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Compartmentalization of the nucleus

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The nucleus is a spatially organized compartment. The most obvious way in which this is achieved is at the level of chromosomes. The positioning of chromosomes with respect to nuclear landmarks and with respect to each other is both non-random and cell-type specific. This suggests that cells possess molecular mechanisms to influence the folding and disposition of chromosomes within the nucleus. The localization of many proteins is also heterogeneous within the nucleus. Therefore, chromosome folding and the localization of proteins leads to a model in which individual genes are positioned in distinct protein environments that can affect their transcriptional state. We focus here on the spatial organization of the nucleus and how it impacts upon gene expression.

Spatial organization

The spatial organization of eukaryotic cells is profoundly important to their function. The most apparent mechanism by which spatial organization is achieved is through membrane-mediated compartmentalization of cells into subcellular organelles having distinct compositions of proteins. Spatial organization is also possible in the absence of compartmentalization. For example, within the cytoplasm, proteinaceous bodies such as P bodies and germ granules concentrate factors to regulate mRNA metabolism.

In the nucleus, functionally related genes are not always arranged linearly or even on the same chromosome, yet genes of common function can colocalize. For example, dozens of active tRNA genes cluster together within the nucleolus [1]. This suggests that the localization of genes might be regulated in a manner that is coupled to their expression. Gene clustering to a subnuclear territory might improve access to transcriptional regulators and promote expression or repression. Consistent with this, individual genes can also reposition themselves with respect to landmarks, and their localization to different parts of the nucleus is associated with either activation or repression. The nucleus lacks membrane-bound compartments; however, changing the location of individual genes and clustering them together could allow cells to rearrange the genome, creating dynamic 'compartments' to fine-tune gene expression. We discuss here the spatial organization of the nucleus; specifically, how chromosomes fold, chromatin interactions within subnuclear domains, and movement of individual genes through specific DNA–protein interactions.

Chromosome folding

Chromosome folding and position within the nucleus might influence gene expression if different parts of chromosomes are exposed to environments with different concentrations of transcriptional regulators. In differentiated cells, chromosomes often form globule-like structures that occupy distinct 'territories' within the volume of the nucleus [2] (Figure 1). Within territories, the position of individual genes with respect to other chromosomes and nuclear landmarks can influence their transcriptional state. In differentiated cells, although expressed genes can localize within territories [3], most active loci are positioned between chromosome territories, often biased toward the nuclear interior. Repressed regions tend to be located within territories or at the nuclear periphery together with heterochromatin [2,4–9].

In organisms such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and in the *Drosophila* embryo, chromosomes are arranged in a Rabl conformation where chromosomes associate with the nuclear envelope in a bundled configuration, with silenced centromeres and telomeres clustering together into a small number of foci at the nuclear envelope [10–12]. An intense area of research is focused on how chromosomes fold to form these highly complex and dynamic conformations and how this influences cell function. Many factors influence chromosome folding, including polymer dynamics, protein–protein and protein–DNA interactions within the nucleus, and gene expression.

To take into account the many components that contribute to chromosome folding, polymer simulations and computer models are often used. A purely deterministic model, assuming that genome folding and organization is predetermined due to interaction of DNA sequences with stable structures such as lamins and nuclear bodies, predicts that every cell would have the same spatial arrangement. However, because different tissues have distinct nuclear organization, dynamic locus-specific rearrangements occur, and nuclear body positions in the nucleus are not fixed a purely deterministic model cannot explain these observations.

