Large-scale chromatin structure and function

Andrew S Belmont*, Steffen Dietzel, Anne C Nye, Yuri G Strukov, and Tudorita Tumbar

Recent results in living cells have now established the existence of levels of chromatin folding above the 30 nm fiber within interphase chromosomes. We discuss the potential functional impact of this large-scale chromatin organization, including its possible role in regulating gene expression.

Addresses
Department of Cell and Structural Biology, University of Illinois, Urbana-Champaign, B107 CLSL, 601 South Goodwin Avenue, Urbana, IL 61801, USA
*e-mail: asbel@uiuc.edu

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Abbreviations

BrUTP	bromouridine triphosphate
ES cells	embryonic stem cells
FISH	fluorescence *in situ* hybridization
GFP	green fluorescent protein
HMG protein	high mobility group protein
LCR	locus control region
SAR/MAR	scaffold-associated region/matrix-associated region
SMC	structural maintenance of chromosomes

Introduction

In the typical textbook model of chromatin structure, DNA folds around nucleosomes, nucleosomes form 10 nm fibers which fold helically into 30 nm chromatin fibers, and these 30 nm fibers form loops organized radially from a prophase chromosome axis that coils to form the fully condensed metaphase chromosome [1].

Several points should be stressed concerning this textbook model. First, there exists a hierarchy in the extent of our knowledge, which parallels the actual chromatin structure hierarchy. Most poorly understood is the large-scale chromatin folding of 10 and 30 nm chromatin fibers into interphase and mitotic chromosomes. Second, this textbook model describes mitotic chromosome organization because it is derived largely from experiments on metaphase chromosomes. Few experiments have directly examined interphase chromosome structure. Third, the basic motivation underlying the investigation of these different levels of chromatin organization has been the expectation of a strong relationship between chromatin structure and DNA function. This expectation has been validated recently for nucleosome structure and spacing with, for instance, the discovery of transcription factors and repressors as components of chromatin modifying activities [2]. For large-scale chromatin structure this expectation has been strengthened by many observations but has not yet been proven.

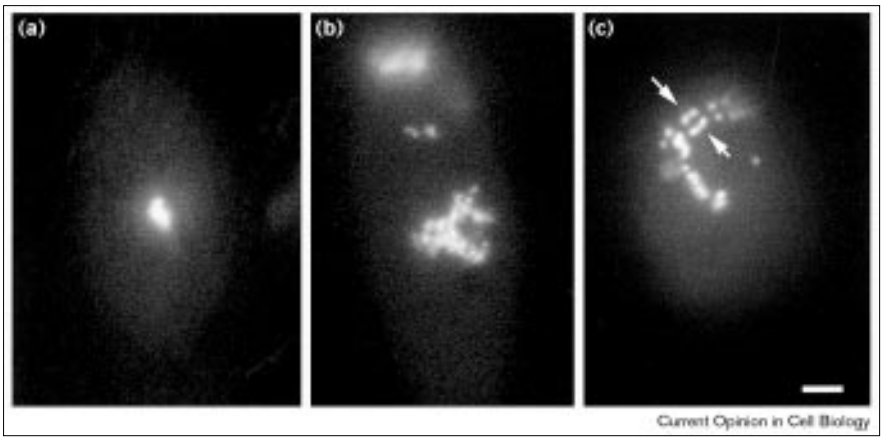
Here we review recent observations demonstrating the compaction of interphase chromatin into stable, larger-scale structures well above the level of the 30 nm chromatin fiber in compaction ratio. We discuss models for this large-scale chromatin organization and evidence for differential compaction in different chromosome regions. We then describe recent experiments implying functional connections between large-scale chromatin organization and regulation of transcription and replication. Finally, we briefly discuss the possible connections between *cis*-acting sequences — such as SAR/MARs (scaffold-associated regions/matrix associated regions), locus control regions (LCRs), and boundary elements — and the establishment of distinct, large-scale chromatin domains. Related topics, concerning nuclear architecture and subnuclear organelles, nuclear matrices or scaffolding, and arrangement and mobility of interphase chromosomes relative to nuclear structure, have been reviewed elsewhere [3–6].

