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Chromatin Organization and Transcriptional Regulation

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Abstract

Cell type specific transcriptional regulation must be adhered to in order to maintain cell identity throughout the lifetime of an organism, yet flexible enough to allow for responses to endogenous and exogenous stimuli. This regulation is mediated not only by molecular factors (e.g. cell type specific transcription factors, histone and DNA modifications), but also on the level of chromatin and genome organization. In this review we focus on recent findings that have contributed to our understanding of higher order chromatin structure and genome organization within the nucleus. We highlight new findings on the dynamic positioning of genes relative to each other, as well as to their chromosome territory and the nuclear lamina, and how the position of genes correlates with their transcriptional activity.

Introduction

Although chromatin was first described 130 years ago [1], the organization and dynamics of chromatin in the interphase nucleus *in vivo*, and how this organization relates to transcriptional regulation, is still not fully understood. Here we review recent advances in electron- and light microscopy, as well as biochemical and molecular biology approaches that have shed new light on this fundamental question in biology.

Higher Order Chromatin Structure

DNA in the eukaryotic cell nucleus exists as a complex with histone proteins. 147bp of DNA are wrapped in 1.7 negatively supercoiled turns around the nucleosome core particle comprised of two H3–H4 and two H2A–H2B histone dimers. Nucleosomes are separated from each other by 10–80bp linker DNA associated with linker histone H1 [reviewed in 2]. This DNA-nucleosome complex forms a 10nm diameter fiber resembling ‘beads on a string’ [3,4] (Fig. 1E). The 10nm chromatin fiber has been shown *in vitro* to form a higher order helical fiber 30nm in diameter (Fig. 1D) containing 6–11 nucleosomes per turn [5,6] which has been proposed to form even higher order chromatin fibers in interphase [7], and a 200–300nm chromonema structure in mitotic chromosomes [8,9]. Two models have been proposed to describe the 30nm fiber (Fig. 1D). First, an interdigitated one-start solenoid structure where each nucleosome interacts with its fifth or sixth neighbor [10]. Secondly, a two-start zigzag ribbon where every second nucleosome interacts [11,12]. In a molecular tweezer experiment using 25-nucleosome repeat arrays *in vitro*, it has been determined that

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the extension characteristics and force of 4pN required to fully extend the array from a 30nm to a 10nm fiber is consistent with a solenoid structure [13].

While it has been extensively studied *in vitro*, evidence for the existence of the 30nm fiber *in vivo* is limited. It has been proposed that the 30nm fiber is the preferred structure in chromatin preparations with low chromatin concentrations and low ionic strength where intra-molecular nucleosome interactions are favored over inter-molecular interactions [reviewed in 14,15]. Moreover, alcohol dehydration and embedding procedures used in electron microscopy sample preparations, as well as the 'Widom 601 nucleosome positioning' sequence used for some of these studies likely favor the formation of the 30nm fiber *in vitro* [reviewed in 14], all factors which call into question its existence *in vivo*.

In interphase cells, the 30nm fiber has so far only been observed in two specialized systems: starfish spermatozooids [16], and chicken erythrocyte nuclei [16,17]. In contrast to the majority of cells, these two model systems are largely transcriptionally inactive, they contain a more highly charged histone H1 isoform, low abundance of non-histone chromatin proteins, and a longer nucleosome repeat length [18], suggesting that the 30nm fiber might be involved in heterochromatic transcriptional repression and compaction [17]. However, this compaction may not be sufficient for transcriptional silencing, as the structure of the 30nm fiber in avian erythrocyte nuclei is loose enough to permit the access of even large proteins to the chromatin fiber [17,19]. Interestingly, in mouse rod photoreceptor cells which have concentric areas of varying chromatin compaction, the central and most compact area shows an amorphous phase with no chromatin fibers, whereas the more peripheral layer with intermediate levels of chromatin compaction shows a 30nm fiber, and the least condensed region shows only the 10nm fiber [20]. This suggests that chromatin within these cells can exist in multiple distinct structures.

