Chromosome segregation: how to open cohesin without cutting the ring?

Yasuto Murayama and Frank Uhlmann*

Chromosome Segregation Laboratory, Cancer Research UK London Research Institute, London, UK.

*Correspondence to: frank.uhlmann@cancer.org.uk

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Chromosome segregation is triggered when the ring-shaped chromosomal cohesin complex is opened by proteolytic cleavage to release pairs of sister chromatids. Even before this dramatic event in anaphase, many cohesin rings lead a dynamic life on chromosomes, and in metazoan cells a good part of them dissociate from chromosome arms during mitotic prophase. Two new papers in *The EMBO Journal* address how chromatin can get into and out of the cohesin ring without requiring its cleavage.

Faithful chromosome segregation during cell division is crucial to safeguard the accurate propagation of genetic information from one cell generation to the next. Following their synthesis during DNA replication, sister chromatids remain tightly connected to one another by the chromosomal cohesin complex, until their separation at anaphase onset. The cohesin complex consists of three core subunits, Smc1, Smc3 and Scc1, that together form a large ring-shaped protein complex that is thought to hold sister chromatids together by topological embrace (Haering *et al*, 2008; Figure 1). This

Figure 1 Cohesin's DNA entry and exit gates, as defined by the behaviour of engineered cohesin gate fusion proteins. **(A)** Rapamycin-induced dimerisation of engineered hinge interaction domains prevents cohesin loading onto DNA. **(B)** A covalent Smc3–Scc1 fusion protein prevents release of cohesin from chromosomes by Wapl in interphase, as well as during the prophase pathway of cohesin removal.

requires the loading of cohesin onto chromosomes by the Scc2/Scc4 complex, a reaction that commences well before DNA replication. At this stage, cohesin persists only transiently on chromosomes and dissociates again with a relatively short half-life. During DNA synthesis in S-phase, cohesin's DNA binding is stabilised by replication-coupled acetylation of its Smc3 subunit, a prerequisite for enduring sister chromatid cohesion (Gerlich *et al*, 2006; Ben-Shahar *et al*, 2008; Chan *et al*, 2012; Lopez-Serra *et al*, 2013). Once cells enter mitosis, cohesin is removed from chromosomes in two waves. During mitotic prophase, much of cohesin dissociates from chromosome arms. Only at centromeres, cohesin is protected from removal, awaiting site-specific proteolysis of the Scc1 subunit by the protease separase to open up cohesin rings and trigger anaphase. But how do cohesin rings come off the chromosome in prophase while remaining intact? A related question is how DNA gets into the ring in the first place? In both cases, the ring must transiently open up to let DNA in or out. Two new papers in *The EMBO Journal* (Buheitel and Stemmann, 2013; Eichinger *et al*, 2013) have added to our understanding of these processes in the *Drosophila* model and in human cells, respectively.

To study how human cohesin entraps chromosomes, Buheitel and Stemmann adopted an approach pioneered by Nasmyth and coworkers in budding yeast. The idea is simple. If the cohesin ring opens up to bind to DNA, then locking possible entry gates by protein engineering should reveal how DNA gets inside. Cohesin's circumference is made up of the Smc1 and Smc3 proteins, which interact with each other on one end at a dimerisation interface known as the hinge, and on the other side via their ATPase head domains (Figure 1). The head interaction is reinforced by the Scc1 subunit, whose N- and C-terminal parts bind to Smc3 and Smc1, respectively. In yeast, locking the hinge interaction by grafting a rapamycin-induced dimerisation interface prevents cohesin loading onto DNA, while locking either the Smc3–Scc1 or Scc1–Smc1 interface does not affect loading (Gruber *et al*, 2006). By depleting human cohesin subunits by RNA interference and replacing them with similarly engineered subunits, Buheitel and Stemmann showed that locking the human cohesin hinge, but not locking either of the Smc head interactions, likewise blocks DNA binding (Figure 1B). This is suggestive of a conserved mode of DNA binding in which the cohesin hinge opens up to entrap DNA.

