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SPOTLIGHT

Condensins under the microscope

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Condensins are key players in mitotic chromosome condensation. Using an elegant combination of state-of-the-art imaging techniques, Walther et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201801048) counted the number of Condensins, examined their behaviors on human mitotic chromosomes, and integrated the quantitative data to propose a new mechanistic model for chromosome condensation.

Genomic information is copied through DNA replication, and for cell division to be effective, the copied information must be faithfully transmitted into two daughter cells. To ensure that faithful transmission occurs, the replicated DNA is condensed into sister chromatids (copied chromosomes). In terms of local organization, it has recently been suggested that nucleosome fibers (10-nm fibers), in which a long strand of negatively charged DNA is wrapped around positively charged core histones, are rather irregularly folded without regular chromatin fibers (Nishino et al., 2012). Higher-order organization necessary to obtain chromosome shape requires two conserved structural maintenance of chromosomes (SMC) protein complexes: Condensins I and II (Hirano et al., 1997; Ono et al., 2003). Condensins I and II share the same pair of SMC ATPase subunits (SMC2 and SMC4) and have distinct sets of non-SMC regulatory proteins (CAP-H, -D2, and -G for Condensin I, and CAP-H2, -D3, and -G2 for Condensin II; Ono et al., 2003). Condensins are also found in the classical chromosome scaffold in histone-depleted chromosomes, which retains the overall size and shape of chromosomes (Paulson and Laemmli, 1977; Ohta et al., 2010). Chromosomes are decondensed without Condensins (Hirano et al., 1997; Ono et al., 2003), which are assumed to actively form and stabilize DNA loops (Goloborodko et al., 2016). Condensin II binds to chromosomes throughout the cell cycle, whereas Condensin I is mainly cytoplasmic during interphase and becomes highly enriched on the axis of mitotic chromosomes after nuclear envelope breakdown. During mitosis, Condensins promote the proper structuring of chromatids. Condensin II is involved in the establishment of the mitotic chromosome axis, whereas Condensin I reduces the size of tethered chromatin loops around the axis (e.g., Green et al., 2012). There are two pressing questions about Condensin biology: (1) How can two Condensins organize the hundreds of megabase-sized DNA molecules (several centimeters long) into human chromosomes? (2) Do Condensins I and II play different roles in the overall compaction process? To answer these questions, we first have to know their copy number and ratio as well as their precise spatial location within a mitotic chromatid. In this issue, Walther et al. address this topic quantitatively using a combination of genome editing, fluorescence correlation spectroscopy (FCS)-calibrated live-cell imaging, and superresolution microscopy stimulated emission depletion (STED).

Walther et al. (2018) first knocked in fluorescent mEGFP tags into Condensin I+II (SMC4), Condensin I (CAP-H/CAP-D2), and Condensin II (CAP-H2/CAP-D3) to homozygosity in a HeLa cell line by genome editing. This resulted in the physiological expression of the tagged Condensin subunits in the cell. FCS-calibrated live-cell imaging was used to count the number of Condensins on chromosomes over mitotic phases. Walther et al. (2018) observed that Condensin I is 1.6–5.6× more abundant than Condensin II during mitosis (Fig. 1) and binds to mitotic chromosomes in two steps: in prometaphase and then early anaphase when it promotes the further compaction of mitotic chromatids. The greater abundance of Condensin I to Condensin II is consistent with other vertebrate studies (e.g., Ohta et al., 2010). How these two "waves" of Condensin I binding are regulated during mitosis, potentially with additional modifications such as phosphorylation, remains an important question. On the other hand, Walther et al. (2018) show that the less-abundant Condensin II does not change its association with chromosomes during mitosis, suggesting very different roles for the two Condensins in the structural organization of mitotic chromosomes. Interestingly, there were twice as many CAP-D3 molecules on chromosomes as CAP-H2, suggesting CAP-D3 might be able to bind mitotic chromosomes independently and additionally to being part of the Condensin holocomplex. More studies are needed to determine whether CAP-D3 has an additional role outside the Condensin II holocomplex.