In an alternative model based on the notion of self-organization, chromosomes fold based upon the function of the particular cell [13]. In this view, protein concentration, affinity for DNA sequences and chromatin modification are important factors in shaping the 3D structure of the genome. Through thermodynamic fluctuations, DNA sequences will come into contact with proteins in the nucleus whose concentration can be regulated. The proteins and DNA will have a particular affinity and interactions with higher affinity will be more stable. Some proteins in the nucleus are distributed heterogeneously and can be part of very stable structures (such as the nuclear lamina), and such interactions could therefore

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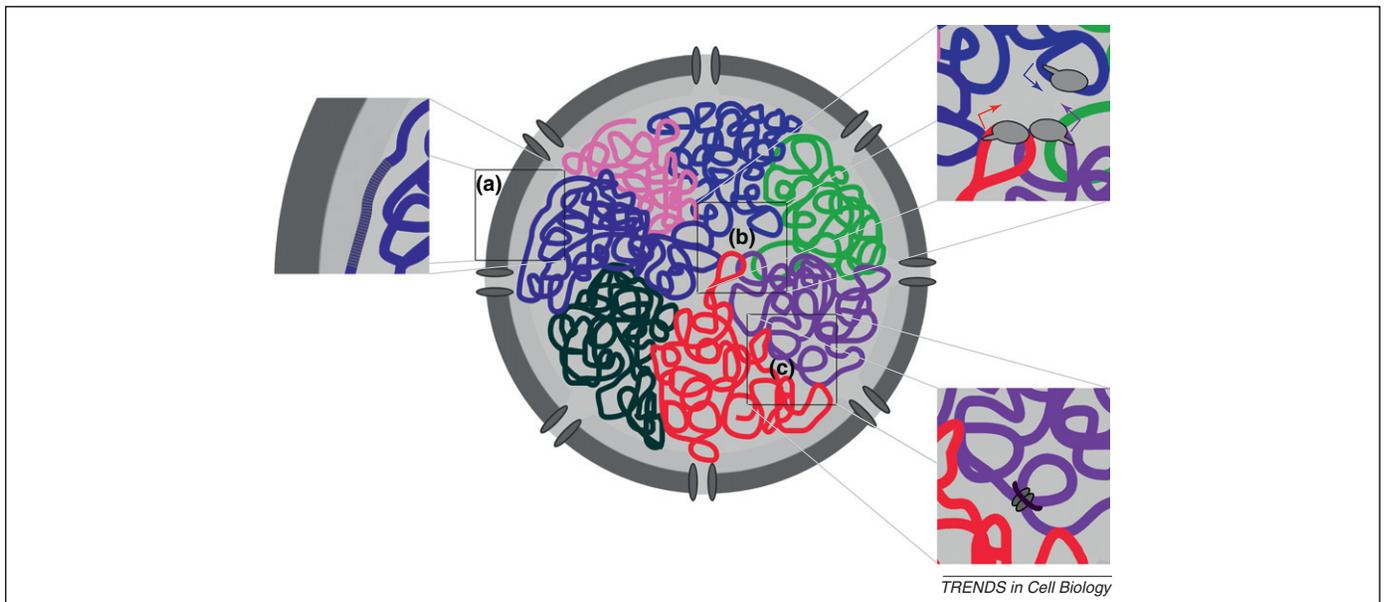
Figure 1. **The nucleus has spatial organization**. Cartoon depicting the arrangement of chromosomes into discrete territories. **(a)** Expanded view of lamin-associated chromatin enriched for dense heterochromatin (hatched pattern). **(b)** Gene kissing at an RNA polymerase II (RNAPII) factory consisting of clusters of active genes from different chromosomes in association with RNAPII (grey). **(c)** Intra-chromosomal loop mediated by CTCF (grey ovals) in association with cohesin (purple ring).

influence the spatial arrangement of chromatin. Chromosome clustering at a location could take place through the interaction of a stable protein complex with either a widespread DNA element or chromatin modification [14].

Recent evidence suggests that chromatin loops represent a fundamental organizing principle influencing chromosome folding [15–18]. At the smallest scale, chromatin is looped around histone octamers, and this not only helps to compact the genome but also can bring distant pieces of DNA into contact. On the kilobase to megabase scale, loops form between regulatory elements and core promoters. Loops have also been proposed to segregate the genome into discrete megabase-length 'compartments' that are either enriched for active or repressive chromatin [15,19].

