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Gene positioning and expression

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Within the nucleus, the genome is spatially organized. Individual chromosomes are non-randomly positioned with respect to each other and with respect to nuclear landmarks [1,2]. Furthermore, the position of individual genes can reflect their expression. Here we discuss two well-characterized examples of gene relocalization associated with transcriptional activation: 1) developmentally regulated genes that move from the nuclear periphery to transcription factories in the nucleoplasm upon induction and 2) genes that are targeted from the nucleoplasm to the nuclear periphery, through interactions with the nuclear pore complex (NPC), upon activation. Finally, we speculate as to the mechanistic and functional commonalities of these phenomena.

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Movement of developmentally regulated genes during differentiation

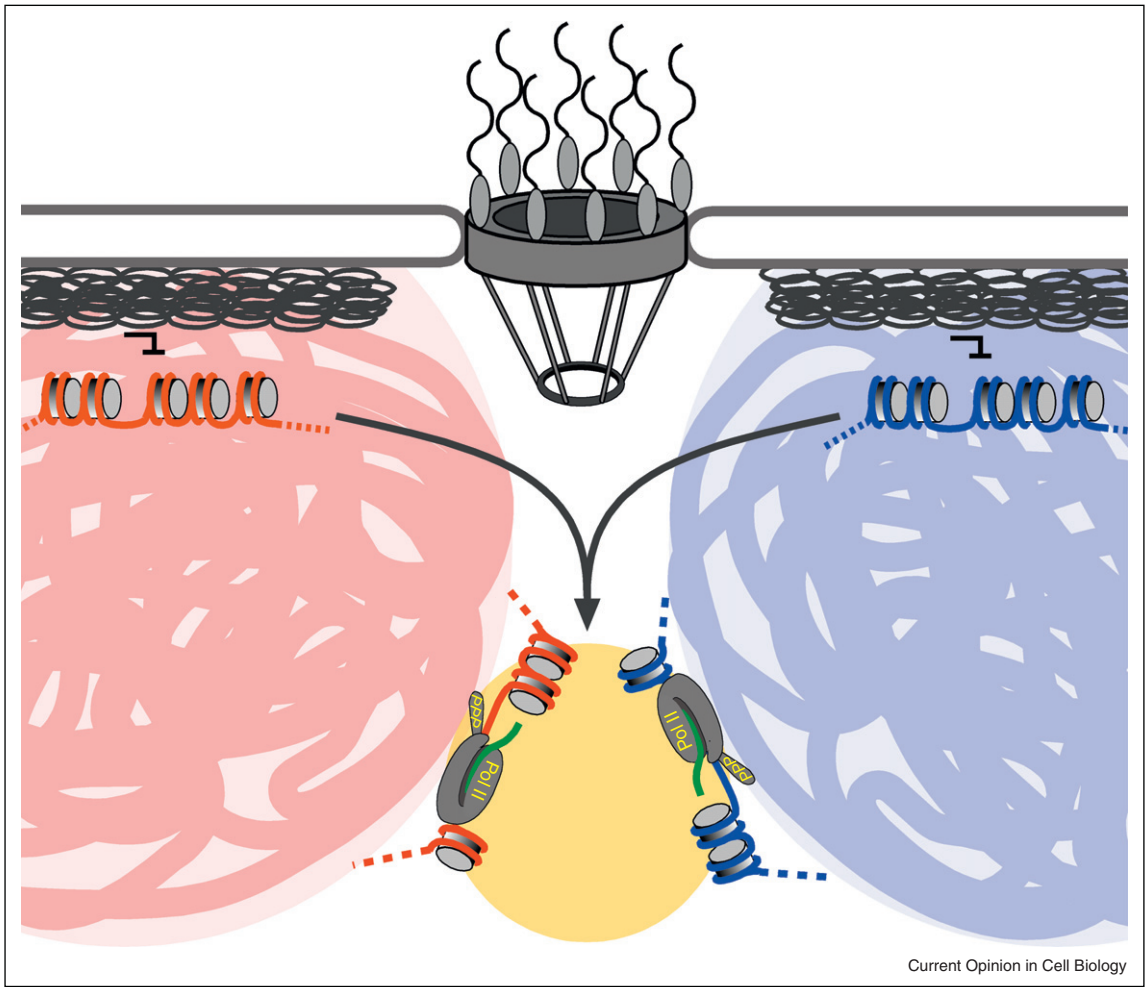
In differentiated metazoan cells, most heterochromatin localizes at the nuclear periphery [3,4]. Likewise, in budding yeast, silent subtelomeric genes localize at nuclear periphery [4–6]. These observations suggest that the nuclear periphery is a transcriptionally repressive environment. Consistent with this model, artificially tethering the yeast mating type locus to the nuclear envelope is sufficient to overcome loss of a *cis*-acting silencing element [7,8] and artificially tethering loci to the nuclear lamina in mammalian cells is sufficient to promote silencing [9•,10•]. This suggests that localization of genes at the nuclear periphery can promote transcriptional silencing.

