

Genetic and epigenetic control of the spatial organization of the genome

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ABSTRACT Eukaryotic genomes are spatially organized within the nucleus by chromosome folding, interchromosomal contacts, and interaction with nuclear structures. This spatial organization is observed in diverse organisms and both reflects and contributes to gene expression and differentiation. This leads to the notion that the arrangement of the genome within the nucleus has been shaped and conserved through evolutionary processes and likely plays an adaptive function. Both DNA-binding proteins and changes in chromatin structure influence the positioning of genes and larger domains within the nucleus. This suggests that the spatial organization of the genome can be genetically encoded by binding sites for DNA-binding proteins and can also involve changes in chromatin structure, potentially through nongenetic mechanisms. Here I briefly discuss the results that support these ideas and their implications for how genomes encode spatial organization.

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SPATIAL ORGANIZATION OF EUKARYOTIC GENOMES

Spatial organization, from organisms to molecules, is a defining feature of life. In multicellular organisms, cells are organized into tissues that are spatially segregated from each other. Cells serve to concentrate and organize biological activities, facilitating metabolism, growth, and division. In eukaryotic cells, membrane-bounded organelles compartmentalize biochemical reactions, permitting greater efficiency and control. Biochemical activities can also be concentrated and regulated through the formation of non-membrane-bounded, phase-separated liquid droplets such as P bodies, germ granules, nucleoli, and others (Courchaine *et al.*, 2016).

Not only is the eukaryotic nucleus segregated from the cytoplasm, but it is also spatially organized within. It has long been appreciated that chromosomes are nonrandomly arranged (Rabl, 1885; Boveri, 1909). During mitosis, chromosome condensation and alignment are critical to facilitate proper segregation (Hirano, 2015). Similarly, during interphase or in postmitotic cells, the spatial organization of decondensed chromosomes is tissue specific,

reflecting different functional states of the genome (Parada *et al.*, 2004; Meaburn and Misteli, 2007; Fraser *et al.*, 2015).

Chromosomes fold and occupy characteristic regions of the interphase nucleus. In unicellular eukaryotes such as yeast, as well as in certain metazoan cells during development, chromosomes associate with the nuclear envelope, and their telomeres and centromeres cluster together at opposite sides of the nucleus (Marshall *et al.*, 1996; Heun *et al.*, 2001; Taddei *et al.*, 2010; Zimmer and Fabre, 2011). In differentiated cells, chromosomes tend to fold and occupy distinct, mostly nonoverlapping “territories” (Cremer *et al.*, 2006; Cremer and Cremer, 2010). The position of chromosomes within the nucleus also correlates with cell type; gene-rich chromosomes that are more highly transcribed tend to be positioned toward the center of the nucleus, and gene-poor chromosomes tend to be positioned toward the nuclear periphery (Parada *et al.*, 2004).

The arrangement of chromosomes with respect to nuclear landmarks such as the nuclear periphery reflects their interaction with stable subnuclear structures. Depending on the cell type, a varying but significant fraction of metazoan genomes is associated with the nuclear lamina. These lamina-associated-domains (LADs), up to 10 Mb in length, localize at the nuclear periphery (Figure 1A; Vogel *et al.*, 2007; Guelen *et al.*, 2008). Similarly, in budding yeast and fission yeast, telomeres and centromeres cluster together and associate with the nuclear envelope (Klein *et al.*, 1992; Funabiki *et al.*, 1993; Palladino *et al.*, 1993; Gotta *et al.*, 1996). Such physical interactions provide a significant constraint on chromosome folding and positioning, leading to a nonrandom arrangement of chromosomes within the nucleus.

Individual genes are often nonrandomly positioned with respect to nuclear structures and with respect to each other and their position

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Abbreviations used: BAC, bacterial artificial chromosome; CTCF, CCCTC-binding factor; LADs, lamin-associated domains; LCR, locus control region; NPC, nuclear pore complex; H3K4me2, dimethylation of histone H3 on lysine 4; H3K9me2, dimethylation of histone H3 on lysine 9; H3K27me3, trimethylation of histone H3 on lysine 27.

