[Cell Cycle 3:1, 26-28; January 2004]; ©2004 Landes Bioscience

Extra Views

Chromosome Shaping by Two Condensins

Tatsuya Hirano

Cold Spring Harbor Laboratory; One Bungtown Road; Cold Spring Harbor, New York USA

Correspondence to: Tatsuya Hirano; Cold Spring Harbor Laboratory, One Bungtown Road; Cold Spring Harbor, New York 11724 USA;Tel.: 516.367.8370; Fax: 516.367.8815; Email: hirano@cshl.org

Received 10/17/03; Accepted 10/20/03

Previously published online as a Cell Cycle E-publication at:
http://www.landesbioscience.com/journals/cc/tocnew31.php?volume=3&Issue=1

KEY WORDS

chromosome condensation, chromosome scaffold, condensin I, condensin II, SMC proteins

ABSTRACT

It remains a big challenge in modern cell biology to determine the mechanism by which a discrete set of rod-shaped chromosomes is assembled from an amorphous mass of interphase chromatin. Recent studies start to shed new lights on how this process is actively supported by a class of multiprotein complexes called condensins. In vertebrate cells, two different condensin complexes make distinct mechanistic contributions to determining the shape and integrity of mitotic chromosomes.

Mitotic chromosome assembly is a critical preparatory process to ensure the faithful and synchronous separation of sister chromatids at the onset of anaphase.[1,2] Strongly implicated in this process is condensin, a multiprotein complex that was originally identified and characterized in *Xenopus* egg cell-free extracts.[3,4] Purified condensin has the ability to introduce positive superhelical tension into DNA in an ATP hydrolysis-dependent manner, and this activity is activated by mitosis-specific phosphorylation mediated by cdc2.[5] Genetic studies in other model organisms have shown that condensin function is required for proper segregation of chromosomes during mitosis.[6-8] While these findings support the idea that condensin is a key (and perhaps the major) component responsible for the formation of mitotic chromosomes, the exact mechanism by which condensin organizes mitotic chromosomes and facilitates their segregation remains elusive.

One of the fundamental obstacles to progress in the field is that we still know very little about how chromatin fibers are organized within mitotic chromosomes.[2,9] Inevitably, the current definition of chromosome "condensation" is relatively vague. Some researchers use this word very narrowly to mean a step(s) in which an extended DNA molecule is converted into a compact structure regardless of its final shape (or functional relevance). However, mitotic chromosome condensation more often refers to the structural reorganization of chromosomes that occurs during mitosis. The goal of this complex process is to actively untangle and refold duplicated genomes so that they can be readily separated at the onset of anaphase. With these functional considerations in mind, it would be helpful to conceptually dissect the assembly of mitotic chromosomes into three distinct steps (Fig. 1A, 1–4). First, catenations of DNA strands from different chromosomes are removed, thereby allowing them to be converted into discrete units (individualization).[10] Second, the fibers are folded and organized into rod-shaped structures (shaping or shape-determination).[11] Finally, the two chromatids within each chromosome are resolved so that they become cytologically distinguishable from each other (resolution).[12] It is important to emphasize that these three steps do not occur independently and must be functionally and mechanistically coordinated with each other. Here I will discuss accumulating lines of evidence that condensin plays important roles in all of these crucial steps of mitotic chromosome assembly.

The first indication that condensin is required for proper chromosome assembly was provided by the *Xenopus* egg cell-free system.[3] In the simplest assay of this system, sperm chromatin was incubated directly with a metaphase (CSF-arrested) extract without preceding DNA replication to produce individual, rod-shaped chromosomes with a single chromatid (i.e., there is no resolution step in this assay). Preincubation of the extract with an antibody against SMC4, a core ATPase subunit of condensin, produced a mass of entangled, prophase-like thin chromatin fibers. Complete depletion of condensin subunits exhibited a severer phenotype, resulting in the formation of a fuzzy, interphase-like chromatin mass (Fig. 1A, 5).[4] Similar results were obtained under a more physiological condition where chromosomes with sister chromatids were assembled after a single round of DNA replication.[13,14] These results show that condensin is required for both the individualization and shaping steps. Addition of the same antibody to the extract after the