If DNA enters cohesin through the hinge, where does it escape during prophase? The prophase removal of cohesin, but not its chromosome loading, depends on the Wapl protein (Gandhi *et al*, 2006; Kueng *et al*, 2006). On the other hand, loading—but probably not prophase dissociation—depends on the Scc2/4 complex. Thus, these two reactions are quite different. Wapl is not only required in prophase, but also promotes cohesin turnover on DNA in G1, before cohesin acetylation in S-phase. Eichinger *et al* therefore investigated the dynamic behaviour of cohesin on *Drosophila* polytene chromosomes. Using fluorescence recovery after photobleaching (FRAP) experiments, they confirmed that Wapl promotes dynamic turnover of cohesin on these chromosomes. In cells from *wapl* mutant flies, turnover was greatly reduced and cohesin accumulated on polytene chromosomes to higher levels. The authors then introduced a covalent

fusion between Smc3 and Scc1, which permanently locks the gate between the two subunits. This fusion protein bound to chromosomes as part of cohesin complexes, but exhibited similarly reduced turnover on chromosomes as seen in *wapl* mutants (Figure 1B). Notably, when the authors re-opened the Smc3–Scc1 fusion through engineered TEV protease cleavage, cohesin turnover resumed. This suggests that DNA exits the *Drosophila* cohesin ring through a gate between its Smc3 and Scc1 subunits, corroborating recent results obtained by this group in budding yeast (Chan *et al*, 2012).

Is the Smc3–Scc1 exit gate also used when cohesin dissociates from chromosomes during prophase? To address this, Eichinger *et al* filmed captivating movies of GFP-marked cohesin in *Drosophila* neuroblasts passing through mitosis. Cohesin dissociation was readily seen in wild-type prophase, but cohesin persisted on chromosome arms in *wapl* mutant flies or when employing the Smc3–Scc1 fusion construct. Buheitel and Stemmann expressed a similar Smc3–Scc1 fusion protein in human cells, which in contrast to wild-type cohesin also stayed along chromosome arms in mitosis. Persistent cohesin led to increased sister chromatid cohesion between chromosome arms, at a time when it would normally be lost. Strikingly, this increased chromosome arm cohesion appears to prevent neither *Drosophila* nor human cells from entering timely anaphase (Kueng *et al*, 2006; Eichinger *et al*, 2013). This implies that separase may have no difficulty in cleaving increased amounts of cohesin along chromosome arms.

The two new studies have lent support to a model in which DNA enters the cohesin ring through an entry gate at the hinge, and exits through a distinct gate at the Smc3 head (Figure 1). Have alternative models be excluded by these experiments? Results obtained with the engineered cohesin gate fusions have to be interpreted cautiously. The grafted hinge dimerisation domain, once closed by rapamycin, may impede not only hinge opening but could also affect another DNA-binding activity of the cohesin hinge. Similarly, the covalent Smc3–Scc1 fusion could prevent Wapl from opening cohesin in ways different from shutting the Smc3–Scc1 interface. Further studies to explore the biochemical behaviour of the wild-type cohesin complex will be crucial to verify, or refute, this appealing cohesin entry and exit gate model.

The two new manuscripts emphasise a number of pressing questions. How does the Scc2/Scc4 complex promote cohesin loading? How does Wapl regulate the Smc3–Scc1 exit gate? Important insight on the latter question comes from a crystallography structure view of the Wapl protein reported in another current *The EMBO Journal* paper (Chatterjee *et al*, 2013). Wapl directly binds to Smc3 in an acetylation-modulated interaction. Could Wapl compete with the Scc1 N-terminus for binding to Smc3, thereby prying the exit gate open? In this model, Smc3 acetylation might turn off Wapl's competition with Scc1, thus allowing the exit gate to shut.

In human cells, Smc3 acetylation recruits sororin to the cohesin complex (Nishiyama *et al*, 2010), which in turn competes with Wapl for interaction with another cohesin subunit, Pds5. Whether and how this affects Wapl's influence on the Smc3–Scc1 exit gate will be important to explore. Sororin is phosphorylated as cells enter mitosis,

which stops it from competing with Wapl. This sets in motion prophase removal, presumably even of acetylated cohesin. At centromeres, sororin prevents premature cohesin removal. There, the shugoshin protein recruits the PP2A phosphatase to counteract sororin phosphorylation and thus to sustain its ability to counteract Wapl (Liu *et al*, 2013). If we thought we

already understood how cohesin works on chromosomes, the fun has only just begun.

Conflict of interest

The authors declare that they have no conflict of interest.

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