species [3••,4,5,81], interactions between and within TADs are dynamic [82–85,86•]. In specific cases, such as the *HoxD* cluster during limb development, small-scale changes within boundaries expose genes to sequentially acting enhancers within adjacent TADs [82,84].

As A-compartment and B-compartment borders consistently coincide with TAD boundaries [4,87••], TADs can be considered the basic units for chromatin segregation. We propose to classify TADs contributing to EC (A-compartment) and to HC (B-compartment) as A-TAD and B-TAD, respectively (Figure 3). Similar to LADs, TADs can be subdivided into constitutive A-TADs or B-TADs (cA-TADs or cB-TADs) and facultative TADs (fTADs), which, as a unit, switch activity and compartments during differentiation, X-inactivation or in response to cues such as hormones [5,86•,87••]. cA-TADs and cB-TADs share features of A-compartment and B-compartment, including differential expression level and enrichment in SINEs and LINEs, and correspond to or constitute ciLADs and cLADs, respectively. fTADs can be classified as fA-TAD or fB-TAD depending on their epigenetic status and accordingly include fLADs, which are dynamic during differentiation (Figure 3) [11,52•]. This classification is supported by data indicating that ciLADs and cLADs are oppositely enriched in SINEs and LINEs, whereas fLADs are equally enriched in both kinds of repeats [11,52•].

Thus, EC/HC segregation is most probably achieved on the TAD level by folding SINE-rich chromatin into cA-TADs and LINE/LTR-rich chromatin into cB-TADs. Homotypic associations between constitutive TADs establish the backbone for chromatin segregation, which is reinforced by binding of cB-TADs to scaffolding structures. fTADs, which probably lack a specific repeat enrichment, adopt a nuclear position depending on their transcriptional status. On a finer scale, segregation of silent/active TADs within EC is achieved by clustering of domains, which are marked by H3K27me3 and associated with Polycomb group proteins [20,53•,83]. An important component of the chromatin backbone is pericentromeric HC, which is excluded from Hi-C and

(Figure 2 Legend Continued) topology of mouse chromosomes X (MMUX) and MMU11 differentially enriched in HC and LADs. cLAD/ciLAD profiles along the chromosome axis of the two chromosomes are shown on the right. cLADs are numerous and distributed homogeneously along MMUX, but fewer and clustered along MMU11. Correspondingly, MMUX (active) is flattened and closely adjacent to the lamina, whereas MMU11 protrudes into the nuclear interior and has a more voluminous shape. cLAD/ciLAD data taken from [52]; LAD profiles are kindly provided by B. van Steensel. Scale bars = 5 μm.