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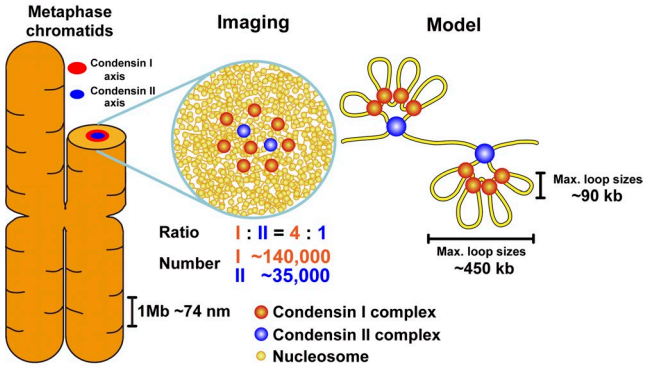

Figure 1. **Quantitative 3D map of Condensins I and II and hierarchical looping model of human mitotic chromosome.** In this schematic, we provide an example of a metaphase chromosome. For details, see Fig. 4 C in Walther et al. (2018). Left: Condensin II (blue) is significantly more restricted to the center of the chromatid (occupying ∼30–35%) than Condensin I (red; up to 50%). Middle: Late metaphase chromosomes, which are laterally compacted from prometaphase ones, have a fourfold higher abundance of Condensin I (∼140,000 copies) than Condensin II (∼35,000 copies). Right: Calculations from the obtained and available data suggest that large Condensin II loops (maximum ∼450 kb) are divided by Condensin I into subloops of a maximum size of ∼90 kb.

Besides counting the numbers of Condensins, the behavioral analysis on Condensins by FRAP provided interesting new insight. Condensin I showed a relatively short chromosomal residence time of ∼2 min, and Condensin II was more stably bound with a longer residence time (>5 min; Walther et al., 2018). The slow binding dynamics and constant abundance of Condensin II on mitotic chromosomes suggest a more structural and stabilizing role, whereas the dynamic stepwise binding and dissociation of Condensin I indicates an actively regulated role in both mitotic compaction and decompaction of chromosomes.

Using STED superresolution microscopy, Walther et al. (2018) took a closer look at the sites of action of Condensins on mitotic chromosomes. They preextracted cells to remove unbound Condensins and chemically fixed them, so whether the chromosomes are structurally preserved must be considered. Nonetheless, the results were very informative. Consistent with earlier research such as by Ono et al. (2003), Condensins I and II differed in their localization within the chromosome axis (Fig. 1). Condensin II was confined more centrally to the axis, and Condensin I occupied a slightly wider area. The chromosome periphery thus seems to be Condensin-free. How can the chromosome periphery be condensed without Condensins? One possibility is that the peripheral regions can be condensed by torsional stress of chromatin loops generated by Condensins around the axis. The other possibility involves another unknown condensation mechanism or mechanisms. Very recently, three papers provided a clue to this issue: even after rapid knockdown of two Condensins, the total mitotic chromosome volume observed by 3D EM, i.e., the compaction state, was similar to that in control cells, although their shapes were abnormal (Samejima et al., 2018), supporting the latter possibility and existence of another mechanism or mechanisms. Ki-67, which locates to the chromosome periphery, was suggested to be an important new player in chromosome compaction (Takagi et al., 2018). Besides other protein factor or factors, a transient increase in free Mg^{2+} observed during mitosis (Maeshima et al., 2018) could be involved in this chromosome compaction mechanism: the nucleosome fiber has a net negative charge, and free Mg^{2+} could decrease the repulsion between two negatively charged nucleosomes and thereby help to condense chromosomes.

An important conclusion of the integration of quantitative imaging and genomics data provided by Walther et al. (2018) is the calculation of the maximum loop sizes in the course of mitotic chromosome condensation: ∼450 kb in prophase, obtained mainly via Condensin II, ∼90 kb in prometaphase and metaphase, and ∼70 kb through the binding of additional Condensin I in anaphase, which is coupled with maximum chromosome compaction upon chromosome segregation. Surprisingly, these maximum loop sizes are comparable with those of DNA loops from the classical metaphase chromosome scaffold (30–90 kb; Paulson and Laemmli, 1977). The revealed loop size reduction and further compaction is particularly advantageous for chromosome transmission during anaphase, which is subject to mechanical shear stress. Tellingly, the authors' loop size estimate is consistent with a recent chromosome conformation capture study and subsequent computational modeling on chicken mitotic chromosomes, which revealed a fine contact probability map of genomic DNA and identified loop structures: large Condensin II–based loops and smaller Condensin I–based loops (Gibcus et al., 2018). How can Condensin I reduce the loop sizes? Even though Condensin I can dynamically move around (∼80% replacement within 10 min) and possesses some motor activity, the loop extrusion by Condensins (Goloborodko et al., 2016) in such condensed chromosomes might pose steric hindrance. It is possible that local nucleosome dynamics in mitotic chromosomes might facilitate the loop extrusion. Further studies using imaging, genomics, biochemistry, and computational modeling will be required to address this interesting question.

