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Coupling 1D modifications and 3D nuclear organization: data, models and function

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Over the past decade, advances in molecular methods have strikingly improved the resolution at which nuclear genome folding can be analyzed. This revealed a wealth of conserved features organizing the one dimensional DNA molecule into tridimensional nuclear domains. In this review, we briefly summarize the main findings and highlight how models based on polymer physics shed light on the principles underlying the formation of these domains. Finally, we discuss the mechanistic similarities allowing self-organization of these structures and the functional importance of these in the maintenance of transcriptional programs.

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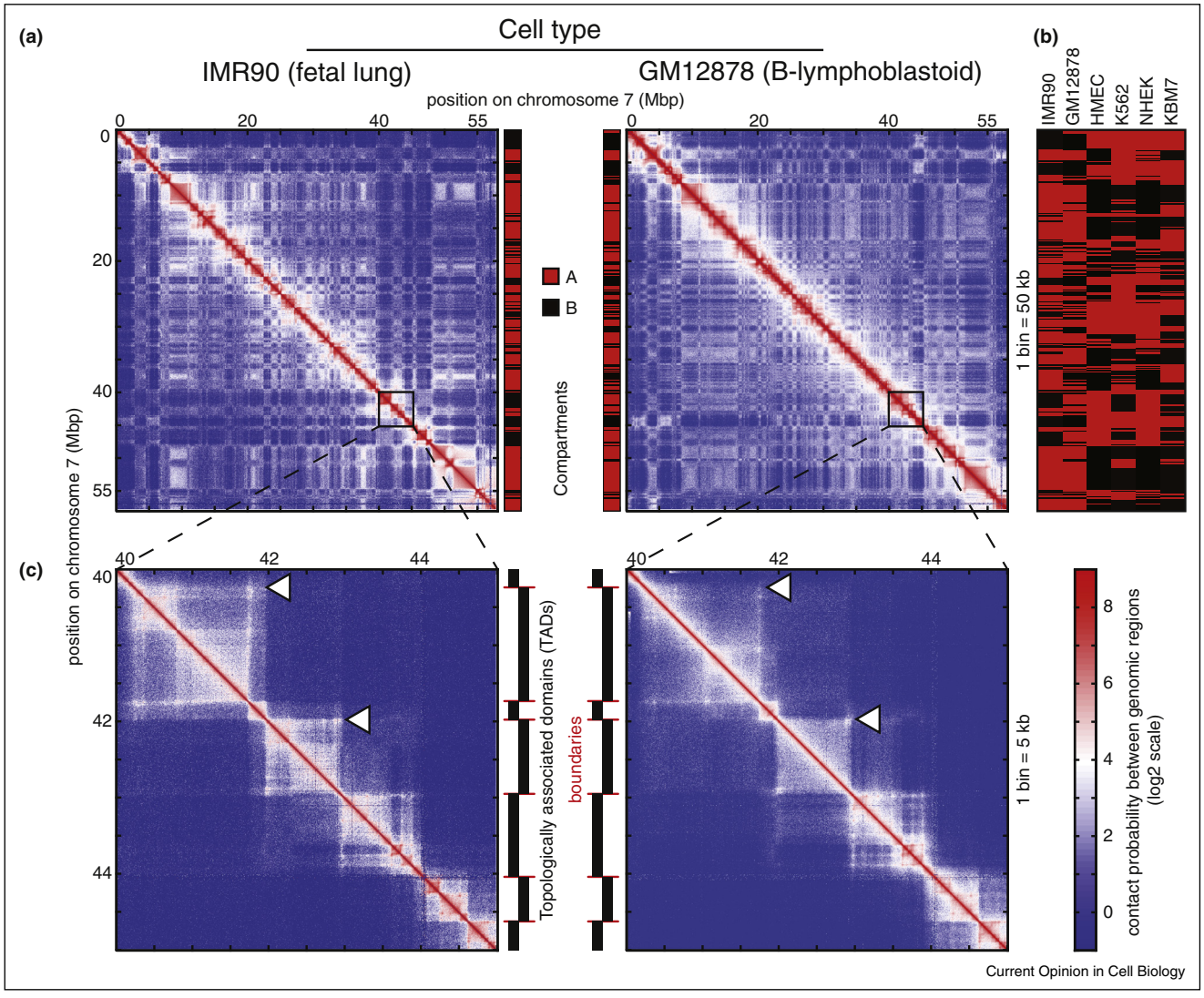
Chromosome conformation capture and superresolution microscopy: 3D compartments in the nucleus

