The Smc5/6 Complex: More Than Repair?

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Through its functions in chromosome replication, segregation, and repair, the Smc5/6 complex has a central role in the maintenance of genome stability. The complex is part of the family of structural maintenance of chromosome protein complexes that also includes cohesin and condensin. Mutations in any of these complexes disrupt chromosome segregation and render cells hypersensitive to different types of DNA damage. The chromosome mis-segregation phenotypes in cohesin and condensin mutants can be attributed to their functions in sister chromatid cohesion and chromosome condensation, respectively. Cohesin-dependent chromatid cohesion is also needed for DNA double-strand break repair, whereas condensin is required for repair of single-strand breaks. How Smc5/6 promotes chromosome stability is largely unknown. Accumulating data suggest that it prevents accumulation of aberrant DNA links between sister chromatids created during repair by homologous recombination. A long-standing idea is that it also has a role in the maintenance of nondamaged chromosomes. Here, we present an overview of the current knowledge of Smc5/6 and discuss a possible nonrepair role of the complex.

Structural maintenance of chromosome (SMC) protein complexes have been implicated in many, if not all, fundamental chromosome-based processes. They regulate chromosome segregation, repair, and transcription, and recent results indicate that these multitasking complexes have an impact on chromosome replication as well (Nasmyth and Haering 2005; Hirano 2006; Terret et al. 2009; Wendt and Peters 2009). The Smc5/6 complex (Smc5/6) is no exception. It is central for correct repair by homologous recombination (HR), is needed for resolution of chromosomes during cell division, and has been implicated in a late step in replication (De Piccoli et al. 2009; Potts 2009). There are also indications that the complex is connected to regulation of gene silencing (Dhillon and Kamakaka 2000; Cuperus and Shore 2002; Zhao and Blobel 2005; Yu et al. 2010). Here, we present current knowledge of the complex's structure and give a summary of its repair functions. We also discuss the evidence for nonrepair roles of the complex. For a more detailed review on the repair function of Smc5/6, we refer to two recent reviews (De Piccoli et al. 2009; Potts 2009).

COMPONENTS AND STRUCTURE OF THE SMC5/6 COMPLEX

Yeast Smc5/6 consists of eight core subunits: Smc5, Smc6, and six nonstructural maintenance of chromosome element (Nse) proteins (Fousteri and Lehmann 2000; Fujioka et al. 2002; Hazbun et al. 2003; McDonald et al. 2003; Morikawa et al. 2004; Pebernard et al. 2004, 2006; Sergeant et al. 2005; Zhao and Blobel 2005). In the budding yeast *Saccharomyces cerevisiae*, Nse proteins are named Nse1, Mms21/Nse2, Nse3, Nse4/Qri2, Nse5, and Kre29/Nse6. In the fission yeast *Schizosaccharomyces pombe*, Smc6 was first identified as Rad18, Smc5 as Spr18, and Nse4 as Rad62 (Verkade et al. 1999; Fousteri

and Lehmann 2000; Morikawa et al. 2004). Six of the human orthologs (hSmc5, hSmc6, hNse1, hNse2/hMms21, hNse3/MAGE1, and hNse4) have been identified and shown to form a complex in vivo (Taylor et al. 2001, 2008; Harvey et al. 2004; Potts and Yu 2005). Here, we call the subunits of the complex Smc5, Smc6, and Nse1 - Nse6. In addition to these eight core subunits, fission yeast Smc5 and Smc6 have been shown to interact with Rad60 (Boddy et al. 2003). Rad60, and its budding yeast homolog Esc2, are also functionally connected to Smc5/6 (see below), but no physical interaction between Esc2 and the *S. cerevisiae* core complex has been reported so far.