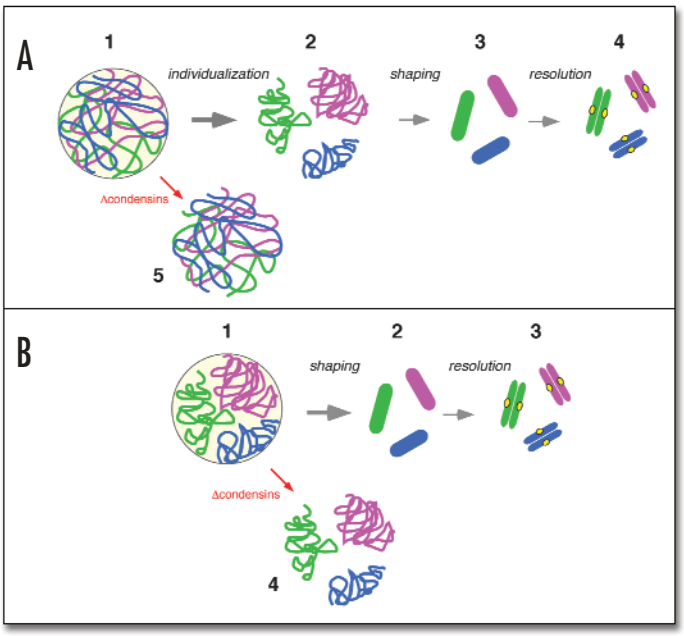

Figure 1. Conceptual pathways of mitotic chromosome assembly. (A) In sperm chromatin or embryonic nuclei, chromatin fibers are heavily entangled with each other (1). First, the entanglement between different fibers must be removed, allowing them to be converted into discrete units (2; individualization). Second, the chromatin fibers are folded and organized into rod-shaped structures (3; shaping). Finally, two chromatids within a chromosome become cytologically distinguishable from each other (4; resolution). In the absence of condensins, the initial individualization step would be compromised, resulting in the formation of an interphase-like chromatin mass (5). (B) In somatic nuclei, chromatin fibers have already been individualized into their "territories"(1). Therefore, depletion of condensins would cause defects in the shaping and resolutions steps (4). Note that the three steps depicted in this conceptual pathway are mechanistically coupled and do not necessarily occur independently of each other.

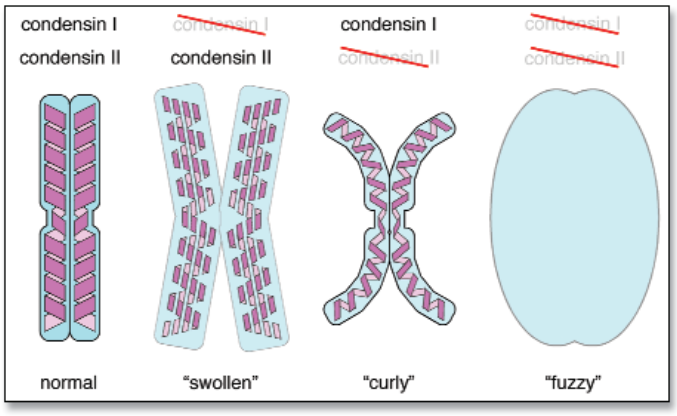

Figure 2. Schematic view of chromosome morphologies observed after depletion of condensin subunits from HeLa cells.[19] The chromosomal DNA is shown in blue, and the chromosome axis stained with anti-SMC2 is shown in magenta. Chromosomes were well organized in control cells containing both condensins I and II ("normal"). Depletion of condensin I- or condensin II-specific subunits produced "swollen" or "curly" chromosomes, respectively. Simultaneous depletion of both condensins resulted in the formation of cloud-like chromosomes with a "fuzzy" appearance.

assembly reaction was completed led to progressive disruption of the rod-shaped chromosomes and converted them into randomly coiled, chromatin balls.[3] While the interpretation of the antibodyblocking experiments may not be straightforward (for instance, steric inhibition by antibody binding cannot be excluded), this result suggests that condensin function is also required for the structural maintenance of rod-shaped chromosomes. The apparent density of chromatin within the observed rod and ball structures was not so different. Therefore, an important role of condensin is to actively organize a chromatin fiber into a rod-shaped chromosome rather than to simply pack it into a random structure.