The past decade has seen major progresses in our understanding of chromosome organization in interphase nuclei with chromosome conformation capture techniques (3C) [1,2], supported by parallel superresolution microscopy studies [3,4••,5•,6]. 3C was designed to characterize long-range chromatin contacts based on chromatin crosslinking, restriction digestion of crosslinked complexes and ligation of digested fragments in their crosslinked chromatin context. Ligation events between restriction fragments not immediately adjacent along the linear genome imply that these fragments were present in the same crosslinked complex. While the original procedure [1] allowed the characterization of contacts between two target loci, later development (termed 4C, 5C, Hi-C) coupled to high throughput sequencing allowed the identification of two-ways contacts of one, many or all fragments of the genome, respectively (for an in-depth technical review, see [2]).

In particular, genome-wide (Hi-C) maps provided a global view of possible contacts between any DNA segments of the genome [7]. Hi-C maps show a characteristic high contact frequency along the diagonal: sequences close to each other along the linear genome have a higher probability of contact. This mainly depends on the distance in base pairs between fragments, a property that can be used to build *de novo* chromosome backbones [8]. At the megabasepair (Mbp) scale, contact maps display a cell-type specific checker board pattern [4••,7,9] (Figure 1a,b). This demonstrates the presence of two mutually exclusive contact compartments, named A and B [7,9] and the checker board pattern implies that sequences in the same compartment (A or B) contact each other more frequently than sequences in different ones (A-B). Strikingly, sequences in A compartments are gene rich, display higher expression than B sequences and A chromatin is generally more accessible and enriched for histone marks specific for active genes. In contrast, B compartments are more densely packed, harbor less genes and more repressed histone marks [7,9,10]. Together, these results demonstrate clustering of functionally similar chromatin, highly reminiscent of previous microscopy and biochemical studies in which spatial segregation between euchromatin (transcriptionally more active) and heterochromatin (transcriptionally more silent) was observed [11].

At higher resolution, Hi-C and 5C experiments highlighted compartmentalization into kbp- to Mbp sized contact domains [3,10], the so-called topologically-associating domains (TAD) (Figure 1b). TADs are defined as highly self-contacting portions of the genome: a sequence inside a TAD has a higher probability to contact sequences inside the same TAD than sequences in neighboring TADs [10]. TADs insulate sequences along the linear genome, thereby segmenting the 1D genome into 3D domains. The presence of TADs or similar 3D domains has been documented in most species in which genome-wide 3C has been carried out ranging from bacteria to mammalian cells and plants [12]. Most characterized promoter-enhancer interactions occur inside the same TAD [10,13•], suggesting that TADs allow insulation of promoters from enhancers located in neighboring TADs. Indeed, deletion of a TAD boundary is sufficient to induce ectopic new contacts with regulatory sequences

Figure 1



Contact maps in human cells. **(a)** Contact frequency map across one third of the right arm of human chromosome 7 in two different cell lines, based on Hi-C data [4••], at 50 kb bin size. The checkerboard contact frequency pattern is cell-type specific and allows the definition of two compartments A and B (middle bars). **(b)** A and B compartments in various cell lines (data from [4••]). **(c)** Conservation of Topologically Associating Domains (TADs) in two cell lines. Contact frequencies are drawn for a bin size of 5 kb. Arrows: 'corner' contacts, in which loci frequently contact each other, as the result of the creation of a loop. These loci are located at the base of the loops.