How are the different subunits organized in the Smc5/6 complex? With the known structural features of cohesin and condensin as starting points, a picture based mostly on the yeast two-hybrid system and biochemical analysis starts to emerge (Fig. 1) (Sergeant et al. 2005; Palecek et al. 2006; Pebernard et al. 2006, 2008a; Duan et al. 2009b). SMC proteins contain a central extended α -helical region that ends in a globular domain on each side. These globular amino and carboxyl termini include a Walker A and Walker B motif, respectively. A third globular domain is found in the middle of the protein; this is called the hinge domain because it is the region in which the SMC protein folds back on itself. This folding brings the Walker motifs of the amino and carboxyl domains together, forming an ATPase that is connected to the hinge via an antiparallel coiled coil. Both cohesin and condensin hydrolyze ATP, and the ATPase activity is needed for their function (Kimura and Hirano 1997; Arumugam et al. 2003; Weitzer et al. 2003). In vitro analysis of *S. pombe* Smc5/6 also indicates that this complex has an ATPase activity that is stimulated by double-strand DNA (Fousteri and Lehmann 2000). Furthermore, point mutations in the ATP-binding domain impair the function of Smc6 and render cells sensitive to DNA-damaging agents (Verkade et al. 1999;

Figure 1. Structure and composition of SMC complexes. (A) An SMC protein is folded at the central hinge domain. This brings the Walker motifs in the amino- and carboxy-terminal globular domains together, creating an ATPase that is connected to the hinge domain via a long stretched coiled coil. (B) General organization of Smc5/6, cohesin, and condensin. Subunits are named according to the budding yeast nomenclature. With the exception of the ATPase activity of SMC proteins, only Smc5/6 contains a protein with enzymatic activity, the Nse2/Mms21 SUMO ligase. Please see text for details.

Fousteri and Lehmann 2000). Like the SMC proteins present in cohesin and condensin, Smc5 and Smc6 interact with each other via their hinge domains (Sergeant et al. 2005; Duan et al. 2009b). Nse2 binds to the coiled-coil domain of Smc5, and a recently published X-ray structure shows that this interaction is mediated by the amino-terminal domain of Nse2 (Duan et al. 2009a). Nse3, Nse4, and Nse1 form a subcomplex that associates with the globular ends of Smc5 and Smc6 (Sergeant et al. 2005; Palecek et al. 2006; Duan et al. 2009b). In the fission yeast complex, both Nse4 and Nse3 bridge Smc5 and Smc6, and Nse1 stabilizes the interaction between Nse3 and Nse4 (Sergeant et al. 2005; Palecek et al. 2006; Pebernard et al. 2008a). Nse5 and Nse6 have been reported to associate as a dimer near the globular heads of the fission yeast complex, but in budding yeast, this subcomplex appears to interact with the hinges of Smc5 and Smc6 (Palecek et al. 2006; Pebernard et al. 2006; Duan et al. 2009b). Whether this difference is relevant in a functional perspective, or is caused by experimental variation, remains to be established. Possibly, the divergent results are a reflection of a flexibility in the SMC arms that allows the hinge to reside in close proximity to the amino and carboxyl termini. However, it is of note that no sequence homology is shared by Nse5 and Nse6 from the two yeasts, and they are only

essential for survival of budding, but not fission, yeast (Pebernard et al. 2006). As it stands today, the idea that Nse5 and Nse6 from the two yeasts are functional homologs is based on the observations that they are stable subunits of the Smc5/6 complex, that mutations of either lead to DNA repair defects in both yeasts, and that the two Nse6 proteins contain similar protein-folding motifs (Hazbun et al. 2003; Zhao and Blobel 2005; Pebernard et al. 2006).