In order to study the decompaction and transcriptional activation of condensed chromatin from human cells that mimics *in vivo* characteristics, Reinberg and colleagues reconstituted 5kb of DNA surrounding the RAR/RXR responsive PEPCK promoter with native histones isolated from HeLa cells, as well as histone H1, the core histone chaperone RSF, and the histone H1 chaperone NAP-1 [21]. This resulted in a highly compacted 30nm chromatin fiber which became decondensed upon transcriptional activation. In contrast, mitotic HeLa S3 chromosomes observed in a close-to-native state by small-angle X-ray scattering and cryo-electron microscopy (cryo-EM) of vitreous sections, fail to show a higher order chromatin structure beyond the 10nm fiber [22,23]. Similarly, cryo-EM of rodent and plant interphase chromatin has been shown to be homogeneous and disorganized [24]. Furthermore, chromatin organization was studied by a combination of electron spectroscopic imaging and electron tomography, which does not involve contrast agents and creates a three dimensional image of chromatin *in situ* [25]. Using this technique, open chromatin or condensed chromatin within chromocenters in mouse embryonic fibroblasts, as well as in mouse spleen lymphocytes and liver tissue cells showed the 10nm fiber, but did not exhibit any evidence for a 30nm or higher order chromatin organization [25]. Therefore, rather than being ordered into a 30nm fiber, chromatin has been described as a dynamic disordered and interdigitated state comparable with a "polymer melt", where nucleosomes that are not linear neighbors on the DNA strand interact within a chromatin region [14,22,23] (Fig. 1D). It has been proposed that these regions represent drops of viscous fluid in which the radial position of genes within these drops may influence their transcriptional activity [14]. This fluid and irregular chromatin arrangement might permit a more dynamic and flexible organization of the genome than the rigid 30nm fiber would provide [14,22], and would consequently facilitate dynamic processes such as transcription, DNA replication, DNA repair and enhancer-promoter interactions [22]. Furthermore, the irregular spacing and concentration of

nucleosomes seen *in vivo* has been shown to be incompatible with the 30nm fiber [26], further supporting the polymer melt model.

In recent years, considerable effort has been made to study chromatin in conditions that are close to the living state and an increasing amount of data suggests that chromatin organization above the 10nm fiber likely does not exist in most mammalian cells. New super-resolution imaging techniques are promising tools to further evaluate the organization and dynamics of chromatin in living cells in the near future.

Genome-wide chromatin interactions

The development of the Chromosome Conformation Capture (3C) and 3C-related genome-wide techniques (circularized chromosome conformation capture (4C), carbon copy chromosome conformation capture (5C), Hi-C) has given us an insight into the structure and long-range interactions of chromatin at the molecular level *in vivo* [reviewed in 27,28]. In yeast, 3C analysis of transcriptionally active chromatin shows local variations in chromatin compaction, but does not support the presence of a 30nm fiber [29]. A seminal study by Dekker and colleagues provided a model of the local chromatin environment of normal human lymphoblasts on the megabase scale as a fractal globule, where chromatin partitions into adjacent regions with minimal interdigitation [30] (Fig. 1B), and that are consistent with the diffusion and binding properties caused by molecular crowding of chromatin binding proteins [31,32]. The fractal globules ultimately associate on the chromosome level to form chromosome territories [30] (Fig. 1 A–B), which can be observed in interphase nuclei using light microscopy techniques. In addition, the fractal globule model suggests a mechanism for the interaction of genomic sites that are distant within a chromosome or on different chromosomes, which might lead to chromosomal translocations in cancer. Interestingly, the three dimensional chromatin structure revealed by Hi-C experiments directly correlates with chromosomal rearrangements and somatic copy number variations reported in human cancer cells [33]. Similarly, the translocation frequency of the *Igh* and *Myc* loci which are located on different chromosomes in mouse B lymphocytes directly correlates to their contact frequency in a 4C-seq experiment [34]. Furthermore, the actual observed intra- and inter-chromosomal translocation frequency has been shown to correlate with the contact probability in a Hi-C experiment in G1 arrested mouse pro-B cells [35].

