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Nuclear organization: taking a position on gene expression

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Eukaryotic genomes are divided into chromosomes that occupy defined regions or territories within the nucleus. These chromosome territories (CTs) are arranged based on the transcriptional activity and chromatin landscape of domains. In general, transcriptionally silent domains reside at the nuclear periphery, whereas active domains locate within the interior. Changes in nuclear position are observed for stress-induced and developmentally regulated tissue-specific genes. Upon activation, these genes move away from a CT to inter-chromosomal space containing nuclear bodies enriched in gene expression machinery. Gene activation is not always accompanied by movement, as positioning is dictated by many determinants, including gene structure and the local genomic environment. Collectively, tissue-specific nuclear organization results from a culmination of inputs that result in proper transcriptional regulation.

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Introduction

The nuclear envelope (NE) is a double membrane that defines the nucleus – the organelle in which processes such as DNA replication, transcription and mRNA processing take place. Advances in cytological methods and molecular genomics have provided insights on organization within the nucleus. Hybridization techniques that allow whole chromosomes to be 'painted' yield snap shot images of chromosomal arrangements, showing that chromosomes are largely confined to specific three-dimensional regions of the nucleus called chromosome territories (CTs, Figure 1a) [1••]. Gene-poor CTs and silenced genes are frequently found in association with the nuclear periphery, a similar location to that of heterochromatin (Figure 1a) [2]. By contrast, gene-rich CTs and active genes map to the nuclear interior. Sequence level organization has been obtained using chromosome conformation capture (3C) technology, which couples chemical cross-linking and massive parallel sequencing to define genome-wide relationships [3–5]. Results from these studies suggest that the genome is arranged as interdigitated CTs rather than randomly inter-twined chromosomes [6]. Emerging from these investigations is a picture of the nucleus as an ordered organelle; the consequences of this organization are just being realized.

Nuclear organization during differentiation

Studies have linked nuclear organization to cellular differentiation. Cultured pluripotent mouse embryonic stem (ES) cells possess dispersed chromatin with limited compaction. Upon differentiation, they show changes in chromatin structure that include large-scale compaction of genomic domains [7]. Consistent with these findings, embryonic development proceeds from a single cell embryo possessing a 'featureless' nucleus with dispersed chromatin, to differentiated cells possessing nuclei with peripherally located compact chromatin domains [8]. Interestingly, an extended and dispersed chromatin meshwork was identified in the eight-cell epiblast, reminiscent of nuclear structures defined in cultured ES cells. In contrast to the 'open' chromatin structure in the epiblast nuclei, lineage-restricted nuclei that give rise to extraembryonic tissues contained a distinctive 'closed' chromatin structure. These investigations indicate that global changes in gene expression correlate with chromatin reorganization, which plays a role in lineage restriction during development.

Transcriptionally active genes contribute to cell-type specific nuclear architecture in differentiated tissues. Several principles governing nuclear organization were uncovered through studies of developmentally regulated genes in *C. elegans* [9••]. Similar to mouse ES cells, nuclei within cells of the *C. elegans* embryo have a dispersed chromatin structure, upon which progressive changes in organization and gene expression occur [9••]. In lineage-restricted cells, active genes were positioned within the interior of the nucleus and silent genes at the periphery. Using transgenes that produced integrated arrays of different size, a hierarchy of subnuclear localization signals was uncovered [9••]. Large arrays produced 'heterochromatic' domains that were anchored to the nuclear periphery, whereas, smaller arrays localized within the nuclear interior. The spatial dynamics of genes within the arrays depended upon properties of the transgene promoter. Arrays with developmentally regulated genes relocated from the periphery to the interior in appropriate cell types. Before relocalization, markers of active transcription were evident, indicating that transcription precedes, and might promote, relocation. Genes with constitutively active promoters did not leave the nuclear periphery,

