Gene Positioning Effects on Expression in Eukaryotes

Huy Q. Nguyen and Giovanni Bosco

Geisel School of Medicine, Dartmouth College, Hanover, New Hampshire 03755; email: giovanni.bosco@dartmouth.edu

Annu. Rev. Genet. 2015. 49:627–46

First published online as a Review in Advance on October 2, 2015

The *Annual Review of Genetics* is online at genet.annualreviews.org

This article's doi: 10.1146/annurev-genet-112414-055008

Keywords

nuclear organization, chromatin looping, transcription

Abstract

The packaging and organization of the genome within the eukaryotic interphase nucleus directly influence how the genes are expressed. An underappreciated aspect of genome structure is that it is highly dynamic and that the physical positioning of a gene can impart control over its transcriptional status. In this review, we assess the current knowledge of how gene positioning at different levels of genome organization can directly influence gene expression during interphase. The levels of organization discussed include chromatin looping, topologically associated domains, chromosome territories, and nuclear compartments. We discuss specific studies demonstrating that gene positioning is a dynamic and highly regulated feature of the eukaryotic genome that allows for the essential spatiotemporal regulation of genes.

INTRODUCTION

An ever-increasing number of studies are contributing to our appreciation that biological information is not just coded in the linear DNA molecule. That DNA is packaged into a massive DNA/RNA/protein complex—the chromosome—is now accepted as fact. However, the idea that chromosome folding is functionally important for how biological information is read and inherited is still relatively new and not without its critics. For example, it has been observed that the three-dimensional (3D) positioning of a gene can impact its expression, and the eukaryotic genome in general is a highly dynamic structure that exhibits different levels of organization. Indeed, gene promoters are organized in such a way that they are separated from their regulatory DNA elements, such as enhancers, and can be brought together by chromatin loop formation to activate transcription. These promoter-enhancer interactions further organize into physically interacting units termed topologically associated domains (TADs). Numerous TADs accumulate as chromosomes fold, forming chromosome territories (CTs). The nucleus can be further compartmentalized by spatially separating transcriptionally active and inactive parts of the genome. Thus, the genome within the nucleus displays different orders of organization that contribute to the spatiotemporal control over transcription. In this review, we present examples and discuss mechanisms of how gene positioning in respect to chromatin looping, TADs, CTs, and nuclear compartments can influence transcription.

CHROMATIN LOOPING

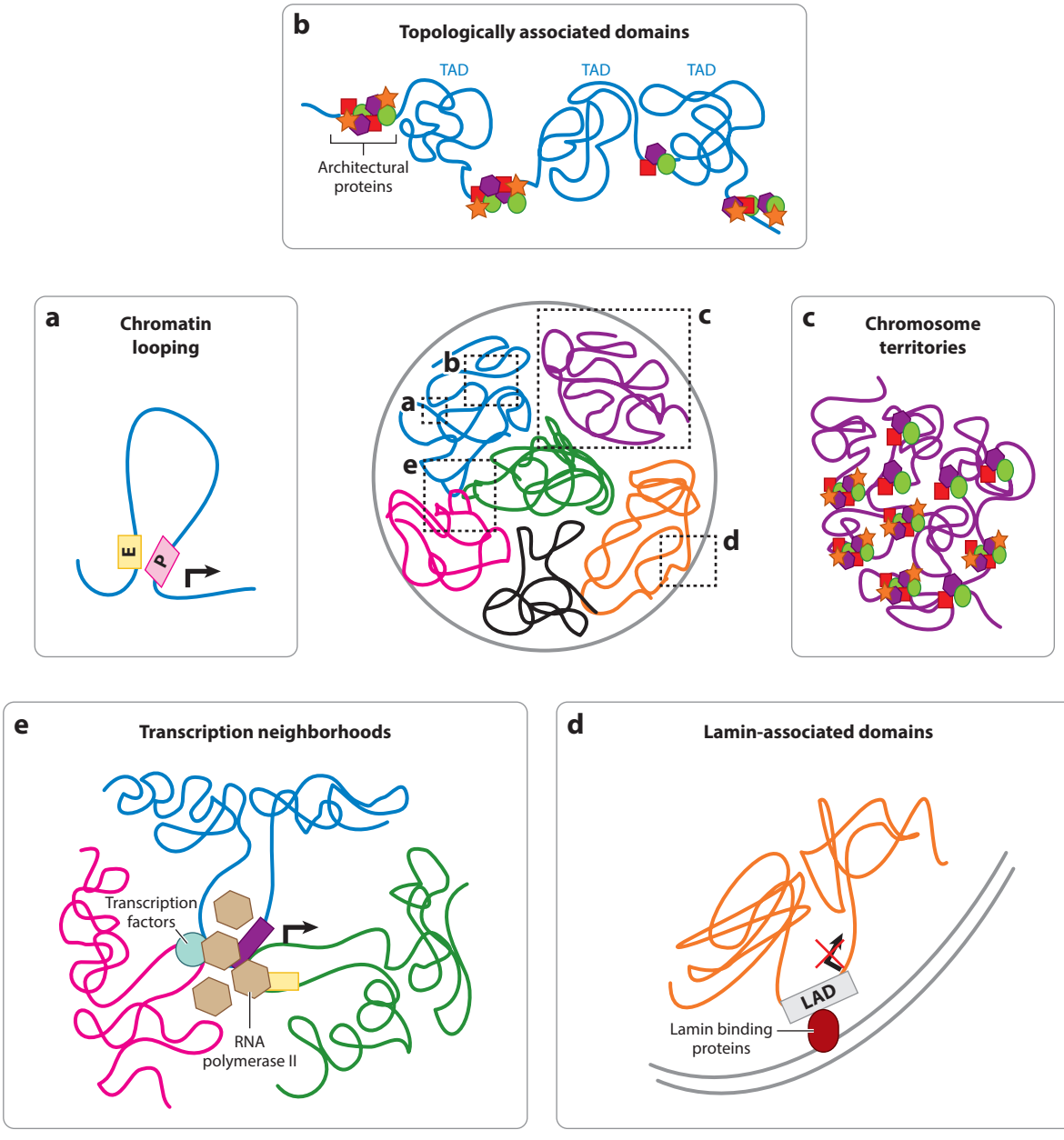
Gene expression requires the physical interaction between gene promoters and their distal regulatory elements, such as enhancers and insulators. In some instances, these genomic elements are separated by kilobases or megabases of DNA on the linear chromosome, which may include other genes and regulatory sequences (7, 20, 28, 52). The linear separation of promoters and enhancers along chromosomes presents a spatial (and possibly mechanical) constraint that must be overcome in order to allow physical interactions required for transcription initiation. Microscopy and chromosome conformation capture (3C)-based techniques have demonstrated that the problem is solved in part by the formation of DNA loops, which position distant DNA elements closer in proximity to one another (41, 78). Chromatin looping enables the chromosome to fold upon itself, bringing distal elements together in the 3D nuclear space (**Figure 1*a***). In this section, we focus on 3D repositioning of genetic elements by chromatin looping and its effects on gene

Figure 1

Diagram of a eukaryotic nucleus depicting different levels of organization. Different chromosomes are depicted by colored lines. Transcription is indicated by a bent black arrow. (*a*) At the submegabase level, initiation of transcription involves the physical interaction between gene regulatory elements, such as promoters (*pink boxes*) and their cognate enhancers (*yellow boxes*). Often times, these regions may be separated by many kilobases along the linear chromosome or even located on different chromosomes. Chromatin looping between these elements facilitates transcription. (*b*) Chromosomes are further segregated into topologically associated domains (TADs) containing many chromatin loops. TADs are separated by architectural proteins (APs) at their borders. Although TADs are separate domains, inter-TAD interactions also occur. The density of APs at TAD boundaries is related to the propensity of TADs to form inter-TAD interactions. (*c*) TADs are further organized at the entire chromosome level, forming chromosome territories (CTs). CTs are nonrandomly organized in the nucleus and occupy distinct regions. (*d*) The nucleus is further segmented into different compartments. Gene position toward the periphery of the nucleus generally results in transcriptional repression. Repression is facilitated by the association of genomic sequences [lamin-associated domains (LADs); *gray boxes*] with the inner nuclear lamina through lamin binding proteins. (*e*) Transcription neighborhoods (TNs) represent an additional nuclear compartment. TNs contain a collection of transcriptional machinery, such as RNA polymerase II, transcription factors, and splicing machinery, and provide an environment for the initiation, hyperactivation, and maintenance of transcription. Multiple genes and regulatory elements are found within TNs, permitting their transcription.

expression. In doing so, we present examples of loop formation, mechanistic details, and the effects of perturbation of normal loop formation.