Some genes localize to the nuclear periphery when repressed, but relocalize upon induction (Figure 1). A number of developmentally induced genes from different organisms and tissue types localize at the nuclear periphery in cells in which they are repressed and away from the nuclear periphery in cells in which they are expressed. This was first reported for the IgH and Igκ loci in mice [11], which localize to the nuclear periphery in hematopoietic progenitor cells and, after induction in pro-B cells, to the nuclear interior. In mice, several other loci relocalize from the nuclear periphery to the nuclear interior upon induction: the GFAP gene during astrocyte differentiation [12], the β-globin locus during erythroid development [13], the C-maf locus during T-cell development [14], the MyoD locus during myoblast development [15], and the Mash1 locus during neural development [16]. In humans, the CFTR gene moves away from the nuclear periphery in cells in which it is expressed [17]. This phenomenon has recently been observed for muscle-specific and gut-specific transgenes during development in *C. elegans* [18••]. Thus, the movement of individual genes from the nuclear periphery to the nuclear interior upon differentiation is a common theme among developmentally induced genes.

Several studies suggest that the interaction of genes with the nuclear lamina at the nuclear periphery promotes repression. Metazoan cells possess a lamina structure at the nuclear periphery, a fibrous mesh made up of lamins and lamin-associated proteins that colocalizes with heterochromatin [19,20]. Genome-wide studies in *Drosophila* show that much of the *Drosophila* genome interacts with lamins and that interaction with lamins correlates with transcriptional repression [21]. During astrocyte differentiation in mice, the association of the genome with the lamina changes in a cell type-specific manner, with genes that become active losing their association with the lamina [22,23••]. Finally, artificially tethering mammalian genes to the nuclear lamina is sufficient to promote transcriptional repression of many neighboring genes [9•,10•,24]. These results suggest that interaction of genes with the nuclear lamina at the nuclear periphery promotes silencing.

How might interaction with the lamina promote repression? Recruitment of lamin A to promoters can repress transcription in both yeast and human cells, suggesting that lamins may directly inhibit transcription [25]. However, it is also possible that the mechanism is less direct. In mammals, histone deacetylases (HDACs) associated with repression interact with inner-nuclear-membrane (INM) proteins such as Emerin [26] and the lamin-associated protein LAP2β [27,28]. This may explain the concentration of hypoacetylated histones at the nuclear periphery [29,30] and the repression of genes artificially tethered to the nuclear lamina [10•]. Consistent with this model, transcriptional repression induced by tethering to the lamina can be relieved by treatment with tricostatin A,

Figure 1



Relocalization of developmentally regulated genes. Repressed genes often associate with the nuclear lamina at the nuclear periphery. Upon activation, these genes are often targeted to the nucleoplasm. Certain co-regulated genes, located on different chromosomes (chromosome territories represented as blue and pink zones) can colocalize with each other at transcription factories (yellow), located between territories. The colocalization of certain genes requires a transcriptional activator (Klf1) that localizes to a subset of transcription factories [37••]. Colocalization may promote expression of co-regulated genes by either concentrating factors that promote their expression or by allowing escape from repressive interactions with the nuclear lamina.

an HDAC inhibitor [10•]. This model is reminiscent of the mechanism by which subtelomeric genes are silenced in budding yeast. In yeast, the Sir proteins that catalyze deacetylation of histones at telomeres are concentrated at the nuclear periphery and anchoring of telomeres to the nuclear envelope seems to promote the establishment and fidelity of silencing of subtelomeric genes [6,31–35]. Thus, localization of genes at the nuclear periphery, coupled with a heterogeneous distribution of repressive factors, could promote repression. Furthermore, relocalization of genes from this environment to a more permissive environment might promote transcription.

