A matter of choice: the establishment of sister chromatid cohesion

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Sister chromatid cohesion is the basis for the recognition of chromosomal DNA replication products for their bipolar segregation in mitosis. Fundamental to sister chromatid cohesion is the ring-shaped cohesin complex, which is loaded onto chromosomes long before the initiation of DNA replication and is thought to hold replicated sister chromatids together by topological embrace. What happens to cohesin when the replication fork approaches, and how cohesin recognizes newly synthesized sister chromatids, is poorly understood. The characterization of a number of cohesion establishment factors has begun to provide hints as to the reactions involved. Cohesin is a member of the evolutionarily conserved family of Smc subunit-based protein complexes that contribute to many aspects of chromosome biology by mediating long-range DNA interactions. I propose that the establishment of cohesion equates to the selective stabilization of those cohesin-mediated DNA interactions that link sister chromatids in the wake of replication forks.

Keywords: DNA replication; sister chromatid cohesion; chromosome segregation; cohesin; Smc complexes

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See Glossary for abbreviations used in this article.

Introduction

Eukaryotic cells inherit much of their genomes in the form of chromosomes during cell division. After DNA replication occurs in S-phase, sister chromatids remain tightly linked in pairs by the cohesin complex. This facilitates their tension-generating bi-orientation on the mitotic spindle, before a protease known as separase cleaves the cohesin complex to liberate sister chromatids for movement towards the spindle poles.

Cohesin is a multisubunit protein complex, shaped like a large proteinaceous ring, which is loaded onto chromosomes—during telophase in human cells and in late G1-phase in budding yeast—by the Scc2/4 cohesin loading complex. During DNA replication in S-phase, when the cohesion between sister chromatids is established, cohesin topologically embraces and entraps replication

products (Haering *et al*, 2008). Sister chromatid cohesion forms the basis not only of chromosome segregation, but also of DNA break repair by homologous recombination during the G2-phase of the cell cycle. In addition, cohesin mediates chromosome condensation and long-range DNA interactions that are part of transcriptional insulators and boundary elements, independently of sister chromatid cohesion. Recent reviews have surveyed the molecular biology of cohesin (Losada & Hirano, 2005; Nasmyth & Haering, 2005; Onn *et al*, 2008). Here, I discuss how cohesin establishes specific and enduring linkages between sister chromatids, by drawing on a comparison with related Smc complexes and their ability to promote DNA interactions.

The ancestors of cohesin

Cohesin is a member of an evolutionarily conserved family of Smc subunit-based protein complexes, which are characterized by a dimer of long flexible Smc subunits that give the complexes their ring shape through head-to-head and tail-to-tail interactions (Anderson *et al*, 2002; Haering *et al*, 2002; Melby *et al*, 1998). The heads of the Smc complexes consist of ATPase domains, the activity of which is essential for their function. In the case of cohesin, mutations in the ATPase prevent it from loading onto chromosomes, suggesting that the ATPase activity mediates an opening reaction through which DNA enters the cohesin ring (Arumugam *et al*, 2003; Weitzer *et al*, 2003). All Smc complexes contain several additional essential subunits. Of note is the conserved kleisin subunit, which binds to the Smc heads directly and stabilizes their interaction (Haering *et al*, 2002); the cleavage of the kleisin Scc1 by separase destroys the cohesin ring in anaphase (Uhlmann *et al*, 2000). Given their striking similarities, and in the absence of evidence to the contrary, we can assume that all Smc complexes act by topologically entrapping more than one strand of DNA in an ATP hydrolysis-dependent reaction.