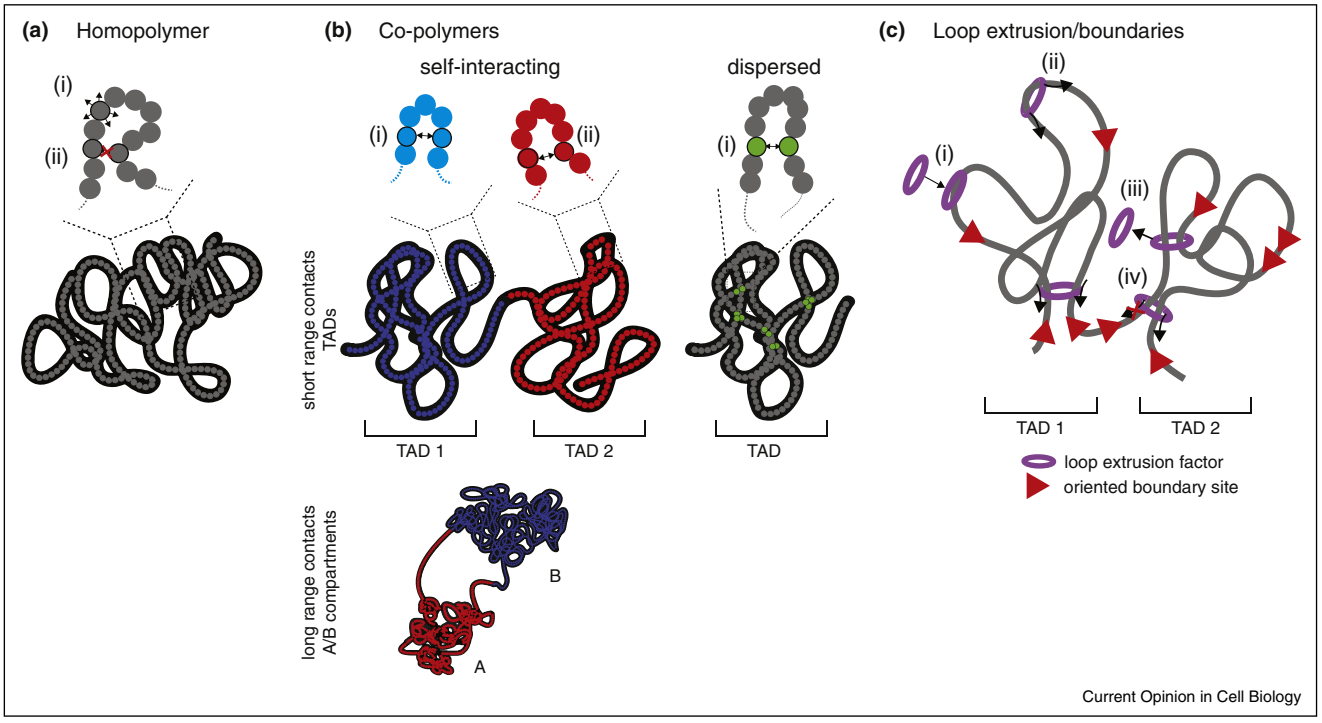
in the neighboring TAD and leads to aberrant gene expression even if not disrupting any regulatory segment [14]. In humans and mouse, this kind of deletion and subsequent gene misregulation leads to limb malformations such as polydactyly, finger malformations or sex reversal [15••,16]. The TAD structure of the genome is remarkably invariant over the course of development, between different cell types (Figure 1c) or even between species in conserved synteny blocks [17••,18–20].

One of the key questions about both A/B compartments and TADs is how these domains are built. Several models based on polymer physics were suggested in the past years, providing an interesting starting point to understand the minimal requirements for the creation of such higher-order chromatin structures.

Modeling chromatin as a polymer

Initial theoretical polymer models of chromosomes considered chromatin as a homogeneous polymer (Figure 2a). In those models, chromatin is described by a chain of connected identical monomers whose dynamics is controlled by thermal forces, excluded volume interactions (monomers cannot overlap) and optionally by the rigidity of the chromatin fiber or by non-specific dynamic interactions between monomers. Such homopolymer models

Figure 2



Polymer models of chromatin folding. **(a)** Homopolymer model: chromatin is constituted by a chain of connected monomers, each monomer representing a stretch of chromatin. Dynamics is controlled by thermal motion (i) and by steric interactions as two beads cannot overlap or cross each other (ii). **(b)** Copolymer model: a state is attributed to each monomer, depending on its underlying genomic or epigenomic information. Transient contacts may occur between loci of the same state (i, ii) or between a locus and the nuclear membrane (not shown). Long blocks of monomers having the same state will collapse into a TAD or may engage long-range interactions with distant regions. Alternative copolymer models were built with the assumption that dispersed monomers have increased affinities, which may lead to loop and TAD formation. **(c)** Loop extrusion model: extruders bind to chromatin (i), actively extrude DNA (ii), until they eventually detach from chromatin (iii). Sliding of extruders along chromatin is stopped or paused when encountering boundary elements in the proper orientation (iv), such as CTCF-bound sites in mammalian cells. This leads to the formation of TADs between convergent sites and boundaries between closely located divergent ones.