After moving away from the nuclear periphery, some developmentally co-regulated genes colocalize, a phenomenon called gene 'kissing' (Figure 1) [36,37••]. Gene kissing can occur between genes on the same chromosome, often megabases apart, or between genes on different chromosomes. The genes colocalize either at foci of active RNA polymerase II called transcription factories [38] or near nuclear 'speckles' [39]. Furthermore, the colocalization of genes on different chromosomes correlates with common translocation sites [40–44]. Gene kissing has been best characterized for genes induced in erythroid lineages in both humans and mice [36,45–47]. Colocalization of the active mouse *Hbb* and *Hba* globin genes with transcription factories has been demonstrated by both immuno-FISH and molecular techniques and requires Klf1, a transcription factor that regulates their expression [37••]. Because there seem to be a limited number of transcription factories per nucleus, kissing may concentrate factors that promote expression of related

genes [37••]. Thus, coupled with transcriptional regulation, certain genes can colocalize in association with subnuclear compartments.

Do these changes in gene positioning represent gene targeting to different subnuclear locations, or does gene positioning represent a downstream consequence of expression? The available data do not resolve this question. Consistent with the possibility that targeting might be specific and controlled by *cis*-acting information, promoters play an essential role in controlling gene positioning. The relocalization of the mouse β-globin locus to a transcription factory requires the Locus Control Region [13]. In *C. elegans*, transgenic promoters for housekeeping genes localize in the nucleoplasm in all cell types, whereas promoters from developmentally regulated genes localize in the nucleoplasm in cells in which they are expressed and at the nuclear periphery in cells in which they are not expressed [18••]. Likewise, the colocalization of co-regulated genes in mouse erythroid cells requires the transcriptional activator Klf1 [37••], but does not require ongoing transcription [48]. Consistent with the idea that gene kissing could concentrate factors that promote expression of related genes, Klf1 also localizes in a punctate pattern within the nucleus that overlaps with the transcription factories with which the genes interact (Figure 1) [37••]. These results are consistent with the possibility that gene positioning and colocalization in the nucleoplasm may be controlled by *cis*-acting DNA elements in the promoters of these genes. However, it is not clear that localization to the nuclear lamina represents targeting. Association of genes with the nuclear lamina may represent a default state for silenced loci. If lamin-associated proteins both bind to hypoacetylated/heterochromatic loci and promote deacetylation/heterochromatinization, silencing might lead to peripheral localization and peripheral localization might stabilize silencing. If so, then this interaction might be blocked by transcription or by activator function. Indeed, when very large transgene arrays are integrated into the *C. elegans* genome they are silenced and their localization does not reflect the promoter sequences in the array. These heterochromatic arrays localize at the nuclear periphery, regardless of the promoters that they possess [18••]. Likewise, a large array of lac repressor binding sites localizes at the nuclear periphery in hamster cells [49]. Tethering an activation domain to this array leads to relocalization of the array from the nuclear periphery to a more internal site [49]. Thus, although it is possible that active genes are targeted to particular sites during differentiation, it remains unclear if localization to the nuclear lamina represents targeting or a default destination for repressed loci.