Prokaryotic Smc complexes compact the bacterial chromosome and promote its resolution and segregation to daughter cells after DNA replication. They may achieve this by establishing long-range DNA interactions between two different Smc binding sites, which are found at places of strong transcription along the chromosome arms and are enriched around *cis*-acting partitioning sequences (Gruber & Errington, 2009; Fig 1A). This ancestral function of Smc complexes is largely recapitulated in eukaryotes by the condensin complex; like its prokaryotic counterpart, condensin promotes the compaction of chromosomes and sister chromatid resolution. The

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Fig 1 | Comparison of Smc complexes and their possible modes of action on chromosomes. (A) Cartoon of the subunit composition of the bacterial Smc complex, its binding sites along the circular bacterial chromosome, and a proposal of how it might compact the chromosome by establishing links between neighbouring binding sites. The movement of bacterial replication products towards opposite cell poles initiates from the *parS* partitioning locus, which is enriched in Smc complexes. Additional binding sites along the chromosome arms correlate with highly expressed genes, such as rRNA (*rrn*) and tRNA (*trn*). (B) The eukaryotic condensin complex has a chromosomal association pattern reminiscent of the bacterial Smc complex. It is enriched at the centromere (CEN) and at highly transcribed loci along the chromosome arms, including rRNA, tRNA and ribosomal protein genes (RPS and RPL). Condensin may similarly promote chromosome compaction by establishing interactions between neighbouring binding sites. The enrichment around the centromere provides additional mechanical stability around this locus during chromosome segregation. (C) Cohesin associates with chromosomes at the same loci as condensin, but shows pronounced translocation on loading in response to transcription, perhaps because of its longer mean residence time on chromosomes. Cohesin also establishes interactions between neighbouring binding sites as part of transcriptional insulators but has specialized in establishing stable interactions between sister chromatids during DNA replication. Brn, barren; Pds, precocious dissociation of sister chromatids; rRNA, ribosomal RNA; Scc, sister chromatid cohesion; Scp, segregation and condensation protein; Smc, structural maintenance of chromosomes; tRNA, transfer RNA; Wapl, wings apart-like; Ycg, yeast CapG; Ycs, yeast condensin subunit.

nucleus (Haeusler *et al*, 2008). Whether the interactions between condensin binding sites follow an underlying organizing principle, or are largely self-organizing according to proximity along the primary DNA sequence, is not known. The function of condensin in sister chromatid resolution, which is probably to facilitate the decatenation of topological DNA links that persist after the termination of DNA replication, is also poorly understood (Bhat *et al*, 1996; D'Ambrosio *et al*, 2008a).

What is so special about cohesin?

The similarities between cohesin and condensin are worth noting. In both budding and fission yeast, cohesin and condensin load onto chromosomes at the same sites (D'Ambrosio *et al*, 2008b; Lengronne *et al*, 2004; Schmidt *et al*, 2009). The loading of cohesin at these sites depends on the Scc2/4 cohesin loading complex, the mechanism of action of which is still unclear (Ciosk *et al*, 2000). Scc2/4 might be the major determinant of these loading sites, to which—at least in budding yeast—Scc2/4 also recruits condensin and a third Smc complex, Smc5/6 (Betts Lindroos *et al*, 2006). This has not yet been seen in other organisms in which condensin might alternatively—or additionally—be able to recognize its loading sites independently of Scc2/4.

A distinguishing feature of cohesin is that, on loading, the complex translocates along the DNA in a manner consistent with downstream movement along RNA polymerase II-transcribed genes (Lengronne *et al*, 2004; Schmidt *et al*, 2009). This translocation is most prominently observed in budding and fission yeast, although not all cohesin translocates in the latter. In *Drosophila*, cohesin largely remains close to its loading complex (Misulovin *et al*, 2008). Although the significance of the translocation reaction

binding pattern of the complexes is strikingly conserved between bacteria and eukaryotes, with enrichment at strongly transcribed regions along chromosome arms and around centromeres, which form the eukaryotic partitioning locus (D'Ambrosio *et al*, 2008b; Jans *et al*, 2009). As in prokaryotes, the mechanism by which condensin compacts chromosomes is still unclear. It is tempting to speculate that condensin promotes interactions between two or more of its binding sites, perhaps by the sequential topological capture of distinct DNA sequences as they come into spatial proximity by Brownian motion (Fig 1B). A proof of principle is provided by the observation of condensin-dependent clustering of tRNA gene loci—a prominent class of condensin binding sites—in the budding yeast

