New Insight into the Mitotic Chromosome Structure
Irregular Folding of Nucleosome Fibers Without 30-nm Chromatin Structure

K. MAESHIMA,1,2 S. HIHARA,1,2 AND H. TAKATA1
1Biological Macromolecules Laboratory, Structural Biology Center, National Institute of Genetics, Mishima, Shizuoka, 411-8540 Japan; 2Department of Genetics, School of Life Science, Graduate University for Advanced Studies (Sokendai), Mishima, Shizuoka, 411-8540 Japan

Correspondence: kmaeshima@lab.nig.ac.jp

Mitotic chromosomes are essential structures for the faithful transmission of replicated genomic DNA into two daughter cells during cell division. A long strand of DNA is wrapped around a core histone and forms a nucleosome. The nucleosome has long been assumed to be folded into 30-nm chromatin fibers. However, how the nucleosome or 30-nm chromatin fiber is organized into mitotic chromosomes remains unclear, although condensins and topoisomerase IIα are implicated in the condensation process. In fact, what do mitotic chromosomes look like in living cells? When frozen hydrated human mitotic cells were observed using cryo-electron microscopy (cryo-EM), higher-order structures including 30-nm chromatin fibers were not found. We thus propose that the nucleosome fibers in the bulk of mitotic chromosomes do not form 30-nm chromatin fibers but instead exist in a highly irregular state that is locally similar to a polymer melt. We provide new insight into mitotic chromosome structure.

The human body is made up of 60 trillion cells that originate from a single fertilized egg. This means that the cells in the body go through ~46 rounds of division (\(2^{46}\) to \(70 \times 10^{12}\)). During the cell divisions (mitosis), chromosomes (compact bundles of DNA) are formed to ensure the faithful transmission of the replicated genomic DNA. The term “chromosomes” was derived from the Greek for “colored body,” reflecting the fact that condensed chromosomes were clearly visible with dyes under a light microscope. Chromosomes have fascinated biologists for more than 100 years, since long before DNA was known to carry genetic information (Olins and Olins 2003). How are the 2 m of genomic DNA in each human cell organized into compact mitotic chromosomes that are 10,000 times shorter? This chapter focuses on the structure of mitotic chromosome with a novel view.

DNA, NUCLEOSOME, AND 30-NM CHROMATIN FIBER

DNA has a negatively charged phosphate backbone that causes electrostatic repulsion between adjacent DNA regions. This property makes it difficult for DNA to fold on itself (Bloomfield 1996; Yoshikawa and Yoshikawa 2002). As shown in Figure 1, for the primary level of folding, a negatively charged DNA molecule is wrapped around the basic core histone octamer, which consists of the two sets of histone H2A, H2B, H3, and H4 proteins, and forms a nucleosome structure (Kornberg and Lorch 1999). The structural details of the nucleosome core are now known at a resolution of 1.9 Å (Davey et al. 2002). In the nucleosome core particle, 147 bp of DNA is wrapped in 1.7 left-handed superhelical turns around the histone octamer. Each nucleosome core is connected by linker DNA. Therefore, the nucleosome fibers were originally described as “beads on a string” (Olins and Olins 2003). Although the core histones have tails with positively charged lysine and arginine residues, only ~60% of the DNA negative charges are neutralized (Strick et al. 2001). Consequently, for further folding, the remaining ~40% of the DNA charge has to be neutralized by other factors, such as linker histone H1 or cations, as we discuss later.

More than 30 years ago, the proposal was made that the nucleosome fiber is folded into “30-nm chromatin fibers” with linker histone H1 or Mg++ ions (Finch and Klug 1976). In fact, purified nucleosomes resemble fibers with a diameter of 30 nm under conventional transmission EM. In the model called the “solenoid,” consecutive nucleosomes are located next to one another in the fiber, folding into a simple one-start helix (Fig. 1, see also Fig. 2A,C). Later, a second chromatin-fiber model of the “two-start helix” was proposed based on EM observations of purified nucleosomes (Fig. 2B,D) (Woodcock et al. 1984). Although some variations exist in this two-start helix model (Bassett et al. 2009), nucleosomes are arranged in a zigzag manner such that a nucleosome in the fiber is bound to the second neighbor but not to the first (Fig. 2B,D).

