Published in final edited form as:

Nat Struct Mol Biol. 2008 September ; 15(9): 899–900. doi:10.1038/nsmb0908-899.

Sister chromatids caught in the cohesin trap

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Abstract

Cohesin is a large ring-shaped protein complex that mediates cohesion between sister chromatids. New experiments show that the sister chromatids of a minichromosome are entrapped by monomeric cohesin rings, thus excluding the possibility that sister chromatid cohesion is mediated by nontopological interactions between cohesin complexes.

During cell division, the two sister chromatids are held together by the cohesin complex until the onset of anaphase, when a protease called separase opens the cohesin rings. This releases cohesin from the chromosomes and allows segregation of sister chromatids to opposite poles. Cohesin-mediated sister chromatid cohesion is essential for proper attachment of sister kinetochores to microtubules emanating from the opposite poles and for subsequent segregation of sister chromatids. The cohesin complex is a tripartite ring in which the Smc1 and Smc3 proteins are connected by their hinge domains on one side and the Scc1 protein (also called Mcd1 or Rad21) closes the ring by connecting the Smc1 and Smc3 head domains on the other side. The ring shape of the cohesin complex, which is large enough to embrace two sister chromatids, led Haering and Nasmyth to propose that the interaction between cohesin complexes and DNA is topological, with one or both sister chromatids trapped inside the cohesin ring; this idea is referred to as the ring or embrace model^{1,2}. The ring model not only provides a simple explanation for how sister chromatids are held together but also explains how separase destroys cohesion at the metaphase-anaphase transition.

Several lines of evidence support the ring model¹⁻⁵. One of the most compelling arguments is that cohesin can be released from a circular minichromosome by linearizing the minichromosome with a restriction enzyme⁶. However, there has been intense debate on the molecular mechanism by which cohesin mediates sister chromatid cohesion, and alternative models have been proposed⁷⁻¹⁰. Oligomerization models suggest that sister chromatid cohesion is generated by association of several chromatin-bound cohesin complexes. The bracelet model suggests that the oligomerization occurs by interaction between head domains of two different Smc heterodimers. Such cohesin complexes are proposed to form filaments that mediate sister chromatid cohesion. The snap model proposes that each Smc complex can bind a single strand of DNA and that sister chromatid cohesion is mediated by the interaction of the coiled-coil or hinge domains between two Smc complexes. Although many observations are consistent with the oligomerization models, compelling experimental evidence to support them is lacking. Nevertheless, these alternative models have greatly stimulated the progress in the field, and it will be important to test whether oligomerization of cohesin complexes contributes to sister chromatid cohesion.

Haering *et al.* now provide the best piece of evidence so far supporting the model that the cohesin ring holds sister chromatids together by entrapping sister DNAs inside its ring¹¹. Importantly, their results are not compatible with the possibility that cohesion between the sister DNAs of circular minichromosomes is mediated by nontopological interactions between cohesin complexes.

If the ring model is true, and the association between the cohesin and sister DNAs is topological rather than physical, then covalently closed cohesin rings should hold sister chromatids together even after protein denaturation. If oligomerization of cohesin complexes is essential for sister chromatid cohesion, protein denaturing conditions should destroy cohesion between sister chromatids (Fig. 1). Haering *et al.* took advantage of the available crystal structures^{1,3} to engineer cysteine residues that efficiently cross-linked the Smc1 and Smc3 hinge domains, as well as the Smc1 head domain and Scc1, in the presence of cross-linking reagents. In addition, a Smc3-Scc1 fusion was used to close the third interface. Though the cysteine substitutions did not interfere with the cohesin's function, the Smc3-Scc1 fusion was only partially functional. To isolate minichromosomes, Haering *et al.* used an elegant protocol developed by Dmitri Ivanov in which velocity gradient sedimentation is used to separate monomeric and cohesed (dimeric) minichromosomes⁵. Interestingly, about 50% of minichromosome dimers were converted into monomers after incubation with 0.5 M KCl, in contrast to a previous report describing cohesin binding to chromatin to be resistant to 1.6 M KCl¹². Importantly, upon cross-linking of the dimeric but not the monomeric fraction, covalently closed cohesin rings caused the appearance of dimeric minichromosomes that were resistant to protein denaturing conditions (1% SDS, 65 °C). If these SDS-resistant dimers represented sister DNAs entrapped by covalently closed cohesin rings, then proteolytic cleavage of the cohesin ring should be sufficient to release the monomeric DNAs. Consistent with this notion, opening of the cohesin ring by proteolytic cleavage at the Smc3-Scc1 interface resulted in strong reduction of minichromosome dimers and increase of monomeric DNAs. Therefore, covalent circularization of cohesin is sufficient to hold sister DNAs together. Further experiments and estimation of the efficiency of the cross-linking allowed the authors to conclude that sister DNAs are trapped within a single cohesin ring.