remains unknown, it might hint at a specific feature of cohesin. Translocation in response to transcription might reflect the long average time that cohesin resides on chromatin, which allows it to hold topologically onto chromosomes while the transcription machinery travels along the relatively short yeast genes. Several measurements of the stability of cohesin on chromosomes (Bernard *et al*, 2008; Gerlich *et al*, 2006b; Kueng *et al*, 2006; McNairn & Gerton, 2009; Rowland *et al*, 2009) suggest an average residence time in the range of minutes, a long time for any molecular interaction. Evidence for translocation has also been obtained for condensin, but the greater dissociation rate of this complex, at least in interphase, might largely obscure this process (D'Ambrosio *et al*, 2008b; Gerlich *et al*, 2006a; Johzuka & Horiuchi, 2007).

Although cohesin has a longer average residence time than condensin, the two complexes could in principle perform a similar reaction, namely the subsequent topological entrapment of more than one strand of DNA (Fig 1C). This has been suggested by recent studies of the contribution of mammalian cohesin to transcriptional regulation, which show that cohesin mediates long-range interactions between neighbouring binding sites that also bind to the CTCF transcriptional insulator (Hadjur *et al*, 2009; Mishiroy *et al*, 2009). According to this finding, cohesin—similarly to all Smc complexes—acts by establishing interactions between distant binding sites. The specificities of the Smc complexes for particular interaction sites could be afforded in part by their unique subunit composition.

Cohesin acetylation and stability on chromosomes

On the basis of these considerations, how does cohesin promote sister chromatid cohesion? I propose that two aspects of cohesin have singled it out to become the crucial mediator of sister chromatid cohesion: its mechanism of targeting to regions where newly replicated sister chromatids interact, and the stabilization of the cohesin-mediated interactions linking DNA replication products.

Once loaded onto chromosomes, cohesin has a slow off-rate that is in the region of minutes. Is this sufficient for promoting enduring sister chromatid cohesion? As chromosomes come under tension from the mitotic spindle, a directional pull is exerted towards separating sister chromatids. Even with a residence time of minutes, sister chromatids would separate slowly but progressively. To prevent this, an even more stable interaction of cohesin with chromosomes is required. Indeed, there is good evidence that at least a subpool of cohesin becomes significantly stabilized on chromosomes after DNA replication. The direct observation of cohesin dynamics on mammalian chromosomes revealed that during the course of S-phase, approximately one-third of all cohesin becomes stably linked to chromosomes, with a mean residence time of at least 6 hours (Gerlich *et al*, 2006b). In addition, evidence from budding yeast suggests that after DNA replication there is no measurable exchange of the functional pool of cohesin that mediates sister chromatid cohesion (Haering *et al*, 2004). In fission yeast, cohesin dissociates from chromosomes if its loading complex is inactivated in the G1-phase, but not after DNA replication in the G2-phase of the cell cycle (Bernard *et al*, 2008).

One hint as to how this nearly permanent stabilization of cohesin is achieved comes from studies of two regulators of the complex. The first is a protein known as Wapl, which binds to cohesin and promotes its dissociation from chromosomes. In the absence of Wapl, cohesin associates more stably with both human and fission yeast chromosomes. In human cells, Wapl promotes the dissociation of

Glossary

Chl	chromosome loss
Csm	chromosome segregation in meiosis
CTCF	CCCTC-binding factor
Ctf	chromosome transmission fidelity
Dcc	defective in sister chromatid cohesion
Eco	establishment of cohesion
Eso	essential for S-chromatin organization
Fen	flap endonuclease
MCM	minichromosome maintenance
Mrc	mediator of the replication checkpoint
PCNA	proliferating cell nuclear antigen
Pds	precocious dissociation of sister chromatids
RFC	replication factor C
Scc	sister chromatid cohesion
Smc	structural maintenance of chromosomes
Tof	topoisomerase-1-associated factor
tRNA	transfer RNA
Wapl	wings apart-like

a substantial pool of cohesin from chromosomes during prophase (Bernard *et al*, 2008; Gandhi *et al*, 2006; Kueng *et al*, 2006).