Early chromatin loop structures were observed using R6K plasmid from *E. coli*. Mukherjee and colleagues found that circular structures were formed from digested DNA fragments in the presence of DNA ligase. The DNA fragments giving rise to such loops were products of a digestion-ligation reaction between two distant DNA sequences presumed to have been brought together in vivo by DNA binding proteins. Protein removal from either of the binding sites before



the digestion-ligation reactions resulted in failure to form DNA loops, demonstrating that DNA binding proteins were required for loop formation (67). Since this early observation, DNA loops have been further explored and characterized. The introduction and development of 3C techniques that involve formaldehyde cross-linking of DNA sequences positioned in close proximity in 3D space, followed by restriction enzyme digestion, ligation, and high-throughput sequencing of the ligation products, have been paramount in the confirmation and identification of DNA loops and interactions at high resolution on a genome-wide scale. It was through 3C and microscopy techniques that chromatin looping was implicated as an important feature of transcription. The latest iteration of 3C, now known as Hi-C, allows for the identification of all chromatin interactions at unprecedented resolution (59). Hi-C has unraveled many interesting properties of enhancer sequences. For example, enhancers can regulate multiple genes and can be located upstream or downstream of a target promoter, bringing up the notion that enhancer competition may exist between different genes. They also rarely interact with the nearest neighboring gene; one estimate reports that only approximately 7% of enhancers actually interact with the nearest promoter. Interactions between enhancers and promoters mostly correspond to increased gene activation, further demonstrating their involvement in transcription (78, 84). Reports vary on the total number of enhancer-promoter chromatin loops that may exist in the nucleus, from thousands to more than one million in human cells (49, 78). However, this vast range is perhaps due to differences in annotation methods and resolution (49, 78, 84). Regardless of the discrepancy in total number of chromatin loops that may exist, it is clear that juxtaposition of enhancers and promoters through chromatin looping occurs and may be a means to position genes and modulate expression. Thus, chromatin loops bridge the spatial gap between a gene and its distant regulatory elements.

The term looping is likely to conjure textbook images of long, floppy, and perfectly shaped ovals of chromatin that are extruded between two distant sequences coming together in space. However, it is worth noting that in most cases in which looping occurs, there is minimal cytological evidence that directly addresses the structure or topology of intervening sequences between interacting promoters and enhancers. The limitation of resolution of light microscopy is a primary reason for this problem. This is also demonstrated by the fact that DNA loop formation was first observed using electron microscopy (41). Because sequences separating enhancer-promoter pairs often contain other genes, these looped-out regions may also be participating in interloop interactions. Although speculative, it is possible that chromatin compaction exerted on looped regions may yield a myriad of topological structures while also providing necessary mechanical forces that facilitate the juxtaposition of distant enhancers and promoters. Thus, the topology of so-called loops and the forces that establish them have largely been ignored, as most studies have exclusively focused on enhancer-promoter interactions and downstream events such as transcription.

Temporal regulation of the transcriptional status of a gene may be enabled by the spatial separation of promoters and enhancers, keeping the gene in the off state. These DNA regulatory elements can function as scaffolds for assembly of DNA-binding proteins. Protein-protein interactions between complexes assembled on enhancers and promoters are thought to be required for transcription status to be in the on state. An example of this phenomenon and how long-distance interactions are facilitated by chromosome looping is seen during erythropoiesis. During mammalian erythropoiesis, terminal differentiation of proerythroblasts into intermediate and late erythroblasts results in the full activation of *α-globin* genes. In order for *α-globin* genes to be fully activated, the promoter must interact with its enhancer, located 40–60 Kb upstream. These elements are physically separated in proerythroblasts when the genes are not being expressed. During erythropoiesis, transcription factor (TF) binding is first observed at the distal enhancer element in uncommitted proerythroblasts, and only at much later stages of differentiation does TF binding occur at the promoter of the *α-globin* gene. Binding of TFs to both sites brings together the

promoter and enhancer and enables full activation of *α-globin* transcription (96). It is inferred from these observations that the long distance along the chromosome between the regulatory elements minimizes the likelihood that these elements would interact in 3D space because of precocious or unregulated events. Presumably, the temporally regulated assembly of TFs on the enhancer and then the promoter, as well as the distance on the linear chromosome between these elements, may conspire to modulate a more precise activation of this gene.

Chromatin insulators are DNA sequences bound by proteins that are important in mediating chromatin loops. Insulators are defined by their ability to block enhancer-promoter interactions and/or to function to prevent the spread of heterochromatin, thereby functioning as boundaries separating adjacent chromatin domains. Chromatin loops can be formed in part by the interactions between two insulator sites to enable transcription by bringing promoters and enhancers into close proximity (39). In vertebrates, the main insulator protein is CCCTC-binding factor (CTCF). Interestingly, Hi-C analysis shows that CTCF binds and anchors all chromatin loops in human and mouse cells, joining a promoter and enhancer at the base of each loop (78). Cohesin complex subunits show extensive genome-wide colocalization with CTCF in mammalian cells (73, 82, 91, 101), and cohesin-mediated looping is required for proper expression of the protocadherin (*PCDH*) genes in neurons. Studies using 3C and shRNA demonstrated that binding of CTCF and cohesin binding to both the promoter and enhancer is required for formation of chromatin loops, enabling interaction of the transcriptional elements and thus permitting proper transcription (44).

In addition to CTCF and cohesin, other insulator proteins have been identified in *Drosophila*, including chromosomal protein 190 (CP190), suppressor of hairy-wing [Su(Hw)], boundary element–associated factor of 32 kD (BEAF-32), and chromator (89). The effect of insulator proteins on gene expression was studied in the well-characterized *Drosophila* ecdysone system, where transcription can be induced by treatment with the hormone ecdysone (80). After ecdysone treatment, CP190 was recruited to the ecdysone-induced protein 75B (*Eip75B*) locus, where *Drosophila* CTCF (dCTCF) and Su(Hw) binding also increases. Binding of these factors resulted in the formation of chromatin loops between the *Eip75B* locus and other insulator sites, leading to increased transcription. In contrast, a genome-wide reduction in CP190 localization to insulator sites was observed when overall transcription was decreased after heat-shock treatment (103). This study demonstrates the role that insulator proteins play in mediating chromatin contacts, which can result in activation or inhibition of transcription in response to different environmental cues.

Inhibition of gene repositioning and chromatin loop formation can also modulate transcription. In *Drosophila*, the Snail repressor blocks chromatin looping by binding to the distal enhancers of target genes, inhibiting the binding of factors that promote loop formation (21). Interestingly, the Levine lab previously observed that Snail binding blocked initiation by RNA polymerase II (RNAPII), but not elongation of paused RNAPII, at developmental genes (10). In the scenario of Snail-repressed genes, RNAPII is present and may require the introduction of additional regulatory elements to begin elongation. Thus, inhibition of chromatin looping, or antilooping, may serve as a potent method to inhibit gene expression, while still maintaining the machinery necessary for transcription to be initiated at the appropriate time.

The above studies have demonstrated that dynamic loop formation and positioning of gene regulatory elements serve as powerful mechanisms for modulating gene expression. However, positioning of genes in which preexisting loops are the default state has also been observed. In human fibroblasts, which are sensitive to tumor necrosis factor alpha (TNFα) signaling, loops were found between TNFα responsive enhancers and their target promoters prior to signaling by TNFα. Treatment with TNFα resulted in upregulation of TNFα-regulated genes but did not noticeably alter the looping at these sites. Although dynamic looping can turn on a gene by repositioning it, preformed loops may be just as important in regulating expression of a different

subset of genes. Preexisting loops were also observed in additional human cell types under different stimuli, demonstrating that these loops represent a fundamental feature of gene positioning. Interactions between stimulus-responsive enhancers and promoters may enable a poised state that is ready to be activated after chemokine stimulation (49). These observations also underscore the fact that physical proximity of enhancers and promoters that results from chromatin looping is not necessarily sufficient for transcriptional activation.

Although bringing genes and their distal regulatory elements together to drive expression may be a primary role and result of chromatin looping, it is also important to note that chromatin looping can result in repression of transcription. An enhancer can affect multiple genes (84), raising the possibility of competition for enhancers among neighboring genes. That is, if enhancer AB can interact with either gene promoter A or gene promoter B, does interaction of promoter A with enhancer AB directly affect transcription of gene B by virtue of sequestration of enhancer AB? Indeed, enhancer competition is present during murine B-cell development. In mice, CTCF looping enables interaction of a specific region of the immunoglobulin heavy chain (*IgH*) gene cluster, termed the intergenic control region 1 (IGCR1),with its distal enhancers. Chromatin looping of IGCR1 with its enhancers effectively inhibits the transcription and rearrangement of different gene segments within the immunoglobulin gene cluster, ensuring normal B-cell development (43). This example illustrates the stoichiometric effect of limited enhancers and how enhancer competition may contribute to complex transcriptional networks.

