Higher-order structure of chromatin and chromosomes

Christopher L Woodcock* and Stefan Dimitrov†

The linear array of nucleosomes that comprises the primary structure of chromatin is folded and condensed to varying degrees in nuclei and chromosomes forming 'higher order structures'. We discuss the recent findings from novel experimental approaches that have yielded significant new information on the different hierarchical levels of chromatin folding and their functional significance.

Addresses
*Biology Department, University of Massachusetts, Amherst, Massachusetts 01003, USA; e-mail: chris@bio.umass.edu
†INSERM U309, Institut Albert Bonniot, Domaine de la Merci, 38706 La Tronche Cedex, Grenoble, France; e-mail: stefan.dimitrov@ujf-grenoble.fr

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Abbreviations
3D three-dimensional
FRAP fluorescence recovery after photobleaching
GFP green fluorescent protein

Introduction

It is becoming increasingly clear that chromatin higher-order structure (i.e. organization beyond the level of the linear array of nucleosomes) plays a critical role in many aspects of gene regulation (e.g. see [1]), perhaps extending even to complex processes such as aging [2]. Moreover, many large-scale and local chromatin-remodeling events involve modulations of the charge balance between histones and DNA [3–7], which induce changes in chromatin compaction. A full understanding of these manifestations of chromatin 'higher-order structure' and their functional significance will require knowledge of the 3D arrangement of components and the mechanisms and dynamics of their assembly and disassembly.

To simplify the discussion, we propose a new hierarchical classification scheme for chromatin based loosely on that used for proteins, in which all levels above the primary structure constitute a form of 'higher order' (Table 1). Until more sequence-specific information is known, it is necessary to differentiate at all levels between global structures (cases where the underlying DNA sequence is not known, and the structural information is generic), and local structures (cases where the underlying DNA sequence and perhaps nucleosome positioning is defined, and the structural information is specific). The scheme allows further expansion as needed — for example, the quaternary level may be required for metaphase chromosomes.

This review focuses on the significant progress that has been reported recently in a few selected areas, concentrating on chromatin secondary structures and mitotic chromosome architecture. Larger-scale chromatin organization and dynamics in the interphase nucleus, representing tertiary and perhaps higher levels, has been reviewed recently [8–12].

Global secondary structures – conformation of arrays of H1-containing nucleosomes

The majority of work on global secondary structures has focused on the '30 nm' chromatin fiber — a ubiquitous conformation adopted, at least *in vitro*, by arrays of nucleosomes containing H1-type linker histones. Evidence concerning the bulk physical and biochemical properties of isolated chromatin together with direct imaging of individual assemblies has led to two principal concepts of fiber architecture: solenoids in which the linker DNA continues the supercoil established in the nucleosome, and zig-zag or crossed linker models in which the linker crosses the fiber (reviewed in [13–18]). Hydrodynamic studies of defined chromatin arrays have helped establish the critical role of the core histone amino termini in the formation of secondary structures, and have clearly demonstrated that chromatin folding, at least *in vitro*, should be viewed in terms of a dynamic equilibrium between compaction levels ([19]; Hayes and Hansen, this issue [pp 124–129]).

Table 1

Proposed hierarchical classification scheme for chromatin structures.

Level of chromatin structure	Examples of global structures	Examples of local structures
Primary – The linear arrangement of features such as nucleosomes on DNA.	The nucleosome repeat length.	Preferred locations of nucleosomes and features such as DH sites on a specific DNA sequence (e.g. [30,73]).
Secondary – Structures formed by interactions of nucleosomes.	The '30 nm' chromatin fiber.	3D architecture of nucleosomes and regulatory proteins on a specific DNA sequence (e.g. [31,33,36•]).
Tertiary – Structures formed by interactions between secondary structures.	Thicker fibers seen in nuclei and postulated to be composed of 30 nm fibers.	Long-distance contacts possibly involving locus control regions, enhancers and promoters [74], or looped chromatin domains [75].