Figure 3



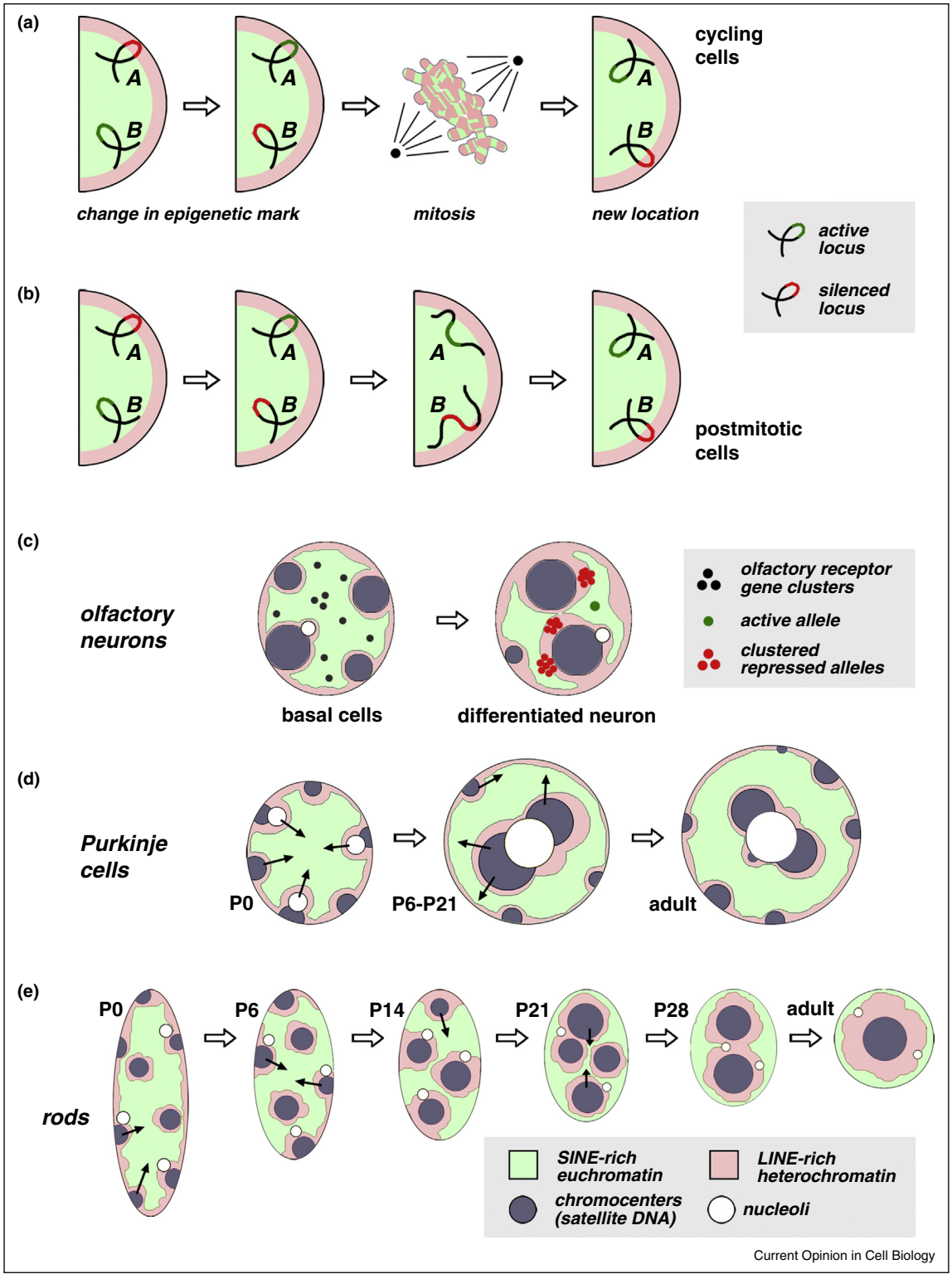
Euchromatic and heterochromatic TADs. EC and HC segregate on the level of TADs. cA-TADs are enriched in genes and SINEs by contrast to gene-depleted and LINE-rich cB-TADs. Binding of cB-TADs to the nuclear lamina or the nucleolus via cLADs strengthens their segregation from cA-TADs. Centromeric or subcentromeric satellite DNA forms especially robust cB-TADs and thereby enhances anchoring of chromosomes to scaffolding structures. Clustering of B-TADs in the repressive zone can be achieved by recruiting LADs to the lamina or as a result of LINE interactions between B-TADs. Clustering of A-TADs is dependent on SINE interactions. fTADs, which change their compartment identity and switch between fA-TAD and fB-TAD status, acquire nuclear positions depending on their transcriptional activity and physical constraints. fLADs within fTADs are recruited to the lamina when repressed and released from the lamina prior transcription activation. Grey dotted lines connect sequential TADs. Two inserted schemes show the hierarchical chromatin folding within a TAD and how it is implemented in gene regulation.

DamID analysis and therefore rarely discussed [13•]. Every chromosome has a stretch of satellite DNA that can be considered an immense cTAD equivalent to cLAD [13•,48], invariably found at the nuclear or nucleolar peripheries [41,62].