Small-scale chromosome looping can occur transiently to regulate transcription of individual genes (Figure 1c). Conditional loop formation often enables distant enhancers to contact a promoter and activate transcription. The β-globin locus control region (LCR), for example, loops back to the promoters of the β-globin genes to activate expression [20,21]. Loops have been observed at numerous genes and can enhance or repress transcription [22–25]. Looping can also affect the position of genes within a chromosome territory (Figure 2). DNA fluorescent *in situ* hybridization (FISH) experiments have revealed that many loci loop out from their chromosome territory upon activation [26,27].

The formation of loops can be regulated by protein–DNA interactions. The CCCTC-binding factor (CTCF) is a DNA–binding insulator protein that influences the interaction of long-distance regulators with promoters. CTCF promotes formation of loops that either enhance or repress transcription depending on the context of the interaction [24]. Intrachromosomal loops can form between CTCF and transcription factors that bind to a promoter or between two CTCF proteins in association with cohesin [28]. Other gene loops are dependent on cohesin alone [29–31]. Thus, CTCF and cohesin play important roles in the formation of intrachromosomal loops that regulate transcription.

Larger looping interactions have been observed using genome-wide derivatives of the 3C technology, which provides a means for quantifying intra-chromosomal and inter-chromosomal interactions. These molecular techniques require computational modeling to use interaction frequencies to approximate 'distances'. Genome-wide 3C recapitulates important aspects of genome organization, providing a snapshot of interaction frequencies in a population of cells, and is quickly advancing the field of chromosome folding. Results suggest that chromosomes are tightly compacted by formation of loops of various sizes, ranging from a few kilobases to several megabases [15,18]. Analysis of the intrachromosomal interactions within human chromosomes suggests that chromosomes are not only densely compacted by looping at the kilobase scale, but are also segregated into discrete megabase-length 'compartments' [15,19]. At the megabase scale, these data are well-described by a 'fractal globule' model that allows chromosome folding without entanglement [15]. This arrangement would allow rapid local unfolding of a region of a chromosome without affecting neighboring regions. It also readily allows subchromosomal domains to be segregated spatially. It will be important in future work to use these molecular techniques to observe changes in inter- and intra-chromosomal interactions throughout development or in response to external stimuli.

Chromosomes can be modeled using physical constraints of the chromatin polymer and loops of various sizes [15,16,32]. In such models, chromatin is described as a polymer of repetitive units (nucleosomes) connected by flexible linkers (naked DNA). The dynamic loop model and the fractal globule model both incorporate loops of all sizes, and this leads to significantly better agreement between the models and experimental observations [15,16]. Earlier linear models or models with fixed loop-sizes do not represent experimental data as well as those that use loops of all sizes. Looping also helps to explain the high level of compaction and existence of chromosome