What caused this difference (Fig. 2)? The Rhodes group demonstrated that the solenoid or zigzag mode of arrangements is defined by the length of the nucleosomal linker DNA (Routh et al. 2008). More recently, Grigoryev et al. (2009) showed that the one-start solenoid and two-start zigzag modes may be simultaneously present in a 30-nm chromatin fiber under certain conditions. The structural
details of the 30-nm chromatin fiber thus remain controversial, although the nucleosome has long been assumed to be folded into 30-nm chromatin fibers before the higher-order organization of mitotic chromosomes or interphase nuclei occurs (Alberts et al. 2007). Only a typical one-start helix (solenoid) model is shown (see also Fig. 2). However, such continuous 30-nm chromatin fibers in native chromosomes are not observed by cryo-EM (Fig. 3A, below), as described in the text. (Reprinted with minor modifications, with permission, from Maeshima et al. 2010 [©Elsevier].)

**UNIFORM TEXTURE OF MITOTIC CHROMOSOMES: IRREGULAR FOLDING?**

What do mitotic chromosomes look like in living cells? One of the best ways to answer this question is by using cryo-EM (Dubochet et al. 1988). Cryo-EM is based on “frozen hydration” by rapid cooling, which ensures immobilization of all macromolecules and small molecules in the sample in a close-to-native state. After sectioning of the samples, the thin, frozen hydrated sections are directly observed under a cryo-EM with no chemical fixation or heavy metal staining. This approach enables direct high-resolution imaging of cell structures in a close-to-native state.

More than 20 years ago, the Dubochet group first observed sections of mammalian mitotic cells using cryo-EM (McDowall et al. 1986). Unexpectedly, the chromosomes showed a homogeneous and grainy texture with ~11-nm spacing (see also Fig. 3A) (McDowall et al. 1986). Positions from the first (N1) to eighth (N8) nucleosome are labeled. (C) In the one-start helix proposed by the Rhodes group, the 30-nm chromatin fiber is an interdigitated solenoid. Essentially, a nucleosome in the fiber interacts with its fifth and sixth neighbors (Robinson and Rhodes 2006). (Blue and orange) Alternative helical gyres cores. (D) In the two-start model proposed by Richmond et al. (Schalch et al. 2005), nucleosomes are arranged in a zigzag manner such that alternate nucleosomes form interacting partners. A nucleosome in the fiber binds to the second-neighbor nucleosome. (Blue and orange) Alternate nucleosome pairs. (Reprinted with minor modifications, with permission, from Maeshima et al. 2010 [©Elsevier].)
To overcome this problem, in collaboration with Eltsov, Frangakis, and Dubochet, we performed a cryo-EM study with compensation for the signal distortions caused by the CTF. For this purpose, several images of the same area were taken at different defocusing values and merged into a single image (Eltsov et al. 2008) to minimize the effects of CTF (Conway and Steven 1999). Even after the CTF correction, we still could not find apparent 30-nm structures detectable in the chromosome areas. In addition, the power spectra (Fourier transform) of the chromosome and cytoplasmic regions showed that an 11-nm peak, but not a 30-nm peak, was prominent in the chromosome region. Our cryo-EM study thus suggests that the 30-nm chromatin fiber is almost absent in the mitotic chromosomes (Eltsov et al. 2008). Consistent with our findings, König et al. (2007) reported the absence of continuous fiber-like structures, such as 30-nm fibers, in Xenopus chromosomes that were assembled in vitro and observed using cryo-substitution and EM tomography.

**CLASSICAL VIEW: ARTIFACT OF SAMPLE PREPARATIONS?**

The original concept of the 30-nm chromatin fiber was based on the observation by conventional transmission EM. Why could we see the 30-nm fibers by conventional EM? The following is a possible explanation.