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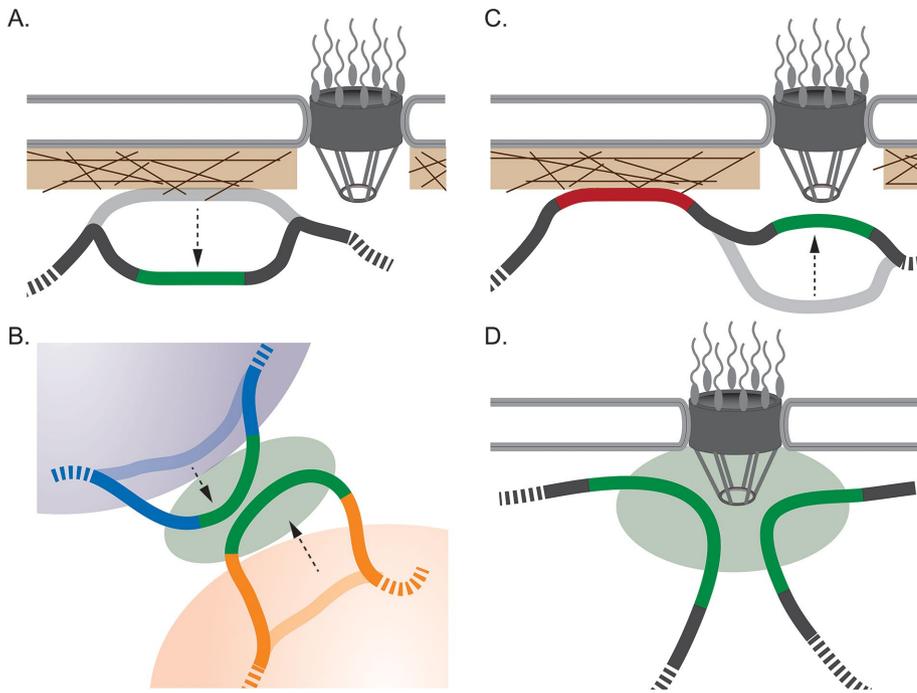


FIGURE 1: Phenomena that lead to nonrandom spatial arrangement of the genome. (A) During development, genes that are induced often move from a peripheral position in association with the lamina, where they are silenced, to a more internal site, where they are expressed (green locus). (B) Gene activation can lead to looping of genes out of their respective chromosome territories, where they can cluster together. (C) Upon activation (green locus), certain genes physically associate with the NPC, causing them to localize to the nuclear periphery. Thus the nuclear periphery is associated with both silencing (red locus at the lamina) and activation/poising (at the NPC). (D) Genes that are targeted to the NPC in budding yeast frequently undergo interallelic clustering and interchromosomal clustering with other genes that are targeted by the same mechanism.

can change upon transcriptional activation or repression. Silenced loci within LADs localize at the nuclear periphery, but such loci are released from the lamina and move to a more internal site upon activation (Figure 1A; Kosak *et al.*, 2002; Zink *et al.*, 2004; Meister *et al.*, 2010; Peric-Hupkes *et al.*, 2010). Similarly, transcriptional induction can lead to a locus looping out from its chromosome territory (Figure 1B; Noordermeer *et al.*, 2008). Heat shock genes reposition near stress granules upon induction (Khanna *et al.*, 2014). Furthermore, in budding yeast, *Drosophila*, *Caenorhabditis elegans*, and mammalian cells, many active genes physically interact with nuclear pore proteins (Casolari *et al.*, 2004, 2005; Brown *et al.*, 2008; Capelson *et al.*, 2010; Kalverda and Fornerod, 2010; Liang *et al.*, 2013; Rohner *et al.*, 2013). Whereas in yeast these interactions apparently occur exclusively at the nuclear periphery (with the nuclear pore complex [NPC]; Figure 1C), in flies and mammals, these interactions can occur either at the nuclear periphery or in the nucleoplasm (with soluble nuclear pore proteins; Capelson *et al.*, 2010; Kalverda *et al.*, 2010; Liang *et al.*, 2013; Light *et al.*, 2013). Interaction with the NPC leads to positioning at the nuclear periphery (Figure 1C). Thus changes in gene expression are frequently coupled to movement of loci with respect to nuclear landmarks.

Coregulated genes sometimes cluster together through interchromosomal interactions (Figure 1, B and D). Clustering has been observed between genes induced during erythropoiesis (Brown *et al.*, 2006; Schoenfelder *et al.*, 2010), genes induced by estrogen (Lin *et al.*, 2009), and genes induced by infection (Apostolou and Thanos, 2008). In yeasts, both tRNA genes and genes that are targeted to the NPC undergo interchromosomal clustering (Figure 1D;

Thompson *et al.*, 2003; Noma *et al.*, 2006; Brickner *et al.*, 2012). These observations suggest that the nucleus might be functionally segregated by creating clusters of genes of related function or with related modes of regulation.