Figure 1

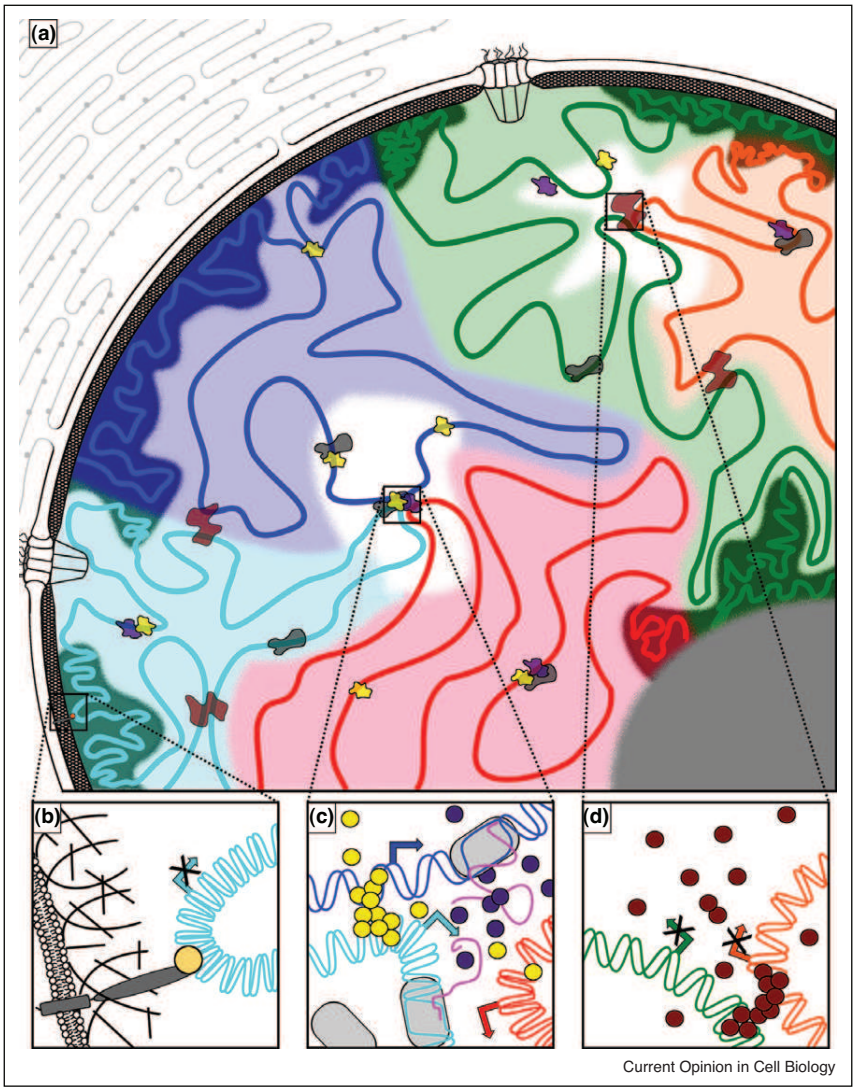


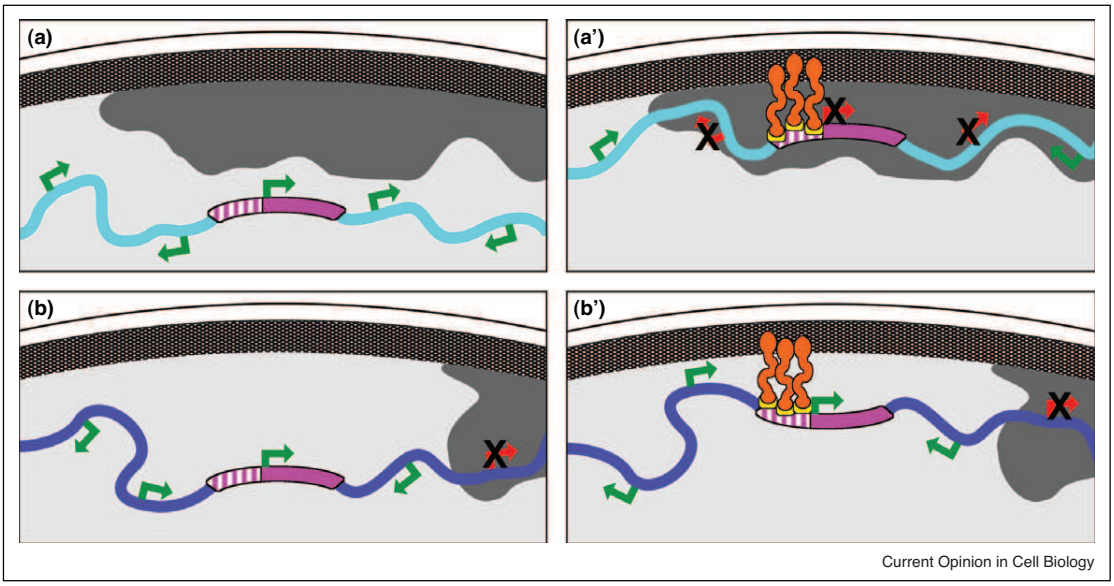
Diagram of a nucleus. **(a)** The nuclear envelope is composed of outer and inner nuclear membranes that are punctuated by nuclear pores (basket structures). Lamins make up the meshwork lining the inner side of the envelope (lattice pattern). Within the nucleus, chromosomes (thick colored lines) occupy specific territories (corresponding colored areas). Interspersed throughout the chromosomes are inter-chromosomal territories (white areas). Heterochromatin primarily occupies the nuclear periphery and is shown as darker patches within the chromosome territories. Nuclear speckles occur throughout inter-chromosomal regions and represent accumulations of transcription factors (yellow), splicing factors (purple), Polycomb proteins (dark red) and RNA polymerase (gray). **(b)** A LEM-domain protein (gray) transverses the nuclear lamina and tethers a chromosome (blue) to the nuclear periphery through interactions with the protein Barrier to Autointegration Factor (BAF; orange circle), silencing a nearby gene (bent arrow marked with black X). **(c)** Active genes (bent arrows) are recruited to a transcription factory within the inter-chromosomal space that is enriched for transcription machinery, RNA polymerase (gray ovals), transcription factors (yellow circles), splicing factors (purple circles) and RNA transcripts (purple lines). **(d)** Polycomb proteins bound at distinct sites dispersed throughout the genome coalesce to form Polycomb bodies (dark red circles).

suggesting that transcription is not a trigger to release from the periphery. Collectively, these studies demonstrated that tissue-specific promoters are major determinants of nuclear organization during development.

The role of nuclear lamina in gene regulation