are sufficient to provide a quantitative and predictive description of chromatin organization in organisms with small genomes and relatively simple nuclear organization such as budding yeast [21–23]. Additionally, these models are able to predict generic aspects of chromosome folding in higher organisms, such as the average evolution of contact frequencies or distances between two loci as a function of their distance in base pairs along the linear genome, as well as chromosome intermingling [24–27]. In particular, large-scale organization of the genome is compatible with unknotted self-similar polymeric conformations, the so-called fractal or crumpled globule model [7,28,29], that are likely to result from the out-of-equilibrium folding of long polymers into a confined space [24]. However, these models fail to describe higher order structures such as A/B compartments or TADs.

Experimental data revealed that TAD epigenomic composition is often uniform for either active or inactive epigenetic marks [4••,9,20,30] while TAD boundaries are enriched for a number of chromatin binding proteins or specific epigenomic states [4••,9,10,30–32]. This suggested that the 1D heterogeneous chromatin landscape along the genome plays a key role in the 3D folding. Motivated by these observations, heteropolymer models started recently to emerge, based on the homopolymer framework but explicitly considering the coupling between chromatin structure and function [33–38,39••,40,41,42••,43,44••,45].

The first major class of models (Figure 2b) considers that chromatin folding is driven by transient specific short-range or contact interactions between genomic loci or with nuclear landmarks such as the nuclear periphery. Mainly two types of polymers were considered: either (co-)polymers where large stretches of monomers have similar short-range interaction properties (Figure 2b, self-interacting) or where individual dispersed monomers have an affinity for each other, sometimes dependent on *trans* acting factors (Figure 2b, dispersed). TAD conformations vary from dense globule to more disordered structures, depending on the strength of interactions, the

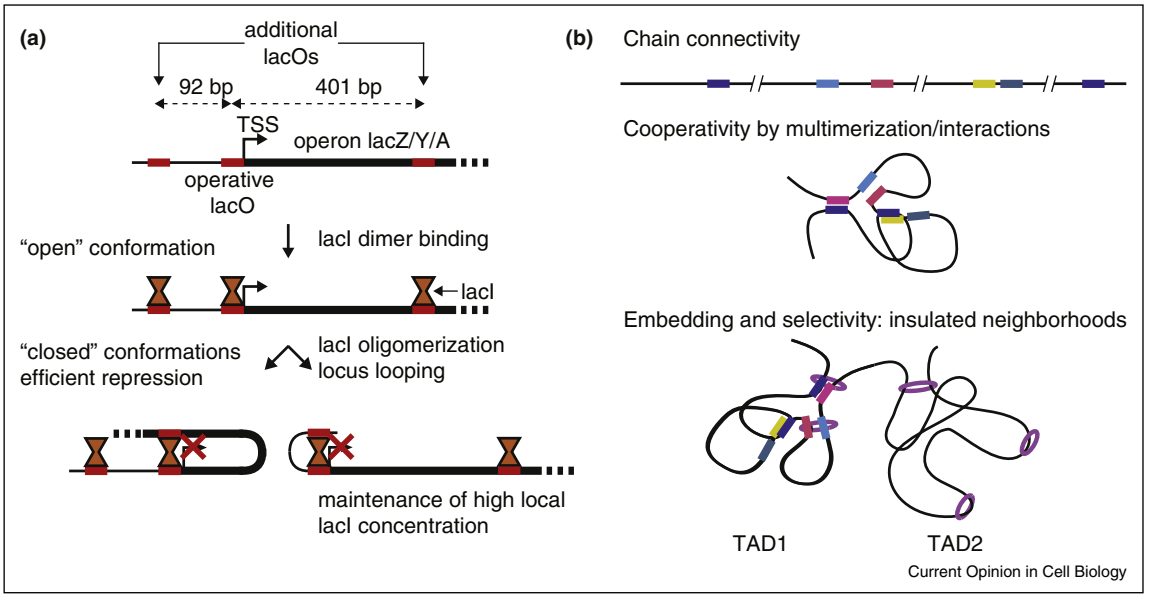
size of the domain and the concentration of the 'bridging' molecules, as observed *in vivo* [33,39••,46–48]. In these frameworks, TAD formation results from an internal collapse due to attractive forces between the monomers composing a TAD. These approaches are experimentally supported by evidence that chromatin-binding proteins (transcription factors, transcription and replication machineries, lamins, cohesins, condensins, heterochromatin-associated complexes such as Polycomb or HP1, and so on) promote directly or effectively physical bridging [49–51]. In *Drosophila*, such heteropolymer models built directly from the 1D epigenomic landscape account well for the checker board patterns observed in Hi-C experiments (Figures 1a and 2b long range contacts) [39••,43,47], as well as the formation and properties of previously observed Polycomb bodies where silent H3K27me-enriched chromatin clusters [5•,52•]. The prediction of long-range interactions is inherent to copolymer models arranged in blocks. Therefore, such models are well adapted to describe A/B compartment formation in mammals [53]. Interestingly, the temporal evolution which can be extracted from the models suggests that the internal collapse of epigenomic domains into TADs is a fast process while long-range contacts between TADs of the same epigenomic state are more slowly formed, more transient and dynamic [34,39••,47]. These predictions are consistent with Hi-C experiments in mammalian cells on synchronized or single cells [54,55]. Short-range interactions such as the ones used in heteropolymer models are however inconsistent with the observation that half of mammalian TADs contains strong loops between convergent CTCF (CCCTC-binding factor) sequence motifs, usually located at domain boundaries [4••,56,57••]. While the formation of these loops can still be explained using dispersed models [34,42••], this first class of models is however inconsistent with the observed preferential looping between convergent CTCF sites and with results obtained by experiments in which boundaries were deleted or inverted [42••,44••].