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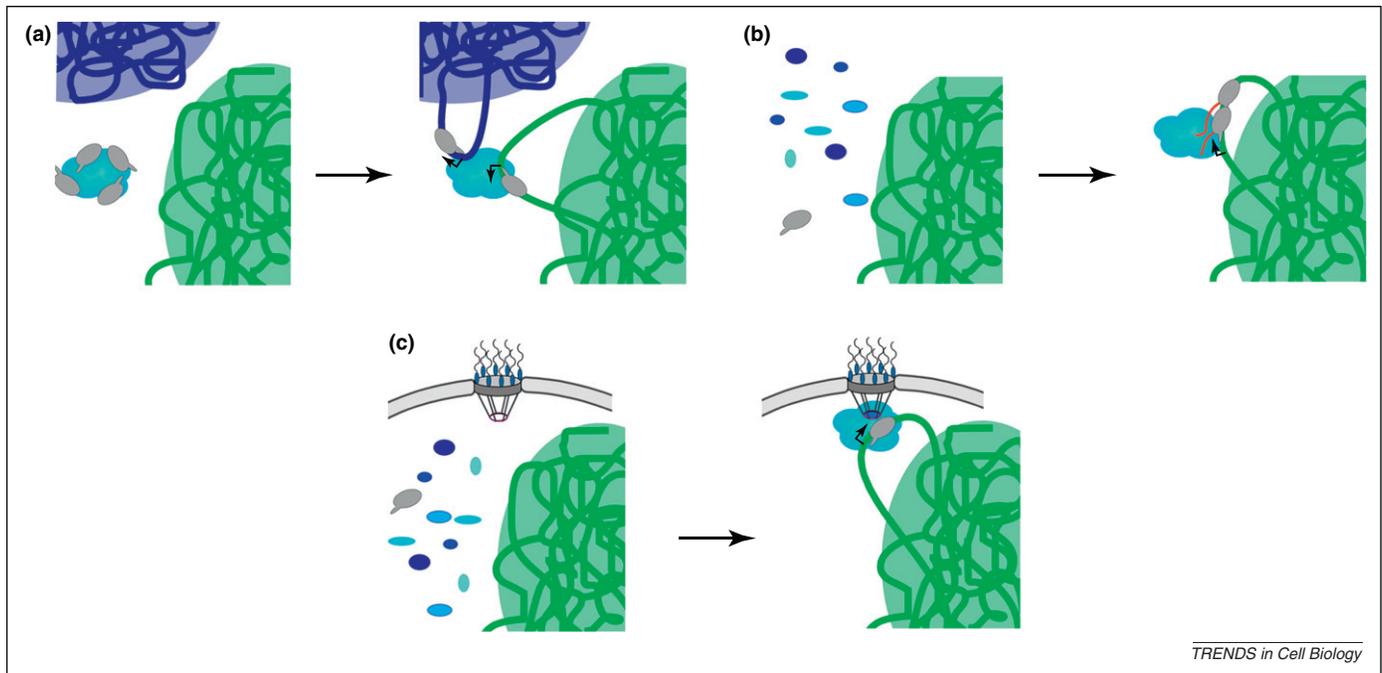
Figure 2. Protein heterogeneities form in the nucleus. **(a)** Chromosomes looping out from their respective territories upon activation to interact with a stable protein body (blue cloud) enriched for RNAPII (grey). **(b)** *De novo* formation of a nuclear body is induced by mRNA (red) production at active sites of transcription. **(c)** Gene targeting to a nuclear pore complex, which serves as an organizing surface to concentrate proteins (blue) and RNAPII (grey).

territories because highly-condensed looped structures create entropic repulsion between polymers [33].

The fractal globule model suggests that a polymer collapses into small globules, which then progressively form larger globules. The scaling component from the end–end distance is to the 1/3 power [15]. The dynamic loop model is scale-independent and assumes that any two monomers will have an equal likelihood of interacting if they can contact each other by diffusion. The interaction will exist for a given period of time and therefore loops of all sizes will form [16,19]. Although both models recapitulate compartmentalized genomes, the organization differs slightly. The fractal globule model not only forms chromosome territories, but also forms genomic territories within chromosomes based upon function. The globular state also allows for easier opening of the chromatin fiber, necessary for genes to loop out from territories. The dynamic loop model has more randomly entangled chromatin domains, but fits FISH data better, although this varies between cells [16]. Models will continue to improve by complementing predictions with FISH and experimental genome-wide 3C data. Future experiments followed by predictive models will help to clarify how folding changes in response to specific events or developmental states.

Through experiments and models it has become abundantly clear that the genome is not simply stuffed into the nucleus. Chromosomes are folded according to their own physical constraints and through specific protein–DNA interactions that help to govern organization and gene expression. Although it is not clear exactly how this folding occurs, loop formation plays an important role in genome compaction, segregation of chromosomes into territories, and regulation of transcription. Polymer models recapitulate particular organizational features of the genome. Future models will need to consider polymer heterogeneity,

the influence of different chromatin states and cell types, and how chromosomes interact with nuclear structures and with each other (see below).