Elegant forced chromatin looping experiments were performed to test whether looping is sufficient to initiate transcription. The Blobel lab found that zinc-finger targeting of the self-association (SA) domain of Ldb1, a transcription cofactor, to the promoter of the *β-globin* gene was sufficient for inducing looping and activation (29). Looping occurs through the interaction of Ldb1 molecules bound at the promoter and distal enhancer. Later investigations by this group found that forced looping through this mechanism was sufficient to reactivate developmentally silenced embryonic and fetal *β-globin* genes in later-stage adult erythroblasts. Reactivation is mediated by forced looping of the distal enhancer, known as the locus control region (LCR), to the promoter of the embryonic globin gene by tethering of the SA domain to this site. Similar results were seen in human erythroblasts. Induced looping of the LCR to the *γ-globin* promoter in adult human erythroblasts activated transcription of the gene, which is normally repressed during this stage. Interestingly, this resulted in the reduced expression of the *β-globin* gene, which normally interacts with the LCR (30). These forced looping experiments show that chromatin looping can drive increased transcription of one gene at the expense of the other as an effect of gene positioning. These observations are consistent with recent studies in *Drosophila* in which an enhancerless reporter gene could be activated by an enhancer on the homologous chromosome in *trans*. Transcriptional activity of the promoter in *cis* to the enhancer decreased when an identical promoter in *trans* was activated, demonstrating that promoters can compete for finite enhancer activity (2).

Presented above is a sampling of the many examples of how gene positioning can affect gene expression, specifically through chromatin looping. There is a clear involvement of insulator proteins and their associated factors, in addition to TFs and their cofactors, in this process. Chromatin looping provides a means by which gene expression can be modulated simply by altering the relative physical position of gene regulatory elements.

TOPOLOGICALLY ASSOCIATED DOMAINS

Chromatin loops are organized into TADs, larger submegabase-sized physical units along the chromosome. TADs are a collection of many chromatin loops, wherein the chromatin within the TAD interacts preferentially with other chromatin within the same TAD (**Figure 1*b***). In this

section, we focus on how gene localization within a TAD and how TAD repositioning of a gene can affect gene expression.

3C-based techniques such as Hi-C have enabled the identification of TADs. TADs have been identified in *Drosophila*, mice, and humans (5, 23, 88). Globular TAD-like structures have also been observed in yeast (65). TADs are characterized by their preference for intra-TAD, rather than inter-TAD, interactions. What defines TAD borders and the relative strength of these borders is not clearly understood; in *Drosophila*, TAD borders seem to be defined by sites that are enriched by numerous architectural proteins (APs), many of which are also insulator proteins (46, 58). In mice and humans, the average size of TADs is 1 Mb, whereas in *Drosophila*, TADs are found to be around 220 kb (23, 32, 89). Furthermore, although intra-TAD interactions are more frequent, inter-TAD interactions can also occur. It is thought that similar TADs may interact and segregate together in the nucleus to form active and inactive chromatin domains (59, 88). TADs enable the establishment and maintenance of a network of interactions between promoters and enhancers and play roles in the positioning of genes, thus influencing gene expression. TAD organization in general is stable and conserved among different cell types and species, indicating that TADs play an important role in mediating nuclear architecture function (32). This is thought to reflect the fact that all cell types must have transcription of housekeeping genes at similar levels. Although the organization may be stable, positioning of TADs remains dynamic and may provide a means by which TADs can influence transcription. Furthermore, those parts of the genome that are organized into cell type–specific TADs are thought to contain physical interactions that accommodate tissue-specific and/or inducible transcriptional units that are coordinately regulated. Here, we present examples of how TAD organization may be regulating expression.

The spatial grouping of many chromatin loops into TADs may provide an additional layer of control over transcription. Previously, it had been hypothesized that a function of TADs is to separate chromatin domains with differential activity during processes such as development (68). Highly expressed and coregulated genes cluster together into units along the linear chromosome, as exemplified by the *Hox* gene family (18, 57). Also, a correlation between the expression levels of genes and TADs was seen in murine X chromosome inactivation, with genes within the same TAD showing coordinated expression (68). Le Dily et al. (55) observed that in response to hormone-induced changes in gene expression, responsive genes were found to be clustered together in specific TADs. Strikingly, genes that were differentially affected after hormone stimulation were segregated into different TADs, suggesting functional significance behind this organization. Indeed, a function of TADs may include the grouping and compartmentalization of genes into functional units that are better suited to respond to certain cues.

Chromatin within TADs can also form inter-TAD interactions to mediate changes in expression. The homeotic *Hoxd* gene cluster forms inter-TAD contacts during mouse limb development. *Hoxd* genes are transcribed colinearly in different waves, such that *Hoxd1–9* are highly expressed early in development to form the arm and forearm, and later in development, *Hoxd10–13* are expressed to form digits. The switch in expression at the different phases is correlated with changes in TAD interactions of the cluster. In the early phase of development, *Hoxd9–11* genes interact with active enhancers located in a telomeric TAD, driving their expression in early limb cells. In the late phase, these *Hoxd* genes form centromeric TAD interactions in late limb cells to form the digits. The switch in TAD interaction is thought to allow the expression of different *Hoxd* genes during different parts of limb formation. Strikingly, it was found that an inversion that places the centromeric TAD further away abrogated the normal inter-TAD regulation of the *Hoxd13* gene by the centromeric TAD and resulted in ectopic interactions with the telomeric TAD. The altered shift from centromeric to telomeric inter-TAD regulation resulted in ectopic transcription and improper limb development (1). This study demonstrates how alterations in gene positioning

through inter-TAD interactions can alter gene expression. Furthermore, regulation of inter-TAD interactions provides precise control over the spatiotemporal activation of genes in processes such as development. However, it is nevertheless important to emphasize that in most cases it is not clear whether transcriptional activation drives inter-TAD interactions that are then required for maintenance of transcription or whether these cell type–specific inter-TAD interactions are a prerequisite for proper transcriptional activation.

TAD repositioning involves the interaction of insulator sequences and their associated APs. In human and mice, TAD boundaries are demarcated by CTCF and cohesin (32). In *Drosophila*, TAD boundaries contain APs, which include dCTCF, Su(Hw), GAGA factor (GAF), BEAF-32, zeste white 5 (Zw5), cohesins, and condensins. Interestingly, the relative occupancy of APs bound to these sites scales with the strength of the TAD borders, with stronger borders associated with TADs that prefer intra-TAD over inter-TAD interactions (95). AP positioning and occupancy are dynamic. An example of this is seen during the response to heat stress in *Drosophila*, where alterations in TAD border strength lead to repression of global transcription. After heat shock, APs are redistributed from TAD borders into the body of the TAD, reorganizing TADs and resulting in weaker borders. An effect of AP redistribution is the increase in long-range inter-TAD contacts and a decrease in short-range intra-TAD interactions. Relocalization of APs is necessary for mediating transcriptional repression. Newly formed contacts and APs function to recruit polycomb to enhancers and promoters, forming polycomb bodies that mediate transcriptional repression (58). The reorganization of TAD interactions through AP redistribution clearly demonstrates the plasticity of genome organization. The dynamic nature of the genome allows it to respond to environmental stressors that may require immediate changes in gene expression.

APs play essential roles in the organization of TADs, orchestration of TAD interactions, and, ultimately, gene regulation. Deletion of insulator sequences or depletion of CTCF/cohesin complexes was found to result in increased inter-TAD interactions at the expense of intra-TAD interactions, leading to altered transcription (68, 94, 106). The importance of APs in TAD regulation and gene expression can be seen after depletion of the cohesin and condensin II subunits Rad21 and Cap-H2. Specifically, depletion of Cap-H2 in *Drosophila* cells results in decreased TAD boundary strength and increased long-range interactions. The opposite effects are seen in Rad21 depletion (58). Interestingly, TAD organization can be further modulated through modification of APs. AP interaction is altered by ubiquitination, sumoylation, and poly(ADP-ribosyl)ation (16, 33, 70, 104). These observations demonstrate the many aspects of TAD organization that can be manipulated to control gene positioning and, therefore, gene expression.

It is clear that organization of genes into TADs impacts gene expression. This organization provides a mechanism by which the landscape of enhancer-promoter interactions can be established while maintaining an appropriate level of plasticity that allows cells to quickly respond to environmental cues. Insulators and APs are crucial both in the establishment and maintenance of TADs, as demonstrated by loss-of-function experiments and the resulting dramatic effects on TAD dynamics. Furthermore, TAD interactions play pivotal roles in many processes, such as development and stress responses, in which alterations in transcriptional profiles are required. Thus, the positioning of genes into functional compartments such as TADs has direct consequences on gene expression.

CHROMOSOME TERRITORIES

Each chromosome is made up of multiple TADs (**Figure 1*c***). One can imagine that inter-TAD interactions can exist between TADs on the same chromosome and between TADs from different chromosomes. One may also expect that TADs adjacent to one another on the linear chromosome

interact more frequently by chance than TADs more distantly spaced on a chromosome. The same logic dictates that interactions between TADs from different chromosomes would be the least frequent interactions to be observed if chromosomes were randomly organized in 3D space. However, we know from very early observations on chromosomes that their organization relative to one another as well as with respect to structures such as the nuclear envelope is not random. Specifically, in many cell types and in many eukaryotic species, each chromosome can occupy a distinct area of the interphase nucleus; these areas are called CTs (26). First observed in the 1900s, CTs have since been confirmed through a plethora of experiments, including whole-chromosome FISH (fluorescence in situ hybridization) and 3C-based techniques (11, 26, 59). CTs have been observed in yeast, *Drosophila*, and mammals (3, 8, 26, 85, 89). In this section, we examine the organization of genes within a CT and also between CTs. Positioning of genes within their CT and the positioning of CTs with respect to each other play influential roles in expression.