The formation of a 30-nm fiber requires the selective binding of nucleosomes, which are close neighbors on the DNA strand. For example, if a nucleosome binds to its first neighbor, it will give rise to a 30-nm fiber such as a typical solenoid (Fig. 1; also see Fig. 2A,C) (Finch and Klug 1976). The binding of the second neighbor will result in a two-start helix or a zigzag ribbon fiber (Fig. 2B,D) (Dorigo et al. 2004). Such intrafiber nucleosomal interactions can occur under dilute conditions, as in in vitro systems (Fig. 4A,B). Indeed, when purified mitotic chromosomes become swollen in a low-salt buffer containing 1–2 mM Mg\(^{2+}\), each chromatin fiber is well separated, and intrafiber nucleosomal interactions become dominant (Fig. 3B,D). After such fibers are fixed and embedded in plastic sections, uniform 30-nm chromatin fibers that diverged radially from the center can clearly be visualized (Fig. 3B,D).

We also assume that formation of 30-nm chromatin fibers might be stabilized through the chemical cross-links (e.g., formaldehyde or glutaraldehyde fixation) of intrafiber nucleosomal associations and further shrinkage with alcohol dehydration during sample preparation in conventional EM. Here, we would like to emphasize the general importance of this effect. As Dubochet al. (2001) pointed out, molecules and particles float and diffuse freely in the aqueous medium of living cells (Fig. 3E). After chemical cross-links such as formaldehyde fixation, molecules adhere together until they form a solid, continuous artificial structure (Fig. 3F). This effect would be facilitated by the extraction of unfixed materials such as small molecules and proteins from the cells during alcohol dehydration or permeabilization with certain detergents (Fig. 3F).

After such processing, no molecule or particle diffuses freely, only artificial structures in which molecules and particles adhere, left in the cells (Fig. 3F). Thus far, this problem has only applied to EM, which has enough resolution to visualize single molecules in cells. However, with the recent progress of super-resolution light microscopy including SIM (structured illumination microscopy), STORM (stochastic optical reconstruction microscopy), PALM (photo-activated localization microscopy), and STED (stimulated emission depletion microscopy) (Hell...
2007), this must pose a common major problem for cell biology. High-resolution imaging in a living or close-to-native state becomes increasingly important to avoid observing such artificial structures in fixed cells (Fig. 3F).

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Above, we discussed the intrafiber nucleosome association in vitro (Fig. 4A,B). However, at high nucleosome concentrations, which occur in vivo, interfiber interactions become increasingly dominant (Fig. 4C,D). Nucleosome fibers are forced to interdigitate with one another, which can interfere with the formation and maintenance of the static 30-nm chromatin fiber and lead to the “polymer melt” state (Fig. 4D).

Some supporting evidence exists for this interfiber nucleosome association. The Hayes group developed an elegant nucleosomal array system to view interfiber nucleosome associations in vitro (Zheng et al. 2005; Kan et al. 2009). They found that internucleosomal interactions via H3 and H4 tails increased significantly at >2 mM Mg++ ions. In addition, the Rippe and Wedemann group performed Monte Carlo computer simulations to pursue the effects of interfiber nucleosome associations (Stehr et al. 2008). The simulation result suggests that the lateral self-interaction of distant fiber regions and the interdigitation of nucleosome fibers indeed become significant with high interfiber nucleosome interaction energies.

NOVEL MODEL OF MITOTIC CHROMOSOME STRUCTURE

Again, how is the long strand of genomic DNA packaged into compact mitotic chromosomes (Fig. 1)? Conventional EM demonstrated that when purified chromosomes become swollen under low-salt conditions, they seem to consist of radial chromatin loops that are somehow tethered centrally by condensin and topoisomerase II (Fig. 3B,D) (Maeshima et al. 2005; see also Maeshima and Laemmli 2003). In addition, considering a structural analogy with meiotic and lambrush chromosomes, we assume that chromatin loops are the fundamental organizing unit of chromosomes (Maeshima and Eltsov 2008). However, our cryo-EM observations show a homogenous grainy texture with no higher-order structures (Fig. 3A) (Eltsov et al. 2008). What causes the different views in Figure 3A and B (see also Fig. 3C,D)?