Within the fractal globule, chromatin is organized into discrete domains. A Hi-C analysis in mouse ES cells identified 2,200 topological domains in which chromatin with a median size of 880kb occupying about 91% of the genome interacts locally [36]. These topological domains are enriched in housekeeping genes and SINE elements, and are separated by topological boundary regions with characteristics of insulator elements, such as CTCF-binding and a segregation of the heterochromatic H3K9me3 mark [36]. This organization of the topological domains is conserved between different human cell types, as well as between human and mouse [36]. A follow up study by the same group using the ChIP-seq technique found a significant overlap of topological domains with cis-regulatory enhancer-promoter units in 19 embryonic and adult mouse tissues and cell types [37]. Similarly, a 4.5Mb region encompassing *Xist* on the X chromosome in mouse ES cells was shown to partition into discrete topologically associating domains (TADs) that are 200kb to 1Mb in size, and are present on both the active and inactive X chromosome in male and female ES cells [38]. While they are enriched in, they do not require H3K27me3, H3K9me2 nor lamina-associated domains (LADs) for their maintenance [38]. Within a TAD, genes are transcriptionally co-regulated, and while the TADs as a whole do not change, the internal TAD contacts rearrange upon ES cell differentiation supporting the link between chromatin structure and transcription [38]. Similarly, a study of the active and inactive X-chromosome in human SATO3 lymphoblast cells revealed that transcription disrupts intrachromosomal

interactions, leading to local chromatin decompaction at promoters [39]. A 5C study as part of the ENCODE project analyzed the interactions of transcriptional start sites (TSS) in 44 regions representing 1% of the genome in three human cell lines [40]. More than 1000 mostly asymmetric long-range interactions with distal elements resembling promoters and enhancers were identified within these regions [40]. However, in contrast to another study [37], ~60% of the interactions were found in only one of the three cell lines analyzed indicating a cell-type specific chromatin folding [40]. Therefore, it remains to be determined how conserved these long-range interactions are between cell types or species. In addition to intrachromosomal contacts, tethered chromosome conformation capture (TCC) experiments in human lymphoblastoid cells revealed that inter-chromosomal contacts are indiscriminate between chromosome territories and their contact probability is a function of their transcriptional activity and position within the territory [41]. Consequently, inter-chromosomal contacts are about 70 times less frequent than intrachromosomal contacts and may be present only in a fraction of cells where both interacting regions are accessible [41] (Figure 1).

The fractal globule model has provided exciting initial insights into genome-wide short- and long-range gene interactions involved in transcriptional regulation and chromosomal translocations in cancer. However, current 3C methodology surveys chromatin topology within dynamic populations of cells. At the single cell level, chromatin interactions are likely to be dynamic, some being stochastic, and their frequency may depend on the cell cycle and additional factors. Therefore, an examination of chromatin topology of single cells is needed to assess cell-to-cell differences as well as changes during the cell cycle and stages of differentiation in order to fully understand the relationship of gene interactions to cellular function.

Gene position in relation to chromosome territories

From the higher order fractal globule structure, chromatin is further organized into chromosome territories, where each chromosome, rather than being intertwined, occupies its own distinct region of the nucleus [reviewed in 42,43]. In order to study the contacts and interdigitation of chromosome territories, Bickmore and colleagues used fluorescently-labeled pooled sequence-capture probes to show that the exons of mouse chromosome 2 predominantly localize at the surface of the chromosome territory [44]. This is consistent with genes looping out of their chromosome territory and allows for interactions with regions of other chromosomes. Pulse-labeling experiments have revealed that only 1% of chromatin from different chromosomes co-localize in interphase cells [45]. Thus it is likely that these inter-chromosomal interactions occur transiently and/or that these are rare events, as has also been proposed by genome-wide mapping of chromosome interactions [41] (Figure 1). The importance of inter-chromosomal interactions for gene regulation still remains to be elucidated, but it has been proposed that some co-regulated genes can colocalize in interchromatin granules or transcription factories [46–48]. In particular, it remains to be demonstrated if looping out from a chromosome territory is an active process preceding transcription, or if it is a consequence of gene activation. Treatment with the histone deacetylase inhibitor TSA results in increased chromatin mobility [49] and an increase in inter-chromosomal co-localization [45], suggesting that gene activation may not be a consequence of gene movement and co-localization, and that the two processes might indeed be independent from each other (Figure 2).