Interaction of chromosomes with nuclear scaffolds

The interaction of chromosomes with stable nuclear structures affects higher-order chromatin folding and gene expression. The most prominent and stable nuclear proteins are those that make up nuclear structures. In higher metazoans, a nuclear lamina is associated with the inner nuclear membrane. It consists of intermediate filament proteins that form a mesh-like structure that provides structural support to the nucleus. Large regions of chromatin, termed lamin-associated domains (LADs), associate with the lamin. These are enriched for Oct1 binding sites, a protein that interacts with lamin-B, and these interactions might provide anchor points for chromosome folding [34].

In metazoan organisms, LADs associate with the nuclear lamina and localize at the nuclear periphery [34,35]. These DNA regions tend to be enriched for repressed chromatin. Lamin interactions with LADs are not fixed; during differentiation, LADs can either gain or lose interaction with lamins as gene expression changes [34,35]. The interaction of chromatin with lamins appears to be regulated through binding of insulator proteins within and surrounding LADs [34,36]. Lamin mutants or depletion of lamins reveal that the lamina is not only important for nuclear structure, but also for tethering heterochromatin to the periphery, the maintenance of heterochromatin, and repressive chromatin marks [37–39]. Lamin mutations also cause diseases such as muscular dystrophy and Hutchinson–Gilford progeria [38,39].

Other filaments within the nucleus, termed the nuclear matrix, are also proposed to provide a scaffold on which

chromosomes can fold, thereby affecting the transcriptional state. The nuclear matrix consists of a fibrogranular ribonucleoprotein (RNP) network that can be visualized using an electron microscope and remains intact when chromatin and soluble proteins are removed. Based on biochemical preparations, the nuclear matrix was hypothesized to be a fibrous skeletal structure that interacts with chromatin to template chromosome folding [40–42]. Nuclear matrix proteins interact with specific DNA sequences termed either scaffold- or matrix-associated regions (S/MARs). MARs are AT-rich, often found in non-coding regions, and are interspersed, which has been proposed to facilitate the formation of chromatin loops. In yeast, MARs are enriched for replication origins and might promote replication coordination [43]. In higher eukaryotic cells, MARs associate with special AT-rich binding protein (SATB1) which recruits histone-modifying enzymes that reinforce a heterochromatic repressed state [44]. The biological role of the matrix *in vivo* is still under debate, however [45]. Chromosome territories can be disrupted upon depletion of nuclear matrix proteins using RNase A and salt extraction, but this method may cause many proteins to precipitate and is therefore hard to interpret [46].

Lamin and matrix proteins are stable nuclear proteins that associate with repressed portions of the genome. The folding that results from the interaction of chromosomes with stable nuclear structures provides important constraints on the physical positioning of genes along these chromosomes, and this influences their access to proteins that affect their expression.

Nuclear body biogenesis and function

If proteins are distributed heterogeneously within the nucleus, subtle changes in chromosome folding can result in dynamic changes in the access of individual genes to different nuclear microenvironments. Proteinaceous nuclear 'bodies' are readily observed by electron microscopy. Many nuclear proteins involved in transcription and mRNA metabolism are heterogeneously distributed [47–50]. For example, proteins that mediate transcriptional silencing in *Saccharomyces cerevisiae* localize to a small number of foci at the nuclear periphery [51]. Likewise, in *Drosophila*, the Polycomb Group transcriptional repressor complex localizes to Polycomb bodies [52]. In many cell types, active RNAPII localizes to discrete nucleoplasmic foci termed transcription factories [47,53]. Although most studies have looked at fixed cells, foci have been visualized in live cells [54,55]. Splicing machinery, DNA repair factors, histone mRNAs and noncoding RNAs have all been shown to localize to discrete bodies [50]. Thus, many biochemical activities essential for gene expression are not uniformly distributed within the nucleus.