EMERGENT PHENOMENA

Nonrandom spatial organization in some cases represents an emergent phenomenon, a functional outcome of transcription, chromatin structure, or polymer biophysics (Misteli, 2008, 2009). Emergent phenomena resulting from self-organization are common and extremely important in biology, and this is a critical consideration in studying nuclear cell biology. Interpreting genetic perturbation of emergent phenomena is more difficult than interpreting genetic perturbation of active targeting mechanisms. For example, if the biophysical process of transcription, chromatin decompaction, or mRNA processing were more energetically costly in the more densely compacted environment within a chromosome territory, this might result in the movement of active genes to the edge of the territory. This movement, however, would not represent active, specific targeting to the interterritorial space. Indeed, modeling chromosomes as polymers and incorporating simple biophysical differences between euchromatin and heterochromatin can recapitulate formation of chromosome territories and the localization of active genes between

chromosome territories (Cook and Marenduzzo, 2009; Heermann, 2011; Wang *et al.*, 2015). Similarly, some subnuclear structures are not stable landmarks but are built around the genes with which they colocalize. For example, nucleoli and histone locus bodies colocalize with the ribosomal DNA and histone genes, respectively. However, such bodies can be initiated to form at an ectopic locus by tethering nucleating factors to that site (Karpen *et al.*, 1988; Kaiser *et al.*, 2008; Mao *et al.*, 2011; Shevtsov and Dundr, 2011). This type of spatial organization, although specific to particular genes and giving rise to a highly biased pattern, reflects an emergent property of certain genes rather than active positioning of genes near nuclear bodies. Distinguishing between nonrandom arrangements that are produced by active targeting mechanisms and those that are produced by emergent phenomena is critical to understanding nuclear architecture.

Here I focus on cases in which the nonrandom positioning of genes and chromosomal domains appears to be conferred by active targeting mechanisms. To define active mechanisms, I draw on the lessons from protein targeting to membrane-bound organelles. Active mechanisms that control subnuclear positioning ought to exhibit specificity and depend on information that is necessary and/or sufficient to promote targeting to stable sites.

DNA-BINDING PROTEINS AND CHROMATIN MODIFICATIONS AFFECT THE SPATIAL ORGANIZATION OF THE GENOME

If spatial organization of the genome is controlled by active mechanisms, then what is the source of the information? Is spatial organization encoded by the DNA sequence, chromatin changes

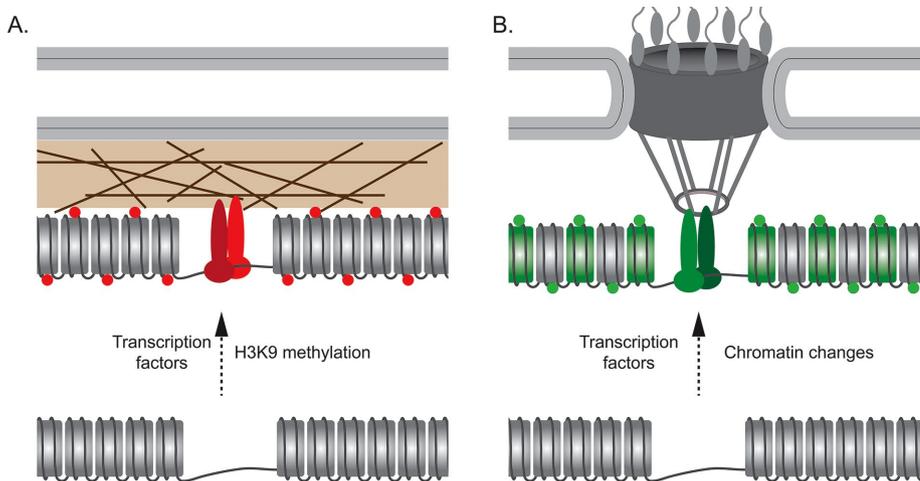


FIGURE 2: Transcription factors and chromatin function to control interactions with the nuclear periphery. (A) Targeting of genes to the nuclear lamina requires both transcription factors such as c-Krox and YY1 and methylation of histone H3 on lysine 9 (red circles). In *C. elegans*, this involves a nuclear envelope–localized protein called CEC-4 that binds to this mark. (B) Targeting to the NPC (or interaction with nuclear pore proteins in the nucleoplasm of *Drosophila* or mammals) requires transcription factors and, in some cases, chromatin changes. Several yeast transcription factors are both necessary and sufficient to cause targeting to the NPC. However, histone acetylation, H3K4 methylation (green circles), and H2A.Z incorporation (green nucleosomes) are also required in certain cases.