Early genetic studies in yeast utilizing in-situ hybridization also supported the idea that condensin is required for proper organization of mitotic chromosomes.[6,7] More recent genetic studies in higher eukaryotic cells have allowed researchers to visualize the morphology of chromosomes after depletion of SMC core subunits of condensin.[15-17] In *Drosophila*[15] and chicken DT40 cells[17] with a greatly reduced level of condensin, individual chromosomes were formed, but they were abnormally fat and showed no discernible sister chromatids (Fig. 1B, 4). The length distribution of these chromosomes were apparently normal, leading the authors to conclude that condensin function is important for the resolution step but may not be required for the axial compaction of chromosomes. These studies raise several interesting questions. First, why are the phenotypes observed in vivo seemingly less drastic than those observed in the *Xenopus* egg extracts? One possible explanation is that chromosomes are assembled from templates that have different degrees of DNA entanglement (or catenation) in the two experimental systems. In somatic interphase nuclei, chromosomes are partitioned into specific "territories" in a largely nonoverlapping fashion,[18] implying that the catenations between different chromosomes have already been dissolved before entering mitosis (Fig. 1B, 1). In contrast, DNA molecules within sperm chromatin (and in early embryonic nuclei) would be heavily catenated with each other (Fig. 1A, 1). If true, depletion of condensin in the cell-free system would emphasize its requirement for the individualization step (Fig. 1A, 5) whereas depletion in the somatic cells would stress its participation in the shaping and resolution steps (Fig. 1B, 4). The second question is whether axial and lateral compactions are regulated by different factors and whether condensin is only responsible for the latter. Given the currently limited knowledge of the chromosome folding mechanism, more detailed structural analyses of the condensin-depleted chromosomes would be required before answering this question. Importantly, however, an elegant assay involving alternate exposures of chromosomes to buffers containing Mg^{2+} or EDTA showed that the structural integrity of chicken DT40 chromosomes is severely compromised in the absence of condensin.[17]

A recent paper by Ono et al. (2003)[19] has reported the most dramatic demonstration of the shape-determining role of condensin. The authors first showed that vertebrate cells have a second condensin complex (condensin II) in addition to the canonical condensin complex (henceforth referred to as condensin I). Condensin II shares the same pair of SMC core subunits with condensin I but contains a unique set of three non-SMC subunits. Depletion of the SMC core subunits by means of RNA interference (RNAi) in HeLa cells produced cloud-like chromosomes with a very fuzzy appearance (Fig. 2, "fuzzy"), reminiscent of chicken DT40 chromosomes depleted of SMC2.[17] Most strikingly, depletion of condensin I- or condensin II-specific subunits resulted in a distinct, highly characteristic defect in chromosome morphology (Fig. 2, "swollen" or "curly" chromosomes, respectively). While the two complexes were equally abundant in HeLa cell nuclear extracts, the relative abundance of condensin I and condensin II in *Xenopus* egg extracts was ~5:1. Consistently,

condensin I function was predominant in the extracts, but depletion of condensin II resulted in the formation of curly or wavy chromosomes similar to those observed in condensin II-depleted HeLa cells. Condensins I and II displayed distinct distributions along the axis of chromosomes assembled in vivo and in vitro. These results show convincingly that condensins I and II make different mechanistic contributions to directly determining the shape of vertebrate chromosomes.

Taken together with the previous antibody blocking experiments in vitro,[3] it is most likely that condensins play dual roles in shape-determination and shape-maintenance of mitotic chromosomes. Both roles of condensins could be supported by their participation in the dynamic assembly of the chromosome scaffold,[11,17] a sub-chromosomal structure that constitutes the central axis of chromatids. Indeed, a biochemically-defined scaffold fraction was found to be destabilized after depletion of SMC2, and other proteins such as topoisomerase II could no longer be recovered into this fraction.[17] It should be emphasized that current models postulate that the "scaffold" is a highly dynamic, mitosis-specific structure,[11,17] and is not a static filamentous structure that is already present in the interphase nucleus.