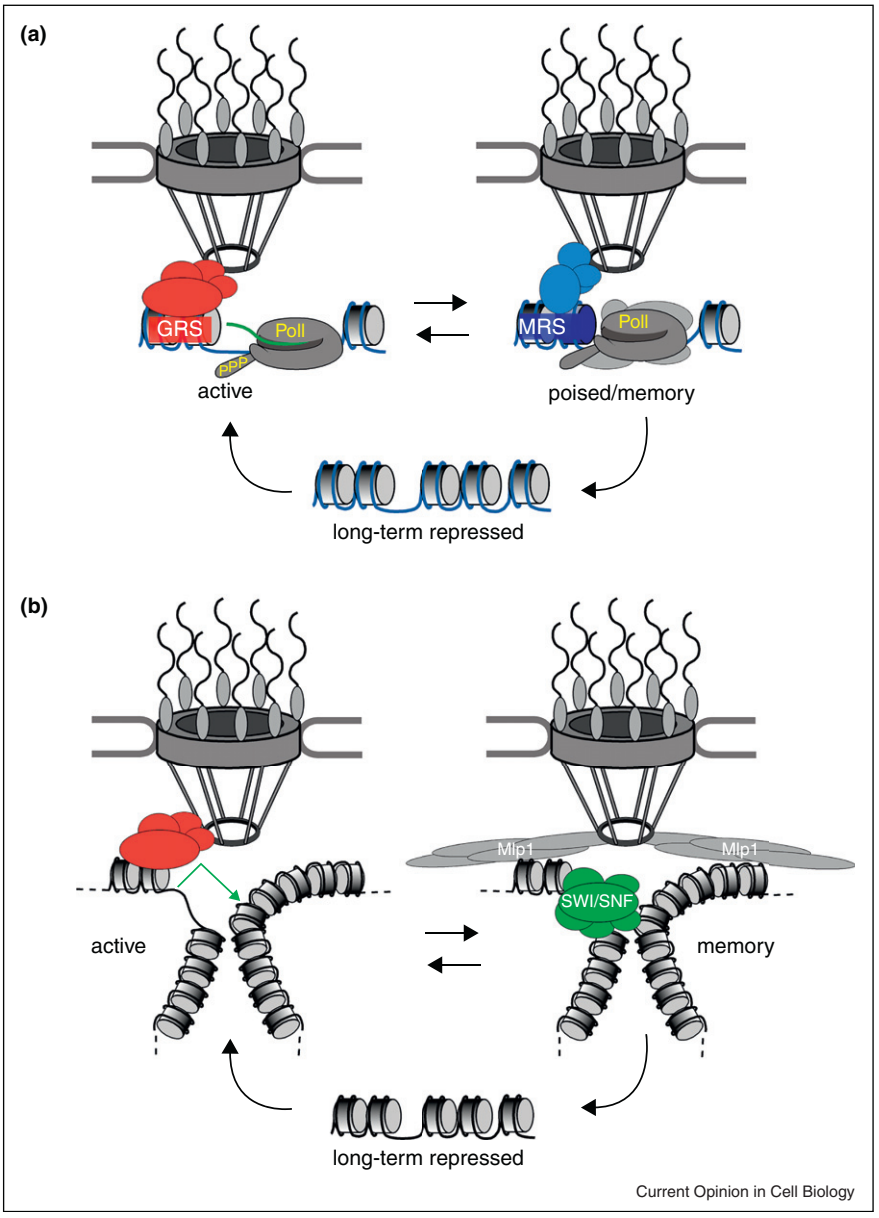
Active genes at the nuclear periphery

Although heterochromatin and silenced genes localize at the nuclear periphery, localization at the nuclear periphery *per se* is not incompatible with transcription. Several of the genes that relocalize from the nuclear periphery to the nucleoplasm during differentiation are induced before relocalization [13,17,52]. Chromatin modifications associated with active transcription, as well as individual active genes have been observed at the nuclear periphery in mouse embryonic stem cells [50•,51]. Furthermore, even genes that are tethered directly to the nuclear lamina are not always repressed. Tethering of endogenous chromosomal loci to the lamina resulted in repression of some, but not all of the neighboring genes [9•,10•,24]. An artificial reporter gene tethered to the lamina is as inducible as the nucleoplasmic form of the gene [24]. Thus, transcription and localization to the nuclear periphery are not always mutually exclusive, suggesting that nuclear positioning can have different effects on different genes.

Some genes are targeted from the nucleoplasm to the nuclear periphery when activated (Figure 2). This phenomenon is best understood in budding yeast. Genome-wide chromatin immunoprecipitation microarray experiments against nuclear pore proteins demonstrated that hundreds of active genes interact with proteins of the nuclear pore complex (NPC) and localize at the nuclear periphery [53,54]. Inducible genes such as *GAL1*, *INO1*, *GAL2*, *HSP104*, and *SUC2* localize in the nucleoplasm when repressed and relocalize to the nuclear periphery upon activation [53–58]. Localization at the nuclear periphery can promote transcription; tethering of *INO1* [55,59] or *HXK1* [57] to the nuclear envelope positively affects how fast or how strongly these genes are expressed and tethering of an artificial promoter to the NPC itself [60] is sufficient to induce transcription. Work from *Drosophila* [61] and mouse [39] raises the possibility that this phenomenon also occurs in metazoans (see below). Thus, some genes localize to the nuclear periphery when active and localization promotes transcription.