To account for that, a second class of models has recently emerged (Figure 2c), based on early speculations on mitotic condensation [58,59]. These models consider the formation of TADs as the results of an active extrusion mechanism [42••,44••]. Protein complexes, putatively structural maintenance of chromosome complexes (SMC; cohesins and condensins) bind to chromatin and extrude dynamically large chromatin loops. Eventually the complexes detach or stop extruding chromatin as they encounter boundary elements. In these models, the local 3D organization of the genome is controlled by the presence and orientation of proteins bound to 1D sequence elements that serve as barriers. Polymer models implementing this mechanism reproduce TAD formation as well as the observed loops between TAD boundaries [42••,44••]. These models are experimentally supported in mammalian cells by the enrichment for cohesins (a possible loop extrusion factor), CTCF and its cognate sequence motif at TAD boundaries, by the high contact frequencies of the sequences at the TAD boundaries ('corners' of the domains, Figure 1b, arrows), as well as by the preferential contacts between convergent CTCF binding sites [4••,10,42••,44••,57••]. Moreover, these models can quantitatively predict the perturbed 3D organization after deletion, inversion or duplication of CTCF sites [14,42••]. Similarly, TADs on the dosage compensated *C. elegans* X chromosome depend on sequence elements (*rex* sites) which are enriched at TAD boundaries, while the formation of these X-specific TADs is achieved by the loading of a condensin-like loop extrusion factor [60]. The loop extrusion models additionally suggest an elegant mechanism for the formation of mitotic chromosomes: the increase in the number of loop extruders coupled to a decrease in the number of boundary elements leads to higher compaction and an array of consecutive loops [61,62] in agreement with Hi-C data obtained for mitotic chromosomes [54]. Loop extrusion models reach their limits when trying to predict long-range (inter-TADs) contacts such as the formation of A/B compartments, or interactions with the nuclear envelope. As these processes are likely driven by epigenomic information, heteropolymer models accounting for both loop extrusion and epigenomically-driven short-range interactions remain to be developed in order to quantitatively describe within the same framework the local and higher-order chromosome organization.