REPAIR FUNCTION OF THE SMC5/6 COMPLEX

In 1995, Lehmann et al. (1995) reported that Smc6, then called Rad18, was an SMC protein needed for repair of ultraviolet (UV) and γ -ray-induced DNA damage in *S. pombe*. Today, it is known that all eight subunits of Smc5/6 are needed for DNA repair in yeast (Fujioka et al. 2002; McDonald et al. 2003; Harvey et al. 2004; Morikawa et al. 2004; Onoda et al. 2004; Pebernard et al. 2004, 2006; Hu et al. 2005; Zhao and Blobel 2005). Repair is also defective in human cells after depletion of *MMS21* by RNA interference (RNAi) or in plants carrying a nonfunctional *RAD18/MIM/SMC6* gene caused by transfer-DNA (T-DNA) insertion (Mengiste et al. 1999; Kozak et al. 2009). Mutations of any of the Smc5/6 subunits, including Rad60/Esc2, render cells hypersensitive to DNA-damaging agents, such as UV, ionizing irradiation, methyl methanesulfonate (MMS), and hydroxyurea (HU) (Verkade et al. 1999; Fujioka et al. 2002; Morishita et al. 2002; Harvey et al. 2004; Morikawa et al. 2004; Onoda et al. 2004; Pebernard et al. 2004, 2006, 2008a; Andrews et al. 2005; Hu et al. 2005; Sergeant et al. 2005; Torres-Rosell et al. 2005b; Zhao and Blobel 2005; Ampatzidou et al. 2006; Cost and Cozzarelli 2006; Miyabe et al. 2006; Santa Maria et al. 2007; Mankouri et al. 2009; Sollier et al. 2009). Epistasis analysis indicates that the complex functions in break repair by HR (Lehmann et al. 1995; Verkade et al. 1999; Morishita et al. 2002; McDonald et al. 2003; Onoda et al. 2004; Pebernard et al. 2004, 2006, 2008a; Cost and Cozzarelli 2006; Santa Maria et al. 2007). See Figure 2 for a schematic overview of how Smc5/6 functions in HR. DNA-damage-responsive checkpoint kinases and cell cycle arrest are normally activated in response to UV, MMS, HU, and/or DNA double-strand breaks (DSBs) in budding and fission yeast *smc6*, *smc5*, and *rad60/esc2* mutants (Verkade et al. 1999; Harvey et al. 2004; Cost and Cozzarelli 2006; Miyabe et al. 2006; Torres-Rosell et al. 2007b; Mankouri et al. 2009). This could be a sign that the Smc5/6 complex is a true repair protein with little effect on the damage checkpoint. However, several studies show that *smc5/6* mutated cells reenter the cell cycle without completing repair, leading to chromosome fragmentation during an aberrant mitosis (Verkade et al. 1999; Harvey et al. 2004; Andrews et al. 2005; Ampatzidou et al. 2006; Miyabe et al. 2006). This could suggest that the complex has a role in checkpoint maintenance. However, this in turn is contradicted by the observation that checkpoint maintenance after replication stalling by HU treatment in *smc6-74*, *smc6-X*, *rad60-1*, and Δ *esc2* mutants is restored by inhibition of HR through deletion of *RAD51*

Figure 2. Smc5/6 at DSB and blocked replication forks. The Smc5/6 complex is needed for repair of DSBs and rescue of damaged forks by HR. Shown are simplified views of DSB repair (*A*) and replication fork rescue (*B*), focusing on the potential roles of Smc5/6. (*A*) In DSB repair, Smc5/6 appears to facilitate sister chromatid recombination via cohesin, which is needed to hold the chromatids in close proximity. Without Smc5/6 and cohesin, chromatids will be misaligned, and this can interfere with strand invasion and Holliday junction (HJ) formation. Based on its known function at blocked replication forks, Smc5/6 might also act in parallel with Mus81/Mms4, Sgs1/Top3/Rmi1, and others in the resolution of DSB-induced double HJs. (*B*) When a replication fork encounters a DNA lesion, it can collapse, which leads to DSB formation and repair by HR. Here, Smc5/6 is thought to work in the resolution of resulting recombination structures. When the fork avoids collapse and remains stable, Smc5/6 has also been proposed to hold the fork in stable recombination-competent conformation (Irmisch et al. 2009). Finally, the fork can bypass a lesion via an HR-dependent template switch mechanism. Here, Smc5/6, as well as Sgs1/Top3/Rmi1, are needed for the resolution of the HR-dependent sister chromatid junctions.