Differentiation and chromatin dynamics

The degree of EC and HC segregation increases with ongoing differentiation. Thus, embryonic stem cells (ESCs) are largely devoid of compact HC domains but possess a hyperdynamic, transcriptionally promiscuous

Figure 4



Two mechanisms of genomic locus repositioning during cell differentiation. **(a)** Repositioning of loci in cycling cells occurs after mitosis. When a new epigenetic mark is set, the locus remains in the same nuclear compartment due to limited chromatin dynamics during interphase, for example, locus *A* in a silencing zone and *B* in a permissive zone. Repositioning takes place in the next interphase, during nuclear re-assembly after mitotic division. **(b)** In postmitotic cells, relocation is the result of changes in long-range chromosome folding, which occur by unknown mechanisms. Three examples of dramatic nuclear reorganization in postmitotic cells are shown in (c)–(e). **(c)** Repositioning and silencing of

and open chromatin configuration [74•,83,88–91], ensuring the higher-order flexibility required for maintenance of the pluripotent state and for developmental progression [92]. As cells differentiate and lose potency, genome segments globally consolidate into larger EC and HC domains replicating synchronously [14,93,94].

During differentiation, the B-compartment largely expands as a result of progressive silencing of genes and their subsequent packaging into HC domains at the nuclear or nucleolar periphery [78,95–97]. Relocation of silenced genes to HC compartments is accomplished by activation of corresponding fLADs [11] primarily due to gain in repressive histone modifications, such as H3K9me2/me3 [9,51••,57] and H3K27me3 [54]. Differentiation-related movements of chromosomal loci away from or towards the HC compartment upon transcriptional activation or repression, correspondingly, are well documented in multiple microscopic studies on the differentiation of human blood cells (reviewed in [57,98,99], and the differentiation of tissues during *C. elegans* development [40••,57,100].

Comparison of interactions between genomic loci in human ESCs and four derivative cell lineages, reveals that about 40% of the genome switches compartment status during cell differentiation. Importantly, while TADs remain invariant during this extensive genome reshaping, inter-locus interactions within them either concertedly increase or decrease, depending on the directional shift from B to A, or from A to B, respectively [87••]. The importance of TAD integrity during compartment switching is stressed by recent findings revealing that disruption of inter-TAD interactions by natural mutations or targeted genome editing causes mis-expression of developmental genes and hence to developmental defects [80••].

How does relocation of TADs occur upon changing their compartment identity? It was suggested that nuclear reorganization is driven by chromatin remodeling rather than transcription [11,51••,101••]. Thus, ectopic chromatin decondensation but not transcription activation drives the repositioning of genes towards the nuclear interior [101••]. Accordingly, TADs with silent genes are unlocked from the lamina prior to activation probably as a result of changes in fLAD chromatin status. Inward and outward movement of activated and inactivated TADs within the nucleus is difficult to conceive considering the limited global chromatin dynamics throughout interphase [102,103]. In cultured cells, the position of peripherally targeted loci changes after cell division [104–106]. This suggests that although activating or inactivating epigenetic marks are set in interphase, relocation of loci to permissive or repressive zones is implemented only during re-establishment of the nuclear architecture following mitosis (Figure 4a).

The majority of repositioning studies are based on cycling cells *in vitro* (e.g., [101••,107–109]) or proliferating cell populations *in vivo* (e.g., [53•,82,84,110–114]), whereas data on postmitotic cells are very limited. For example, silenced olfactory receptor genes [115] are sequestered to repressive nuclear compartments in postmitotic olfactory sensory neurons [42,116] (Figure 4c). The dramatic repositioning of large chromosome regions in Purkinje nuclei involves progressive fusion of nucleoli and dynamic fusion and fission of chromocenters [117] (Figure 4d). Differentiation of mouse rods is accompanied by chromocenter fusion, release of LINE-rich HC from the periphery and its accumulation around the internal chromocenter [28,118] (Figure 4e). Clustering and relocation of the loci in postmitotic neurons apparently occur as a result of changes in long-range chromosome folding (Figure 4b). Although the underlying mechanisms remain largely elusive, it is highly conceivable that chromatin types with similar repeats tend to unite. In this respect, rods represent an important subject for further studies, because EC/HC segregation in their nuclei cannot be facilitated by scaffolding [28,58••] but rather relies on other mechanisms, including the proposed repeat affinity.