Gene positioning within CTs impacts expression. In humans, regions that contain transcriptionally active genes tend to localize toward the periphery of their CT. In contrast, noncoding regions reside toward the interior of their CT (35, 54, 61, 86). For example, in human female amniotic fluid cell nuclei, the *ANT2* (adenine translocase 2) gene is expressed on the active X chromosome (Xa) and repressed on the inactive X (Xi). FISH analysis has shown that actively transcribed *ANT2* is localized at the periphery of Xa but is buried within the CT of Xi (31). Localization of active genes at the CT periphery is thought to allow for mobility and positioning of genes and regulatory regions to areas accessible to the transcriptional machinery (9, 98). It has been observed in human and mouse cells that genomic regions with a higher density of active genes are more likely to reposition and loop out and away from the center of their CT. Furthermore, repositioning decreases when transcription is inhibited, implicating that active transcription may be required to maintain the looping out of a gene (60). It should be noted that although in general transcriptionally active genes tend to be at the edges of CTs, an exception to this generality is observed for those chromosomes that typically reside toward the periphery of the nucleus. As discussed below, genes apposed to the nuclear lamina tend to be transcriptionally silent; thus, CTs found at the nuclear periphery must somehow localize their silent genes toward the lamina while placing their active genes on the opposite face of the CT toward the interior of the nucleus.

Clearly, there exists a relationship between positioning of a gene in respect to its CT and gene expression. Many studies have shown that active genes tend to loop away from their CT when activated (38, 99, 102). However, it is unclear whether gene repositioning is a cause or consequence of changes in expression. During murine thymocyte development, *CD8* expression coincides with the locus being repositioned outside of its CT. Repositioning of *CD8* is accompanied by the clustering of its *cis*-regulatory elements (located within 40 kb of the *CD8* promoter), forming an active chromatin hub. In cells not expressing *CD8*, the gene remains within its CT and is not clustered with the regulatory elements (53). Chambeyron and colleagues noticed that upon *Hox* gene activation during human embryonic stem cell (ESC) differentiation, the *Hox* locus decondenses and loops out from its CT (19). Follow-up studies found that *Hox* gene repositioning to the outside of the CT also brings adjacent genes with it. Although *Hox* expression increases when looped out, the expression levels of the adjacent genes do not change. This observation argues that repositioning of a gene outside of its CT is not sufficient to alter transcription (66). Although looping away from the CT may not be sufficient to activate expression in all contexts, it is possible that the looping is necessary for activation. Looping may enable the locus to be accessible to TFs that may be unable to access the interior of the CT. Along these lines, gene repositioning may or may not be necessary for transcription initiation, but it may function to increase the efficiency of transcription elongation and/or reinitiation. Moving the gene to a transcriptionally conducive environment may help maintain high transcription levels regardless of the gene-specific

requirements for transcription initiation. For example, the *β-globin* locus is frequently found looped away from its CT in proerythroblasts, a stage that precedes its activation. This looped-out position is dependent on the presence of its distal enhancer elements (LCR) (77). Similarly, the *β-globin* locus in human hematopoiesis is also looped out prior to activation. Extrusion of the *β-globin* gene from its CT involves GATA-1 and GATA-1 coregulators such as FOG-1. Extrusion from the CT mediated by these factors is necessary for activation of transcription, as depletion of these components resulted in transcriptional repression and *β-globin* repositioning into its CT. Surprisingly, RNAi depletion of FOG1 after transcriptional activation did not result in changes in transcription or positioning of the locus back into its CT, demonstrating that after a gene is looped out of its CT to be activated, other mechanisms may take over to maintain active transcription (56). These findings support the idea that chromatin looped out from its CT enables a poised state for activation. Although relocating a gene outside of a CT may not be sufficient to activate it, relocation is still necessary for making the locus available for interaction with factors important for transcriptional activation.

Although CTs occupy distinct regions, there is a considerable amount of intermingling that occurs between them (12). Localization of active genes at the interface of different CTs and chromatin looping may enable loci from different CTs to interact, forming interchromosomal contacts and ultimately resulting in changes in expression. In naïve murine T-cells, the promoter region of interferon gamma (*IFNγ*), located on chromosome 10, interacts with the regulatory region of the T-helper cell 2 cytokine locus (TH2-LCR) on chromosome 11. This interchromosomal association is thought to poise the *IFNγ* for activation upon stimulation. Naïve T-cell differentiation into TH1 and TH2 cells results in the reduction of *IFNγ* promoter and TH2-LCR interchromosomal interactions and the increase of intrachromosomal interactions between *IFNγ* promoter and *IFNγ*-specific enhancers. Furthermore, the interchromosomal contacts formed before differentiation are important for downstream *IFNγ* expression, as ablation of these contacts inhibits transcription of *IFNγ* (90). CT intermingling has also been observed in instances of gene repression, as exemplified in mouse olfactory neurons. In this system, the monogenic and monoallelic expression of one of approximately 2,800 olfactory receptor (OR) genes are crucial. Silencing of OR genes located on different chromosomes is achieved by the clustering of the genes into heterochromatic foci. These interchromosomal interactions are mediated by the downregulation of lamin B receptor (LBR), as ectopic LBR expression results in the perturbation of OR aggregates. Furthermore, disruption of the interchromosomal interactions results in misregulation of both OR transcription and olfactory neuron specificity (25). Thus, these examples show CT intermingling as being important for the juxtaposition of regulatory elements residing on different chromosomes, and this intermingling of chromatin from different CTs is critical for proper expression of genes.

The requirement for intermingling and interchromosomal contacts in mediating gene positioning and expression was assessed by experiments in which these contacts were abrogated. In response to TNF signaling in humans, the *SAMD4A*, *TNFAIP2*, and *SLC6A5* genes come together to form multigene complexes where they are activated by the NF-κB TF. These multigene complexes contain intra- and interchromosomal contacts of regions from different CTs. The physical interaction of the genes is required for coexpression upon induction. Fanucchi and colleagues assessed the requirement of chromosomal interactions by cleverly disrupting the multigene complex through the use of transcription activator–like effector nucleases (TALENs), forming specific double-strand breaks (DSBs) at regions where the genes interact (34, 83). The authors observed that breakage of chromosomes affected the participation of the genes in the multigene complex. Interestingly, disruption of individual gene interactions also suppressed expression of the other genes in an ordered manner, with disruption of *SAMD4A* having the strongest effect, repressing both *TNFAIP2* and *SLC6A5* transcription. In contrast, disruption

of *TNFAIP2* affected only *SLC6A5*, and disruption of *SLC6A5* had no effect on transcription of the other two. Thus, *SAMD4A* appears to function as the most important participant in this multigene complex, leading the authors to propose that *SAMD4A* acts as the main scaffolding platform that brings additional required proteins to the multigene complex to mediate expression (34). These results demonstrate not only the requirement for the intermingling of chromosomes but also the role of the genes in the positioning and expression of other genes.

The nonrandom organization of chromosomes into CTs provides an additional mechanism to modulate gene positioning and gene expression. Positioning of genes at the periphery of CTs can increase both mobility and accessibility of the genes. Increased mobility allows for increased interaction with distal regulatory elements in a less confined space, while increased accessibility may allow the binding of factors required for activation or repression of the gene. These same principles apply to the interaction of genes in *trans* and from different CTs, and the studies mentioned above underscore the vital role that gene positioning and CT organization play on gene expression.

NUCLEAR COMPARTMENTS

The nucleus is a highly compartmentalized area in which gene positioning in specific compartments can have differential effects on expression. In this section, we focus on how radial positioning and gene positioning in nuclear compartments, specifically at the nuclear lamina and within transcription neighborhoods, can modulate gene expression. We use the term radial positioning to describe the 3D position of a gene within the nucleus, specifically, the gene's position in relation to the center or the periphery (nuclear envelope) of the nucleus.

Radial positioning of a gene has been associated with its transcriptional status. In general, the periphery of the nucleus is considered a repressive area, whereas the interior or center of the nucleus is associated with being transcriptionally permissive. One notable exception is that genes associated with nuclear pore complexes, also at the periphery of the nucleus, tend to be transcriptionally active (17, 50). CTs are nonrandomly oriented in the nucleus, with gene-dense chromosomes frequently found to be more centrally localized and gene-poor chromosomes residing nearer the periphery (27, 86). Specifically, the gene-dense human chromosome 19 is localized more internally as compared to the peripherally localized gene-poor chromosome 18 (92, 93). Elegant studies using an inducible transgene in human cells showed that upon transcriptional activation, the transgene relocalized from the periphery to the nuclear interior. This long-range movement of the transgene was found to be necessary for expression and is dependent on actin and myosin (22). Altogether, these studies suggest that the compartmentalization of the nucleus may play a functional role in gene expression.