(Ampatzidou et al. 2006; Miyabe et al. 2006; Mankouri et al. 2009). Moreover, cell survival of *smc6-9*, *smc5-31*, *smc5-33*, and $\Delta nse6$ mutants after DNA damage, or after stress due to mutations in other repair proteins, increases in $\Delta rad51$ or $\Delta rad52$ genetic backgrounds (Torres-Rosell et al. 2005b; Cost and Cozzarelli 2006; Miyabe et al. 2006; Pebernard et al. 2006; Sollier et al. 2009). This suggests that in the absence of Smc5/6 function, repair by HR is properly initiated and continues to a point at which the checkpoint is tuned down but is not correctly finished. The ensuing resumption of cell cycle progression with incompletely repaired chromosomes leads to a catastrophic anaphase and an increase in cell death. When recombination is inhibited by deletion of genes needed at an early stage of HR, the damage is no longer processed to a checkpoint-invisible structure in *smc5/6* mutants. This leads to a prolonged cell cycle delay and allows cells to perform HR-independent repair that increases cell survival. Indeed, an emerging view is that Smc5/6 acts in late steps of HR and is needed for correct resolution of recombination structures that form between sister chromatids at blocked replication forks (see, e.g., Ampatzidou et al. 2006; Miyabe et al. 2006; Pebernard et al. 2006). This is supported by the following observations. First, aberrant Rad51-dependent recombination structures accumulate at MMS-blocked forks in budding yeast *smc6*, *nse2*, and *rad60/esc2* mutants (Branzei et al. 2006; Mankouri et al. 2009; Sollier et al. 2009). Second, similar structures are found in the recombination-prone rDNA gene repeats in *smc6-9* cells (Torres-Rosell et al. 2005b). Third, cells having a dysfunctional Smc5/6 complex or a defective Rad60/Esc2 protein become more slow growing or die when combined with deletions of the genes coding for the Sgs1 helicase or the Mus81 endonuclease, both involved in the resolution of recombination junctions (Morikawa et al. 2004; Pebernard et al. 2004, 2006, 2008a; Torres-Rosell et al. 2005b; Miyabe et al. 2006; Raffa et al. 2006; Mankouri et al. 2009; Prudden et al. 2009; Sollier et al. 2009). Mus81 is also needed for the suppression of fission yeast *smc6-74* by Brc1 overexpression (Verkade et al. 1999; Sheedy et al. 2005). Brc1, homologous to Esc4 in budding yeast, encodes a six-BRCT (BRCA1 amino-terminal)-domain protein needed for normal resistance to ionizing irradiation, MMS, HU, and camptothecin (CPT) (Verkade et al. 1999; Rouse 2004; Sheedy et al. 2005; Roberts et al. 2006, 2008). Similar to the Smc5/6 complex, Brc1/Esc4 appears to have a central role for efficient restart of replication after DNA damage, possibly functioning as a docking platform for other repair factors (Rouse 2004; Sheedy et al. 2005; Chin et al. 2006; Roberts et al. 2006, 2008; Lee et al. 2007).

So how does the Smc5/6 complex prevent the accumulation of, and/or resolve, recombination intermediates? Studies of both human and yeast cells suggest that it could work together with cohesin, at least during DSB repair. As stated, cohesin is essential for sister chromatid cohesion, and this tethering of chromatids is essential for correct chromosome segregation. Cohesin is also recruited for DNA breaks, is specifically important for DSB repair by sister chromatid recombination, and appears to facilitate

this process by holding the damaged and undamaged sisters in close proximity (Sjögren and Nasmyth 2001; Kim et al. 2002; Strom et al. 2004, 2007; Unal et al. 2004, 2007). The Smc5/6 complex also accumulates at site-specific DSBs and is specifically needed for sister chromatid recombination (De Piccoli et al. 2006; Lindroos et al. 2006; Potts et al. 2006). Furthermore, knockdown of Nse2 or Smc5 inhibits the recruitment of cohesin to a DSB in human cells, and damage-induced chromatid cohesion is defective in budding yeast *smc6-56* mutants (Potts et al. 2006; Strom et al. 2007). This could indicate that the Smc5/6 complex, via cohesin, organizes sister chromatids in a way that allows resolution of the recombination intermediates at blocked replication forks. However, in contrast to *smc6* or *nse2* mutated cells, cohesin malfunction does not lead to an accumulation of aberrant recombination structures during replication in the presence of MMS (Sollier et al. 2009). Thus, the role of Smc5/6 at replication forks appears to be separate from its function in DSB repair outside the S phase. Another possible function for Smc5/6 could be to position Nse2 (Mms21) at specific chromosomal regions. In 2005, three independent research groups identified Nse2 as a small ubiquitin-like modifier (SUMO) ligase required for DNA repair and able to stimulate sumoylation of itself, Smc5, Smc6, and the repair proteins Yku70 and Trax in vivo (Andrews et al. 2005; Potts and Yu 2005; Zhao and Blobel 2005). Later, Nse2 was also shown to prevent accumulation of aberrant recombination structures at MMS-blocked replication forks (Branzei et al. 2006) and to trigger MMS-induced sumoylation and change in nuclear localization of Nse4 (Pebernard et al. 2008b). The Nse2 ligase activity is also essential for stabilization of human telomeres maintained by a so-called alternative lengthening of telomeres (ALT) mechanism that depends on HR (Potts and Yu 2007). Smc5/6 has been shown to localize to (ALT) telomeres and collapsed replication forks and to change its chromosomal interaction pattern in response to MMS (Torres-Rosell et al. 2005b; Lindroos et al. 2006; Pebernard et al. 2008b). Therefore, it can be envisaged that the role of the non-Nse2 subunits of Smc5/6 is to identify and bind to specific chromosomal structures present at damaged forks and telomeres, thereby placing the SUMO ligase activity where it is needed. If this is true, how sumoylation of the different Nse2 substrates would facilitate sister recombination and prevent formation of aberrant recombination structures remains, however, to be determined.