The second regulator is the acetyltransferase Eco1, which early studies have identified as a cohesion establishment factor, a protein that is required for the generation of stable sister chromatid cohesion but is not part of the cohesin complex (Ivanov *et al*, 2002; Skibbens *et al*, 1999; Tóth *et al*, 1999). In fission yeast, the Eco1 orthologue Eso1 is required for the stabilization of cohesin as cells progress through S-phase (Bernard *et al*, 2008). The mechanism of action of Eco1 has since been unveiled: it associates with the DNA replication fork, probably through an interaction with the multi-functional DNA polymerase processivity factor PCNA (Lengronne *et al*, 2006; Moldovan *et al*, 2006), and acetylates the cohesin subunit Smc3 during DNA replication when the fork passes cohesin binding sites (Ben-Shahar *et al*, 2008; Ünal *et al*, 2008; Zhang *et al*, 2008). Smc3 acetylation explains the essential function of Eco1 in the establishment of cohesion, as indicated by the analysis of non-acetylatable and acetylation-mimicking mutations of two conserved Smc3 acetylation sites. However, it is not only acetylation-mimicking mutations in Smc3 that make Eco1 dispensable for sister chromatid cohesion; the deletion of Wapl from budding yeast cells has the same effect (Ben-Shahar *et al*, 2008; Rowland *et al*, 2009; Sutani *et al*, 2009), suggesting that Smc3 acetylation counteracts Wapl. Considering the role of Wapl in promoting cohesin dissociation from chromosomes, one simple hypothesis is that the role of Smc3 acetylation in the establishment of cohesion is to generate a stable Wapl-resistant cohesin pool (Fig 2A). In addition to acetylation-mimicking mutations in Smc3, a number of other point mutations in Smc3 and the cohesin subunits Scc3 and Pds5 allow for the establishment of cohesion in the absence of Eco1 (Rowland *et al*, 2009; Sutani *et al*, 2009). These mutations could demarcate surfaces of interaction that mediate the destabilization of cohesin by Wapl. This hypothesis does not exclude the fact that Smc3 acetylation could have other consequences for the cohesin complex that facilitate the establishment of cohesion (Ben-Shahar *et al*, 2008; Rowland *et al*, 2009).

In the absence of Eco1 and Wapl, sister chromatid cohesion is functional, albeit compromised. There is no pronounced cohesin

Fig 2 | Establishment of sister chromatid cohesion. (A) Establishment of cohesion during DNA replication. Replication fork-associated Eco1 acetylates Smc3, which renders cohesin resistant to the destabilizing activity of Wapl, a prerequisite for enduring sister chromatid cohesion. How Wapl destabilizes cohesin, and what further impact it has on the establishment of cohesion, is not known. Several additional cohesion establishment factors are depicted at the replication fork. Their mechanism of action is unknown, but they could aid the passage of the replication fork through cohesin rings, potentially by coordinating lagging strand loop release with the encounter of cohesin. (B) Cohesion establishment in response to DNA breaks in G2. The ability of cohesin to link two of its binding sites might establish interactions between sister chromatids because of their proximity in G2. In response to DNA damage, Eco1 stabilizes these links by Scc1 acetylation, which might be aided by the recruitment of Eco1 to break sites and genome-wide Scc1 phosphorylation by the damage-activated Chk1 kinase. Ac, acetyl group; Chl, chromosome loss; Csm, chromosome segregation in meiosis; Ctf, chromosome transmission fidelity; Eco, establishment of cohesion; Fen, flap endonuclease; Mrc, mediator of the replication checkpoint; PCNA, proliferating cell nuclear antigen; RFC, replication factor C; Scc, sister chromatid cohesion; Smc, structural maintenance of chromosomes; Tof, topoisomerase-1-associated factor; Wapl, wings apart-like.

removal in prophase during budding yeast mitosis, and whether the main function of Wapl is to add robustness to the establishment of cohesion or is unrelated to sister chromatid cohesion remains to be clarified. An additional regulator of cohesin stability in human cells, Sororin, has not yet been identified in yeast (Rankin *et al*, 2005; Schmitz *et al*, 2007). Whether Sororin impacts on Smc3 acetylation and acts as a cohesion establishment factor, or whether it has an independent role in maintaining cohesin stability after DNA replication, will be interesting to address.