(potentially independent of the DNA sequence), or a combination of both? Work from several experimental systems reveals that both sequence-specific DNA-binding proteins and chromatin modifications play essential roles in controlling the spatial organization of genes and chromosomal domains. Binding sites for sequence-specific DNA-binding proteins such as CTCF and transcription factors serve as the genetic information that affects chromosome folding and spatial positioning. Non-genetic chromatin changes such as histone methylation and variant histones also play essential roles in controlling the positioning of individual genes and chromosomal domains within the nucleus.

Transcription factors

Spatial positioning of individual genes with respect to stable nuclear landmarks can be controlled by *cis*-acting DNA elements that recruit sequence-specific DNA-binding proteins. In *Drosophila*, the *gypsy* insulator element is sufficient to induce targeting of a locus to the nuclear periphery (Gerasimova *et al.*, 2000). For developmentally induced genes that localize at the nuclear periphery in association with the nuclear lamina before induction (Kosak *et al.*, 2002), the positioning at the periphery can be determined by *cis*-acting DNA sequences. For example, insertion of the *IgH* and *Cyp3a* genes at ectopic sites in the genome is sufficient to reposition the ectopic locus to the nuclear periphery (Zullo *et al.*, 2012). The transcription factors cKrox and YY1 are critical for this targeting (Zullo *et al.*, 2012; Harr *et al.*, 2015). Similarly, the β -globin gene possesses *cis*-acting DNA elements that are necessary for targeting to the nuclear periphery (Bian *et al.*, 2013). This suggests that transcription factor-binding sites play a critical role in targeting to the nuclear lamina (Figure 2A).

Hundreds of genes that interact with nuclear pore proteins have been identified in yeast, flies, and mammalian cells (Casolari *et al.*, 2004, 2005; Brown *et al.*, 2008; Kalverda *et al.*, 2010; Liang *et al.*, 2013). Initially, it was believed that this may represent an emergent product of transcription by which NPC-associated mRNA export fac-

tors might create an affinity sink for actively transcribed genes (Casolari *et al.*, 2005; Dieppois *et al.*, 2006; Taddei *et al.*, 2006). However, targeting to the NPC in yeast does not require active transcription (Schmid *et al.*, 2006; Brickner *et al.*, 2007, 2016; Ahmed *et al.*, 2010; Light *et al.*, 2010; Randise-Hinchliff *et al.*, 2016). Furthermore, binding sites for certain transcription factors are enriched among genes that interact with pore proteins (Casolari *et al.*, 2004, 2005; Brown *et al.*, 2008; Liang *et al.*, 2013). In *Drosophila*, GAGA factor-binding sites are also enriched for nuclear pore protein binding, and the transcription factor MBD-R2 physically interacts with the nuclear pore protein Nup98 and promotes interaction of Nup98 with target genes (Capelson *et al.*, 2010; Kalverda *et al.*, 2010; Pascual-Garcia *et al.*, 2014). Thus transcription factor-binding sites correlate with interactions with nuclear pore proteins. However, in most cases, the functional significance of these transcription factors in controlling interaction with nuclear pore proteins or spatial positioning of target genes has not yet been tested.

In budding yeast, transcription factor-binding sites have been functionally identified as both necessary and sufficient to target genes to the nuclear periphery (Ahmed *et al.*, 2010; Light *et al.*, 2010; Brickner *et al.*, 2012, 2016; D'Urso *et al.*, 2016; Randise-Hinchliff *et al.*, 2016). Not every yeast transcription factor has this ability, suggesting that a subset of transcription factors affects gene positioning. Five transcription factors have been identified (Put3, Cbf1, Gcn4, Ste12, and Sfl1) that are both necessary for targeting endogenous loci to the periphery and whose binding sites function as "DNA zip codes" that are sufficient to target an ectopic site to the periphery (Figure 2B). In several of these cases (Gcn4, Ste12, and Sfl1), the transcription factors cause peripheral targeting when tethered to an ectopic site through a heterologous DNA-binding domain, confirming that they are indeed sufficient to confer positioning at the periphery (D'Urso *et al.*, 2016; Randise-Hinchliff *et al.*, 2016). This suggests that controlling gene positioning is an underappreciated function of some transcription factors (Figure 2B).