Gene position in relation to the nuclear periphery

Beyond the organization of chromatin in chromosome territories, the radial position of genes within the nucleus has been implicated in gene regulation. In particular, it has been

suggested that the low transcriptional activity of perinuclear heterochromatin is a consequence of nuclear lamina-mediated gene silencing [50]. The nuclear lamina which is comprised of a meshwork of type V intermediate filament proteins (lamins) and other associated proteins (reviewed in [51]) provides the interface between the inner nuclear membrane, nuclear pore complex and the nearby chromatin. Associations of large regions of chromatin, termed lamin associated domains (LADs) with the nuclear lamina is generally associated with transcriptional repression [52], however relocation to the periphery is not always sufficient for gene silencing [53], nor is it necessary as many inactive loci are located within the nucleoplasm away from the nuclear periphery. Nonetheless the association with, and disassociation of gene loci from the nuclear lamina and corresponding changes in transcriptional status, for example during embryonic stem cell differentiation [52], implicates this nuclear compartment in the regulation of gene expression.

Recent studies have advanced our understanding of how genes relocate to and from the nuclear periphery. In *S. cerevisiae* the *INO1* gene relocates to the nuclear pore complex (NPC) upon transcriptional activation [54]. This relocation is controlled by two upstream 8bp and 20bp DNA elements termed 'DNA zip codes' which are sufficient for relocation and clustering at the NPC [55], suggesting that the genome itself encodes for its spatial organization. DNA elements can also mediate gene repositioning in mammalian cells. The *IgH* and *Cyp3a* loci are located within LADs that dissociate from the nuclear lamina in cell types in which these genes are actively transcribed [56]. Integration of BACs containing these genomic regions into a control locus relocates the locus to the nuclear periphery [57]. Through a series of truncation experiments, Singh and colleagues identified a 4–6kb minimal sequence element at these loci that is sufficient to target the surrounding DNA region to the nuclear periphery and consequently attenuate transcription of a reporter gene [57]. This sequence element is enriched with the *GAGA* motif, which when inserted as 10 copies in a 400bp array, is sufficient to target a DNA locus to the lamina. The sequestration at the lamina could be partially inhibited through knockdown of either the zinc finger protein cKrox, which binds the *GAGA* motif, or the histone deacetylase HDAC3 [57]. Therefore, chromatin modifications, in addition to the DNA sequence elements, may also be involved in positioning genes at the nuclear periphery. This is further supported by findings implicating histone deacetylases in targeting the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene to the nuclear periphery in non-expressing cells [58]. In addition to histone acetylation levels, histone H3 lysine 9 methylation has also been suggested to influence DNA positioning at the nuclear lamina in *C. elegans* embryos [59]. An RNAi screen identified 29 factors that, when knocked-down, led to activation of a peripheral repressed reporter array. Interestingly, only 2 of these factors resulted in additional movement of the array into the nucleoplasm, demonstrating that movement is not required for gene activation. Conversely, movement of a reporter gene under an inactive promoter from the periphery was not accompanied by transcriptional activation [59]. Therefore, while there is a correlation between gene expression levels and nuclear periphery positioning, the two processes are not necessarily dependent on each other. Additional characterization of the factors that are required for gene positioning relative to the nuclear periphery and other nuclear structures represents an interesting area for future research.

Transcription dependent gene movement

When considering gene positioning, either relative to topological associated domains, chromatin territories, or a nuclear structure such as the lamina, an important consideration is whether changes in gene position occur prior to or following changes in gene expression (Figure 2). For example, the *HOX* gene cluster in mammals change during differentiation from a single domain marked by H3K27me3 to a bimodal domain in which the active *Hox* genes occupy a separate region rich in H3K4me3 distinct from the inactive regions [60].

