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Cohesin: building loops, but not compartments

Judith HI Haarhuis & Benjamin D Rowland

DNA is subjected to major cellular events, such as transcription, replication and DNA repair. To control these processes, the architecture of the DNA is tightly regulated. Recent work, including two studies in this issue of *The EMBO Journal*, provides compelling evidence that cohesin structures chromosomes through the processive enlargement of loops. While cohesin promotes chromosomal looping, it rather counteracts nuclear compartmentalization.

See also: **J Gassler *et al*** (December 2017) and **G Wutz *et al*** (December 2017)

The genome of a diploid human cell is almost 4 metres long, yet the diameter of a nucleus is only a few micrometres. Within this confined setting, the DNA needs to be folded in such a way that it allows for dynamic processes such as transcription and the DNA damage response. High-resolution chromatin conformation capture experiments have revealed that the genome is organized into loops and compartments (Rao *et al*, 2014). Loops are *cis* contacts ranging up to a few megabases in size. The genome can roughly be divided into two compartments that reflect actively transcribed regions (A) and more repressed regions (B). How the loops are formed has long remained a mystery, and whether loops and compartments share any functional relationship was unknown.

Cohesin is a ring-shaped protein complex that can entrap DNA inside its lumen. It holds together the sister chromatids from S phase until mitosis to ensure proper chromosome segregation. However, cohesin can also hold together DNA elements *in cis* to bring together two distant loci. It was already proposed 16 years ago that long-range loops may be formed by the processive enlargement of tiny loops by the extrusion of DNA through cohesin rings (Nasmyth, 2001). More recently, *in silico* polymer simulations have revealed that loop extrusion by cohesin within defined genomic regions could in principle fully explain genome organization at the megabase scale (Sanborn *et al*, 2015; Fudenberg *et al*, 2016). Based upon the loop extrusion model, one can make a number of predictions. First, loops should simply be cohesin dependent. But more importantly, the duration with which cohesin embraces DNA should determine the length of the loops.

Genome organization by loop extrusion

The past year has seen a number of key publications that together provide overwhelming experimental evidence in support of the loop extrusion model. The cohesin dependency was tested directly through the use of different elegant systems that all allowed the efficient depletion of cohesin's SCC1 subunit (also known as RAD21). SCC1-deficient cells lacked virtually all loops genome-wide (Fig 1A). Cohesin therefore is essential for the formation and/or maintenance of loops (Gassler *et al*, 2017; Rao *et al*, 2017; Wutz *et al*, 2017). Re-introduction of SCC1 in depleted cells led to the re-appearance of loops within an hour (Rao *et al*, 2017). Cohesin-dependent loop formation apparently is a very rapid process.

Important as these findings are, they merely tell us that cohesin is required for loops. It does not provide insight into the mechanism by which the loops are formed. Cohesin binds to chromatin in a dynamic manner that involves a constant cycle of DNA entrapment and release. Cohesin's release factor WAPL is essential for this turnover. WAPL binds to cohesin's PDS5 subunit and opens a DNA exit gate to release cohesin from DNA. If cohesin were to form loops through a processive mechanism, the duration with which it entraps DNA may well determine how far loops can be enlarged. Three studies have now tested this hypothesis in different ways (Gassler *et al*, 2017; Haarhuis *et al*, 2017; Wutz *et al*, 2017). WAPL deficiency led to a far longer residence time of cohesin on the DNA. Importantly, this stabilization of cohesin on DNA led to longer loops genome-wide (Fig 1A). The duration with which cohesin embraces DNA therefore indeed does determine the length of chromatin loops. This is the strongest evidence yet that cohesin structures the genome by loop extrusion. WAPL-mediated cohesin turnover ensures that the 3D genome is kept dynamic by the continuous formation, loss and re-formation of chromatin loops.