Transcription factors and their binding sites also control interchromosomal clustering of coregulated genes. Before differentiation of T helper cells, the interferon γ gene clusters with a locus control region (LCR) on another chromosome, and this is regulated by the DNase-hypersensitive region of the LCR (Spilianakis *et al.*, 2005). Clustering of the Interferon β gene with sites on other chromosomes in response to virus infection requires the NF- κ B transcription factor (Apostolou and Thanos, 2008). Clustering of coregulated genes during erythropoiesis requires the Klf1 transcription factor (Schoenfelder *et al.*, 2010), and clustering of bacterial artificial chromosomes carrying the *HSP70* gene near nuclear speckles requires the *HSP70* promoter (Hu *et al.*, 2010). Similarly, clustering of tRNA genes in both budding yeast and fission yeast requires the sequence-specific transcription factor TFIIC (Noma *et al.*, 2006; Haeusler *et al.*, 2008). In budding yeast, transcription factors that control positioning to the nuclear periphery are also necessary for specific interchromosomal clustering in association with the nuclear pore (Brickner *et al.*, 2012, 2015; Randise-Hinchliff *et al.*, 2016). Of

importance, insertion of their DNA-binding sites at an ectopic site in the genome can promote clustering with endogenous genes that possess the same binding site (Brickner *et al.*, 2012, 2016; Randise-Hinchliff *et al.*, 2016). This suggests that genomes encode both peripheral localization and interchromosomal clustering through cis-acting DNA elements such as transcription factor-binding sites.

Chromatin

Chromatin modifications often correlate with spatial position and, in some cases, are required for the normal spatial arrangement of the genome. Subtelomeric regions are silenced by the yeast histone deacetylase Sir2. Histone deacetylation recruits Sir4, which binds to both hypoacetylated histones and the nuclear envelope membrane protein Esc1, providing a physical link between chromatin state and a subnuclear structure (Taddei and Gasser, 2012). Lamina-associated domains tend to be transcriptionally repressed and methylated on histone H3 lysine 9 (Guelen *et al.*, 2008; Wen *et al.*, 2009; Yokochi *et al.*, 2009; Kind *et al.*, 2013). Loss of the H3K9 methyl transferase G9a leads to loss of peripheral H3K9me2 but does not obviously alter the peripheral localization of all genes (Yokochi *et al.*, 2009). However, association of lamina-targeted BACs or endogenous LADs in human cells or lamina-associated repetitive arrays in *C. elegans* is dependent on H3K9 methylation (Towbin *et al.*, 2012; Gonzalez-Sandoval *et al.*, 2015). Thus, together with the transcription factors mentioned earlier, H3K9 methylation is required for the interaction with certain loci with the nuclear periphery (Figure 2A).

In *C. elegans*, H3K9 methylation may function alone in targeting to the lamina. The system in which this requirement was discovered used long arrays of transgenes (Meister *et al.*, 2010). The H3K9 methylation of these arrays is apparently a nonspecific product of their repetitive nature. Targeting to the nuclear lamina and H3K9 methylation are mutually reinforcing events: H3K9 methylation is both promoted by targeting to the nuclear lamina and required for targeting to the lamina. A nuclear envelope-associated protein called CEC-4 binds to methylated H3K9 through a chromodomain to mediate localization of heterochromatic repetitive arrays to the nuclear lamina (Gonzalez-Sandoval *et al.*, 2015). This suggests that H3K9 methylation alone can target such arrays to the nuclear lamina, independent of their sequence and presumably independent of DNA-binding proteins.

The intrachromosomal looping and clustering of Polycomb targets leads to formation of Polycomb bodies. This requires the Polycomb group protein PC (Lanzuolo *et al.*, 2007). However, mutations that disrupt Polycomb response elements, which reduce recruitment of Polycomb group proteins, do not strongly affect either H3K27me3 or Polycomb body formation. This suggests that H3K27 methylation, rather than a DNA-binding protein, may facilitate the formation of Polycomb bodies.