In summary, Walther et al. (2018) offer various new imaging and quantification tools and ideas for chromosome analysis, and the results provide new mechanistic insight into mitotic chromosome condensation. Like all great studies, the analysis by Walther et al. (2018) has opened up important new questions and highlighted areas in need of more investigation. Further interdisciplinary studies combining quantitative live-cell imaging on Condensins and chromatin, chromosome EM techniques, genomics, and computational modeling (e.g., Gibcus et al., 2018) will be needed to shine a more definitive light on mitotic chromosome organization and condensation.

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References

Gibcus, J.H., K. Samejima, A. Goloborodko, I. Samejima, N. Naumova, J. Nuebler, M.T. Kanemaki, L. Xie, J.R. Paulson, W.C. Earnshaw, et al. 2018. A pathway for mitotic chromosome formation. *Science.* 359:eaao6135. https://doi.org/10.1126/science.aao6135

Goloborodko, A., J.F. Marko, and L.A. Mirny. 2016. Chromosome Compaction by Active Loop Extrusion. *Biophys. J.* 110:2162–2168. https://doi.org/10.1016/j.bpj.2016.02.041

Green, L.C., P. Kalitsis, T.M. Chang, M. Cipetic, J.H. Kim, O. Marshall, L. Turnbull, C.B. Whitchurch, P. Vagnarelli, K. Samejima, et al. 2012. Contrasting roles of condensin I and condensin II in mitotic chromosome formation. *J. Cell Sci.* 125:1591–1604. https://doi.org/10.1242/jcs.097790

Hirano, T., R. Kobayashi, and M. Hirano. 1997. Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E and a Xenopus homolog of the Drosophila Barren protein. *Cell.* 89:511–521. https://doi.org/10.1016/S0092-8674(00)80233-0

Maeshima, K., T. Matsuda, Y. Shindo, H. Imamura, S. Tamura, R. Imai, S. Kawakami, R. Nagashima, T. Soga, H. Noji, et al. 2018. A Transient Rise in Free Mg(2+) Ions Released from ATP-Mg Hydrolysis Contributes to Mitotic Chromosome Condensation. *Curr. Biol.* 28:444–451.

Nishino, Y., M. Eltsov, Y. Joti, K. Ito, H. Takata, Y. Takahashi, S. Hihara, A.S. Frangakis, N. Imamoto, T. Ishikawa, and K. Maeshima. 2012. Human mitotic chromosomes consist predominantly of irregularly folded nucleosome fibres without a 30-nm chromatin structure. *EMBO J.* 31:1644–1653. https://doi.org/10.1038/emboj.2012.35

Ohta, S., J.C. Bukowski-Wills, L. Sanchez-Pulido, F.L. Alves, L. Wood, Z.A. Chen, M. Platani, L. Fischer, D.F. Hudson, C.P. Ponting, et al. 2010. The protein composition of mitotic chromosomes determined using multiclassifier combinatorial proteomics. *Cell.* 142:810–821. https://doi.org/10.1016/j.cell.2010.07.047

Ono, T., A. Losada, M. Hirano, M.P. Myers, A.F. Neuwald, and T. Hirano. 2003. Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. *Cell.* 115:109–121. https://doi.org/10.1016/S0092-8674(03)00724-4

Paulson, J.R., and U.K. Laemmli. 1977. The structure of histone-depleted metaphase chromosomes. *Cell.* 12:817–828. https://doi.org/10.1016/0092-8674(77)90280-X

Samejima, K., D.G. Booth, H. Ogawa, J.R. Paulson, L. Xie, C.A. Watson, M. Platani, M.T. Kanemaki, and W.C. Earnshaw. 2018. Functional analysis after rapid degradation of condensins and 3D-EM reveals chromatin volume is uncoupled from chromosome architecture in mitosis. *J. Cell Sci.* 131:jcs210187. https://doi.org/10.1242/jcs.210187

Takagi, M., T. Ono, T. Natsume, C. Sakamoto, M. Nakao, N. Saitoh, M.T. Kanemaki, T. Hirano, and N. Imamoto. 2018. Ki-67 and condensins support the integrity of mitotic chromosomes through distinct mechanisms. *J. Cell Sci.* 131:jcs212092. https://doi.org/10.1242/jcs.212092

Walther, N., M.J. Hossain, A.Z. Politi, B. Koch, M. Kueblbeck, Ø. Ødegård-Fougner, M. Lampe, and J. Ellenberg. 2018. A quantitative map of human Condensins provides new insights into mitotic chromosome architecture. *J. Cell Biol.* https://doi.org/10.1083/jcb.201801048