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Manipulating nuclear architecture

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Abstract

The eukaryotic genome is highly organized in the nucleus. Genes can be localized to specific nuclear compartments in a manner reflecting their activity. A plethora of recent report has described multiple levels of chromosomal folding that can be related to gene-specific expression states. Here we discuss studies designed to probe the causal impact of genome organization on gene expression. The picture that emerges is that of a reciprocal relationship in which nuclear organization is not only shaped by gene expression states but also directly influences them.

Introduction

A rapidly growing number of imaging and chromosome conformation capture (3C) based studies have revealed fundamental principles that govern the hierarchical organization of the chromosome. Individual chromosomes occupy distinct territories but show substantial intermingling allowing for interchromosomal contacts [1]. Moreover, active “open” and inactive “closed” chromatin appears to be partitioned into separate sub-nuclear domains [2]. Gene activity is also linked correlatively with numerous additional nuclear “neighborhoods” [3]. For example, chromatin associated with the nuclear lamina (lamina-associated domains, LADs) is typically gene poor, and genes proximal to the nuclear lamina tend to be repressed and marked by repressive chromatin modifications [4]. Another repressive nuclear environment includes nucleolus-associated domains (NADs) that consist of mostly repressive chromatin surrounding the sites of ribosomal synthesis [5]. Gene rich chromosomes tend to be located towards the center of the nucleus, and a correlation between interior positioning of genes in the nucleus and their activity has been observed in select cases [6]. However, such correlation does not seem to hold for many genes, and the nuclear periphery is not entirely restrictive to transcription [1,7]. While the nuclear lamina tends to

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be associated with heterochromatin, the immediate vicinity of the nuclear pores seems to be euchromatic, suggesting that the nuclear periphery contains distinct subdomains.

Individual chromosomes are further folded into so-called topological domains, regions with a median size of under 1MB within which long range looped cis-interactions occur [8–11]. Strikingly, these domains are similar between cell types and even between species. Most tissue-specific long range interactions between enhancers and promoters occur inside of topological domains, are mostly less than 100 kb in distance [12], and are established by gene-specific transcription factors and their co-activators [13,14]. Looped chromatin interactions are also observed at repressed genes [13,14]. Finally, intragenic physical contacts between promoter and terminator sequences have been found in yeast [15] and some mammalian genes [16,17].

Vast improvements have been made not only in imaging technologies but also in 3C (chromosome conformation capture) based methods, which have benefited from ever increasing sequencing power and computational prowess. These developments have narrowed the gap between chromatin interactions that can be detected in single cells by microscopy and those that are measured at the population level by 3C based methods, which assess relative proximity of chromatin fragments based on their crosslinking frequencies. It is expected that this gap will be narrowed further and perhaps closed in the not too distant future. These improvements will certainly produce more detailed descriptions of chromatin interactions as well as gene positions relative to other genes and nuclear compartments.

Several fundamental questions exist that are not addressed by descriptive examination only. 1) Do active and silent genes move to their respective sub-nuclear compartments as a result of their activation/ repression, or does their nuclear environment determine their transcription activities? 2) Are distinct nuclear neighborhoods shaped by genes with similar activities or do they pre-exist prior to contacts with chromatin? 3) Are long range looped genomic interactions cause or consequence of gene activation/ repression? 4) Do chromatin interactions directly participate in the transcription process, and conversely do gene expression states feed back to chromatin interactions? This review focuses on studies that go beyond correlative evidence to examine cause-effect relationships of gene activity and positioning or looping by specifically manipulating nuclear topology. The reports discussed here encompass different model organisms which can differ significantly in their nuclear structure, but they nevertheless provide examples of approaches to manipulating nuclear topology, revealing insights into fundamental principles of nuclear organization.

Nuclear positioning

Does location of a gene near a nuclear structure or neighborhood influence its activity, or is gene location an epiphenomenon of its activity? Several studies addressed this question by examining the consequences of forced gene positioning to the nuclear pore or the nuclear lamina.