Consistent with the physical association of genes with the NPC, a number of NPC proteins, mRNA transport factors or NPC-associated factors are required for peripheral targeting of *INO1*, *GAL1* [59,62,63••], *GAL2* and *HSP104* [58]. This suggested that the change in localization of these genes to the nuclear periphery might represent a consequence of transcription, perhaps through a bridging interaction of NPC-associated mRNA transport factors with the gene [54,58]. Consistent with this possibility, the interaction of some genes with the NPC is RNase sensitive [54], the targeting of *HXK1* to the nuclear periphery requires the 3′UTR [57] and the targeting of *GAL10* and *HSP104* to the NPC requires the mRNA transport receptor Mex67 [58]. However, experiments with a temperature sensitive allele of RNA polymerase II showed that the interaction of *GAL1* with the NPC [64] and the targeting of *INO1* to the nuclear periphery [59] are independent of mRNA production. Therefore, the localization of some genes at the nuclear periphery is independent of transcription, suggesting that

Figure 2



Genes associated with the Nuclear Pore Complex at the nuclear periphery. **(a)** The mechanism of *INO1* targeting to the NPC. In yeast, active genes such as *INO1* contain zip codes (Gene Recruitment Sequences, or GRSs) in their promoters that can localize at the nuclear periphery through interaction with the NPC, perhaps through the interaction adaptor proteins (X). After repression, some genes like INO1 remain associated with the NPC by a separate mechanism. Localization of recently repressed INO1 requires a different zip code (Memory Recruitment Sequence, or MRS), a different interaction with the NPC (perhaps through adaptor proteins) and leads to promoter poising in association with unphosphorylated RNA polymerase II. **(b)** The mechanism of *GAL* gene transcriptional memory. After repression, *GAL* genes remain looped, with the 5′ and 3′ ends of the gene associated. This looping requires the Tpr homologue Mlp1 and, along with the SWI/SNF chromatin remodeler, promotes faster reactivation of the *GAL* genes [74•,75•,76].

these genes might be targeted to the nuclear periphery in a manner that is coupled to transcription, but not dependent on transcription.

Consistent with this idea, genes possess *cis*-acting targeting elements that control localization. The targeting of the *GAL2* gene to the nuclear periphery requires the promoter, but not the coding sequence or 3′UTR [58]. The targeting of *INO1* to the nuclear pore complex is controlled by two *cis*-acting DNA sequences called Gene Recruitment Sequences (GRS I and GRS II) in its promoter (Figure 2) [63••]. These sequences are distinct

from the Upstream Activating Sequences that control *INO1* transcription [65–67]. Importantly, these GRS elements function as 'DNA zip codes'; when integrated at an ectopic locus, they are sufficient to confer both peripheral localization and a physical interaction with the NPC [63••]. Mutations in the GRS elements block peripheral targeting of *INO1* and another GRS-targeted gene, *TSA2*, leading to a defect in transcription [63••]. This supports the idea that targeting to the NPC promotes transcription. Genome-wide, GRS I-containing promoters are enriched for genes that interact with the NPC and that are induced by protein folding stress [63••]. Finally, the GRS I element, when introduced into the genome of the highly divergent yeast *Schizosaccharomyces pombe*, functions as a DNA zip code to confer peripheral localization [63••]. This suggests that GRS I-mediated targeting to the NPC is an ancient mechanism, having been conserved for between 400 million and one billion years.

Several complementary studies examining the interaction of nucleoporins with the *Drosophila melanogaster* genome suggest that genes interact with nuclear pore proteins in flies [61,68,69••,70••]. Intriguingly, these studies identified at least two distinct types of genes: those that interact with the NPC at the nuclear periphery and those that interact with nucleoporins in the nucleoplasm. The genes that interact with nucleoporins in the nucleoplasm tended to be more active, developmentally important genes, whereas the NPC-associated genes were less active. Depletion of nucleoporins led to a defect in the transcription of the genes in the nucleoplasm [68,69••,70••]. This suggests that interactions with nucleoporins can also occur away from the pore and that interactions with nucleoporins in the nucleoplasm and at the NPC may have different effects on transcription.

