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Chromatin organization and transcriptional regulation

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Cell type specific transcriptional regulation must be adhered to in order to maintain cell identity throughout the lifetime of an organism, yet it must be 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.

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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 microscopy and light microscopy, as well as biochemical and molecular biology approaches that have shed new light on this fundamental question in biology.

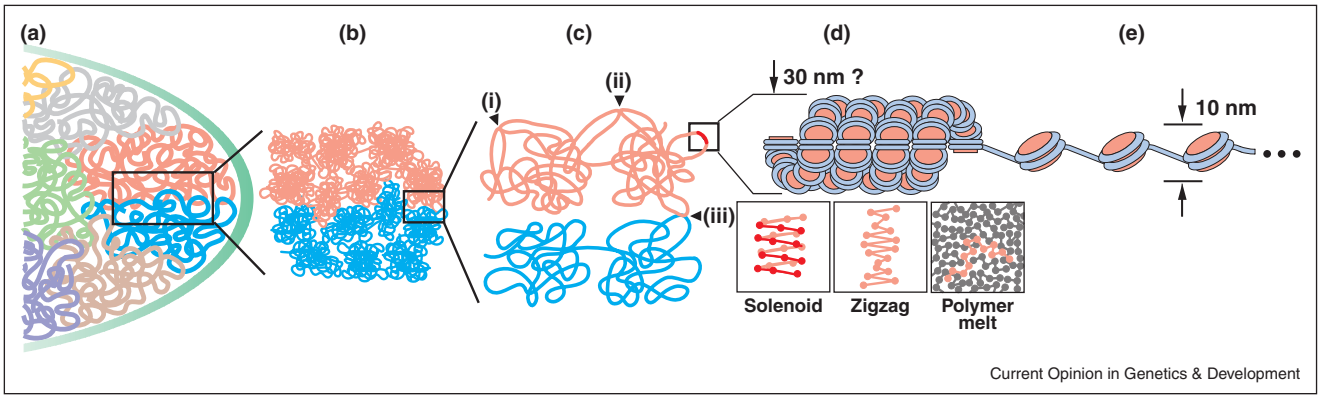
Higher order chromatin structure

DNA in the eukaryotic cell nucleus exists as a complex with histone proteins. 147 bp 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 10–80 bp linker DNA associated with linker histone H1 (reviewed in [2]). This DNA–nucleosome complex forms a 10 nm diameter fiber resembling 'beads on a string' [3,4] (Figure 1e). The 10 nm chromatin fiber has been shown *in vitro* to form a higher order helical fiber 30 nm in diameter (Figure 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 200–300 nm chromonema structure in mitotic chromosomes [8,9]. Two models have been proposed to describe the 30 nm fiber (Figure 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 the extension characteristics and force of 4 pN required to fully extend the array from a 30 nm to a 10 nm fiber is consistent with a solenoid structure [13].

While it has been extensively studied *in vitro*, evidence for the existence of the 30 nm fiber *in vivo* is limited. It has been proposed that the 30 nm 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 probably favor the formation of the 30 nm fiber *in vitro* (reviewed in [14]), all factors which call into question its existence *in vivo*.

In interphase cells, the 30 nm fiber has so far only been observed in two specialized systems: starfish spermatozoids [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 30 nm 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 30 nm 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 30 nm fiber, and the least condensed region shows only the 10 nm

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 (iii) between adjacent chromosome territories (very rare). **(d)** Chromatin may form a 30 nm fiber with a solenoid zigzag, or polymer melt organization (see text). **(e)** Chromatin is resolved as a 10 nm 'beads on a string' fiber consisting of nucleosomes.

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 5 kb 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 30 nm chromatin fiber which became decondensed upon transcriptional activation. By 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 10 nm 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 10 nm fiber, but did not exhibit any evidence for a 30 nm or higher order chromatin organization [25••]. Therefore, rather than being ordered into a 30 nm 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] (Figure 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 30 nm 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 30 nm 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 10 nm fiber probably 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, and does not support the presence of a 30 nm fiber [29]. A seminal study by Dekker and colleagues provided a model of the local chromatin environment of normal human lymphoblasts