In 1998 the Sternglanz group showed that in yeast anchoring a silencing -defective mating type locus to the nuclear periphery restored gene silencing [18]. This study was not only a prominent example of nuclear organization in regulating transcription but, importantly,

illustrated a gain -of-function approach to test the hierarchical relationship of gene positioning and activity. Similar strategies were adopted in mammalian and *Drosophila* cells using stably integrated test genes containing LacO elements along with constructs expressing LacI DNA binding domains fused to lamina-associated proteins. This allowed efficient tethering to the inner nuclear membrane [19–21]. Interestingly, forced gene re-positioning required traversal through mitosis, suggesting that the breakdown and subsequent reassembly of the nuclear envelope might enable changes in gene positioning. The effects on reporter gene expression as well as neighboring endogenous genes varied among these studies with some being repressed upon anchoring to the nuclear lamina but others remaining active. In a follow-up study, the Singh group identified GAGA motif-enriched sequences that when integrated at ectopic sites are sufficient for association with LADs at the nuclear periphery and inhibition of transcription [22]. Moreover, they identified the mammalian GAGA factor cKrox as critical for targeting. Of note, cKrox remained bound to chromatin during mitosis, suggesting that it might be involved in the re-association with LADs in newly assembled nuclei following mitosis.

In *C.elegans*, downregulation of lamin A or reduction in histone H3 lysine 9 (H3K9) methylation detaches heterochromatic genes from the nuclear periphery and, in some cases, increases their expression [23,24]. However, gene activation was neither sufficient nor required for release from the periphery [24]. Likewise, reduction in lamin A levels in mammalian cells leads to relocalization of a set of genes [25] but again, this does not automatically augment their expression but might instead poise them for activation. Interestingly, a mutation in lamin A found in patients with Emery-Dreifuss muscular dystrophy can increase retention of muscle specific genes at the periphery and alter their expression [23]. In concert, the above studies are compatible with a generally repressive microenvironment near the nuclear lamina, but they also indicate that gene specific regulatory elements can overcome the repressive transcriptional milieu at the nuclear periphery, and that gene positioning and activity can be uncoupled [26].

The lamina-proximal heterochromatin is punctuated by nuclear pores. In yeast active genes reside proximal to the nuclear pores [27,28] while in mammalian systems active genes did not exhibit such positioning preferences [29]. In yeast, anchoring of an inducible gene to the nuclear envelope optimized its induction by both lowering basal expression and increasing induced levels of transcription presumably by promoting association with nuclear pores [30] consistent with the view that the nuclear pore environment favors gene expression. Notably, tethering the yeast *INO1* gene to the nuclear periphery allows this gene to be more rapidly reactivated upon re-stimulation following an intermittent phase of repression [31]. This points to a potential link of nuclear positioning with a form of transcriptional memory. What mediates targeting of genes to the nuclear pore? RNA processing factors have been implicated in this function in yeast, suggesting that targeting can occur as a consequence of mRNA production [32–34]. However, select short promoter-upstream DNA sequences are capable of conveying association with the nuclear pore when introduced at an ectopic gene locus [35]. Notably genes with similar DNA targeting sequences tend to be coregulated and cluster at nuclear pores even if the genes are located on different chromosomes [36]. These reports suggest that in yeast the nuclear pore contributes to genome organization in general and perhaps even the clustering of groups of genes with shared properties.

An example on how the nuclear environment can impact on gene positioning in mammalian cells came from studies of the murine and human α -globin gene locus in erythroid cells. The latter seems to be imbedded in less condensed chromatin than the former, is more frequently positioned away from its chromosome territory, and tends to associate more readily with other erythroid expressed genes [37]. Upon engraftment into murine erythroid cells the human α -globin locus behaves very much like its murine counterpart with regard to positioning and chromatin environment. However, expression of the human locus remains high with levels being comparable to its endogenous murine counterpart. Hence, while the surroundings of a gene locus can influence its position, the relevance of gene position for gene function, if any, remains unclear in this case.

Compartment boundaries

The juxtaposition of repressive (lamina associated) and active (nuclear pore associated) domains at the nuclear periphery raises the possibility that domain boundaries might be at or near the nuclear pores. A very elegant screen to identify boundary activities in yeast yielded components of nucleo-cytoplasmic transport machinery [38]. Boundary activity, as defined as capacity to block the spreading of heterochromatin, was dependent on association with the nuclear pore complex. Tethering of a nuclear pore complex protein to a modified gene relocated the gene to the nuclear pore and recapitulated boundary activity [38].