A very recent publication gives yet another explanation on how Smc5/6 could act at blocked replication forks (Chen et al. 2009). In this study, it is shown that the complex physically interacts with the Mph1 helicase. Moreover, both MMS and HU hypersensitivity and accumulation of recombination structures at MMS-blocked replication forks in *smc6* and *nse2* mutants are suppressed by deletion of *MPH1* or by mutations inhibiting its helicase activity (Chen et al. 2009). These investigators propose that Smc5/6 modulates Mph1 to prevent recombination at blocked forks and/or that the complex helps to resolve recombination structures formed by Mph1. Interestingly, *mph1* Δ suppresses the lethality of *smc6* and *nse2* deletions, allowing slow growth of the double mutants (Chen et al. 2009). This

indicates that a failure to prevent recombination at naturally occurring fork blocks could be one part of the essential role of the Smc5/6 complex. Is the other part, which remains nonfunctional and, therefore, prevents wild-type growth of *smc6Δmph1Δ* and *nse2Δmph1Δ* mutants, caused by a function in a nonrepair pathway?

THE SMC5/6 COMPLEX: MORE THAN REPAIR?

The first hint that Smc5/6 does something other than repair by HR is provided by the fact that Smc5, Smc6, Nse1, Nse2, Nse3, and Nse4 are essential for survival of yeast cells. Generally, deletion of genes essential for recombination as such (e.g., *RAD51*) or involved in the resolution of recombination products (e.g., *SGS1*) does not lead to lethality. However, HR is essential for survival of higher eukaryotes, so it is possible that the repair function of Smc5/6 is unique in such a way that it also becomes vital for yeast. The isolation of an *S. pombe* separation-of-function mutation in *SMC6* that permits cell growth but is defective in repair has also been used as an argument for a nonrepair function of Smc5/6 (Fousteri and Lehmann 2000). Cells with mutations that reduce/inactivate the SUMO ligase activity of Nse2 are also viable but damage hypersensitive (Andrews et al. 2005; Zhao and Blobel 2005). The weak point of these findings, as an indicator of a nonrepair function of Smc5/6, is that the mutants might only be partially defective, with remaining repair activity sustaining survival. Nor does the nuclear morphology of Smc5/6-depleted cells provide a definitive answer as to whether the complex is solely involved in repair. Yeast cells lacking a functional complex accumulate fragmented chromosomes and/or other types of abnormal nuclear structures (Lehmann et al. 1995; Verkade et al. 1999; Fousteri and Lehmann 2000; McDonald et al. 2003). They also activate the DNA-damage checkpoint (Hu et al. 2005; Torres-Rosell et al. 2005a,b). These phenotypes could, in principle, be caused by defective repair of spontaneous damage only, but could also hide a nonrepair function that, when defective, leads to chromosome breakage. When conditional *smc5* or *smc6* mutants are released into non-permissive conditions from a G₁ cell cycle arrest, the damage checkpoint remains inactivated until after the first passage through mitosis (Torres-Rosell et al. 2005b). This could be caused by channeling of spontaneous damage into persistent checkpoint-invisible recombination links between sister chromatids that will lead to chromosome breakage during anaphase and/or cytokinesis. In line with an S-phase-specific role, Smc5/6 is indeed needed during replication for timely cell division (Torres-Rosell et al. 2005b, 2007a; Lindroos et al. 2006). However, no aberrant recombination structures are detected at replication forks in temperature-sensitive *smc6* mutants grown at high temperature in the absence of damaging agents (Sollier et al. 2009). This argues for a nonrepair function during S phase that prevents damage formation following chromosome segregation and/or cell division.