Even in yeast, there are multiple mechanisms by which genes can be targeted to the NPC and these mechanisms have distinct effects on transcription. Several genes that localize at the nuclear periphery when they are active remain at the nuclear periphery after they are repressed (Figure 2) [59]. In fact, the *INO1* and *GAL1* genes remain at the nuclear periphery in the population through several cell divisions [59]. Thus, localization of these repressed genes at the nuclear periphery represents an epigenetic form of 'transcriptional memory'. Localization at the nuclear periphery correlates with a distinct mechanism of activation, suggesting that the function of this form of memory is to prime genes for reactivation [59,71]. In the case of *INO1*, the mechanism by which the recently repressed gene is localized at the nuclear periphery is distinct from the mechanism by which the active gene is localized at the nuclear periphery [72,73••]. Whereas localization of active *INO1* to the nuclear periphery requires the GRS DNA zip codes, localization of recently repressed *INO1* to the nuclear periphery requires a DNA zip code called the Memory Recruitment Sequence (MRS; Figure 2). In the context of the *INO1* promoter, the MRS only functions after *INO1* has been repressed and the two targeting mechanisms are independent [73••]. The targeting mediated by these two elements requires different NPC proteins, is regulated differently through the cell cycle [72] and leads to distinct biochemical interactions with the NPC [73••]. Whereas GRS-mediated targeting of active *INO1* to the NPC promotes robust transcription, MRS-mediated targeting of recently repressed *INO1* to the NPC alters the chromatin state of the promoter and primes the gene for reactivation [73••].

We do not understand how the interaction of genes with the NPC or nucleoporins promotes transcription or alters chromatin structure. In yeast, nucleoporins interact with the promoters of genes like *GAL1* and *INO1* and this interaction is important for transcription [63••,64]. It is possible that, as with the Sir proteins, factors that promote transcription are concentrated at the NPC and targeting improves the efficiency of recruiting such factors. Alternatively, perhaps the NPC provides a stable surface on which three-dimensional events such as chromatin remodeling or gene looping are more efficient. Although there is no evidence that gene looping requires interaction with the NPC during transcription, the memory of recent *GAL* gene transcription involves a stable gene loop in association with the NPC (Figure 2b) [74•,75•]. This interaction requires the Tpr homologue Mlp1 [75•] and is distinct from the mechanism used by the *INO1* gene, requiring the SWI/SNF chromatin remodeling complex (Figure 2b) [76]. Because looping does not persist as long as *GAL* gene transcriptional memory, it remains to be seen if there are multiple mechanisms by which memory is conferred [71]. Finally, because nucleoporins can promote transcription in the nucleoplasm, it is also possible that these proteins have a novel, direct function in transcription. If so, then perhaps these functions could be carried out in association with the NPC in some organisms and in the nucleoplasm in other organisms.

Concluding remarks

The two phenomena highlighted in this brief review represent the best-characterized changes in gene positioning associated with changes in gene expression. Given the lack of true compartmentalization of the nucleus, we propose that the spatial organization of the nucleus is achieved by: 1) protein-protein interactions that create spatial heterogeneities within the nucleus (e.g. Sir protein foci that promote silencing of subtelomeric genes or transcription factories that could promote transcription of coregulated genes; Figure 1), 2) the folding of chromosomes such that individual genes can access the appropriate subnuclear compartments (e.g. Figure 1), 3) the stabilization of both chromatin conformation and

subnuclear compartments through interaction with surfaces such as the nuclear envelope (e.g. the lamina or the NPC), and 4) molecular mechanisms that allow regulated movement of genes from one compartment to another. The two phenomena discussed here illustrate these ideas. If the nuclear lamina both concentrates repressive factors and interacts with heterochromatin, it could create a positive feedback mechanism to both package and repress large parts of the genome (Figure 1). The NPC and transcription factories may represent stable sites to which co-regulated genes are targeted and achieve more robust transcription through colocalization and concentration of shared factors (Figures 1 and 2). Thus, subnuclear compartments need not always be pre-existing entities but might be produced through conditional interactions among genes. A better understanding of the molecular basis for localization to these sites, and how localization impacts transcription, will allow us to answer a number of fascinating questions. How do DNA elements confer conditional localization to particular subnuclear sites? Why is the expression of different genes affected differently by localization to the same subnuclear compartment? To what extent is localization really important? In other words, are genes targeted to particular places/factories within the nucleus and, if so, does this matter for their proper expression? The answers to these questions will provide important insights into how genomes function within cells.

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