Are these bodies stable assemblies that serve as factories for biochemical processes (Figure 2a)? Or are they formed as a product of transcription, due to the accumulation of RNA or proteins involved in post-transcriptional events (Figure 2b)? Although these models are not mutually exclusive (i.e. post-transcriptional assemblies might produce stable factories), they suggest different ways in which spatial organization could impact upon transcription. If nuclear bodies are stable sites that contain a

limiting factor important for gene expression, then the movement of genes to such bodies would represent an important step in regulating transcription. Alternatively, if they can form when and where they are needed, the movement of genes might not be as crucial as the proper nucleation and assembly of such bodies.

Some nuclear bodies are suggested to be stable and non-dynamic structures. Foci of active RNAPII are thought to be stable sites of transcription that are not disrupted by either blocking transcription or extraction of the nuclear matrix [48,49]. Likewise, interchromatin granule clusters are interspersed throughout the nucleoplasm and contain splicing machinery, transcription elongation factors, and histone-modifying enzymes. In response to estrogen, coregulated genes occasionally colocalize at interchromatin granules [56]. This is consistent with the idea that some subnuclear bodies are stable sites to which genes can be targeted (Figure 2a). It is important to note, however, that although the position of a nuclear body may be relatively immobile, the constituents of the body are readily exchanged, as assessed by photobleaching assays [57,58].

Recent experiments show that some nuclear bodies can be formed *de novo* (Figure 2b). Artificially tethering premRNA or proteins involved in histone mRNA processing to chromatin is sufficient to nucleate formation of a histone locus body (HLB) [59]. Likewise, tethering coding mRNA, non-coding RNA or repetitive non-coding RNA is sufficient to induce the formation of nuclear speckles, paraspeckles or nuclear stress bodies, respectively [59]. Finally, tethering proteins found in Cajal bodies to chromatin is sufficient to nucleate Cajal body (CB) formation [60]. The formation of protein bodies as needed at sites of transcription would not necessitate any change in gene localization to allow access to a body.

Nuclear bodies, such as CB and promyelocytic leukemia nuclear bodies (PML-NBs), exhibit constrained movement within the interchromatin space [61,62]. The impact of ATP on nuclear body mobility is unclear. One study showed that when ATP is depleted or transcription is blocked, CB movement increases [61]. In another study, however, PML-NB movement decreased after ATP depletion, suggesting that some PML-NBs undergo ATP-dependent motion [63]. The results of both experiments are complicated by the fact that ATP depletion affects chromatin compaction and mobility, with doubtless, but unpredictable, effects on the structure and dynamics of the interchromatin space.

Nuclear bodies offer a mechanism by which to concentrate and spatially segregate nuclear activities into subcompartments. This type of organization, which also occurs in the cytoplasm, could improve the efficiency and fidelity of protein–DNA or protein–RNA interactions that are important for gene expression. Genes can associate dynamically with such bodies, subject to additional levels of regulation; the gene can either move to the body or the body can form *de novo* at the gene.

Movement of individual genes within the nucleus

The localization of individual genes can reflect their expression state [26,64–66]. In differentiated cells, euchromatin is concentrated towards the nuclear interior

whereas heterochromatin lies near the nuclear periphery. Association with the lamina can promote silencing; artificially tethering a locus to the nuclear lamina is sufficient to repress transcription of neighboring genes [6,67]. Being tethered at the nuclear lamina is not repressive for every gene, however; some genes remain transcriptionally active at the periphery [6,68,69].

Many localization studies focus on inducible or developmentally regulated genes, allowing one to correlate localization with expression state. These genes tend to be important for either cell survival or proper differentiation and must be activated quickly and precisely. Many genes localize at the nuclear periphery when repressed and then move to the nuclear interior upon transcriptional activation [5,65,70], suggesting that one way to tightly control expression is through altering the subnuclear position of the gene. The immunoglobulin loci, β-globin, Hox, CFTR and Mash-1 all move from the periphery to the nucleoplasm upon induction [5,26,65,70]. Tissue-specific changes in gene localization also occur during development in live worms [71]. Transgenes with either gut- or muscle-specific promoters localize at the nuclear periphery before they are induced in the embryo, or in tissues in which they are repressed in the adult. In cells in which these transgenes are expressed, however, they localize in the nucleoplasm [71]. These observations strongly support the conclusion that tissue-specific genes interact with the lamina in tissues in which they are repressed and escape this interaction in tissues in which they are expressed.