Evidence supports the idea that the nuclear periphery may function as a repressive compartment (**Figure 1*d***). This nuclear compartment consists of the inner nuclear membrane, lamina, and resident proteins. Genes positioned at the nuclear periphery have been found to contain repressive histone marks. These repressive histone marks include methylated histones such as H3K27me3 and H3K9me2, and are associated with inactive or repressed genes (42, 76). A *Drosophila* genome-wide screen using DamID identified 500 genes that interact with the lamina. These genes were found to be within lamin-associated domains (LADs), are transcriptionally silent and late replicating, and lack active histone marks. Induction of gene transcription resulted in reduced lamin binding. Specifically, developmentally coregulated genes tend to cluster and associate with the lamina, and are released during differentiation when the genes are activated (76). Interestingly, the same DamID method in mouse ESCs found that the DNA-lamina (lamin B1) contacts drastically changed during each step of differentiation and lineage commitment. Gene dissociation from the lamina did not automatically result in gene activation. Genes that dissociated from the

lamina during the transition from ESCs to neural progenitor cells remained dissociated and were later activated during differentiation into astrocytes. These results suggest that release from the lamina transitioned the genes to an intermediate state, where the gene was poised for activation in the next stage of differentiation (75). These DamID experiments further illustrate the repressive properties of the nuclear periphery and how gene positioning in this zone may silence them.

Is repositioning of a gene at the nuclear periphery sufficient to silence that gene? Elegant studies that repositioned transgenes and endogenous genes to the nuclear periphery and lamina resulted in transcriptional repression of most genes (37, 79, 107). Interestingly, tethering of a transgene to the lamina functioned to also silence neighboring genes, further demonstrating the repressive nature of the nuclear periphery (37). Recruitment of lamina-associating DNA sequences (LASs) to the lamina involves binding of the zinc-finger TF cKrox to DNA and interaction with HDAC3. Knockdown of one of these components results in reduced DNA-lamina associations (107). More recently, the YY1 (yin-yang 1) zinc-finger TF was found to be involved in recruitment of DNA to the lamina and is sufficient for this process (45). Thus, positioning of a gene at the nuclear periphery, or more specifically, the nuclear lamina, has profound effects on its expression. The process is also dynamic, as association with the periphery can be reversed to allow for gene activation.

TRANSCRIPTION NEIGHBORHOODS

In addition to the nuclear periphery serving as a functional compartment, transcription has been observed to occur predominantly within compartments termed transcription factories or transcription neighborhoods (TNs) (**Figure 1*e***). These neighborhoods harbor a collection of transcription machinery including RNAPII, splicing machinery, TFs, and nuclear bodies. Recruitment of genes into TNs is thought to be conducive to gene expression, but exactly how such neighborhoods facilitate any part of transcription is not understood. In this section, we examine the effects of gene positioning into transcription neighborhoods and effects on expression.

TNs have been observed mostly in the space between CTs, termed the interchromatin domain (ICD). The ICD is believed to provide the optimal environment for transcription, lending the space needed for the colocalization of intermingling genes with their regulatory elements along with transcriptional machinery, while decreasing the chance of ectopic interactions with other DNA (47). This idea is consistent with the presence of phosphorylated and active RNAPII, TF IIIH, nuclear RNAs, and intermingling chromosomes from different CTs (12, 13, 97, 105). The formation of phosphorylated RNAPII clusters is indicative of ongoing transcription. Also, active genes have been found to loop out of their CTs to associate with RNAPII in the ICD (24, 62, 63). RNAPII is believed to be a rate-limiting component of transcription, as the total number of RNAPII units found in a cell is less than the total number of actively transcribed genes. This also suggests that multiple genes may associate in a single TN (48). Thus, the accumulation of transcription machinery into a distinct, accessible compartment may allow the activation of genes simply by gene repositioning into these neighborhoods, and genes may be brought there by the RNAPII itself. It is important to note that accessibility of the ICD is inferred by the lack of DNA staining when using light microscopy and chromatin density in electron micrographs. What the actual molecular constituents of the ICD are and how densely they are packed are unknown.

During B-cell development into plasma cells, immunoglobulin genes are highly transcribed, producing enormous amounts of antibody molecules. Immunostaining coupled with DNA/RNA FISH revealed that actively transcribing immunoglobulin genes (*IgK*, *IgH*, and *Igλ*) from three different chromosomes colocalize at TNs, as marked by RNAPII (74). Further ChIP-3C-seq experiments with antibodies against RNAPII demonstrated that enhancer elements act in *trans* (interchromosomally) at these TNs to promote both the positioning of the different genes and their

activation. These immunoglobulin TNs were also found positioned in the ICD. It was speculated that the existence of the TNs in this space allowed for rapid and efficient export of immunoglobulin transcripts out of the nucleus for translation, demonstrating additional functionality of TNs in the ICD (74).

Positioning into or out of TNs corresponds to the switch between genes being on or off (71). Moreover, induction of the *Myc* proto-oncogene in mouse B lymphocytes resulted in the repositioning into a preassembled TN preoccupied by the highly transcribed *Igh* gene located on a different chromosome. It has been speculated that the preassembled *Igh* TN contains the necessary components needed for rapid and efficient activation of *Myc* upon repositioning of the *Myc* gene into the TN (72). TNs have also been found to exist in the absence of transcription. Active and phosphorylated RNAPII clusters were still observed after the inhibition of transcription with drug treatment or heat shock in mammalian cells, suggesting a role for TNs independent of transcriptional activity. Also, inhibition of transcription resulted in the disassociation of active genes from RNAPII in TNs (64). Along these lines, Ferrai and colleagues identified two types of TNs: poised transcription factories and active factories (36). These TNs differ in that the poised transcription factories contain RNAPII phosphorylated on Ser5 but not Ser2 of the C-terminal domain, whereas active factories are phosphorylated at both residues. They found that the inducible *uPA* gene associates with poised factories prior to induction and is located within a CT, and repositions out of the CT after activation and associates with active factories (36). Therefore, one possibility is that the TNs may provide the necessary environment that anticipates transcriptional activation and thereby ensures efficient temporal activation of genes.

Although TNs ubiquitously contain RNAPII, specialized TNs have also been identified that contain unique TFs that enable specificity. In mouse erythroid cells, specific globin genes are regulated by Klf1 (Kruppel-like factor 1), a TF necessary for erythropoiesis (6). When active, Klf1-regulated genes on different chromosomes were found to colocalize at Klf1-containing TNs in a Klf1-dependent manner. The authors noted that many of the genes repositioned to these TNs were not dependent on Klf1 for expression but that increased and prolonged expression did require Klf1. Strikingly, ectopic Klf1-regulated transgenes were able to be recruited to endogenous Klf1 TNs that contained endogenous Klf1-regulated genes. A function of specialized TNs may be to provide a synergistic effect on expression by clustering specific TFs and regulatory elements, increasing their local concentration, and perhaps driving transcription initiation by mass action (87).

Additional components of TNs include nuclear speckles, also known as splicing speckles, which are involved in RNA splicing. Active murine globin genes from different chromosomes are found outside of their CTs and are associated with nuclear speckles involved in RNA processing (14, 15). Coordinately expressed genes in human erythroid cells were found to be positioned in close spatial proximity with each other, active RNAPII, and SC35 splicing speckles after induction. Disassembly of SC35 speckles through overexpression of the serine-arginine protein kinase Cdc2-like kinase (Clk2) resulted in both the disassociation of the normally clustered coregulated genes and the decrease in their transcription (81). In addition to providing an optimal compartment for gene expression, the proteins inside TNs may serve as maintenance factors that facilitate persistent localization of genes within specific TNs.

As evidenced by these studies, compartmentalization of the nucleus into transcription neighborhoods has dramatic effects on gene positioning and expression. The grouping of transcriptional machinery, TFs, and regulatory elements not only provides transcriptionally conducive areas in which all the primary players are present but also functions to recruit coregulated genes. As a result, recruited genes are both activated and hyperactivated in a stimulus response–specific manner. Thus, gene localization in respect to TNs demonstrates that gene positioning has direct effects on gene expression.

DIRECTED MOVEMENT OF CHROMATIN

In many of the examples discussed above, the change in physical positions of genes and even very large regions of the genome occurs in interphase cells. That is to say that the relative repositioning of genes in interphase is not necessarily a consequence of how chromosomes happened to segregate and organize in the previous mitosis. Indeed, in many cases gene repositioning occurs in terminally differentiated cells that have withdrawn from the cell cycle. This means that one or more force-generating mechanisms must exist within the interphase nucleus in order to physically move chromatin. Because the repositioning of specific genes for the purpose of activating transcription, for example in and out of a specific TN, cannot be random, it is inferred that molecular machines must exert directed force and perform work on the chromatin fiber. With the exception of a few studies in which the activity of actin and myosin was demonstrated to drive such movements of chromatin inside the nucleus (22), there are no clear candidates for what these molecular machines might be. Although both actin and myosin are now thought to normally reside in the nucleus (40, 51, 69), filamentous actin (F-actin) is typically not found in the nucleus (4, 40, 100). Therefore, the absence of nuclear F-actin makes it unlikely that conventional actin-myosin force-generating filaments could explain many of the gene repositioning events that have been observed. One possibility is that the movement of RNA polymerase molecules could provide the force to move chromatin, but this seems an unlikely general principle for directed movement of chromatin because clear examples exist where repositioning of a gene is not dependent on transcription and may even precede transcription. Thus, it remains a mystery as to what the molecular machines that drive gene movements to specific places inside the eukaryotic nucleus might be. Lastly, it is difficult to imagine how any region of the genome can be physically moved without causing some level of tension on adjacent chromatin sections. Although some chromatin segments may freely move and reposition, other segments may be tethered or compacted. It is interesting to speculate that the relative elasticity and compaction of chromatin may also be important parameters that must be regulated during active gene repositioning (**Figure 2**). Such dynamic regulation of elasticity and compaction may be required not only to permit movement of genes but may also be equally important in retrieving genes back into their default positions.