Peripheral localization of heterochromatin depends on proteins in the nuclear lamina, a network of proteins that line the inner side of the NE (Figure 1a and b) [10•]. Genomic sites that contact the nuclear lamina have been mapped by DamID [11] and are called lamina-associated domains (LADs) [12]. LADs range in size from tens of kilobases to several megabases, are relatively gene poor, have low transcriptional activity and are enriched in repressive chromatin marks. LADs make up nearly 40% of human and mouse genomes. Lamin–DNA interactions

Figure 2



Effects of tethering to the nuclear envelope. **(a)** A transgene (pink) with LacI binding sites (pink stripes) is inserted into a chromosome (thick blue line) and expressed (green arrow), along with neighboring 'bystander' genes. **(a′)** The same transgene moved to silent chromatin territories (dark gray) at the nuclear periphery and silenced (red arrow with black X) upon association with a LacI-NE protein fusion protein. **(b)** The same transgene inserted into a different chromosome is expressed both before and **(b′)** after tethering to the NE. In each panel the lattice pattern represents the nuclear lamina, orange lines represent the nuclear envelope protein, yellow circles indicate the LacI DNA binding domains, striped pink region represents LacI binding motif repeats; pink box represents the transgene reporter, thick light and dark blue lines represent chromosomes, green arrows indicate active genes and red arrows silenced genes.

are dynamic throughout development. Comparisons of LADs defined in mouse ES cells and terminally differentiated astrocytes suggested that changes in lamina–DNA contacts involve single genes or small gene clusters and occur cumulatively during differentiation [12]. Detachment of repressed genes from the lamina correlates with activation or serves as a predictor of activation upon differentiation for a subset of genes. These observations imply that movement of genes away from the nuclear periphery may unlock a gene for expression in a future developmental stage.