Although heterochromatin is concentrated at the nuclear periphery, it seems to be excluded from the area near nuclear pores [72]. Work in yeast suggests that some active genes localize at the nuclear periphery through an interaction with the nuclear pore complex (NPC) [73–75]. Chromatin immunoprecipitation/DNA microarray experiments suggest that hundreds of active genes interact with nuclear pore proteins and mRNA export factors [64,76,77]. Furthermore, many inducible genes are recruited to the nuclear periphery, near nuclear pores, upon induction [64,66,76–78]. Although mRNA may mediate the interaction of some genes with the NPC, for others it is not required [64,76,79–81]. For example, targeting the *INO1* gene to nuclear pores does not require mRNA production, but instead requires small DNA sequences termed gene recruitment sequences (GRS I and II) in the promoter [76]. These elements are distinct from the known upstream activating sequences in the promoter, suggesting that, although targeting is normally coupled to transcription, it is mediated by separate elements. Importantly, insertion of the GRS elements at an ectopic site is sufficient to target this locus to the nuclear periphery [76]. In other words, these elements function as 'DNA zip codes'; they are sufficient to confer localization to a particular place in the nucleus, independent of chromosomal context. This suggests that genomes code for their spatial organization.

One hypothesis for why genes are recruited to NPCs is that this clusters genes of common function together, promoting transcription and efficient mRNA export. Genome-wide, the GRS I element is over-represented in the promoters of genes that interact with the NPC and in the promoters of stress-inducible genes. One of these genes,

TSA2, also requires the GRS I to be targeted to the nuclear periphery [76]. Finally, when the GRS I element is inserted into the genome of a highly divergent yeast species, *Schizosaccharomyces pombe*, it is able to target the *URA4* locus to the nuclear periphery [76]. This suggests that the targeting mechanism mediated by the GRS I is ancient, having been conserved for 400 million to 1 billion years [76].

Work in yeast suggests that gene recruitment to the nuclear pore complex promotes robust transcriptional induction of some genes [76,80]. In male flies, the overexpressed X chromosome also associates with nuclear pore proteins and proper dosage compensation requires the nuclear pore proteins Nup153 and Megator [82]. Recent studies have identified many genes in flies that interact with nuclear pore proteins, and loss of these interactions leads to defects in transcription. Unlike yeast, however, many of the genes that interact with nuclear pore proteins in flies do not localize at the nuclear periphery [73,74]. Thus, nuclear pore proteins can interact with chromatin away from the nuclear pore complex to promote transcription. It remains to be seen how the biochemical function of non-NPC nuclear pore proteins in promoting transcription relates to the function of NPC nuclear pore proteins in promoting transcription.

In addition to changing their localization with respect to subnuclear structures, some coregulated genes colocalize with each other upon induction, a phenomenon termed 'gene kissing' (Figure 1b). This phenomenon occurs in T cell development and in response to estrogen signaling [22,56]. A recent study found that in the erythroid lineage, coinduced genes frequently colocalize with each other and share RNAPII transcription factories [20,83]. The colocalized genes interact with each other and with active RNAPII [25,83], and the colocalization is dependent on the transcription factor Klf1. Not all of the clustered genes contained binding sites for Klf1, however, suggesting another factor could play a role [83]. Nonetheless, these results suggest that coregulated genes can colocalize to share transcription factors and other common proteins for efficient expression.

Individual loci within the nucleus take on preferential arrangements to optimize expression. Thus, DNA elements, proteins, and the transcription machinery play a role in controlling gene localization and inter-chromosomal interactions. What remains to be elucidated is the mechanism for gene repositioning, the proteins involved, and the precision of gene targeting.