In metazoans, boundary elements and enhancer blocking insulator sequences are thought to organize the genome structure via chromatin looping and clustering at specific nuclear compartments [39]. For example, the *Drosophila* gypsy insulator functions in part by moving the insulator site to the nuclear periphery [40]. Moreover, when integrated on different chromosomes gypsy insulators mediate juxtaposition of these chromosomes. The chicken HS4 enhancer blocking insulator, when inserted ectopically into a human cell line, is tethered to the surface of the nucleolus [41]. Tethering is depended on an intact CTCF binding site, raising the possibility that CTCF-mediated interaction with the peri-nucleolar milieu might aid in spatially configuring enhancer elements to block their function.

The above experiments support the idea that sub-nuclear localization is not simply a reflection of gene activity but can influence gene activity and the function of boundary and enhancer blocking insulator elements.

Chromatin looping

Distal enhancers typically form contacts with target promoters via looping [12,42]. Mechanistic studies mostly involving loss of function approaches identified a number of factors essential for these contacts [13]. However, they failed to distinguish whether looped interactions are a prerequisite for gene transcription or merely a reflection thereof.

Early studies aimed at examining the consequences of enforced enhancer -promoter chromatin looping used in vitro transcription assays or transfected human cells in which a promoter and enhancer were located on separated plasmids [43,44]. Juxtaposition of the two plasmids via a streptavidin -biotin bridge or a dimerizing transcription factor activated transcription. Forced enhancer recruitment to a promoter also enabled long-range activation of a reporter gene in yeast [45]. In another study using an engineered IFN- β enhancer driven

reporter gene, it was shown that forced enhancer-promoter looping via a prokaryotic DNA binding protein was capable of stimulating enhancer dependent transcription [46]. Interestingly, interspersed transcription factor binding sites could function as enhancer decoys by forming a non-productive loop [46]. This result echoes an experiment carried out by nature in which a SNP created a binding site for the erythroid transcription factor GATA1 at an intergenic region upstream of the human α -globin genes [47]. This nucleated binding of a GATA1 transcription factor complex and initiation of transcription at the expense of the normally initiated α -globin genes [47]. Although a direct test of non-productive looping was impossible in this study for lack of sufficient human material, the results were consistent with a decoy loop model, and further strengthened the idea that looping can not only direct activation but also attenuation of transcription.

Another mode of repression through controlled chromatin looping has been demonstrated with a designer reporter gene in which an enhancer was flanked by inducible dimerizing transcription factors. Induction of dimer formation insulated the enhancer from the promoter and diminished enhancer activity [48]. Similarly, introduction of the β -globin HS5 insulator between the human β -globin genes and the locus control region (LCR) led to loop formation between the ectopic HS5 and its endogenous counterpart upstream of the LCR [49]. Interaction of the two flanking HS5 elements reduced LCR- β -globin gene looping and reduced β -globin transcription. Thus, chromatin looping can trigger gene silencing by at least two mechanisms, enhancer insulation and enhancer competition, both of which likely account for normal insulator function [39].

A looped long-range interaction has also been described among silencer elements within the coding region of the Kit gene. However, its role, if any during gene repression remains unresolved [50]. Enforced looping similar to experiments outlined below could be envisioned to examine the causality of looping within the coding region and transcriptional silencing.

The studies discussed so far involved modified reporter genes or artificial episomal constructs. To examine the causality of chromatin looping and transcription activation at a native gene locus, Deng *et al* employed designer zinc finger proteins to tether the presumed looping factor Ldb1 to the native murine β -globin promoter in proerythroblasts in which the β -globin locus was inactive and unlooped [51]. Ldb1 recruitment triggered a long-range interaction between the LCR and the β -globin promoter and strongly activated β -globin transcription. This established Ldb1 as sufficient for initiating an LCR- β -globin loop and demonstrated that forced enhancer-promoter looping can cause transcription.