A role for NE association in gene repression was revealed by studies of testes-specific genes in Drosophila [13••]. These genes are arranged into large clusters (20–200 kb) containing multiple testes-expressed genes that associate with B-type lamins in somatic cells. Loss of the B-type lamin increases somatic expression of the genes in two testes-specific clusters, correlating with release of the cluster from the NE. Transcriptional de-repression in the absence of lamin was limited to genes within the testes-specific gene clusters. These studies provide direct evidence that association with the NE can confer cell-type specific transcriptional repression.

Effects of tethering to the NE

Parameters that define the transcriptional outcome resulting from NE association are unclear. To gain insights into these determinants, several groups developed systems that deliberately place genes near the nuclear periphery. In these studies, a two part system was used consisting of (1) multiple binding sites for the *E. coli* Lac I repressor protein positioned upstream of a reporter gene and (2) a transgene expressing a protein consisting of the Lac I DNA binding domain fused with either a lamin or a NE protein [14,15••,16,17]. Expression of the fusion protein causes peripheral localization of the reporter gene (Figure 2). Expression of the reporter and adjacent endogenous genes, referred to as bystanders [18], was used to monitor the transcriptional impact of the new position. In some cases, the reporter gene and the bystanders were repressed (Figure 2a′), while in other cases they remained active (Figure 2b′). These data suggest that promoter properties contribute to the impact of the periphery on transcription. Even so, insertion of the same reporter gene at multiple genomic locations showed distinct transcriptional outcomes, indicating that genomic environment (gene density, gene activity and epigenetic marks) also contributes to the transcriptional outcome (Figure 2a and b).

Effects of nuclear pore proteins on gene positioning

Multiple constituents of the NE contribute to genome organization. In addition to lamins, components of the nuclear pore complex (NPC) show chromatin association (Figure 1a). The interactions between nuclear pore

proteins and chromatin are complex and not confined to the NE. In Drosophila, Nucleoporin Associated Regions (NARs), ranging in size from 5-kb to 500-kb, make up approximately 25% of the genome [19]. Many of the NARs localize to the nuclear interior and contain active genes that regulate development [20••,21•]. Interactions between nuclear pore components and genes within the *S. cerevisae* genome occur at the NE [22–24]. Yeast genes activated by nutrient availability localize to the nuclear periphery before activation: a process that requires specific sequences, termed 'zip codes', located in the promoter region that interact with nuclear pore proteins [22,24]. Visitation to the NE establishes a 'transcriptional memory', which allows for rapid activation upon subsequent stimulation. Memory of prior activation is linked to specific DNA elements and the incorporation of the histone variant H2A.Z [23]. Collectively, these data demonstrate that nuclear pore proteins associate with active genes; however, positioning of this interaction – peripheral or internal – varies depending on the organism or additional variables.

Contribution of transcriptional machinery to nuclear organization

The interchromosomal space located between CTs contains a variety of nuclear substructures, referred to by many names including foci, speckles, bodies and spots (Figure 1a). The number and composition of these bodies depends on cell type [25]. Nuclear bodies are enriched in specific factors, such as those involved in transcription and RNA processing. While the function of these bodies has been challenging to discern, recent studies demonstrate an important role in nuclear organization.

Actively transcribing RNA Polymerase II exhibits a non-uniform distribution within interchromosomal spaces (Figure 1a). These 'transcription factories' have varying composition, with some enriched for RNA splicing factors, such as SC35 [26] (Figure 1c). Localization within factories depends on gene structure, as demonstrated by plasmid transfection experiments [27]. For example, plasmids with differing promoter types distributed into distinct factories. Plasmids containing genes with introns accumulated in SC35 rich factories, whereas plasmids with intronless genes localized to factories devoid of SC35. Transfected plasmids were found in factories with the endogenous genes, suggesting that co-assembly is driven by shared transcription factors. Similar conclusions were drawn from studies of integrated BAC arrays containing human *Hsp70* genes [28••]. These arrays associated with the endogenous genes in SC35-containing factories; localization depended only upon a small promoter fragment and the process of transcription, regardless of the transcription level. Transcription factors may be central to the co-localization into a factory. For example, mouse *globin* genes preferentially co-localize in transcription factories with hundreds of other transcribed genomic loci that are regulated by the transcription factor Klf1 [29••].