CTCF sets the boundaries

Cohesin primarily loops together CTCF sites. CTCF binds to DNA via a consensus motif that has a specific orientation. Intriguingly, loops are almost exclusively found between CTCF sites pointing towards each other (Rao *et al*, 2014). By using a system that acutely depletes CTCF, two papers have now investigated the genome-wide role of CTCF. They show that CTCF is required for the formation of loops between CTCF sites (Fig 1A; Nora *et al*, 2017; Wutz *et al*, 2017). The formation of loops *per se* is not affected by

Division of Gene Regulation, The Netherlands Cancer Institute, Amsterdam, The Netherlands. E-mail: b.rowland@nki.nl
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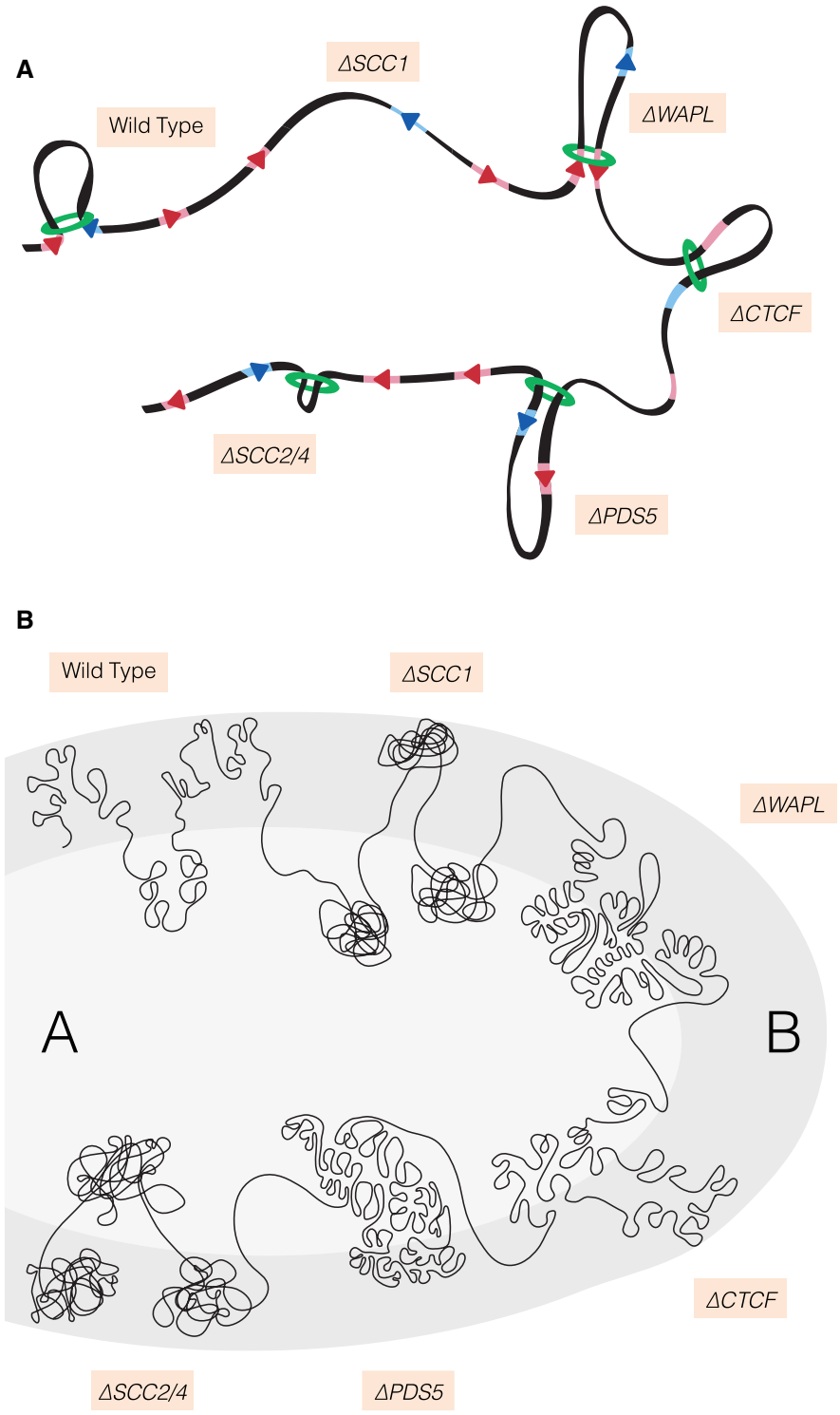