So, are there any indications of such a function? Interestingly, although bulk replication seems to be unperturbed

in budding yeast *smc5/6* mutants as determined by fluorescence-activated cell sorter (FACS) analysis, detailed observations using pulsed field gel electrophoresis (PFGE) of chromosomes isolated from *smc6* mutants indicate that Smc5/6 facilitates a late step in replication of both normal and rDNA-containing chromosomes (Lindroos et al. 2006; Torres-Rosell et al. 2007a; Kegel 2011). The late replication delay remains unchanged after deletion of *RAD52* or *RAD51* genes, supporting the notion that the role of Smc5/6 during unperturbed replication is not to repair DNA damage and/or prevent accumulation of aberrant recombination structures (Torres-Rosell et al. 2007a; Kegel 2011). Additional evidence that Smc5/6 has a nonrepair role during replication comes from investigations of its chromosomal-binding pattern. In budding yeast, the complex is recruited to chromosomes during replication and reaches maximal levels of chromosomal association in G₂/M cells (Lindroos et al. 2006). In fission yeast and frog, the Smc5/6 chromosomal interaction also increases during replication (Tsuyama et al. 2006; Pebernard et al. 2008b). Because the association is detected in unchallenged cells, and, at least, in budding yeast, it shows no consistent correlation with mapped fragile sites or replication slow zones, it is likely to be independent of DNA damage (Lindroos et al. 2006). Moreover, the Smc5/6 complex is still recruited to *S. cerevisiae* chromosomes in the absence of Mre11, which is needed for the localization of the complex to DNA breaks (Lindroos et al. 2006). Instead, the association is under the control of Scc2, which also regulates cohesin's and condensin's chromosomal interaction during an unchallenged cell cycle (Ciosk et al. 2000; Lindroos et al. 2006; D'Ambrosio et al. 2008).

THE SMC5/6 COMPLEX AND CHROMOSOME TOPOLOGY

Taken together, it appears that Smc5/6 has a function apart from its role in repair. This facilitates a late step in replication and prevents chromosome fragmentation during passage through mitosis. What could this function be? Intriguingly, when budding yeast Smc5/6 associates with chromosomes during S phase, it does so in a length-dependent manner; the frequency of interaction sites increases in linear correlation with chromosome length (Lindroos et al. 2006). How could a protein complex be preferentially recruited to longer chromosomes? What mark and/or structure would be determined by chromosome size? Possibly, the reason for the length-dependent association pattern is that Smc5/6 directly or indirectly senses topological stress that accumulates during replication.

For better understanding of this idea, a short introduction of replication-induced topological stress is required (Fig. 3A). This stress is formed as a result of the separation of the two parental strands in the DNA duplex during the passage of the replication fork. The strand separation will cause the DNA ahead of the fork to become overwound or positively supercoiled. To allow complete chromosome duplication, these supercoils must be removed. The main players in this process are enzymes called topoisomerases, which either are type I or type II enzymes (Wang 2002).

Figure 3. (A) Schematic of replication-induced topological challenges. During replication, the DNA ahead of the advancing replication fork becomes overwound due to separation of the two parental DNA strands. This tightly wound, or positively supercoiled, DNA (+Sc) must be removed to avoid replication inhibition. This is performed by topoisomerases (Top1 and Top2 that release the tension via transient formation of DNA breaks (please see text for details). Another mechanism to avoid accumulation of supercoiled DNA ahead of the fork is to allow the advancing fork to rotate with the turn of the helix. This rotation will lead to sister chromatid intertwinement (SCI) behind. (B) A potential role for Smc5/6 is the resolution of superhelical tension. Smc5/6 sequesters SCIs behind the fork and thereby facilitates the rotation mechanism (Smc5/6 wt). If Smc5/6 is non-functional, the rotation mechanism is inhibited; supercoils accumulate ahead of the fork, leading to a replication delay (*smc5/6* mutant).

