GENOME ORGANIZATION

Human cohesin extrudes interphase DNA to make loops

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Three-dimensional organization of the eukaryotic genome regulates key processes such as transcription, DNA replication and cell division, and as such is thought to be important for cell differentiation and development. In interphase, DNA is organized into loops and topologically associating domains (TADs). Loops and TADs depend on cohesin, but the mechanism by which cohesin contributes to the formation of these structures was unknown. Reporting in *Science*, Peters and colleagues now show that cohesin generates loops by extrusion of DNA fibres.

Cohesin belongs to the family of SMC (structural maintenance of chromosomes) protein complexes — also including condensin and SMC5–SMC6 — which are ring-shaped ATPases that can encircle DNA. Condensin is known to extrude loops in vitro, which has been associated with the folding of DNA in mitotic chromosomes. This, in combination with other observations — for example, that cohesin co-localizes with the architectural protein CTCF at TAD boundaries and loop 'anchors', and that the longer cohesin resides on chromatin, the longer the loops are — has led to the hypothesis that cohesin extrudes DNA loops until it encounters convergently oriented loop anchor DNA sequences that are bound by CTCF.

To test this hypothesis, the authors analysed whether purified human cohesin is capable of generating loops in vitro. They tethered DNA molecules to a glass surface and imaged them by total internal reflection microscopy in the presence of cohesin, in conditions similar to those that had been previously reported to enable condensin to perform loop extrusion. These experiments led to the finding that cohesin actively forms loops but can do so only in the presence of the regulatory HAWK (HEAT repeat proteins associated with kleisins) NIPBL–MAU2, which stimulates cohesin's ATPase activity. Loops formed at a mean rate of 0.8–1 kb per second (similarly to condensin) by the incorporation of DNA from both ends in a symmetrical manner (unlike cohesin, which extrudes DNA asymmetrically).

Next, the authors found that cohesin functions as a monomer and confirmed that cohesin and NIPBL–MAU2 are located at the base of loops, which is consistent with a model whereby cohesin-dependent DNA loops are formed by extrusion. Furthermore, as substituting ATP with CTP or introducing ATP in the absence of NIPBL–MAU2 led to the loss of loops, the authors concluded that the cohesin ATPase activity, combined with a dynamic interaction between NIPBL–MAU2 and cohesin, have a direct role in enabling cohesin to extrude and maintain DNA loops.

SMC complexes contain two elongated SMC proteins that are connected via their hinge and a kleisin subunit, forming a ring-like structure, and cohesin is thought to mediate sister chromatid cohesion by entrapping the two sister DNA molecules inside this ring. So how do cohesin and DNA interact to create the loops? They could topologically entrap two DNA molecules, as during cohesion establishment, or 'pseudo-topologically', whereby a single DNA loop is threaded through the ring, or without any encircling of DNA. Topological cohesin–DNA interactions are resistant to high-salt concentration, but loop extrusion was disrupted in these conditions, suggesting non-topological DNA entrapment. Moreover, an engineered form of cohesin that forms a ring but in which all surfaces are linked to prevent topological DNA entrapment also enabled loop extrusion, raising the possibility that looping does not require entrapment.

This study provides insights into how cohesin contributes to the 3D organization of interphase chromatin. The results indicate that single cohesin complexes can catalyse the formation of DNA loops by extrusion in an ATP-dependent manner and in the presence of NIPBL–MAU2, by interacting with DNA either non- or pseudo-topologically.

The mechanism of loop extrusion remains to be elucidated. Key open questions include understanding which parts of cohesin bind to DNA, and how DNA binding events are coordinated during cohesin's NIPBL–MAU2-dependent ATPase cycle.

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ORIGINAL ARTICLE Davidson, I. F. et al. DNA loop extrusion by human cohesin. *Science* https://doi.org/10.1126/science.aaz3418 (2019)
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