In vivo confirmation of interphase large-scale chromatin organization

Correlative light and electron microscopy indicated the existence of discrete, large-scale chromatin fibers formed by the folding of 10 nm and 30 nm chromatin fibers and with estimated packing ratios of hundreds to thousands [7–9]. Fluorescence *in situ* hybridization (FISH) directly measured packing ratios in this range [10] and demonstrated that interphase chromosomes formed compact, well-defined territories [11]. Recent *in vivo* imaging more clearly demonstrates this large-scale chromatin organization. HeLa cells constitutively expressing a green fluorescent protein (GFP)-tagged histone H2B show similar fine nuclear chromosomal substructure [12•] to that observed previously by DNA staining of aldehyde fixed cells [7]. Microinjection of fluorescent nucleotides followed by long chases of several cell generations with random chromosome segregation produced cells with a small number of spatially distinct, labeled compact chromosome territories [13••]. Subchromosome regions measuring ~0.2–0.4 μm in diameter were visualized.

Combining tagging of chromosome sites — by engineering cell lines that express GFP-tagged lac repressor proteins which bind to introduced lac operator direct repeats — with gene amplification has allowed selective visualization of small, amplified chromosome regions in living cells [14,15••]. Different cell clones contain amplified chromosome regions of characteristic condensation, ranging from highly condensed to highly extended (Figure 1). The most typical conformation shows confinement to compact territories but with obvious internal substructure suggestive of fibrillar folding. Immunogold staining and electron microscopy revealed ~100 nm large-scale chromatin fibers,

Figure 1



Visualization of amplified chromosome regions using lac operator arrays and GFP-tagged lac repressor binding. Different cell clones after gene amplification show amplified chromosome regions of characteristic condensation ranging from **(a)** highly condensed to **(c)** highly extended. Most clones show an appearance similar to **(b)** in which the amplified chromosome regions are compact but show internal substructure suggestive of fibrillar folding. (a–c) are images from living cells expressing the GFP-tagged lac repressor. No image processing was used other than reducing contrast to enhance the low nuclear background fluorescence. The clone shown in (c) shows highly extended fibers or fiber segments. In some cells, symmetrical staining patterns (arrows) suggest paired sister chromatids. Scale bar = 2 μm.

similar to the large-scale chromatin organization of bulk chromatin [14]. Significantly, distinct fibers could be unambiguously traced for over 5 μm in length in living cells by light microscopy, providing a striking demonstration of the existence of large-scale chromatin fibers.

Direct visualization of extended large-scale chromatin fibers *in vivo* revealed changes in length of less than 15% over periods of several hours (G Li, A Belmont, unpublished data), thus demonstrating stability of large-scale chromatin folding. Small, local conformational changes were seen and frequently appeared to occur though bending or coiling of pre-existing structures. A general immobility of interphase chromosomes has been observed (as reviewed elsewhere [6]). Subchromosomal domains in general were stable with respect to shape and condensation over periods of several hours [13••].

Models of large-scale chromatin folding

Structural analysis has led to the proposal of four models of large-scale chromatin organization. The differences between these four models reflect the technical difficulties associated with investigating large-scale chromatin structure and the uncertainties introduced by various extraction and preparation methods traditionally used to study chromosome structure.

A giant-loop, random-walk model was proposed for interphase chromosomes based on statistical analysis of the mean separation between two chromosome sites as a function of genomic distance [16]. Numerical modeling has shown the compatibility between these measurements and polymer based models that include large-scale chromatin organization above the 30 nm fiber level [17•]. A well known radial-loop, helical-coil model of mitotic chromosome structure [18,19] has been extended to interphase chromosomes by postulating a particular loop geometry that might, under certain circumstances, give rise to a fiber, with an elliptical 60–90 nm cross-section [20,21].

A third model proposes a successive, helical coiling of 10 nm fibers into 30–50 nm diameter tubes and of these into 200 nm diameter tubes, which coil into ~600 nm metaphase chromatids [22].