Type I topoisomerases (e.g., *S. cerevisiae* Top1 and Top3) relieve superhelical tension by creating single-strand nicks in the DNA, whereas Type II topoisomerases (e.g., *S. cerevisiae* Top2) transfer one DNA helix through a transient DSB created by the isomerase. Another way to prevent the accumulation of positive supercoils ahead of an advancing replication fork is to allow the replication fork to advance in a rotating manner, following the turn of the DNA helix (Postow et al. 2001; Wang 2002). A rotational movement of the fork will prevent the accumulation of supercoils and instead lead to the formation of sister chromatid intertwinings (or catenations) behind (Fig. 3A). This type of resolution has been proposed to be especially important at the site of replication termination when the two converging forks limit the accesses of topoisomerases.

If the budding yeast Smc5/6 chromosomal-binding pattern is a reflection of higher topological tension on longer chromosomes, it is expected that topoisomerases should be more important for replication of longer chromosomes. And we have indeed found a length-dependent inhibition of late replication in cells lacking functional type I topoisomerases (Kegel 2011). In addition, *smc6* and *nse2-sumo-ligase* mutants show the same type of replication delay, indicating that Smc5/6 not only senses topological strain but also has a role in its resolution. Moreover, investigations of the association of Smc5/6 with chromosomes with altered topology and plasmid topology assays suggest that Smc5/6 works in the pathway that facilitates fork rotation and transition of positive supercoils ahead of the fork to chromatid intertwinings behind (Fig. 3B) (Kegel 2011). In line with a topological function for the complex, overexpression of Smc6 leads to changes in *S. pombe* nuclear architecture that are similar to those observed in cells lacking topoisomerases of types I and II (Uemura and Yanagida 1984; Harvey et al. 2004). In addition, the fission yeast *smc6-74* mutant is synthetically lethal with temperature-

sensitive *top2-191* at semipermissive temperatures (Verkade et al. 1999). And budding yeast *nse2-sumo-ligase* and *smc6-56* mutants show synthetic growth defects when combined with mutations in Top2 (Takahashi et al. 2008; A Kegel, unpubl.). Intriguingly, an *smc6-9*-dependent replication delay in an rDNA-containing budding yeast chromosome is diminished when transcription is inhibited (Torres-Rosell et al. 2007a). Additionally, localization of *S. pombe* Nse4 to transfer RNA (tRNA) genes requires ongoing transcription, and the budding yeast Smc5/6 preferentially interacts with intergenic regions downstream from open reading frames (Lindroos et al. 2006; Pebernard et al. 2008b). Because the transcription process also induces superhelical tension, it might be that Smc5/6 influences both replication- and transcription-induced topological strain.

Similarly to the function of Smc5/6 during repair, evidence shows that the complex also acts together with cohesin in unchallenged cells. The previously mentioned lethality of the *smc6-74 top2-191* mutant appears to be due to a misregulation of cohesin's chromosomal association, because moderate overexpression of the protease separase, which removes cohesin from chromosomes at anaphase, increases survival of the mutated cells (Outwin et al. 2009). Moreover, Nse2 is required for sumoylation of the SMC protein subunits in cohesin (Takahashi et al. 2008). However, the relevance of the functional connections between the two complexes awaits confirmation. This becomes especially clear in light of other recently published data showing that knockdown of Smc5 and Mms21 leads to a cohesion defect in human cells (Behlke-Steinert et al. 2009). Although this is in accordance with the findings that Smc5/6 positively regulates cohesin after DNA damage (Potts et al. 2006; Strom et al. 2007), it is definitively not a phenotype that is expected to be rescued by a factor that removes cohesin from chromosomes (Outwin et al. 2009). Potentially, these discrepancies are due

to differences in experimental setups. Detailed analysis of chromosome stability and dynamics after complete depletion of each of the Smc5/6 subunits in defined stages of the cell cycle is still lacking and will improve the understanding of the complex. But even without this analysis, current data support a nonrepair topological function of Smc5/6 that allows full replication of chromosomes and prevents chromosome fragmentation during mitosis. This, together with the absence of functional repair, can explain why cells lacking the Smc5/6 complex cannot survive.

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