Spatial chromatin compartments: 'nano-reactors' for gene regulation

The main question raised by the presence of nuclear compartments at different scales (A/B compartments, TADs, loops and so on) is whether this spatial organization of the genome is a by-product of genome activity or whether it is participating in the regulation of genome function. A popular hypothesis is that molecular crowding and spatial confinement increase binding affinities to the regulated sequences. This 'high concentration' paradigm has been formalized for the bacterial lac operon system and the necessity of DNA loops for efficient regulation has been demonstrated *in vivo* [63–67]. The lac operon is transcriptionally controlled by the binding of the lacI repressor to its lacO target site located 5′ of the controlled genes (Figure 3a). The association of the repressor to the functional lacO site (and the subsequent repression of transcription) is reinforced by the presence of additional lacO motifs in the vicinity. The ability of the tetrameric lacI repressor to bind simultaneously two operator sites thus favors DNA looping and the formation of a biochemical nano-reactor (Figure 3a) by enhancing the repression level, decreasing the sensitivity to number of repressors and reducing intrinsic noise. Nuclear compartments would similarly correspond to nano-reactors: a few number of reacting biomolecules co-localize in space, increasing their local concentration and thus promoting their

Figure 3



Nuclear compartments as self-assembled biochemical nano-reactors. **(a)** Schematic working principle of the *E. coli* lac operon nano-reactor. Dispersed lacO sites are located around the functional lacO site (5′ of the transcribed region). lacI binding to these additional sites locally increases lacI concentration while lacI oligomerization leads to locus looping for efficient transcriptional repression. **(b)** Conserved features of eukaryotic nano-reactors leading to the formation of nuclear compartments. Dispersed binding sites for DNA-binding proteins are present on the same polymeric molecule, thereby providing a basal level of insulation as loci located on the same molecule are more likely to contact each other by looping. Multimerization of binding factors and/or interactions with common factors which themselves oligomerize lead to the formation of 'neighborhoods'. Finally, the formation of loops directed by sequence motifs on the 1D genome allows the insulation from each ''neighborhood'' and the formation of nuclear compartments.

biochemical activity on chromatin. Although proteins and sequences involved in the formation of specific compartments are different, the mechanisms driving spatial confinement may be similar to the lac operon case, based on three major features: (i) chain connectivity: the polymeric nature of chromatin induces a generic confinement as dispersed sequences on the same chain have a high probability to contact each other by looping. (ii) cooperativity: multimerization of DNA or chromatin binding proteins promote sequestration and physical bridging between target sequences. (iii) embedding and selectivity: the formation of TADs leads to 'insulated neighborhoods' [57••], thus contributing to the structural but selective confinement of active or repressed modules.

At loop scales, enhancers recruit transcription factors, which in turn interact with the transcriptional mediator, thus establishing a transient physical contact with the regulated promoter leading to enhanced recruitment and stabilization of the transcriptional machinery at promoters [68] (Figure 3b). Interestingly, enhancer/promoter looping is observed even in the absence of transcriptional activity, suggesting that these loops constitute structural elements organizing the genome [13•,19]. At larger scales, multimerization of Polycomb proteins in *Drosophila* [52•,69] induces *cis* clustering of individual epigenomic domains as well as *trans* clustering between distant domains (e.g., ANT-C and BX-C, located 10 Mb away from each other) [70]. This clustering of Polycomb-repressed genes into nuclear Polycomb bodies correlates with gene repression level [70]. Similar colocalisation of PcG repressed genes or HP1-regulated domains is observed in mammals, suggesting conservation of the regulatory logic [68]. At very large scale, the A/B compartments increase confinement of the TADs with similar transcriptional activity, providing a basal level of confinement and selectivity [17••,20].

Nuclear organization is known to increase over the course of development, with the appearance of numerous compartments (for review [71]). The creation of these is initiated by transcription factors binding specific genomic sites, which recruit chromatin-modifying enzymes [72–74]. This leads to spreading of specific chromatin marks *in cis* as well as *in trans* due to the spatial confinement by the insulated neighborhood [75–79]. Multimerizing proteins with specific affinities to these marks [50,80] then reinforce and enhance contacts between similar domains. Nucleation by a small number of factors coupled to self-assembly of the local chromatin therefore leads to the formation of nuclear compartments which further enhance, stabilize and/or perpetuate the active or repressed environment.

Ultimately, the assembly of nano-reactors is a conversion from a 'hard-wired' transcriptional program in which transcription factors determine the activity of genes to a 'soft-wired' self-reinforcing state in which spatially highly concentrated proteins maintain gene expression states, well in line with the fact that initiating transcription factors can disappear while cells maintain their fate.

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