A number of recent studies not involving direct manipulation of chromatin loops also support that chromatin looping can guide transcriptional processes. The long non-coding RNA Xist is transcribed from the X-inactivation center of the silenced X chromosome and ultimately spreads broadly across the entire chromosome. However, initially, upon activation of Xist production, Xist localizes focally to numerous distal regions before assuming a more uniform distribution pattern. Focal Xist enrichments are not defined by specific sequences but by their spatial proximity to the Xist locus [52]. Importantly, when expressed from an ectopic genomic integration site early Xist localization again reflected

proximity contacts of the ectopic site. Therefore, it is thought that long range interactions direct the initial localization of Xist followed by local spreading. Another example for looped chromatin interactions guiding transcriptional repression comes from studies showing that a gene loop can mediate association of the chromatin remodeling factor Iswi to its contacted target sites in yeast [53]. These reports provide prominent examples of a hierarchical order descending from the 3D structure of the genome to transcriptional silencing.

Conclusions/Open questions

The studies discussed above contribute to a model best described as a reciprocal relationship, rather than a uni-directional one, between nuclear architecture and gene activity. Upon regulatory cues, nuclear factors organize the conformation and positioning of the genome, which can in turn feed back on gene activity and chromatin state. Critical goals for the future will be to identify the factors that are *directly* involved in organizing chromatin folding and positioning and to elucidate the mechanisms by which they do so. In theory, similar to the approaches discussed in this review, tethering of genes to any nuclear neighborhood (Figure 1A) or forcing looping between genes and various regulatory elements (Figure 1B) could be used to investigate functional roles of nuclear compartmentalization and chromatin folding in gene activity. To alter the positioning or folding of a specific genomic region, integrated DNA sequences with corresponding binding protein (e.g. LacO/ LacI system) or engineered DNA-binding proteins such as artificial zinc fingers, TALE proteins or CRISPR complexes targeting endogenous DNA sequences can be fused to proteins that associate with specific nuclear compartments, constitutive looping factors, or inducible dimerization moieties. In this context it deserves to be explored whether gene expression patterns can be altered via these strategies not just out of scientific curiosity but also in therapeutic settings in which changes in gene expression are desirable. Finally, it will be important to understand how genes and their associated nuclear factors interpret the cues provided by positional and conformational information to influence gene activity.

Another major unresolved question is whether gene positioning or looping serve any epigenetic (i.e. heritable) function [54,55]. In other words, it will be critical to understand whether and how any positional information and chromatin looping is propagated through the mitotic phase of the cell cycle. During mitosis the nuclear envelope breaks down, chromosomes condense, and most transcriptional regulators, including those needed for looping, are evicted from chromatin. Additionally, radial positioning of chromosomes does not appear to be heritable through mitosis [56]. Tantalizing evidence in favor of mitotically stable topological information showed that a LAD-associated test gene was positioned at the periphery of mitotic chromosomes, suggesting that might serve to reposition itself within a LAD in the G1 phase of the cell cycle [22]. However, recent single cell studies indicate that LADs are reshuffled following mitosis [57]. Hence, at first glance, any memory function for higher order gene organization or positioning seems difficult to envision in light of these dramatic changes in chromosomal topology during mitosis. The easiest solution is that certain histone modifications and nuclear factors that remain stably associated with mitotic chromatin [58,59] initiate chromatin folding and gene localization in cells emerging from mitosis. However, not all spatial information might be lost during mitosis. For example,

some inducible genes in yeast (that undergo a closed mitosis which leaves the nucleus intact) move to the nuclear periphery and, even following repression, can remain localized there for several cell division cycles [55]. This localization pattern correlated with accelerated reactivation kinetics upon re-induction. In addition, certain chromatin loops at the imprinted Igf2-H19 locus that depend on CTCF might persist through mitosis [60]. How common mitotically stable loops are will be the subject of future work. Moreover, it will be necessary to determine whether these topological features per se or the factors that cause them underlie epigenetic function. These questions might be amenable through specific manipulation of chromatin organization.

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A Targeting to nuclear compartments

B

Figure 1.

Manipulations of gene positioning and looping. (A) Targeting a gene or chromatin domain to the nuclear periphery or other nuclear compartments by DNA-binding protein factors. (B) Forced chromatin looping to produce various outcomes. Juxtaposition of enhancers or silencers with promoters might activate or repress transcription, respectively. Repression might also be achieved by insulating enhancers or inducing competing unproductive loops.