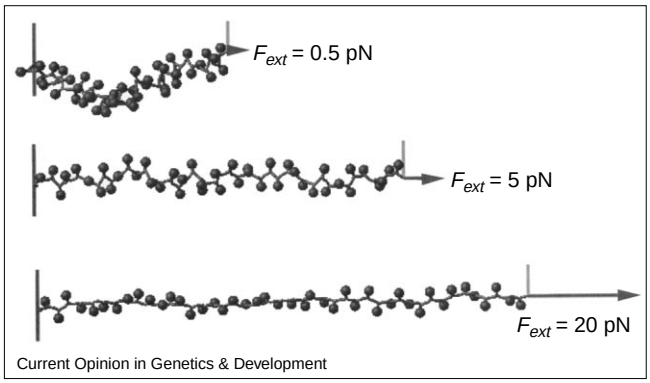
In terms of the architecture of chromatin secondary structures, recent new approaches, coupled with sophisticated modeling, have established constraints on possible chromatin architectures. Rydberg *et al.* [20••] have utilized the physics of DNA breakage by ionizing radiation in which a single hit results in a shower of secondary particles that induce spatially correlated single-strand breaks. Secondary hits thus tend to concentrate within nucleosomes that are neighbors in 3D space and the observed lengths of single-stranded DNA fragments can be compared with predictions of model structures. After irradiating living cells, the predicted major peak of DNA fragment size occurred at 78 bases resulting from two hits within a single nucleosome. Of more interest in terms of chromatin secondary structure was the distribution of DNA fragments in the 300–1000 base range, and comparison of these data with theoretical predictions based on generic solenoid and zig-zag models. The fragment sizes showed a remarkably good fit to predictions of zig-zag secondary structures but the predicted peak at ~1000 bases corresponding to one turn of a six-nucleosome/turn solenoid was not observed. Control experiments showed that permeabilized cells exposed to low salt showed no peaks corresponding to chromatin secondary structures, confirming that this treatment effectively abolishes all but the primary structure.

The fact that these experiments can be performed on living cells makes the strategy especially significant. It is well established that chromatin conformation is greatly influenced by the ionic milieu, yet the *in nucleo* levels of cations and polyamines are not known with any certainty. Thus, any congruence between *in vivo* and *in vitro* data is particularly valuable.

In a completely different approach, Cui and Bustamente [21••] used molecular tweezers (capture and manipulation by laser beam) to grasp the ends of isolated chromatin fibers and document force–length relationships during stretching and relaxation. Molecular modeling based on these data, the known mechanical properties of DNA, and the structure of the chromatosome [22••], converged on an irregular, fluctuating zig-zag structure (Figure 1), similar to that predicted from electron cryo-microscopy [23••]. An independent modeling study [24] starting from the two-angle zig-zag model [25], derived mechanical properties similar to those measured in [21••]. Data from atomic force microscopy also support a zig-zag secondary chromatin structure [16,17] and the instrument can, in principle, also be used to generate force–length relationships of chromatin fibers. To date, however, the forces recorded using atomic force microscopy [26] have been much larger than those measured with molecular tweezers or predicted by theory, perhaps because of poorly understood interactions between chromatin and substrate.

Importantly, the force–length relationships reported by Cui and Bustamente [21••] reveal a weak inter-nucleosomal attraction which allows chromatin to become highly compact at intermediate salt concentrations. Identifying the molecular basis of this attraction, which may involve the core histones [27] and/or occur between histone amino termini and linker DNA (Hayes and Hansen, this issue [pp 124–129]), will be an important future goal.

Figure 1



Models of chromatin secondary structure at different levels of extension (F_{ext}), based on force–distance measurements of individual chromatin fibers. (F_{ext} is the extension force applied.) Modified from [22••].

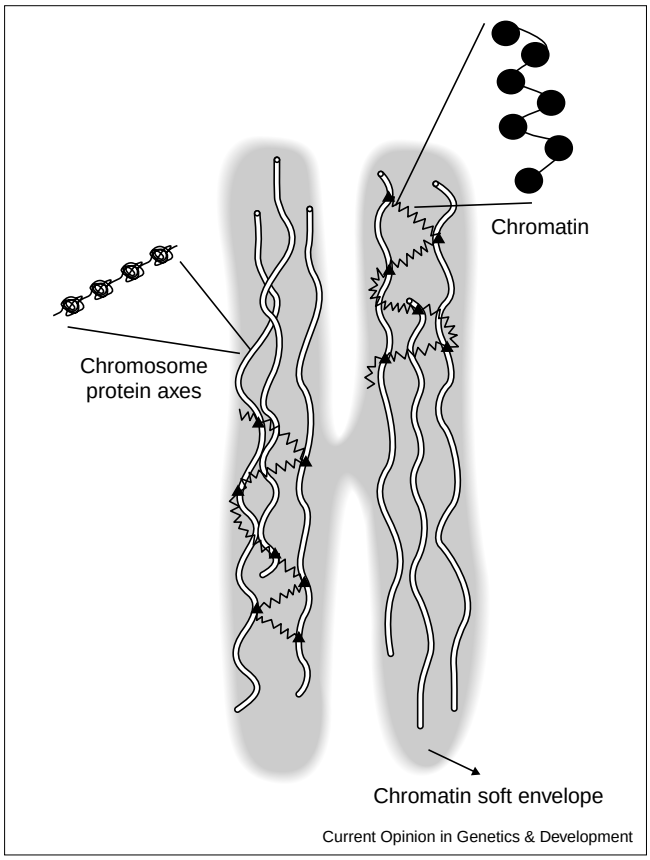
At present, the weight of evidence from these new approaches, and also from detailed analyses of the products of chromatin digestion *in nucleo* [28] favor a zig-zag conformation. It is clear that native chromatin can have a more extreme compaction level than predicted from simple close-packing of 30 nm fibers [29•] — whether this results from an altered secondary chromatin structure in the highly compact state, or from a tertiary structure, perhaps involving the side-to-side interdigitation of 30 nm fibers [15], remains to be seen.