How does cohesin choose sister chromatids?

One could imagine that the mere proximity of DNA replication products, as they emerge from the replication fork, gives cohesin—or any other Smc complex (Lam *et al*, 2006)—a high likelihood of establishing interactions between them by sequential topological embrace. Given the localization of Eco1 at the replication fork, the cohesin rings that link replication products have a good chance to become acetylated and thus stabilized. In principle, this mechanism might suffice to establish stable sister chromatid cohesion, and

there is no reason to believe that it does not take place. However, there is evidence that this is not the only, and maybe not the main, mechanism by which sister chromatids are linked.

The possibility that contacts between two sister chromatids are established when they are in close proximity, immediately after their synthesis, requires that cohesin is loaded onto at least one of the two newly synthesized replication products after the replication fork has passed. This would require the renewed action of the cohesin loading complex Scc2/4 and renewed ATP hydrolysis. By contrast, Scc2/4 and a motif in the cohesin ATPase that is required for swift loading of cohesin onto DNA before S-phase are not strictly required for the establishment of sister chromatid cohesion (Lengronne *et al*, 2006). This of course does not exclude the possibility that an as yet uncharacterized alternative cohesin loading reaction takes place close to replication forks. However, an attractive alternative would be that no new DNA contacts need to be made during the establishment of cohesion and that the two replication products are left within the cohesin ring after the replisome passes through its centre (Haering *et al*, 2002).

Additional establishment factors at the fork

In addition to Eco1, several non-essential cohesion establishment factors have been identified in budding yeast, many of which are known to be linked to the replication fork machinery (Hanna *et al*, 2001; Mayer *et al*, 2001, 2004; Petronczki *et al*, 2004; Skibbens, 2004; Warren *et al*, 2004). These factors have been divided by genetic epistasis analysis into two groups (Xu *et al*, 2007); the components of either group can be deleted without loss of cell viability, but the simultaneous inactivation of factors in both groups is lethal. Both epistasis groups are required for the establishment of sister chromatid cohesion during S phase, but not for the maintenance of cohesion once it is established (Xu *et al*, 2007). What can we learn from these factors about the cohesion establishment process?

The first group includes the Ctf18, Ctf8 and Dcc1 subunits of an alternative RFC complex (RFC^{Ctf18}), which is able to load PCNA onto DNA (Bermudez *et al*, 2003; Mayer *et al*, 2001). The absence of RFC^{Ctf18} leads to reduced PCNA levels in the vicinity of replication forks (Lengronne *et al*, 2006). Cohesion defects have also been seen after the mutation of RFC core subunits and PCNA itself, and genetic interactions between PCNA and Eco1 have been documented (Moldovan *et al*, 2006; Skibbens *et al*, 1999). If PCNA recruits Eco1 to replication forks, RFC^{Ctf18} could act upstream from Eco1. However, other explanations cannot be excluded, as PCNA is the landing platform for a large number of enzymatic activities at replication forks that are involved in processes such as lesion bypass, Okazaki fragment maturation and chromatin assembly. The RFC^{Ctf18} epistasis group also contains the replication checkpoint mediator protein Mrc1, the presence of which at the fork—but not its checkpoint activity—contributes to the establishment of cohesion in an unknown manner (Katou *et al*, 2003; Xu *et al*, 2004).