Targeting of certain yeast genes to the NPC requires the SAGA histone acetyltransferase and is inhibited by the Rpd3(L) histone deacetylase (Cabal *et al.*, 2006; Luthra *et al.*, 2007; Randise-Hinchliff *et al.*, 2016). Furthermore, targeting of yeast *INO1* to the nuclear periphery after repression (a phenomenon called epigenetic transcriptional memory) requires both local incorporation of H2A.Z and dimethylation of H3K4 (Brickner *et al.*, 2007; Light *et al.*, 2010, 2013). Therefore chromatin modifications also play essential roles in targeting to the nuclear pore complex (Figure 2B).

In addition to active and poised genes, the NPC in budding yeast and *Drosophila* also interacts with persistent double-strand breaks, eroded telomeres, and collapsed replication forks, and this interaction facilitates DNA repair (Nagai *et al.*, 2008; Kalocsay *et al.*, 2009; Ryu *et al.*, 2015). Relocalization of DSBs to the NPC requires

both H2A.Z and SUMOylation of either chromatin or repair factors, leading to a physical interaction with the SUMO-dependent ubiquitin ligase Slx5/Slx8, which interacts with the NPC component Nup84 (Nagai *et al.*, 2008; Kalocsay *et al.*, 2009; Churikov *et al.*, 2016; Horigome *et al.*, 2016). Thus positioning of DNA damage, which is unlikely to be dependent on DNA sequence, is mediated by changes in chromatin and associated proteins.

Coordinating the function of DNA-binding proteins and chromatin

Because transcription factors and chromatin changes influence each other and can have indirect effects, in most cases, it remains to be determined whether either of these mechanisms alone is sufficient to confer spatial positioning. Furthermore, the function of transcription factors themselves might be regulated by posttranslational modifications such as acetylation and SUMOylation (Texari *et al.*, 2013). In cases in which both DNA-binding proteins and chromatin modifications play essential roles in controlling the spatial organization of the genome (i.e., neither is sufficient), they might function in the same linear pathway or by a combinatorial mechanism.

The requirement for histone acetylation or methylation in targeting genes to the NPC (Figure 2B) may represent either a direct role for chromatin in mediating targeting to the NPC or a role for chromatin in regulating transcription factor binding/function. In support of this idea, Rpd3(L) regulates Put3 DNA binding, and SAGA is required for targeting of certain genes to the periphery because histone acetylation promotes binding of transcription factors. In support of this idea, SAGA is required for peripheral targeting of genes whose subnuclear localization is regulated by transcription factor occupancy but is not required for peripheral targeting of genes whose subnuclear localization is regulated downstream of transcription factor occupancy (Randise-Hinchliff *et al.*, 2016). This suggests that histone acetylation (or other functions of SAGA) may regulate transcription factor occupancy by regulating either DNA binding or transcription factor abundance.

The histone modifications associated with transcriptional memory are necessary but not sufficient to confer targeting to the nuclear periphery. Mutants lacking H2A.Z or unable to methylate H3K4 fail to target genes to the NPC during memory (Brickner *et al.*, 2007; Light *et al.*, 2010, 2013). However, neither H2A.Z nor H3K4me2 is sufficient to cause targeting to the NPC (Light *et al.*, 2010; D'Urso *et al.*, 2016). This suggests that targeting to the NPC requires both a transcription factor and the correct chromatin state and that they may function together (Figure 2B). Future work will distinguish a direct role for chromatin in affecting gene positioning in this system from a regulatory role.

CONCLUSION

Nuclear cell biology is an important and challenging area. Future work will seek to develop a more complete understanding of the molecular and evolutionary mechanisms that control the spatial organization of the genome. To make progress, we must first address two important issues. First, we must distinguish spatial organization that is a product of emergent properties of nuclear biology from spatial organization that is the product of active mechanisms. Both types of phenomena contribute to the spatial organization of the genome, and they need to be integrated to understand the whole. Second, we must carefully define the relationship between DNA-binding proteins and chromatin changes in determining spatial organization. Subnuclear positioning of genes and domains is controlled by DNA-binding proteins, chromatin structure, and, in some cases, both. This suggests that both DNA sequence-dependent

and DNA sequence-independent mechanisms affect the spatial organization of the genome. Understanding the evolutionary processes that can select for such mechanisms poses an exciting and important problem for future work.

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