CHROMOSOME ORGANIZATION

Reeling it in: how DNA topology drives loop extrusion by condensin

Structural maintenance of chromosomes (SMC) complexes such as condensin regulate chromosome organization by extruding loops. A new study uses single-molecule imaging of condensin on supercoiled DNA to understand how condensins navigate the under- and overwound DNA states common throughout the genome.

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A key challenge in the field of chromosome biology is understanding how long, linear strands of chromatin are effectively compacted, segregated in mitosis and organized within the tiny volume of the nucleus. To this end, SMC complexes such as condensins and cohesins have long been known to organize chromosomes at various scales in mitosis and interphase, respectively. Recent work has shown that condensin and cohesin organize chromosomes by extruding DNA loops[1–4]. However, previous studies of loop extrusion have generally considered extrusion on naked DNA in vitro. It has therefore remained unclear how SMC complexes might navigate DNA in the cell, which is chromatinized and subjected to torsional stresses. In particular, processes such as DNA replication and transcription are known to cause under- or overwinding of the DNA strand, known respectively as negative and positive supercoiling[5–7]. A new study in *Nature Structural & Molecular Biology* brings new insights into how extrusion and supercoiling interact, and has shown that condensin preferentially binds and coalesces positively supercoiled DNA in vitro[8] (Fig. 1).

Condensins, along with topoisomerase II, are known to be essential molecular components required for chromatin condensation during mitosis[9]. Whereas topoisomerase II relieves torsional stress and supercoiling, condensin can introduce topological stresses and has loop-extrusion activity similar to that of its cousin cohesin[2,4]. In addition to the stresses introduced by condensin itself, positive supercoiling is naturally abundant because of processes such as transcription and replication[5–7]. Moreover, positive supercoiling can manifest as a loop-like structure known as a plectoneme, similar to the winding of a string when you twist its end. It has been previously suggested that condensin may be able to stabilize plectonemes and that cohesin preferentially compacts positively supercoiled DNA[10,11]. It has also been shown that condensins are capable of generating positively supercoiled DNA themselves[12,13]. Some natural questions, therefore, are: how does condensin interact with and induce positively supercoiled DNA, and what are the in vivo implications of this?