We assume that in mitotic chromosomes, the nucleosome fibers fold irregularly toward the center, which contains abundant condensins (Fig. 3C). Therefore, the compact native chromosome would be made up primarily of an irregular nucleosome fiber network cross-linked by condensins (Fig. 3C). To make chromosomes long and rod shaped (but not spherical), the formation of an axial structure is essential. Whatever we call this axis (i.e., “scaffold,” “core,” or “glue”; Kireeva et al. 2004), condensins very likely have a primary role in its formation. In our model, first condensins bind to specific sites in the genomic DNA to make loops. Second, condensins form self-assembled structures like an axis (Fig. 3C). Indeed, an immuno-EM study of condensins showed a traceable condensing array near the center of the chromosome cross sections (Maeshima et al. 2005), suggesting the existence of an assembled structure of condensins. Finally, note that in our model, the nucleosomal fibers in native chromosomes are almost randomly oriented and locally the nucleosome fibers are “melted” (Fig. 3C).

IMPLICATIONS OF INTERPHASE CHROMATIN STRUCTURE

We next consider interphase nuclei from mitotic chromosomes. Unexpectedly, interphase nuclei in most higher eukaryote cell types examined by cryo-EM also do not contain regular 30-nm chromatin fiber (Dubochet and Sartori Blanc 2001; Bouchet-Marquis et al. 2006; Fakan and van Driel 2007). A study reported that only specific cell types have nuclei that contain many 30-nm chromatin fibers (e.g., starfish spermatozoids and chicken erythrocyte nuclei; Woodcock 1994), possibly for robust gene silencing in the inactive nuclei. Surprisingly, typical heterochromatin regions in mammalian or plant nuclei that have been visualized by cryo-EM look very similar to mitotic chromosomes (Bouchet-Marquis et al. 2006; Fakan and van Driel 2007). Therefore, the melt may show the representative state of compacted chromatin in vivo. This can provide another view of mitotic chromosome condensation (Fig. 5); in interphase nuclei, numerous compact chromatin melt domains (chromatin clumps) are already formed (Fig. 5A) (Rouquette et al. 2009). In addition to charge neutralization by cations, the “physical force” such as the macromolecular crowding effect (depletion attraction or entropic force) (Asakura and Oosawa 1954; Marenduzzo et al. 2006) can be a major contributor to the formation of melt domains (Fig. 5A) because nuclear macromolecule concentration...
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The concept of polymer melt implies dynamic polymer chains (de Gennes 1979); nucleosome fibers may be constantly moving and rearranging at the local level (Fig. 4E). During mitosis, this dynamic movement could be a driving force of chromosome condensation to assemble the chromatin melt domains. Recently, the Jun group proposed that the entropic force of a dynamic, random flexible polymer chain could drive bacterial chromosome segregation (Jun and Wright 2010). With a DNA decatenation enzyme such as topoisomerase II, the segregation process of the dynamic random polymer chain would be facilitated. In interphase nuclei, because the folding of the nucleosome fiber determines DNA accessibility (Tremethick 2007; Bassett et al. 2009), these dynamics may have several advantages in a template-directed biological process such as transcriptional regulation and DNA replication (Fig. 4E). For example, in gene regulation, dynamic movement of nucleosome fibers will help with targeting of transcription factors and complexes because target sequences are exposed more often, whereas regular folding structures such as 30-nm chromatin fibers or larger fibers can hide target sequences. Dynamic irregular folding can easily form loops, facilitating interactions between promoter and enhancer sequences.

CONCLUSIONS

We propose that nucleosome fibers in the bulk of mitotic chromosomes exist in a highly disordered state that is locally similar to a polymer melt, without forming 30-nm chromatin fibers. We also postulate that a similar state exists in the majority of active interphase nuclei. The concept of the polymer melt implies dynamic polymer chains. This may have several advantages in chromosome condensation and segregation during mitosis and the gene transcription and DNA replication processes during interphase.

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