DISCUSSION

The studies described above demonstrate the direct role that gene positioning plays in the regulation of gene expression and how global chromosome folding impinges on local regulation. Although this is only a very small selection of many studies, it is evident that different levels of genome and nuclear organization do indeed contribute to spatiotemporal control of transcription. The maintenance of proper gene expression is of the utmost importance for the proper development and viability of an organism, underscoring the importance of proper gene positioning within the organizational states. The requirement for chromatin looping to mediate interactions between enhancers and promoters that are physically separated allows the genes to be selectively expressed or silenced simply by directed movement. The requirement for multiple factors to enable looping further decreases the chances of ectopic interactions and misexpression of genes. Furthermore, the organization of chromatin loops into TADs enables the coregulation of multiple genes. Further sequestration of chromosomes into distinct territories allows for spatial regulation of genes. Lastly, compartmentalization of the nucleus provides a method to quickly and efficiently repress or activate genes. Thus, gene positioning in respect to each level of organization depicted above presents a multitude of avenues by which transcription can be modulated.

Much progress has been made toward elucidating positioning effects on expression, but many open questions remain to be answered. Although numerous proteins involved in chromatin

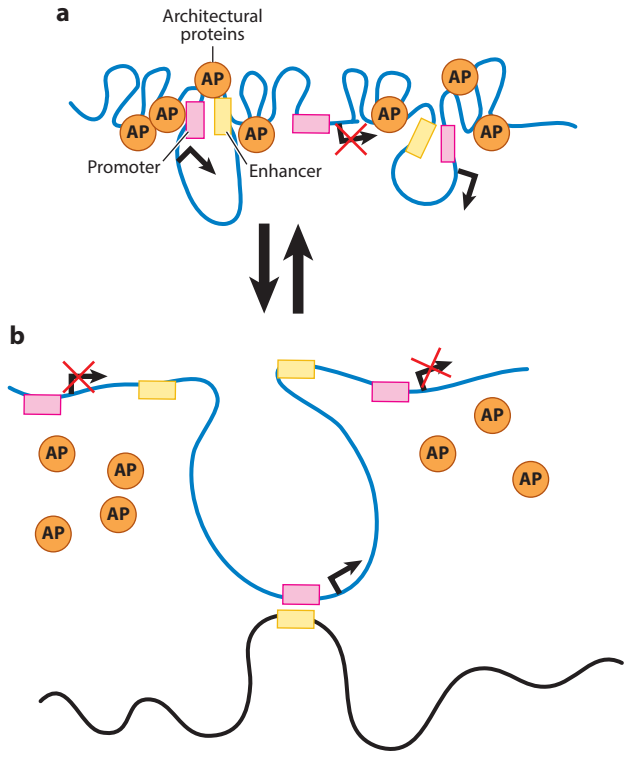


Figure 2

Directed chromatin movement. Speculative mechanism of directed movement of specific chromatin regions. (*a*) The local compaction of chromatin, possibly by architectural proteins (APs) such as CTCF (CCCTC-binding factor), cohesin, or condensin (*orange circles*), permits interaction between specific promoters (*pink rectangles*) and enhancers (*yellow rectangles*), enabling transcription of specific genes. (*b*) The looping out of specific genomic regions such as promoters to form long-range interactions with distal enhancers requires adequate chromatin slack. This slack may be provided by the decompaction of neighboring regions. Consequently, previously interacting enhancer/promoter loops may need to be sacrificed to permit new loop interactions. Decompaction may be mediated by the loss of factors previously compacting the local chromatin.

looping have been identified, such as CTCF and cohesins, additional components underlying the mechanisms behind genome organization and directed chromatin movement remain unknown. By identifying additional components that function in directing genome organization, a more complete understanding of organizational effects on gene expression can be achieved. Another interesting but unexplored aspect is the consequence of chromatin positioning for the rest of the genome. When a region of the genome is looped out, what happens to the rest of the genome not actively participating in this event? One possibility is that when a region is looped out to form distal interactions, the adjacent genomic regions must provide the slack needed for this movement (**Figure 2**). In addition to serving as a mechanism to promote transcription, gene looping may inhibit other chromatin loops and transcription. This may be an additional function behind the organization of coregulated genes into domains. One could imagine that when these coregulated genes need to be expressed and looped out, the slack needed to loop out is afforded by regions of the genome that are not coexpressed. In addition, an issue that requires further investigation is the direct effect of genome organization on cellular processes. The recent advent of the CRISPR/Cas9 gene-editing technique provides a means to further investigate genome organizational effects. By

using CRISPR/Cas9, units of genome organization such as TADs can be manipulated to test direct effects on cellular processes that include gene expression. For example, AP binding can be manipulated by way of introducing or deleting genetic motifs underlying their binding. A result of this may be the alteration of chromatin looping and also TAD organization. Although studies that have depleted APs have provided essential information about organizational effects on gene expression, the weakness in these methods is that the APs may be functioning in mechanisms independent of genome organization. Thus, gene-editing tools aimed at manipulating TAD organization would provide less ambiguous conclusions.

Although many questions remain to be answered and many ideas remain to be explored, there is no disputing the essential role that gene positioning plays in gene regulation. The future seems bright, as the further development of 3C, super-high-resolution microscopy, and genome-editing techniques will provide the necessary tools to paint a clearer picture.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We would like to thank Heather Wallace for critical reading of the manuscript and all members of the Bosco lab for stimulating discussions and suggestions. Research in the Bosco laboratory is supported by NIH R01 GM069462 and American Federation of Aging Research Foundation Glenn/AFAR BIG Award.

LITERATURE CITED

1. Andrey G, Montavon T, Mascrez B, Gonzalez F, Noordermeer D, et al. 2013. A switch between topological domains underlies *HoxD* genes collinearity in mouse limbs. *Science* 340:1234167
2. Bateman JR, Johnson JE, Locke MN. 2012. Comparing enhancer action in *cis* and in *trans*. *Genetics* 191:1143–55
3. Berger AB, Cabal GG, Fabre E, Duong T, Buc H, et al. 2008. High-resolution statistical mapping reveals gene territories in live yeast. *Nat. Methods* 5:1031–37
4. Bettinger BT, Gilbert DM, Amberg DC. 2004. Actin up in the nucleus. *Nat. Rev. Mol. Cell Biol.* 5:410–15
5. Bickmore WA, van Steensel B. 2013. Genome architecture: domain organization of interphase chromosomes. *Cell* 152:1270–84
6. Bieker JJ. 2001. Krüppel-like factors: three fingers in many pies. *J. Biol. Chem.* 276(37):34355–58
7. Blackwood EM, Kadonaga JT. 1998. Going the distance: a current view of enhancer action. *Science* 281:60–63
8. Bolzer A, Kreth G, Solovei I, Koehler D, Saracoglu K, et al. 2005. Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. *PLOS Biol.* 3:e157
9. Bornfleth H, Edelmann P, Zink D, Cremer T, Cremer C. 1999. Quantitative motion analysis of subchromosomal foci in living cells using four-dimensional microscopy. *Biophys. J.* 77:2871–86
10. Bothma JP, Magliocco J, Levine M. 2011. The snail repressor inhibits release, not elongation, of paused Pol II in the *Drosophila* embryo. *Curr. Biol.* 21:1571–77
11. Boveri T. 1909. *Die blastomerenkerne von* Ascaris megalocephala *und die theorie der chromosomenindividualität*. *Arch Zellforsch.* 1909:181–268
12. Branco MR, Pombo A. 2006. Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. *PLOS Biol.* 4:e138