Chromatin dynamics

To form inter- or intra-chromosomal interactions, or to change the localization of a gene with respect to a nuclear substructure, the folding and disposition of chromosomes must change. In fact, watching an individual locus demonstrates that chromosomes are never static; there are constant movements and fluctuations on small scales. Is all movement in the nucleus just random diffusion? How do longer-range rearrangements occur?

The dynamics of individual loci can be observed in live cells by tracking a tagged locus with respect to landmarks [84–86]. In yeast, *Drosophila*, and humans the typical locus

exhibits diffusive movement within a constrained sphere of 0.5–1.0 μm [84,87,88]. Interaction with structures in the nucleus can limit mobility. In yeast grown in the presence of glucose the *GAL1* gene is in the nucleoplasm; when transcribed it relocates to the nuclear periphery [77,89]. Under activating conditions the gene slides along the nuclear envelope and exhibits a more constrained localization, whereas in repressive conditions the gene visits a larger volume of the nucleus [85,86]. In higher eukaryotes, interaction with the nuclear lamina can further constrain diffusion [90]. These results suggest that interaction with subnuclear structures constrains the mobility of genes, leading to more confined 'gene territories' [67].

The movement of genes appears to be a random walk driven by Brownian motion [84]. Some loci will occasionally jump up to 0.5 μm, however, in an ATP-dependent manner [91]. It was shown that an inducible array of transcriptional activators, upon induction, relocates from the nuclear periphery to the nuclear interior through a rapid, curvilinear movement. This movement was disrupted by transient transfection of mutated actin or myosin I [92]. These results suggest that there are different types of chromatin movement: Brownian motion/constrained diffusion and directed movement. How these two mechanisms are exploited and regulated is unclear, however, and will be an important area of focus for future work.

Concluding remarks

It has become clear that nuclear organization impacts upon gene regulation. Chromosomes are folded and segregated within the nucleus. Protein assemblies that facilitate gene expression, repression, and RNA processing are heterogeneously distributed. Chromosomes dynamically change their folding to reposition genes to enhance *trans* interactions with protein bodies or structures, to loop genes out from a territory, or to form inter-chromosomal contacts with coregulated genes. One advantage of the flexible compartmentalization of the nucleus is that it allows dynamic associations of loci and proteinaceous bodies. During development or in response to external stimuli, transcriptional regulation is coupled to the spatial arrangement of genes within the nucleus. The periphery is often a repressive location, and as such genes that are silent are frequently associated with the nuclear envelope, but upon transcriptional activation are relocated to the nucleoplasm. Some inducible genes are repressed in the nucleoplasm, however, and then recruited to NPCs upon induction.

Proteinaceous bodies in the nucleus could serve as conditional subnuclear 'organelles' that promote more efficient biochemical reactions than could be achieved in a diffusion-limited environment. It is still unclear whether DNA is recruited to stable nuclear bodies, if bodies are formed *de novo* where needed, or both. Because chromosomes have constrained mobility, only having stable nuclear bodies might not be feasible. Thus, both DNA and proteins may be able to move to find each other in the nucleoplasm and, in cases where they cannot colocalize, new bodies can form.

The spatial organization of the nucleus may provide cells with an additional layer of regulatory control, through controlling the position of individual genes and their access to regulatory factors. How chromosome territories form and how the localization of individual genes in the nucleus is determined is still unclear. DNA elements that are recognized by proteins contribute to intra-chromosomal looping, lamin interactions and NPC interactions. This suggests that the genome encodes its folding and spatial organization. New 3C methods along with computer simulations offer important insight into genome organization. Advances in single-cell experiments will reveal cell–cell variability and improve our understanding of the functional impact of gene localization. Finally, understanding the molecular mechanisms that mediate this spatial organization, and how the encoded information can be regulated, represent important challenges for the future.

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