The second epistasis group is assembled around Ctf4, a central component of the replisome progression complex that mediates protein interactions between the MCM DNA helicase and the DNA polymerase α /primase complex at the replication fork (Gambus *et al*, 2006; Hanna *et al*, 2001; Tanaka *et al*, 2009; Zhu *et al*, 2007). This group also contains the Ctf4-interacting replication pausing regulators Tof1 and Csm3, the role of which during undisturbed S-phase progression is unclear. These proteins could act in a second pathway to recruit Eco1 to replication forks independently of PCNA. Alternatively, the Ctf4-dependent link of DNA helicase and primase could regulate the initiation of Okazaki fragment synthesis during lagging strand replication, as occurs in prokaryotic replication systems (Tougu & Marians, 1996). An implication of lagging strand reactions in the establishment of cohesion comes from another establishment factor in this epistasis group, the Chl1 helicase (Farina *et al*, 2008; Gerring *et al*, 1990; Petronczki *et al*, 2004; Skibbens, 2004), which stimulates the Fen1 flap endonuclease, a key enzyme during Okazaki fragment processing. Notably, mutation or depletion of Fen1 also causes a sister chromatid cohesion defect (Farina *et al*, 2008; Yuen *et al*, 2007).

How do these contributors relate to cohesion establishment? No stable interactions between any of these factors and cohesin—which could have indicated their participation in a potential cohesin opening reaction as part of cohesion establishment—have been documented. Could the replication fork traverse through cohesin rings to leave replication products trapped inside? If this were the case, the geometry of the replisome would probably be crucial to its success in passing through cohesin without disrupting the ring. A model of a eukaryotic replication fork, based on knowledge of prokaryotic DNA replication, suggests that fork passage might be possible (Lengronne

Sidebar A | In need of answers

- (i) How do Smc complexes link distant strands of DNA? Evidence has shown that single cohesin rings link replicated circular minichromosomes. Is this the universal mechanism, as assumed here, by which all Smc complexes mediate DNA interactions? Two DNA strands could be subsequently embraced by the same Smc complex, but alternative models in which interactions of more than one Smc complex with each other underlie these long-range interactions have been proposed.
- (ii) What happens to cohesin as the replisome passes? Many aspects of the model in which Eco1-dependent cohesin acetylation by the passing replication fork stabilizes sister chromatid cohesion remain to be tested. Does acetylation change additional features of cohesin that contribute to the establishment of cohesion? What is the precise molecular impact of Wapl on cohesin?
- (iii) How does cohesin choose sister chromatids? Does the replisome indeed traverse through the cohesin ring? A firm answer to these questions will have to await the reconstitution of cohesin loading onto DNA *in vitro*, followed by replication using an established *in vitro* replication system.

et al, 2006). However, our understanding of the actual arrangement of eukaryotic fork components and the stability of their interactions within the replisome is still incomplete. If the trombone model for Okazaki fragment synthesis holds true for eukaryotes, a potential obstacle to the passage of replication forks through cohesin rings could be the lagging strand loop (Hamdan *et al*, 2009; Fig 2A). A potential role of the Ctf4 epistasis group could thus be to coordinate fork passage through cohesin rings with Okazaki fragment synthesis, so that loops would be released at the time of cohesin passage. Whether an interaction between cohesin and the MCM helicase is relevant in this process remains to be clarified (Ryu *et al*, 2006). This is clearly only one possible way in which these diverse replication proteins could impact on the replication fork to facilitate the establishment of cohesion. We also have to consider why cohesin translocates along chromosomes in response to transcription, but not in response to a passing replication fork (Lengronne *et al*, 2006).

The establishment of cohesion in G2

Sister chromatid cohesion must be established during DNA replication. Once replication is complete, cohesin can still be loaded onto chromosomes but will no longer be able to establish sister chromatid cohesion (Uhlmann & Nasmyth, 1998). There are probably two reasons for this. First, if sister chromatids are not held together when synthesized, they will be free to diffuse away from each other. Once separate, there is no known mechanism that can bring sister chromatids back together. Even homology-based mechanisms that recognize sister chromatids during mitotic repair by homologous recombination appear to depend on, rather than to generate, sister chromatid cohesion (Sjögren & Nasmyth, 2001). Second, even if sister chromatids are correctly paired, cohesin added in the G2-phase of the cell cycle is unable to generate stable cohesive links (Haering *et al*, 2004; Lengronne *et al*, 2006). The reason for this becomes apparent from a notable exception.