13. Bridger JM, Kalla C, Wodrich H, Weitz S, King JA, et al. 2005. Nuclear RNAs confined to a reticular compartment between chromosome territories. *Exp. Cell Res.* 302:180–93
14. Brown JM, Green J, das Neves RP, Wallace HA, Smith AJ, et al. 2008. Association between active genes occurs at nuclear speckles and is modulated by chromatin environment. *J. Cell Biol.* 182:1083–97
15. Brown JM, Leach J, Reittie JE, Atzberger A, Lee-Prudhoe J, et al. 2006. Coregulated human globin genes are frequently in spatial proximity when active. *J. Cell Biol.* 172:177–87
16. Capelson M, Corces VG. 2006. SUMO conjugation attenuates the activity of the gypsy chromatin insulator. *EMBO J.* 25:1906–14
17. Capelson M, Liang Y, Schulte R, Mair W, Wagner U, Hetzer MW. 2010. Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. *Cell* 140:372–83
18. Caron H, van Schaik B, van der Mee M, Baas F, Riggins G, et al. 2001. The human transcriptome map: clustering of highly expressed genes in chromosomal domains. *Science* 291:1289–92
19. Chambeyron S, Bickmore WA. 2004. Chromatin decondensation and nuclear reorganization of the *HoxB* locus upon induction of transcription. *Genes Dev.* 18:1119–30
20. Chepelev I, Wei G, Wangsa D, Tang Q, Zhao K. 2012. Characterization of genome-wide enhancer-promoter interactions reveals co-expression of interacting genes and modes of higher order chromatin organization. *Cell Res.* 22:490–503
21. Chopra VS, Kong N, Levine M. 2012. Transcriptional repression via antilooping in the *Drosophila* embryo. *PNAS* 109:9460–64
22. Chuang CH, Carpenter AE, Fuchsova B, Johnson T, de Lanerolle P, Belmont AS. 2006. Long-range directional movement of an interphase chromosome site. *Curr. Biol.* 16:825–31
23. Ciabrelli F, Cavalli G. 2015. Chromatin-driven behavior of topologically associating domains. *J. Mol. Biol.* 427:608–25
24. Cisse II, Izeddin I, Causse SZ, Boudarene L, Senecal A, et al. 2013. Real-time dynamics of RNA polymerase II clustering in live human cells. *Science* 341:664–67
25. Clowney EJ, LeGros MA, Mosley CP, Clowney FG, Markenskoff-Papadimitriou EC, et al. 2012. Nuclear aggregation of olfactory receptor genes governs their monogenic expression. *Cell* 151:724–37
26. Cremer T, Cremer M. 2010. Chromosome territories. *Cold Spring Harb. Perspect. Biol.* 2:a003889
27. Croft JA, Bridger JM, Boyle S, Perry P, Teague P, Bickmore WA. 1999. Differences in the localization and morphology of chromosomes in the human nucleus. *J. Cell Biol.* 145:1119–31
28. Cullen KE, Kladde MP, Seyfred MA. 1993. Interaction between transcription regulatory regions of prolactin chromatin. *Science* 261:203–6
29. Deng W, Lee J, Wang H, Miller J, Reik A, et al. 2012. Controlling long-range genomic interactions at a native locus by targeted tethering of a looping factor. *Cell* 149:1233–44
30. Deng W, Rupon JW, Krivega I, Breda L, Motta I, et al. 2014. Reactivation of developmentally silenced globin genes by forced chromatin looping. *Cell* 158:849–60
31. Dietzel S, Schiebel K, Little G, Edelmann P, Rappold GA, et al. 1999. The 3D positioning of ANT2 and ANT3 genes within female X chromosome territories correlates with gene activity. *Exp. Cell Res.* 252:363–75
32. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, et al. 2012. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485:376–80
33. El-Kady A, Klenova E. 2005. Regulation of the transcription factor, CTCF, by phosphorylation with protein kinase CK2. *FEBS Lett.* 579:1424–34
34. Fanucchi S, Shibayama Y, Burd S, Weinberg MS, Mhlanga MM. 2013. Chromosomal contact permits transcription between coregulated genes. *Cell* 155:606–20
35. Federico C, Cantarella CD, Di Mare P, Tosi S, Saccone S. 2008. The radial arrangement of the human chromosome 7 in the lymphocyte cell nucleus is associated with chromosomal band gene density. *Chromosoma* 117:399–410
36. Ferrai C, Xie SQ, Luraghi P, Munari D, Ramirez F, et al. 2010. Poised transcription factories prime silent uPA gene prior to activation. *PLOS Biol.* 8:e1000270
37. Finlan LE, Sproul D, Thomson I, Boyle S, Kerr E, et al. 2008. Recruitment to the nuclear periphery can alter expression of genes in human cells. *PLOS Genet.* 4:e1000039

38. Galiova G, Bartova E, Kozubek S. 2004. Nuclear topography of beta-like globin gene cluster in IL-3-stimulated human leukemic K-562 cells. *Blood Cells Mol. Dis.* 33:4–14
39. Gaszner M, Felsenfeld G. 2006. Insulators: exploiting transcriptional and epigenetic mechanisms. *Nat. Rev. Genet.* 7:703–13
40. Gonsior SM, Platz S, Buchmeier S, Scheer U, Jockusch BM, Hinssen H. 1999. Conformational difference between nuclear and cytoplasmic actin as detected by a monoclonal antibody. *J. Cell Sci.* 112(Pt. 6):797–809
41. Griffith J, Hochschild A, Ptashne M. 1986. DNA loops induced by cooperative binding of lambda repressor. *Nature* 322:750–52
42. Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, et al. 2008. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature* 453:948–51
43. Guo C, Yoon HS, Franklin A, Jain S, Ebert A, et al. 2011. CTCF-binding elements mediate control of V(D)J recombination. *Nature* 477:424–30
44. Guo Y, Monahan K, Wu H, Gertz J, Varley KE, et al. 2012. CTCF/cohesin-mediated DNA looping is required for protocadherin alpha promoter choice. *PNAS* 109:21081–86
45. Harr JC, Luperchio TR, Wong X, Cohen E, Wheelan SJ, Reddy KL. 2015. Directed targeting of chromatin to the nuclear lamina is mediated by chromatin state and A-type lamins. *J. Cell Biol.* 208:33–52
46. Hou C, Li L, Qin ZS, Corces VG. 2012. Gene density, transcription, and insulators contribute to the partition of the *Drosophila* genome into physical domains. *Mol. Cell* 48:471–84
47. Iborra FJ, Pombo A, Jackson DA, Cook PR. 1996. Active RNA polymerases are localized within discrete transcription "factories" in human nuclei. *J. Cell. Sci.* 109:1427–36
48. Jackson DA, Iborra FJ, Manders EM, Cook PR. 1998. Numbers and organization of RNA polymerases, nascent transcripts, and transcription units in HeLa nuclei. *Mol. Biol. Cell* 9:1523–36
49. Jin F, Li Y, Dixon JR, Selvaraj S, Ye Z, et al. 2013. A high-resolution map of the three-dimensional chromatin interactome in human cells. *Nature* 503:290–94
50. Kalverda B, Pickersgill H, Shloma VV, Fornerod M. 2010. Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. *Cell* 140:360–71
51. Kapoor P, Shen X. 2014. Mechanisms of nuclear actin in chromatin-remodeling complexes. *Trends Cell Biol.* 24:238–46
52. Kleinjan DA, van Heyningen V. 2005. Long-range control of gene expression: emerging mechanisms and disruption in disease. *Am. J. Hum. Genet.* 76:8–32
53. Ktistaki E, Garefalaki A, Williams A, Andrews SR, Bell DM, et al. 2010. CD8 locus nuclear dynamics during thymocyte development. *J. Immunol.* 184:5686–95
54. Kurz A, Lampel S, Nickolenko JE, Bradl J, Benner A, et al. 1996. Active and inactive genes localize preferentially in the periphery of chromosome territories. *J. Cell Biol.* 135:1195–205
55. Le Dily F, Bau D, Pohl A, Vicent GP, Serra F, et al. 2014. Distinct structural transitions of chromatin topological domains correlate with coordinated hormone-induced gene regulation. *Genes Dev.* 28:2151–62
56. Lee HY, Johnson KD, Boyer ME, Bresnick EH. 2011. Relocalizing genetic loci into specific subnuclear neighborhoods. *J. Biol. Chem.* 286:18834–44
57. Lewis EB. 1978. A gene complex controlling segmentation in *Drosophila*. *Nature* 276:565–70
58. Li L, Lyu X, Hou C, Takenaka N, Nguyen HQ, et al. 2015. Widespread rearrangement of 3D chromatin organization underlies polycomb-mediated stress-induced silencing. *Mol. Cell* 58:216–31
59. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, et al. 2009. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326:289–93
60. Mahy NL, Perry PE, Bickmore WA. 2002. Gene density and transcription influence the localization of chromatin outside of chromosome territories detectable by FISH. *J. Cell Biol.* 159:753–63
61. Mahy NL, Perry PE, Gilchrist S, Baldock RA, Bickmore WA. 2002. Spatial organization of active and inactive genes and noncoding DNA within chromosome territories. *J. Cell Biol.* 157:579–89
62. Malyavantham KS, Bhattacharya S, Alonso WD, Acharya R, Berezney R. 2008. Spatio-temporal dynamics of replication and transcription sites in the mammalian cell nucleus. *Chromosoma* 117:553–67