Figure 1. Cohesin-dependent loop formation.
(A) Depletion of the core cohesin subunit SCC1 results in loss of loops. Cohesin is depicted as a green ring. Loss of the SCC2/SCC4 complex results in fewer and shorter loops, while removal of WAPL or PDS5 results in longer loops. CTCF (triangles) binds to CTCF sites (red for forward-oriented and blue for reverse-oriented sites) and acts as a boundary element that halts cohesin. (B) Cohesin-dependent loops negatively affect compartmentalization. Compartments are less well defined if there is an increase in looping. Conversely, a decrease in looping results in a clearer distinction between compartments (light grey is A compartment, and dark grey is B compartment). The A compartment generally contains actively transcribed genes and lies in the inner nucleus, while the B compartment is more repressed and lies in the nuclear periphery.

CTCF depletion, as this rather is cohesin dependent (Gassler *et al*, 2017; Rao *et al*, 2017; Wutz *et al*, 2017). The available data would support the model that cohesin forms loops until it encounters an inwards-pointing CTCF site. Here, CTCF then acts as a boundary that stops cohesin.

Thanks to WAPL-mediated turnover, cohesin creates dynamic loops, but CTCF then determines the genomic regions within which these loops can be enlarged. How CTCF creates a boundary for cohesin is unclear. Possible scenarios would be that CTCF forms a physical barrier, that cohesin has affinity for CTCF, or that CTCF locally slows down cohesin's "extrusion activity". Interestingly, Wutz *et al* (2017) show that PDS5 is required to maintain CTCF boundary integrity. PDS5 deficiency stabilizes cohesin on DNA, which leads to longer loops, but in contrast to WAPL-deficient cells, cohesin now less frequently loops together CTCF sites (Fig 1A). How PDS5 in fact controls CTCF's boundary function remains an interesting question for the future.

A processivity factor?

Cohesin's loading onto DNA to a large degree is dependent on the cohesin loader complex consisting of SCC2 and SCC4 (also known as NIPBL and MAU2, respectively). In correspondence with the finding that cohesin is required for looping, these loading factors are also important for looping (Haarhuis *et al*, 2017; Schwarzer *et al*, 2017). Strikingly though, the SCC2/SCC4 complex is not only important for the amount of loops, but also for the length of the loops (Fig 1A; Haarhuis *et al*, 2017). What actually drives cohesin-dependent loop enlargement remains unknown. Cohesin harbours an ABC-like ATPase machinery whose activity is dependent on SCC2 (Murayama & Uhlmann, 2014). This raises the fascinating possibility that SCC2 controls cohesin's enzymatic activity and thereby its processivity on DNA to drive the enlargement of chromatin loops. Further studies will be needed to decipher how exactly SCC2 controls loop formation.

Cohesin counteracts nuclear compartmentalization

Whereas deficiency for either cohesin or one of its loader subunits impaired loop formation, this did not abrogate the

formation of compartments (Gassler *et al*, 2017; Haarhuis *et al*, 2017; Rao *et al*, 2017; Schwarzer *et al*, 2017; Wutz *et al*, 2017). In fact, these cells displayed a more pronounced distinction between the A and B compartments. Conversely, compartmentalization was less pronounced in WAPL-deficient cells (Gassler *et al*, 2017; Haarhuis *et al*, 2017; Wutz *et al*, 2017). Together this would suggest that there are two distinct modes of genome organization. One mode entails cohesin-dependent loop formation, and the other is nuclear compartmentalization. Loop formation does however affect the latter mode, but in a negative sense (Fig 1B). It may well be that cohesin-mediated looping limits the macro-scale mobility of DNA within the nucleus and thereby antagonizes the forces that drive compartmentalization. Cohesin-mediated loop formation could also be used to relocate DNA from one compartment to another to control gene expression.

All in all, these different papers have provided major insight into different aspects of chromosomal organization. We have learned a great deal about the basic principles behind loop formation, what determines which DNA elements are bridged together, what may drive loop formation, and even how all of this affects nuclear organization. It has been quite a year for chromosome biology.

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