Outlook

The nucleus possesses well defined compartments, which are not demarcated by membranes. We have proposed in this review that attraction forces between repeats of different types drive segregation of EC and HC to achieve nuclear compartmentalization. Thus, chromatin arrangement in the nucleus is blueprinted on the linear chromosome level and is further reinforced by scaffolding nuclear structures, which selectively bind chromatin enriched in certain repeat types. One can speculate that EC/HC segregation is so important that during evolution it imposed a selection pressure on interspersed repeats to

(Figure 4 Legend Continued) thousands of olfactory receptor genes take place in postmitotic olfactory neurons during their differentiation [42]. In undifferentiated neurons (so-called basal cells), clusters of olfactory receptor genes are distributed throughout the entire nucleoplasm. During differentiation, repressed alleles relocate and cluster in large foci positioned in the HC zone around chromocenters, whereas a single active allele localizes to the EC zone. **(d)** Postmitotic reorganization of nuclei of Purkinje cells in the cerebellum takes four weeks and is accompanied by an increase in the nuclear volume [117]. Initially, nucleoli and chromocenters move inwards and fuse, their number being lowest at P6. In later stages some subcentromeric regions dissociate from the central clusters and move to the nuclear periphery, thereby doubling the number of chromocenters. **(e)** Nuclear inversion in rod cells is a slow process, which is completed in about six weeks and is accompanied by a reduction of the nuclear volume [28]. Rod photoreceptors stop proliferation at P5–P6 when they still have a conventional nuclear organization with HC adjacent to the nuclear periphery. In postmitotic rods, chromocenters and LINE-rich HC gradually dissociate from the periphery and fuse in the nuclear interior, whereas EC relocates to a thin peripheral shell.

accumulate in either gene-rich or gene-poor chromosomal regions.

Albeit in agreement with recent genome studies, our hypothesis is so far missing direct experimental evidence. It can be unequivocally tested by integration of an ectopic chromosome, consisting of regions enriched in various repeats, into wild type cells with subsequent analysis of its interaction partners and topology. No less challenging is the task of elucidating the main players involved in recognition of repetitive sequences. The question remains open: what proteins recognize repeats and do they act alone or together with non-coding RNAs?

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- •• of outstanding interest

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The authors introduce a modification of the Hi-C method with much higher resolution. The new approach allows further subdivision of A and B compartments into six subcompartments and identification of about 10,000 loop domains (apparently corresponding to subTADs) with a median size of 185 kb, which are conserved between cell types and species. Loop formation is highly correlated with presence of convergent CTCF-binding sites at the base of the loops.

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One of few studies on the role of pericentromeric satellite repeats in the organization of the mammalian genome. PADs represent silenced chromatin which is scarcely assembled around chromocenters in ESC; in differentiated cells, PADs progressively increase and consolidate in larger blocks closely overlapping with constitutive LADs.

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List of acronyms

CTCF: CCCTC-binding factor
DamID: DNA adenine methyltransferase identification
DNMT: DNA methyltransferase
EC: euchromatin
ESC: embryonic stem cell
HC: heterochromatin
HDAC: histone deacetylase
Hi-C: chromosome conformation capture (3C) combined with deep sequencing
HMT: histone methyltransferase
HP1: heterochromatin protein 1
HSA: *Homo sapiens*
LAD: lamina-associated domain
LBR: lamin B receptor
LINE: long interspersed nuclear element
LTR: long terminal repeat
MECP2: methyl CpG binding protein 2
MMU: *Mus musculus*
NAD: nucleolus-associated domain
PAD: pericentromere-associated domain
SINE: short interspersed nuclear element
TAD: topologically associated domain