Transcriptional repressors contribute to nuclear organization. This has been well documented for Polycomb group (PcG) proteins. PcGs play a role in the developmental repression of *Hox* gene clusters, which display an evolutionarily conserved chromosome arrangement and span megabases of DNA. Extensive studies of *Hox* genes in Drosophila demonstrated that transcriptional repression depends upon PcG proteins that associate with Polycomb Response Elements (PREs) distributed throughout the gene clusters [30]. Repression correlates with coalescence of PREs into nuclear foci, termed Polycomb bodies that are present in the nuclei of most, but not all, tissues (Figure 1d) [31]. Polycomb bodies represent a gathering of PRE elements from distant genomic sites scattered throughout the genome [32,33••,34]. This arrangement strengthens gene repression, presumably by causing local increases in repressive proteins, and fosters nuclear compartmentalization of similarly regulated genes [33••].

Insulator proteins also contribute to nuclear organization by establishing long-range and inter-chromosomal associations [35]. The insulator binding protein CCCTC binding factor (CTCF) is a multiple zinc finger protein with a highly conserved DNA binding domain. CTCF is essential, as depletion in pre-implantation mouse embryos causes early embryonic lethality [36,37]. CTCF binds tens of thousands of sites in mammalian genomes [38]. CTCF participates in inter-chromosomal and intra-chromosomal interactions that regulate transcription. Interactions between CTCF and partner proteins such as cohesin and lamins might be crucial for positioning genes into subnuclear compartments. Emerging evidence suggests that even though CTCF binding is constitutive, CTCF-dependent long-range interactions might be regulated [39,40]. Looping between CTCF sites appears to be responsible for establishing higher order chromatin structures that form the foundation of tissue-specific nuclear organization.

Nuclear positioning and disease

The relative positioning of CTs can impact human disease. For example, chromosomal translocations that are commonly associated with human pathologies result from chromosome fusions [41]. These translocations arise from double-strand breaks that are repaired through non-homologous end joining between neighboring chromosomes. A consequence of translocation is the mis-positioning of many genes within the affected chromosomal region, which might contribute to the disease pathology. As CT organization differs among tissues, such translocation events are associated with tissue-specific diseases, such as blood disorders [42]. Since translocation events do not always interfere with the normal nuclear position and

expression of genes within the rearranged chromosomes [43], genes at the breakpoint represent the major contributors to disease.

Altered positioning of CTs is commonly found in tumor cells [1••]. Emerging evidence suggests that early events in cancer may involve changes in the position of individual genes, not complete CTs. Studies of breast cancer cells in a three-dimensional *in vitro* cell culture model revealed changes in the position of many genes [44]. The absence of transcriptional changes associated with movement of these genes suggests that these genes are not responsible for tumorogenesis, but may be useful markers for cancer detection. Changes in gene positioning have been observed in a patient with cardiomyopathy [45]. Here, expression of a mutant A-type lamin caused genes within clusters on chromosome 13 to be mis-localized to the nuclear interior and misexpressed. Taken together, these data demonstrate that nuclear organization breaks down in disease states.

Conclusions and perspectives

The combination of cytology and new molecular genomic approaches to study protein–DNA interactions has revealed that the nucleus is an ordered, yet dynamic environment. Whole chromosomes are arranged in territories and inter-chromosomal spatial relationships are confined [46]; however, CTs and genes have the ability to change position. The mechanisms by which movement occurs within the nucleoplasm are largely unknown. Emerging evidence suggests that the myriad of nuclear bodies are likely to function as 'magnets' that attract commonly regulated genes to shared nuclear positions.

General rules have been established that predict the transcriptional status of a gene based upon nuclear positioning. Determinants include DNA sequence content, gene density, promoter strength, epigenetic chromatin modifications and chromatin folding. However, there are many exceptions to these rules. Exceptions should be treasured, as they provide future impetus to address whether nuclear position matters.

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