Local secondary structures

Chromatin-mapping studies of individual genes have revealed very specific local primary structures comprising positioned nucleosomes, DNase I hypersensitive sites, and binding sites for regulatory proteins and complexes (e.g. see [30,31]). It appears likely that in many cases the functional unit is not the linear array of elements but a local secondary chromatin structure in which one or more nucleosomes, together with regulatory and/or transcriptional complexes, form a distinct 3D assemblage in the nucleus (e.g. see [32,33]).

A full understanding of such units will require a 3D molecular level model of the local chromatin structure and its modulations and will probably emerge, as in other fields [34], through the fitting of X-ray structures of individual components into envelopes derived from lower-resolution microscopy techniques. This type of approach requires the isolation from bulk chromatin of the nucleosomal array in question, and techniques for achieving this have recently been developed. Site-specific recombination in yeast was used to produce circular chromatin

Figure 2



A model for the structure of mitotic chromosome based on elasticity measurements. The chromosome contains a few rigid axes to which the 'soft' chromatin is anchored. The axes are very thin (<20 nm), have a great latent length, and are built of proteins or protein complexes with elastic properties similar to that of titin (i.e. formed of repetitive domains), which can be unfolded upon application of force. Potential candidates are titin itself [62•] and SMC (structural maintenance of chromosomes) complexes.

arrays containing the silent *HMR* locus which retained associated Sir proteins and a repressive chromatin structure *in vitro* [35]. Another successful yeast strategy was the use of minichromosome plasmids containing a portion of the *STE6* gene which is differentially expressed according to mating type. In minichromosomes isolated from the repressed α strain, the Tup1p corepressor was confined to the *STE6* nucleosomes, each of which contained two molecules [36•]. With X-ray structures of the nucleosome core particle [27] and large portions of Tup1p and its corepressor Ssn6 published [37–39], all the factors required for the goal of relating repression to a specific chromatin secondary structure are available. Reconstitution in *Drosophila* embryo extracts has been used successfully to create MMTV (mouse mammary tumor virus) promoter chromatin *in vitro* [40•]. The reconstituted material bound glucocorticoid receptor, inducing ATP-dependent chromatin remodeling. These advances will most likely lead to a better understanding of unique sequence chromatin secondary structures.

Structure of mitotic chromosomes

Despite the efforts of generations of cell biologists, the basic architecture of mitotic chromosomes as well as the hierarchical level of chromatin structure they represent (see Table 1) are poorly understood. Significant advances in our knowledge of mitotic chromosome condensation and structure, however, have recently come from two complementary approaches: yeast genetics and biochemical manipulations of mitotic extracts prepared from *Xenopus* eggs. This has led to the discovery of new macromolecular complexes that play a fundamental role in chromosome assembly and to a novel view of mitotic chromosome structure. Here we briefly describe the condensin complex (for a recent review, see [41]) and focus on the role of histone amino termini in chromosome assembly and on newly developed biophysical techniques for studying chromosome structure.

Chromosome condensation: the condensin complex

A pivotal finding regarding mitotic chromosome condensation came from the identification of the condensin complex [42,43] as a key player in the process. Condensin is required for proper chromosome condensation and segregation [43–45] and its property of inducing ATP-dependent positive supercoiling in closed circular DNA [46,47•] has led to the suggestion that chromosome condensation results from the generation of a global positive writhe. At present, however, the mechanism by which condensin acts on the chromatin template is unclear.