In response to a double-stranded DNA break in G2, cohesin is recruited to the break site and reinforces sister chromatid cohesion in an Eco1-dependent manner, which is a prerequisite for efficient break repair by homologous recombination (Ström *et al*, 2004, 2007; Ünal *et al*, 2007). RFC^{Ctf18} is recruited to the break

site and could facilitate the establishment of cohesion by engaging PCNA along with Eco1 (Ogiwara *et al*, 2007). Notably, cohesin is now enabled to establish sister chromatid cohesion all along the genome, not just around the break site. The overexpression of Eco1 in G2 cells has the same effect, allowing cohesin to establish new genome-wide sister chromatid cohesion.

A possible explanation for these findings is related to the generic ability of cohesin to link two distant binding sites, perhaps by sequential embrace (Hadjur *et al*, 2009; Mishiro *et al*, 2009). If sister chromatid cohesion pre-exists, cohesin will have a good chance of linking sister chromatids. This, however, will not provide additional sister chromatid cohesion unless Eco1 stabilizes these links (Fig 2B), which might be achieved by recruiting Eco1 to break sites. In addition, it has been suggested that the checkpoint kinase Chk1 phosphorylates the cohesin subunit Scc1 in response to DNA damage, thereby promoting Scc1 acetylation by Eco1 (Heidinger-Pauli *et al*, 2008, 2009). Although the capture of sister chromatids might have occurred through different means, the acetylation of Scc1 seems to counteract Wapl, as does replication fork-dependent Smc3 acetylation. The genome-wide reinforcement of sister chromatid cohesion that is achieved in this manner warrants an accurate chromosome segregation as cells exit from the checkpoint arrest (Ünal *et al*, 2007). At the break site itself, additional sister chromatid cohesion could facilitate recombination reactions and cohesin could alternatively, or additionally, promote interactions between the two sides of the broken DNA strand.

A second exception in which cohesion is established after the completion of DNA replication is evident during the elastic centromere breathing that accompanies the bi-orientation of sister chromatid pairs on the mitotic spindle. Sister sequences transiently split during this process, which is accompanied by a loss of cohesin from centromeres (Eckert *et al*, 2007; Ocampo-Hafalla *et al*, 2007). Cohesin is reloaded again when centromeres reassociate, a process which is probably facilitated by a prominent cohesin loading site that exists in the centromere (Lengronne *et al*, 2004). Notably, Eco1 is required for efficient centromere re-association, suggesting that the re-establishment of cohesion is part of the mechanism that rejoins sister centromeres. The cohesin acetylation site that is targeted in this context remains to be identified. These findings suggest that although initial sister chromatid cohesion must be established during DNA replication, its subsequent modulation is an integral part of the response to DNA damage and to the elastic counterforce of sister centromeres to the mitotic spindle.

Conclusions

Cohesin is one of at least three essential Smc complexes in eukaryotes. Based on their structural resemblance and the emerging similarities in how they load onto chromosomes, it is not surprising that Smc complexes have overlapping roles in the processes of sister chromatid cohesion, chromosome condensation, transcriptional regulation and DNA repair. The crucial step in all of these processes could be the linkage of distant DNA binding sites by topological embrace. However, individual complexes might have additional roles, such as engaging in specific protein interactions or recruiting additional enzymatic activities (Andrews *et al*, 2005; Chang *et al*, 2005; Nonaka *et al*, 2002). An important specialization of the cohesin complex is its marked stabilization on chromosomes, which is a prerequisite to mediate enduring sister chromatid cohesion, and correlates with its acetylation. A second unique feature

might lie in its open ring structure, compared to the more pin-like appearance of condensin (Anderson *et al*, 2002). This could facilitate the passage of the replication fork through cohesin rings as an efficient means to trap replication products, in addition to the generic ability of cohesin to mediate DNA contacts by sequential loading onto more than one binding site. Molecular genetics approaches will continue to increase our understanding of the many roles of Smc complexes in chromosome biology. Ultimately, their molecular mechanism will only be elucidated by using biochemical assays to study their loading onto DNA and by observing their behaviour during the passage of a replication fork.

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