63. Malyavantham KS, Bhattacharya S, Barbeitos M, Mukherjee L, Xu J, et al. 2008. Identifying functional neighborhoods within the cell nucleus: proximity analysis of early S-phase replicating chromatin domains to sites of transcription, RNA polymerase II, HP1γ, matrin 3 and SAF-A. *J. Cell Biochem.* 105:391–403
64. Mitchell JA, Fraser P. 2008. Transcription factories are nuclear subcompartments that remain in the absence of transcription. *Genes Dev.* 22:20–25
65. Mizuguchi T, Fudenberg G, Mehta S, Belton JM, Taneja N, et al. 2014. Cohesin-dependent globules and heterochromatin shape 3D genome architecture in *S. pombe*. *Nature* 516:432–35
66. Morey C, Kress C, Bickmore WA. 2009. Lack of bystander activation shows that localization exterior to chromosome territories is not sufficient to up-regulate gene expression. *Genome Res.* 19:1184–94
67. Mukherjee S, Erickson H, Bastia D. 1988. Detection of DNA looping due to simultaneous interaction of a DNA-binding protein with two spatially separated binding sites on DNA. *PNAS* 85:6287–91
68. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, et al. 2012. Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* 485:381–85
69. Nowak G, Pestic-Dragovich L, Hozak P, Philimonenko A, Simerly C, et al. 1997. Evidence for the presence of myosin I in the nucleus. *J. Biol. Chem.* 272:17176–81
70. Ong CT, Van Bortle K, Ramos E, Corces VG. 2013. Poly(ADP-ribosyl)ation regulates insulator function and intrachromosomal interactions in *Drosophila*. *Cell* 155:148–59
71. Osborne CS, Chakalova L, Brown KE, Carter D, Horton A, et al. 2004. Active genes dynamically colocalize to shared sites of ongoing transcription. *Nat. Genet.* 36:1065–71
72. Osborne CS, Chakalova L, Mitchell JA, Horton A, Wood AL, et al. 2007. Myc dynamically and preferentially relocates to a transcription factory occupied by Igh. *PLOS Biol.* 5:e192
73. Parelho V, Hadjur S, Spivakov M, Leleu M, Sauer S, et al. 2008. Cohesins functionally associate with CTCF on mammalian chromosome arms. *Cell* 132:422–33
74. Park SK, Xiang Y, Feng X, Garrard WT. 2014. Pronounced cohabitation of active immunoglobulin genes from three different chromosomes in transcription factories during maximal antibody synthesis. *Genes Dev.* 28:1159–64
75. Peric-Hupkes D, Meuleman W, Pagie L, Bruggeman SW, Solovei I, et al. 2010. Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. *Mol. Cell* 38:603–13
76. Pickersgill H, Kalverda B, de Wit E, Talhout W, Fornerod M, van Steensel B. 2006. Characterization of the *Drosophila melanogaster* genome at the nuclear lamina. *Nat. Genet.* 38:1005–14
77. Ragoczy T, Telling A, Sawado T, Groudine M, Kosak ST. 2003. A genetic analysis of chromosome territory looping: diverse roles for distal regulatory elements. *Chromosome Res.* 11:513–25
78. Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, et al. 2014. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 159:1665–80
79. Reddy KL, Zullo JM, Bertolino E, Singh H. 2008. Transcriptional repression mediated by repositioning of genes to the nuclear lamina. *Nature* 452:243–47
80. Riddiford LM, Cherbas P, Truman JW. 2000. Ecdysone receptors and their biological actions. *Vitam. Horm.* 60:1–73
81. Rieder D, Ploner C, Krogsdam AM, Stocker G, Fischer M, et al. 2014. Co-expressed genes prepositioned in spatial neighborhoods stochastically associate with SC35 speckles and RNA polymerase II factories. *Cell Mol. Life Sci.* 71:1741–59
82. Rubio ED, Reiss DJ, Welcsh PL, Disteche CM, Filippova GN, et al. 2008. CTCF physically links cohesin to chromatin. *PNAS* 105:8309–14
83. Sanjana NE, Cong L, Zhou Y, Cunniff M, Feng G, et al. 2012. A transcription activator-like effector (TALE) toolbox for genome engineering. *Nat. Protoc.* 7(1):171–92
84. Sanyal A, Lajoie BR, Jain G, Dekker J. 2012. The long-range interaction landscape of gene promoters. *Nature* 489:109–13
85. Schardin M, Cremer T, Hager HD, Lang M. 1985. Specific staining of human chromosomes in Chinese hamster × man hybrid cell lines demonstrates interphase chromosome territories. *Hum. Genet.* 71:281–87
86. Scheuermann MO, Tajbakhsh J, Kurz A, Saracoglu K, Eils R, Lichter P. 2004. Topology of genes and nontranscribed sequences in human interphase nuclei. *Exp. Cell Res.* 301:266–79

87. Schoenfelder S, Sexton T, Chakalova L, Cope NF, Horton A, et al. 2010. Preferential associations between co-regulated genes reveal a transcriptional interactome in erythroid cells. *Nat. Genet.* 42:53–61
88. Sexton T, Cavalli G. 2015. The role of chromosome domains in shaping the functional genome. *Cell* 160:1049–59
89. Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, et al. 2012. Three-dimensional folding and functional organization principles of the *Drosophila* genome. *Cell* 148:458–72
90. Spilianakis CG, Lalioti MD, Town T, Lee GR, Flavell RA. 2005. Interchromosomal associations between alternatively expressed loci. *Nature* 435:637–45
91. Stedman W, Kang H, Lin S, Kissil JL, Bartolomei MS, Lieberman PM. 2008. Cohesins localize with CTCF at the KSHV latency control region and at cellular c-myc and H19/Igf2 insulators. *EMBO J.* 27:654–66
92. Tanabe H, Habermann FA, Solovei I, Cremer M, Cremer T. 2002. Non-random radial arrangements of interphase chromosome territories: evolutionary considerations and functional implications. *Mutat. Res.* 504:37–45
93. Tanabe H, Muller S, Neusser M, von Hase J, Calcagno E, et al. 2002. Evolutionary conservation of chromosome territory arrangements in cell nuclei from higher primates. *PNAS* 99:4424–29
94. Tark-Dame M, Jerabek H, Manders EM, Heermann DW, van Driel R. 2014. Depletion of the chromatin looping proteins CTCF and cohesin causes chromatin compaction: insight into chromatin folding by polymer modelling. *PLOS Comput. Biol.* 10:e1003877
95. Van Bortle K, Nichols MH, Li L, Ong CT, Takenaka N, et al. 2014. Insulator function and topological domain border strength scale with architectural protein occupancy. *Genome Biol.* 15:R82
96. Vernimmen D, De Gobbi M, Sloane-Stanley JA, Wood WG, Higgs DR. 2007. Long-range chromosomal interactions regulate the timing of the transition between poised and active gene expression. *EMBO J.* 26:2041–51
97. Verschure PJ, Van Der Kraan I, Enserink JM, Mone MJ, Manders EM, Van Driel R. 2002. Large-scale chromatin organization and the localization of proteins involved in gene expression in human cells. *J. Histochem. Cytochem.* 50:1303–12
98. Verschure PJ, van Der Kraan I, Manders EM, van Driel R. 1999. Spatial relationship between transcription sites and chromosome territories. *J. Cell Biol.* 147:13–24
99. Volpi EV, Chevret E, Jones T, Vatcheva R, Williamson J, et al. 2000. Large-scale chromatin organization of the major histocompatibility complex and other regions of human chromosome 6 and its response to interferon in interphase nuclei. *J. Cell Sci.* 113(Pt. 9):1565–76
100. Wasser M, Chia W. 2000. The EAST protein of *Drosophila* controls an expandable nuclear endoskeleton. *Nat. Cell Biol.* 2:268–75
101. Wendt KS, Yoshida K, Itoh T, Bando M, Koch B, et al. 2008. Cohesin mediates transcriptional insulation by CCCTC-binding factor. *Nature* 451:796–801
102. Williams RR, Broad S, Sheer D, Ragoussis J. 2002. Subchromosomal positioning of the epidermal differentiation complex (EDC) in keratinocyte and lymphoblast interphase nuclei. *Exp. Cell Res.* 272:163–75
103. Wood AM, Van Bortle K, Ramos E, Takenaka N, Rohrbaugh M, et al. 2011. Regulation of chromatin organization and inducible gene expression by a *Drosophila* insulator. *Mol. Cell* 44:29–38
104. Yu W, Ginjala V, Pant V, Chernukhin I, Whitehead J, et al. 2004. Poly(ADP-ribosyl)ation regulates CTCF-dependent chromatin insulation. *Nat. Genet.* 36:1105–10
105. Zirbel RM, Mathieu UR, Kurz A, Cremer T, Lichter P. 1993. Evidence for a nuclear compartment of transcription and splicing located at chromosome domain boundaries. *Chromosome Res.* 1:93–106
106. Zuin J, Dixon JR, van der Reijden MI, Ye Z, Kolovos P, et al. 2014. Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. *PNAS* 111:996–1001
107. Zullo JM, Demarco IA, Pique-Regi R, Gaffney DJ, Epstein CB, et al. 2012. DNA sequence-dependent compartmentalization and silencing of chromatin at the nuclear lamina. *Cell* 149:1474–87