In this short article, I have first made an attempt to reconcile the apparent discrepancy in the phenotypes observed after condensin-depletion in vivo and in vitro. I have then discussed most recent evidence that vertebrate cells have two different condensin complexes that make distinct contributions to mitotic chromosome architecture. The discovery of condensin II raises an exciting array of new questions about chromosome structure and evolution. What is the precise geometry of the two condensin complexes within metaphase chromatids, and how do they functionally interact with each other? Are condensins I and II recruited to specific regions in a sequence-specific manner? Is there any relationship between the distribution of the two complexes and classical chromosome banding? Yeasts have condensin I but not condensin II, but why does condensin II apparently substitute for condensin I in *C. elegans*? Is this related to the unique, holocentric nature of *C. elegans* chromosomes? Future investigation into these questions will undoubtedly enrich our understanding of mitotic chromosome architecture and dynamics.

References

1. Nasmyth K. Segregating sister genomes: The molecular biology of chromosome separation. Science 2002; 297:559-65.
2. Swedlow JR, Hirano T. The making of the mitotic chromosome: Modern insights into classical questions. Mol Cell 2003; 11:557-69.
3. Hirano T, Mitchison TJ. A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. Cell 1994; 79:449-58.
4. Hirano T, Kobayashi R, Hirano M. Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E and a Xenopus homolog of the Drosophila Barren protein. Cell 1997; 89:511-21.
5. Kimura K, Hirano M, Kobayashi R, Hirano T. Phosphorylation and activation of 13S condensin by Cdc2 in vitro. Science 1998; 282:487-90.
6. Saka Y, Sutani T, Yamashita Y, Saitoh S, Takeuchi M, Nakaseko Y, et al. Fission yeast cut3 and cut14, members of a ubiquitous protein family, are required for chromosome condensation and segregation in mitosis. EMBO J 1994; 13:4938-52.
7. Strunnikov AV, Hogan E, Koshland D. SMC2, a Saccharomyces cerevisiae gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family. Genes Dev 1995; 9:587-99.
8. Bhat MA, Philp AV, Glover DM, Bellen HJ. Chromatid segregation at anaphase requires the barren product, a novel chromosome associated protein that interacts with topoisomerase II. Cell 1996; 87:1103-14.
9. Koshland D, Strunnikov A. Mitotic chromosome condensation. Annu Rev Cell Dev Biol 1996; 12:305-33.
10. Gimenez-Abian JF, Clarke DJ, Devlin J, Gimenez-Abian MI, De la Torre C, Johnson RT, et al. Premiototic chromosome individualization in mammalian cells depends on topoisomerase II activity. Chromosoma 2000; 109:235-344.
11. Maeshima K, Laemmli UK. A two-step scaffolding model for mitotic chromosome assembly. Dev Cell 2003; 4:467-80.
12. Losada A, Hirano M, Hirano T. Cohesin release is required for sister chromatid resolution, but not for condensin-mediated compaction, at the onset of mitosis. Genes & Dev 2002; 16:3004-16.
13. Cuvier O, Hirano T. A role of topoisomerase II in linking DNA replication to chromosome condensation. J Cell Biol 2003; 160:645-55.
14. Wignall SM, Deehan R, Maresca TJ, Heald R. The condensin complex is required for proper spindle assembly and chromosome segregation in Xenopus egg extracts. J Cell Biol 2003; 161:1041-51.
15. Steffensen S, Coelho PA, Cobbe N, Vass S, Costa M, Hassan B, et al. A role for Drosophila SMC4 in the resolution of sister chromatids in mitosis. Curr Biol 2001; 11:295-307.
16. Hagstrom KA, Holmes VF, Cozzarelli NR, Meyer BJ. C. elegans condensin promotes mitotic chromosome architecture, centromere organization, and sister chromatid segregation during mitosis and meiosis. Genes Dev 2002; 16:729-42.
17. Hudson DF, Vagnarelli P, Gassmann R, Earnshaw WC. Condensin is required for nonhistone protein assembly and structural integrity of vertebrate chromosomes. Dev Cell 2003; 5:323-36.
18. Cremer T, Cremer C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2001; 2:292-301.
19. Ono T, Losada A, Hirano M, Myers MP, Neuwald AF, Hirano T. Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. Cell 2003; 115:109-21.