Core histone amino termini, but not histone H1, are essential players in mitotic chromosome condensation

Linker histone H1 is heavily phosphorylated at the beginning of mitosis and dephosphorylated after anaphase, suggesting that the histone and its phosphorylation state could be causally involved in chromosome compaction [48]. Neither chromosome condensation nor nuclear assembly are affected by the absence of linker histones in either *Xenopus* extracts [49,50], or *Tetrahymena* [51], however, thus arguing against a causal role of histone H1 in these processes. Rather, the core histone amino termini appear to play a critical role in chromosome condensation [52]. Chromosome assembly in *Xenopus* extracts was inhibited by adding intact but not 'tailless' exogenous nucleosomes to the reaction, the efficiency of inhibition being different for each individual histone tail. These data suggest that chromosome assembly factors are recruited by nucleosomes through the histone tails. Neither topoisomerase II nor condensins were found associated with exogenous nucleosomes, indicating that other critical assembly factors remain to be identified.

Mitotic chromosome condensation requires phosphorylation of histone H3 tail at serine 10

Histone H3 exhibits site-specific phosphorylation at serine 10 during mitosis [53]. The use of phosphoepitope-specific antibody has demonstrated that this histone H3 modification is coupled tightly to the initiation of chromosome

condensation but is not required for the maintenance of chromosome compaction [54]. Moreover, a causal relationship between histone H3 phosphorylation and chromosome condensation has been identified in *Tetrahymena* [55••]. A mutant in which histone H3 cannot be phosphorylated exhibited abnormal chromosome condensation and segregation, demonstrating that phosphorylation of histone H3 at serine 10 is required for proper chromosome dynamics. A second mitotis-specific phosphorylation site at serine 28 of histone H3 was identified recently [56] but its role has yet to be determined. Two candidate kinases responsible for the mitotic phosphorylation of histone H3 serine 10 have been proposed: the NIMA kinase [57] and the Ipl1/aurora kinase [58].

Mechanical properties of chromosomes

Promising biophysical approaches for quantifying the elasticity properties of mitotic chromosomes have been developed recently, providing important insights into their underlying structure. Using a novel micropipette technique [59,60], mitotic chromosomes in cultured newt lung cells were found to be highly extensible objects, exhibiting reversible deformation upon stretching up to 10 times their original length. Further informative studies have used 'chromosomes' assembled in *Xenopus* extracts in which it was possible to measure both the longitudinal deformability and the bending rigidity of individual chromosomes [61••]. These chromosomes also were found to be highly extensible — they could be stretched up to 100 times their original length without disruption. They were also very flexible, the measured chromosome persistence length being only a few fold larger than the diameter. The relationship between the measured longitudinal deformability and the bending rigidity was remarkable in that chromosome rigidity was 2000 times less than that calculated from the experimental force–extension curve. These data best fit a model in which chromosomes are constructed of thin elastically-deformable rigid axes surrounded by a soft chromatin envelope (Figure 2). The elastic properties of the axes can be approximated by titin-like molecules and genetic evidence has been presented indicating that mutations in a *Drosophila* titin homolog disrupt chromosome condensation and mitosis [62•]. Native and *in vitro* assembled chromosomes had very similar elastic properties, suggesting an essentially identical underlying structure, quite different from the widely discussed scaffold loop [63] and hierarchical helical folding [64] models of chromosome structure.

Conclusions

Further application of the novel approaches discussed above promises to inspire a fresh look at many aspects of chromatin secondary and tertiary structure. Problems such as local secondary structure and chromosome architecture for which no incisive techniques were previously available, now appear amenable to rapid advances. As we progress from an essentially one-dimensional concept of nucleosomal arrays with bound regulatory complexes to a higher-resolution 3D view, it will be essential to consider nuclear structures and events in the context of the conformational dynamics of mononucleosomes [65] and polynucleosomes (see Hayes and Hansen, this issue [pp 124–129]). The extremely rapid flux of nuclear components that is now becoming apparent from *in vivo* observations [66,67•,68] is a further indication that chromatin should be viewed in terms of its dynamic equilibrium system rather than as a static structure. Modeling, especially using time-resolved approaches [22••,69] will become increasingly important in this endeavor, and is now being creatively applied to tertiary [70–72] as well as secondary chromatin structures.

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