Human mitotic chromosome structure: what happened to the 30-nm fibre?

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The EMBO Journal (2012) 31, 1621–1623. doi:10.1038/emboj.2012.66; Published online 13 March 2012

The long-standing view of chromosome packaging is that 10-nm beads-on-a string chromatin fibres fold into higher-order 30-nm fibres, which further twist and coil to form highly condensed chromosomes. After four decades of intense pursuit of the structure and properties of 30-nm chromatin fibres, work of Nishino *et al* (2012) in this issue of The EMBO Journal demonstrates that regular 30-nm fibres are absent from human mitotic chromosomes. The emerging view is that chromosome-level condensation can be achieved through packaging of 10-nm fibres in a fractal manner.

At the heart of a human chromosome is a single contiguous DNA molecule ranging in size from 50 to 250 million base pairs. This raises a question that has fascinated scientists for many years: how is chromosomal DNA condensed so that it all fits into the cell nucleus? The nucleosome, which consists of ~147 bp of DNA wrapped 1.75 times around an octamer of core histone proteins, is the first level of chromosomal DNA packaging (Figure 1). Arrays of nucleosomes spaced at ~10–60 bp intervals along chromosomal DNA make up the next level of organization and are known as 10-nm 'beads-on-a string' chromatin fibres (Figure 1). *In vitro* studies of short chromatin fragments isolated from nuclei, and more recently of reconstituted oligonucleosomes, have established unequivocally that 10-nm beads-on-a string chromatin fibres fold in the presence of cations into 'higher-order' structures that are ~30 nm in diameter (Hansen, 2002; Tremethick, 2007). The precise structure of the 30-nm fibre remains a point of contention, with the most commonly proposed models being a two-start helix and a one-start solenoid (Tremethick, 2007). Based on the huge body of *in vitro* data, it has long been assumed that the 30-nm chromatin fibre is a requisite intermediate in the packaging of 10-nm chromatin fibres into chromosomes. In what can be thought of as the continuous folding model of chromosome structure, 10-nm beads-on-a string chromatin fibres first form

folded 30-nm fibres, which further twist and coil until chromosomal-level compaction is achieved (e.g., see Hansen, 2002). Despite the nearly universal belief in the importance of the 30-nm fibre as a structural module within chromosomes, there have been few direct investigations of this question *in situ* and *in vivo*. To address this deficiency, Nishino *et al* (2012) used the techniques of cryo-electron

Figure 1 A new model for explaining how the DNA of mitotic chromosomes is packaged and condensed. In this model, the fundamental unit of chromosome packaging is the 10-nm beads-on-a string chromatin fibre. In all, 10-nm chromatin fibres are not folded into higher-order 30-nm structures. Rather, 10-nm fibres are packaged in a fractal manner such that the structural features of chromosomes are similar at many magnifications. To achieve fractal packaging, the contiguous chromosomal 10-nm fibre must be able to fold back and interact with itself at many levels. Local and at-a-distance contacts between 10-nm fibre segments are mediated by extensive nucleosome–nucleosome interactions.

microscopy (cryo-EM) and small angle X-ray scattering (SAXS) to probe for the existence of 30-nm structures in isolated human mitotic chromosomes.

An SAXS experiment allows determination of the size of periodic structures within biological samples. SAXS analysis of human mitotic chromosomes yielded evidence for regular ~30-, ~11-, and ~6-nm structures. The 11- and 6-nm structures are believed to result from edge-to-edge and face-to-face nucleosome orientations within packaged chromatin fibres, respectively. To determine the nature of the 30-nm structure observed by SAXS, the chromosomes were examined by cryo-EM. No folded 30-nm chromatin fibres were observed in the interior of the chromosomes. However, the images unexpectedly showed that the chromosome surface was coated with electron-dense granules the size of ribosomes, and western blotting and immunostaining confirmed the presence of ribosomal contaminants. When the ribosomes were stripped from the chromosome surface, no 30-nm structures were detected by SAXS. As a positive control, SAXS and cryo-EM analyses were able to observe folded 30-nm chromatin fibre structures in chicken erythrocyte nuclei, which lack ribosomal contaminants (Nishino *et al*, 2012). Hence, 30-nm fibres should have been detected in the human mitotic chromosomes if they were there. The conclusion from the combined SAXS and cryo-EM data was that regularly folded 30-nm chromatin fibres are not present in human mitotic chromosomes. This conclusion is in line with other recent studies that have questioned whether mammalian chromosomes contain regular 30-nm chromatin fibres (see Maeshima *et al*, 2010; Fussner *et al*, 2011).