Finally, a folded chromonema model is based on *in vivo* light microscopy combined with transmission electron microscopy ultrastructural analysis of folding intermediates during the transition into and out of mitosis. In this model an ~100 nm diameter chromonema fiber folds into a 200–300 nm diameter prophase chromatid, which coils to form the metaphase chromosome [8,9,14,15••].

Differential large-scale chromatin compaction and functional correlations

If the large-scale level of chromatin organization plays an important functional role other than general chromosome condensation, we would expect to see reproducible folding differences for specific chromosome loci. We have already mentioned reproducible differences in compaction for various amplified chromosome regions (Figure 1); however, the cause and significance of these differences remain unknown.

There are long-standing observations of heterochromatin associated condensation and reduced nuclease and methylase accessibility for different chromosome regions. Attributing this variation in enzyme accessibility to the correct level(s) of chromatin folding has been problematic. Comparison of inactive and active X chromosome domains using FISH revealed characteristic differences in geometry but not volume with the active X chromosome territory showing a more convoluted and larger surface area [23]. This result is difficult to reconcile with previous light and electron microscopy showing denser staining, implying a

smaller volume, for the inactive X chromosome. Structural changes associated with DNA denaturation or measurement errors related to light microscopy resolution limitations possibly could obscure actual volume differences. These caveats not withstanding, this experiment does place constraints on the differences in structure of active and inactive X chromosome and rules out significant volume changes of several fold or more.

Interestingly, dosage compensation in *C. elegans* involves an SMC (structural maintenance of chromosomes) complex containing components shared with the mitotic SMC condensin complex. This implies that there is a possible functional link between interphase chromosome condensation and transcriptional repression [24••]. FISH measurements of interphase distances between pairs of probes separated by 0.1–1.5 mega basepairs (Mbp) did show compaction differences in human fibroblasts for two different R and G banded regions, with the gene enriched R bands showing less compaction [25••]. A dramatic extension of large-scale chromatin fibers — including the decondensation of a ~90 Mbp heterochromatic chromosome arm into a ~30 μm long chromonema fiber — has been seen after targeting of the acidic activation domain of VP16 via a lac repressor fusion protein to amplified chromosome regions containing large numbers of lac operator repeats (T Tumbar, G Sudlow, A Belmont, unpublished data). This targeting was accompanied by transcriptional activation but the extension of large-scale chromatin fibers was not dependent on RNA polymerase II activity. The dramatic conformational changes in this artificial system may represent an exaggeration of a natural physiological response that would normally be localized to a smaller region surrounding endogenous loci during transcriptional activation.

Large-scale chromatin organization might affect transcription by presenting an accessibility barrier to large protein complexes. An interchromosome domain compartment (ICD) model was proposed in which active genes map to the surface of chromosome territories [11]. This was motivated by the uniform, compact appearance of chromosome territories by FISH and by the preferential distribution towards the periphery of chromosome territories demonstrated so far for a small number of genes [26]. The idea of a compact chromosome territory that might limit accessibility is contradicted, however, by the actual chromatin distribution observed at higher resolution by both light and electron microscopy [9]. More recently, a chromosome painting study that combined confocal microscopy and deconvolution, revealed considerable internal substructure within chromosome territories that is more compatible with previous ultrastructural work (PJ Verschure, I van der Kraan, R van Driel, personal communication). Moreover, sites of transcription, marked by incorporation of BrUTP, occurred at the edge or between chromosome subdomains within a chromosome territory. These results are more consistent with previous electron microscopy localization of uridine and BrUTP incorporation, which showed heavy labeling at the edge of condensed, large-scale chromatin domains [27–29], and a recent light microscopy FISH study, which showed BrUTP incorporation within the interior of chromosome territories [30•]. If there are accessibility limitations these are more likely to map to the periphery of large-scale chromatin fibers, or larger chromosome domains formed by the folding of these fibers, rather than to the surface of chromosome territories.