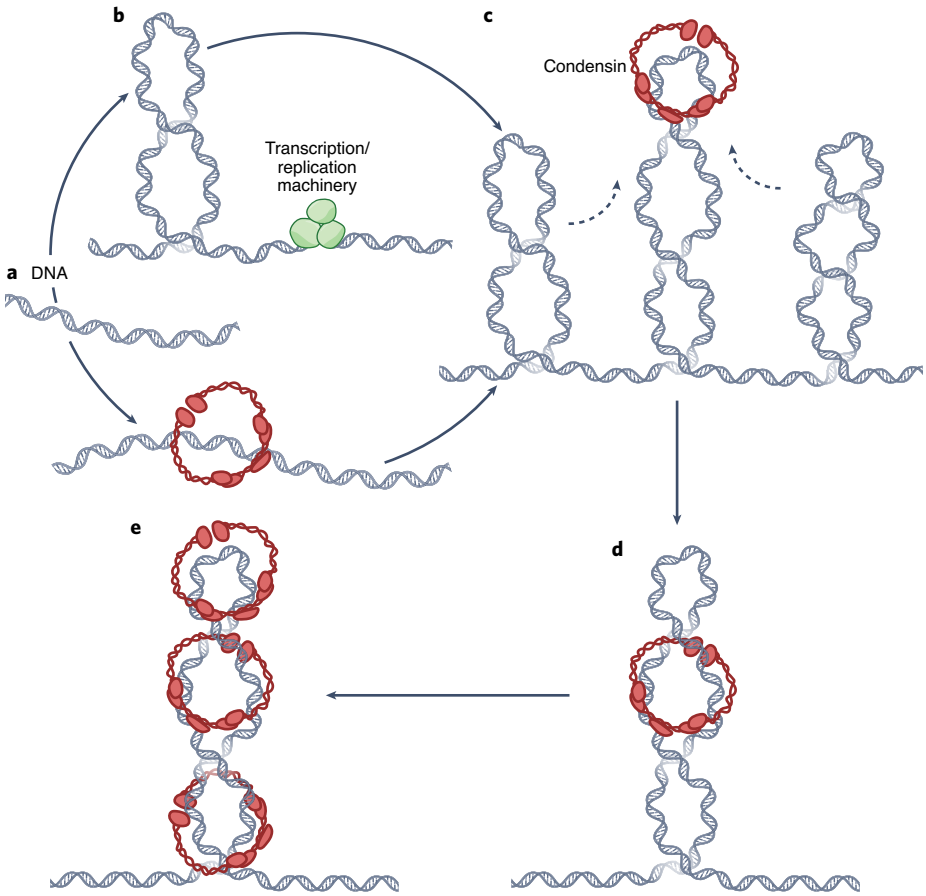

Fig. 1 | Condensin induces and coalesces positively supercoiled DNA, which acts as a binding site for additional condensins. a, Unbound DNA has a relaxed conformation. **b**, Positive supercoiling is induced by transcriptional or replicative machinery or by condensin itself. **c**, Condensin preferentially binds at the tip of positively supercoiled plectonemes and coalesces nearby plectonemes into a single plectoneme. **d**, Condensin extrudes DNA and moves down the coiled loop. **e**, Additional condensins preferentially bind the positively supercoiled DNA.

Through a single-molecule imaging approach, which they had used previously to demonstrate condensin's loop-extrusion

activity[4], Kim et al. now show that condensin preferentially loads at the tip of plectonemes on positively supercoiled DNA. They used an in vitro system in which 42-kilobase-pair stretches of DNA are attached to a coverslip at both ends. The addition of a DNA intercalating agent (SYTOX orange) to the stretched DNA allowed the authors to create and image positively supercoiled plectonemes using highly inclined and laminated optical sheet microscopy (HILO)[14].

Upon addition of condensin, the plectonemes seemed to coalesce into a single loop as condensin processed down the molecule. Moreover, 70% of DNA-binding events co-localized with a plectoneme, and the majority of binding events happened at the tip. This suggested that condensin has a high affinity for positively supercoiled loops. Compared to relaxed or negatively supercoiled DNA, condensin also had a four times greater propensity to bind positively supercoiled DNA, suggesting that this phenomenon is specific to positively supercoiled plectonemes. Overall, the authors nicely demonstrated preferential loading of condensins at the tips of plectonemes, which may have implications for cohesin and condensin loading site selection in cells.

In addition to demonstrating condensin's preference for plectonemic DNA, the authors used atomic force microscopy to show that condensin causes entanglement of circular DNA in vitro, which is consistent with a role for condensin in generating positive supercoils[12,13]. Using relaxed, circular pieces of DNA, they found that the fraction of entangled DNA molecules substantially increases with the addition of condensin and ATP, but not condensin alone. This feature of condensin, along with its preference for plectonemic DNA, suggests a potential mechanism for condensin to recruit other condensin molecules.

Using their single-molecule imaging approach, the authors were able to quantify multiple condensin-binding events along the same piece of DNA. They observed that condensins bound at different plectonemes often merged into a single loop upon extrusion. Further, approximately 40% of the loops involved multiple condensin-binding events at a single plectoneme, often near the tip. This result is remarkably consistent with not only their previous findings, but also existing work demonstrating a preference of cohesin for positively supercoiled DNA[10].

Taken together, these data suggest a model whereby condensin preferentially binds to the tip of plectonemes, extrudes DNA and coalesces positively supercoiled DNA (Fig. 1c,d). Moreover, the data are consistent with a role for condensin in generating positively supercoiled loops, and with the possibility that the resolution of DNA into a single plectoneme may provide a binding site for the recruitment of even more condensins. Overall, Kim et al. provide a strong case for condensin's preference for, and extrusion of, positively supercoiled DNA in vitro by visualizing condensin's interaction for the first time.

The authors effectively set the stage for future work examining the interplay between DNA supercoiling and SMC complexes. The creation of positive supercoils that cooperatively drive the binding of additional condensins is an attractive explanation reconciling condensin's loop-extrusion activity with chromosomal compaction during mitosis that could be further explored. For example, the mechanism by which condensin generates supercoiled DNA is so far unelucidated. Additionally, the question of how topoisomerase II is involved with this mechanism remains. Specifically, can condensin generate positive supercoils even in the presence of topisomerase II, and can supercoiled loops effectively recruit topoisomerase?

Finally, although the authors elucidated how condensin navigates supercoiled DNA in vitro, how SMC complexes such as cohesin and condensin extrudes chromatin in vivo is still unclear. Toward this goal, extending the single-molecule imaging approaches to visualize SMC-mediated loop extrusion in vivo will be an important future step[15]. ❐

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Published online: 14 July 2022

https://doi.org/10.1038/s41594-022-00805-8

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Acknowledgements

We thank S. Vos and S. Nemsick for insightful comments on this piece. D.N.N. and A.S.H. are supported by the Broad Institute of MIT and Harvard. A.S.H. additionally acknowledges support from US National Institutes of Health grants R00GM130896, DP2GM140938, R33CA257878 and UM1HG011536, National Science Foundation grant 2036037, the Mathers Foundation and a Pew-Stewart Cancer Research Scholar grant.

Competing interests

The authors declare no competing interests.