Recent studies of sister chromatid cohesion have revealed its remarkable complexity, with multiple levels of regulation and many players involved^{10,13-16}. Although the observations from Haering *et al.* provide strong evidence for the topological interaction between cohesin and chromatin, it will be important to apply additional approaches, such as *in vitro* reconstitution and emerging microscopy techniques, to shed more light into this fundamental aspect of biology. It remains to be tested whether this mechanism is unique to yeast minichromosomes or applicable to chromosomes in higher eukaryotes. It will also be interesting to analyze whether other related protein complexes, such as condensin, have similar activities. Moreover, binding of cohesin to chromatin can be very dynamic¹⁷, but a stable association of cohesin with chromatin is required for sister chromatid cohesion. Thus, a major challenge will be to find out how cohesin associates stably with chromatin—is this simply due to the passage of DNA through the cohesin ring? Recent evidence suggests that acetylation of the Smc3 subunit by the Eco1 acetyltransferase has an important role in stabilizing sister chromatid cohesion¹⁸. However, the function of Eco1 can be bypassed and sister chromatid cohesion can be established in the absence of the Eco1 acetyltransferase¹⁹. New findings suggest that cohesin has an important role in cells with unreplicated chromosomes, where it regulates gene expression²⁰⁻²⁵. It will be exciting to explore whether this involves trapping individual chromatin fibers by cohesin rings, or whether it reflects a new function of cohesin distinct from its ability to mediate sister chromatid cohesion.

Acknowledgments

This work was supported by Austrian Science Fund grants (P18955, P20444, F3403) and the (European Community's) Seventh Framework Programme (*FP7/2007-2013*) under grant agreement number PIEF-GA-2008-220518.

References

1. Haering CH, Lowe J, Hochwagen A, Nasmyth K. *Mol. Cell.* 2002; 9:773–788. [PubMed: 11983169]
2. Gruber S, Haering CH, Nasmyth K. *Cell.* 2003; 112:765–777. [PubMed: 12654244]
3. Haering CH, et al. *Mol. Cell.* 2004; 15:951–964. [PubMed: 15383284]
4. Gruber S, et al. *Cell.* 2006; 127:523–537. [PubMed: 17081975]
5. Ivanov D, Nasmyth K. *Mol. Cell.* 2007; 27:300–310. [PubMed: 17643378]
6. Ivanov D, Nasmyth K. *Cell.* 2005; 122:849–860. [PubMed: 16179255]
7. Huang CE, Milutinovich M, Koshland D. *Phil. Trans. R. Soc. Lond. B.* 2005; 360:537–542. [PubMed: 15897179]
8. Milutinovich M, Koshland DE. *Science.* 2003; 300:1101–1102. [PubMed: 12750506]
9. Guacci V. *Genes Cells.* 2007; 12:693–708. [PubMed: 17573771]
10. Diaz-Martinez LA, Gimenez-Abian JF, Clarke DJ. *J. Cell Sci.* 2008; 121:2107–2114. [PubMed: 18565823]
11. Haering CH, Farcas AM, Arumugam P, Metson J, Nasmyth K. *Nature.* 2008; 454:297–301. [PubMed: 18596691]
12. Ciosk R, et al. *Mol. Cell.* 2000; 5:243–254. [PubMed: 10882066]
13. Onn I, Heidinger-Pauli JM, Guacci V, Unal E, Koshland DE. *Annu. Rev. Cell Dev. Biol.* published online, doi:10.1146/annurev.cellbio.24.110707.175350 (10 July 2008).
14. McNairn AJ, Gerton JL. *Trends Genet.* 2008; 24:382–389. [PubMed: 18602182]
15. Losada A. *Biochim. Biophys. Acta.* published online, doi:10.1016/j.bbcn.2008.04.003 (24 April 2008).
16. Yanagida M. *Phil. Trans. R. Soc. Lond. B.* 2005; 360:609–621. [PubMed: 15897183]
17. Gerlich D, Koch B, Dupeux F, Peters JM, Ellenberg J. *Curr. Biol.* 2006; 16:1571–1578. [PubMed: 16890534]
18. Unal E, et al. *Science.* 2008; 321:566–569. [PubMed: 18653894]
19. Ben-Shahar TR, et al. *Science.* 2008; 321:563–566. [PubMed: 18653893]
20. Pauli A, et al. *Dev. Cell.* 2008; 14:239–251. [PubMed: 18267092]
21. Schuldiner O, et al. *Dev. Cell.* 2008; 14:227–238. [PubMed: 18267091]
22. Wendt KS, et al. *Nature.* 2008; 451:796–801. [PubMed: 18235444]
23. Parelho V, et al. *Cell.* 2008; 132:422–433. [PubMed: 18237772]
24. Misulovin Z, et al. *Chromosoma.* 2008; 117:89–102. [PubMed: 17965872]
25. Stedman W, et al. *EMBO J.* 2008; 27:654–666. [PubMed: 18219272]

Figure 1.

Covalently closed cohesin rings hold minichromosome chromatids together after protein denaturation. **(a–c)** Models for sister chromatid cohesion: **a** and **b** represent the ring model, in which cohesin topologically encircles sister chromatids; **c** represents the oligomerization model, in which sister chromatid cohesion is generated by association of several chromatin-bound cohesin complexes. Haering *et al.*¹¹ tested the models by using cross-linking agents to create covalently closed cohesin rings (**b,c**). They argued that if the ring model is correct, covalently closed cohesin rings should hold sister chromatids together even after protein denaturation (**b**). On the other hand, the oligomerization model predicts that protein denaturation would destroy sister chromatid cohesion (**c**). Treatment with 1% SDS at elevated temperature (65 °C) unfolds the secondary structure of proteins. This results in disruption of protein-protein interactions, whereas covalent bonds remain intact. Haering *et al.*¹¹ observed that upon cross-linking, covalently closed cohesin rings rendered dimeric minichromosomes resistant to these protein denaturing conditions. This observation is consistent with the ring model (**a,b**), but not with the oligomerization model (**c**).