Dramatic changes in overall nuclear morphology and intranuclear DNA distribution accompany differentiation, mitogen stimulation, and transformation, although the functional significance of these changes remains unclear. Cell cycle changes in chromatin structure have been well documented, although most work has focused on lower levels of organization. Because of the tremendous heterogeneity in large-scale chromatin compaction between different chromosomal regions within the same nucleus, it has never been obvious how tightly correlated structural changes in large-scale chromatin organization are to cell cycle progression. Examining the cell cycle dynamics of a heterochromatic, amplified chromosome arm has demonstrated a precise choreography of changes in large-scale chromatin organization, which is tightly correlated with DNA replication [15••]. Cell cycle variation in overall compaction of individual chromosome arms, estimated by the distance between proximal and distal loci, was demonstrated by FISH in *Drosophila* larval interphase nuclei [31••].

Cis-acting sequences and propagation of changes in large-scale chromatin organization

The regulation of large-scale chromatin structure is believed to be mediated by *cis*-acting sequences capable of acting over tens to hundreds of kilobases. Potential candidate sequences include SAR/MAR sequences, boundary elements, enhancers that interact with distant promoters, and LCRs; all have been reviewed elsewhere [32–35]. These sequences have been defined largely through biochemical and functional assays and postulated as having a structural role.

Recent studies have provided further evidence for long-range propagation of chromatin changes involving *cis*-acting sequences. For example, there is now evidence of additional sequences causing increased chromatin accessibility located even further upstream than the known LCR sequences in the mouse and human β-globin loci [36••,37••]. Silencing of transgene repeats occurs as an all or none event, suggesting a cooperative effect which might involve changes in chromatin structure over distances exceeding 100 kilobases; insulator sequences from the chicken β-globin gene prevent this silencing and might act as boundaries to propagation of distinct chromatin structures [38••]. A synthetic protein designed to bind tightly to SAR/MAR sequences has been shown to inhibit mitotic chromosome condensation [39] and to reduce position effect variegation spreading from a SAR rich block of heterochromatin [40•]. Interestingly, *in vivo* cross-linking experiments indicate a broad distribution of binding sites

for two homeodomain transcription factors throughout their genetic targets [41]. This raises the possibility that an 'open' large-scale chromatin state might be created by distribution of transcription factor binding regions over a large DNA region. In fact, the globin LCR, consisting of widely separated DNase1 hypersensitive sites, containing clusters of transcription factor binding sites, might represent one specific example of a more general phenomenon.

Recent molecular studies, however, have led to alternative explanations for the action of these *cis* sequences that are not directly related to large-scale chromatin structure. Enhancers and/or LCRs might interact directly with promoters by DNA looping without a requirement for special chromatin structures. SAR/MAR sequences stimulate transcription and have been shown to interact with enhancers to increase local chromatin accessibility [42]. Boundary sequences have been proposed to act as promoter decoys rather than boundaries to propagation of distinct chromatin structures [43]. Direct testing of the proposed structural roles of these *cis*-acting sequences is needed to resolve these conflicting models.

Conclusions

Although it is now clear that there exists a stable, large-scale chromatin structure within interphase nuclei, the structural details and functional significance of this level of chromatin organization remain to be established. Experiments that go beyond correlative studies and directly test the causal relationship between large-scale chromatin organization and function are needed. Investigation into the basic ultrastructural motifs underlying this large-scale chromatin organization must be integrated with studies of chromosome biochemistry, including the molecular function of SMC complexes involved in mitotic chromosome condensation, dosage compensation, and sister chromatid cohesion [44], and the possible action of chromatin remodeling complexes [45] at this level of chromatin organization. New methods for directly assaying large-scale chromatin compaction and ultrastructure need to be applied to specific chromosomal loci to allow identification and characterization of *cis* and *trans* factors regulating large-scale chromatin organization. Finally, most structural studies of large-scale chromatin organization have focused on a small number of transformed, immortal cell lines in culture. Studies that examine changes in large-scale chromatin organization during cell differentiation within whole organisms will be valuable in establishing the functional significance underlying this level of chromatin architecture, especially when coupled to genome projects which will establish gene arrangement and expression patterns as a function of chromosome location.

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