However, it is unknown whether the structural changes that accompany gene activation are necessary for transcription to occur, or whether they are a secondary event stabilizing the gene expression program in the cells. The two alleles of the imprinted *Kcnq1* locus have recently been shown to associate in early embryogenesis at sites of high RNA polymerase II occupancy [61]. This suggests a role for gene transcription in mediating the pairing, however cause and effect again remain unknown. Similarly, the long noncoding RNAs TUG1 and MALAT1/NEAT2 have been implicated in the relocation of growth control genes between Polycomb bodies and interchromatin granule clusters [62]. Long range chromosomal interactions between the *ifrry* cytokine gene and its receptor genes *ifrryR1* and *ifrryR2* are also associated with gene expression [63]. This interaction persists following inhibition of transcription with the RNA polymerase II inhibitor α -amanitin, implying that gene transcription is not required to maintain the intergenic interactions. However, it remains to be determined whether transcription is required for their establishment. Along these lines, chromatin looping may directly affect transcription, rather than being the result of transcriptional co-regulation. This was shown by zinc-finger mediated tethering of the GATA1 associated protein Ldb1, or merely its self-association domain, to the β -globin promoter in erythroid cells [64]. This led to the formation of a chromatin loop between the promoter and the locus control region (LCR), and to expression of the β -globin gene in the absence of GATA1 [64]. In order to untangle the interconnection of gene transcription and gene movement, live cell systems, in which one can follow the activation or silencing of individual endogenous genes with respect to their chromosome territory or a nuclear compartment, will be required. These types of experiments will be critical to extending our understanding of the role of nuclear organization in the regulation of gene expression.

Outlook

In recent years, light- and electron microscopy approaches, as well as the emergence of genome-wide 3C-related studies have broadened our understanding of the three-dimensional organization of chromatin within the nuclear space, and how it relates to transcriptional regulation. However, many fundamental questions remain unanswered. Although increasing evidence from experiments that are close to the native chromatin state do not support the 40 year old concept of higher order chromatin structure, there is still a lack of understanding with regard to the structure of chromatin in the living cell, and whether or not a 30nm fiber or even higher order chromatin organization exists in live interphase mammalian cells. Chromatin may have very different structures within a cell depending on multiple factors, such as the radial position within the nucleus, the cell cycle stage, the differentiation state of the cell, transcriptional activity, nucleosome occupancy, DNA and histone modifications, histone variants, long-range chromatin interactions, or any combination of these factors.

While 3C-related techniques have provided significant insight into genome-wide chromatin association frequencies within a population of cells, these techniques currently do not tell us how dynamic such interactions are in and among single cells. It remains to be determined what the frequency and duration of these interactions are, how they relate to the cell cycle and differentiation, and if they are the cause or consequence of transcriptional regulation.

While recent advances in imaging and molecular approaches have provided significant insights into chromatin organization and gene interactions, future studies examining individual living and fixed cells will provide the basis for further advances.

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Figure 1.

Chromatin organization in the mammalian nucleus. **(A)** Chromosomes are organized in chromosome territories. **(B)** Chromosome territories are comprised of fractal globules, and fractal globules from adjacent chromosome territories can interdigitate. **(C)** Chromatin fibers interact (i) within a fractal globule (frequent), (ii) between fractal globules of the same chromosome territory (rare), or between adjacent chromosome territories (very rare). **(D)** Chromatin may form a 30nm fiber with a zigzag, solenoid or polymer melt organization (see text). **(E)** Chromatin is resolved as a 10nm 'beads on a string' fiber consisting of nucleosomes.

A. Activation precedes movement **B. Activation depends on prior movement** **C. Activation and movement are independent events** **Figure 2.**

Transcriptional activity influences chromatin topology. **(A)** Transcriptional activation of a gene may precede its movement within the nucleus. **(B)** An inactive gene may get activated subsequent to its movement to a site that is favorable to transcriptional activation. **(C)** Transcriptional activation and gene movement may be independent of each other. Red, inactive gene; green, active gene.