Nishino *et al* (2012) next used ultra-SAXS (USAXS) to probe mitotic chromosomes for the presence of periodic structures between 50–1000 nm. No regular structures were observed in this range. The cryo-EM, SAXS, and USAXS data collectively indicate that no periodic structures beyond the 10-nm fibre exist in bulk human mitotic chromosomes. If chromosomes are devoid of regular 30-nm fibres, and 10-nm fibres are not continuously folded into successively higher-order structures, how is chromosomal-level compaction achieved? Both Fussner *et al* (2011) and Nishino *et al* (2012) have proposed that chromosome condensation is achieved by packaging of 10-nm beads-on-a string chromatin fibres in a fractal manner. A fractal organization means that the chromosome structure is self-similar at all scales, that is, chromosomes have structural features that are similar at many magnifications (Figure 1). Evidence for a fractal structure of human interphase chromosomes has recently been obtained (Bancaud *et al*, 2009; Lieberman-Aiden *et al*, 2009).

Is there a molecular basis for a fractal chromosome organization built from the 10-nm fibre? As it turns out, short chromatin fragments do more than fold into 30-nm structures *in vitro*. They also self-associate, or oligomerize (Hansen, 2002). Because the self-association phenomenon often has been dismissed as ‘precipitation’, the potential importance of this structural transition frequently has been overlooked. However, in the light of the new data supporting a fractal chromosome organization, it is worth re-evaluating the relevance of self-association. Detailed biochemical studies of short reconstituted oligonucleosomes have demonstrated that self-association is induced by physiological levels of polyvalent cations. Self-association is freely reversible and highly cooperative (Hansen, 2002). Individual nucleosome

core particles oligomerize (Liu *et al*, 2011), indicating that self-association is mediated by intermolecular nucleosome–nucleosome interactions. Self-association is an intrinsic property of 10-nm fibres, that is, it does not require prior formation of 30-nm fibres (Hansen, 2002). Both 30-nm fibre formation (Dorigo *et al*, 2003) and self-association (Kan *et al*, 2009) are mediated by the N-terminal tail domain of histone H4. Consequently, self-association potentially will prevent formation of 30-nm fibres by sequestering the H4 N-terminal domain. The self-association transition is influenced by many factors that are known to regulate genome function *in vivo*. For example, core histone tail domain acetylation (Szerlong *et al*, 2010; Liu *et al*, 2011) and nucleosome-free gaps (Hansen, 2002) disrupt self-association, while linker histones promote self-association (Hansen, 2002). All told, a condensation mechanism based on the *in vitro* phenomenon of chromatin fibre self-association is able to explain how a fractal organization of 10-nm fibres can be achieved, how the packing density of 10-nm fibres in a given chromosomal region can be increased or decreased, and why 30-nm fibres do not form during the packaging process.

Should the 30-nm fibre be abandoned all together? Probably not. For one thing, 30-nm fibres are present in the chromosomes of certain cell types such as chicken erythrocytes (Nishino *et al*, 2012). In the case of human mitotic chromosomes, microscopy and SAXS are powerful methods for detecting bulk periodic structures, but they cannot be used to rule out the presence of short stretches of 30-nm fibres (or small amounts of other regularly folded hierarchies for that matter). And of course, there is the overwhelming body of data documenting that the 30-nm fibre is a real *in vitro* entity. For now, it seems best to acknowledge that it is possible that 30-nm fibres are present in chromosomes under certain specific conditions, but that they are not required to achieve large-scale condensation of human mitotic chromosomal DNA.

The work of Nishino *et al* (2012) and recent related studies (see Maeshima *et al*, 2010; Fussner *et al*, 2011) have called into question the continuous folding model of chromatin and chromosome condensation. This model is deeply ingrained, to the point where it has become dogma. Nevertheless, a new paradigm appears to be in order, at least for human mitotic chromosomes. The foundation of the revised paradigm is built on two key tenets: regularly folded 30-nm fibres are largely absent from highly condensed mitotic chromosomes and chromosome condensation occurs primarily by packaging of 10-nm beads-on-a string chromatin fibres in a fractal manner. These tenets would fundamentally alter the way that we view chromatin fibre packaging within chromosomes. Why is it so important to unravel the mysteries of chromosome packaging? In biology, structure is inexorably linked to function. Consequently, if we are to fully understand how processes such as transcription, replication, and repair of chromosomal DNA take place in a chromatin milieu, we must understand the rules that govern how the genome is organized, packaged, and condensed. In this respect, nothing has changed at all.

Conflict of interest

The author declares that he has no conflict of interest.

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