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Functional interplay between cohesin and Smc5/6 complexes

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Abstract

Chromosomes are subjected to massive re-engineering as they are replicated, transcribed, repaired, condensed and segregated into daughter cells. Among the engineers are three large protein complexes collectively known as the Structural Maintenance of Chromosomes (SMC) complexes: cohesin, condensin and Smc5/6. As their names suggest, cohesin controls sister chromatid cohesion, condensin controls chromosome condensation, and while precise functions for Smc5/6 have remained somewhat elusive, most reports have focused on the control of recombinational DNA repair. Here, we focus on cohesin and Smc5/6 function. It is becoming increasingly clear that the functional repertoires of these complexes is greater than sister chromatid cohesion and recombination. These SMC complexes are emerging as inter-related and cooperating factors that control chromosome dynamics throughout interphase. However, they also release their embrace of sister chromatids to enable their segregation at anaphase, resetting the dynamic cycle of SMC-chromosome interactions.

Keywords

Cohesin; Smc5/6; Mitosis; SMC complexes

Introduction

The structural maintenance of chromosomes (SMC) complexes are critical in chromosome organization and dynamics throughout the eukaryotic cell cycle. The components of these essential complexes were initially identified and characterized in both fission and budding yeasts, in mammalian cells, and in *Xenopus* oocyte extracts, but have now been characterized in diverse systems. These are cohesin, condensin and Smc5/6. For the first two, their names derive from the initial phenotypes caused by genetic inactivation or depletion, meaning that cohesin is required for the cohesion between sister chromatids, and condensin is required with type II topoisomerases for chromosome condensation. A more descriptive name for the Smc5/6 complex is lacking because its function has been more difficult to tease out, and while many studies have focused on roles in recombination, similar defects can be ascribed to cohesin and condensin mutants.

Each complex contains two large SMC proteins: Smc1 and 3 in cohesin, Smc2 and 4 in condensin, and Smc5 and 6 in Smc5/6, plus a number of non-SMC subunits. Through a

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combination of structural studies, electron microscopy and protein-protein interactions, a related architecture for each complex has emerged. Each SMC protein contains N- and C-terminal globular domains, respectively containing Walker A and B ATPase motifs. A long coiled-coil domain that is split by a flexible hinge separates these globular domains. Each protein folds at the hinge, enabling ATP to bridge the Walker A and B motifs and hold the globular domains together. The SMC pairs form heterodimers that interact through their hinge domains, forming a V-shaped structure. A kleisin protein, Scc1/Rad21 in cohesin, Barren/CAP-H in condensin, and Nse4 in Smc5/6, closes the “V”. Each complex has several additional components and regulators that dock onto this common scaffold, and provide specificity of function.

Here, we focus on cohesin and the Smc5/6 complex. These are chosen because there appears to be significant overlap of their location and function, as well as crosstalk between these two complexes. Similar interactions with condensin may well be identified, but at this stage we refer the reader to excellent reviews on condensin and do not consider it further (Hirano 2005; Piazza, et al. 2013; Thadani, et al. 2012).

The cohesin cycle

A cycle of cohesin association with chromosomes has been extensively studied, particularly in the budding yeast *Saccharomyces cerevisiae*, leading to a detailed model of how this complex affects chromosome structure (for a detailed review of the cohesin cycle see (Nasmyth 2011; Nasmyth, et al. 2009)). The base cohesin ring (Smc1, Smc3 and Scc1) has an additional component, Scc3, that docks onto the cohesin complex through Scc1 (Fig 1A). Scc3 reversibly interacts with two cohesin regulators, Wapl/Rad61 and Pds5, that modulate cohesin’s cohesiveness (Thadani, et al. 2012). Cohesin is rapidly loaded onto chromosomes immediately after sister chromatid segregation at anaphase by a loader complex of Scc2 and Scc4 (Bermudez, et al. 2012; Ciosk, et al. 2000; Kogut, et al. 2009; Watrin, et al. 2006). In *S. pombe* the loader contacts the cohesin ring in multiple sites including evolutionarily conserved regions within Smc1, Smc 3 and Scc3 (Murayama, et al. 2014). To be loaded, the cohesin ring needs to be transiently opened. This requires ATP hydrolysis by the Smc subunits (Arumugam, et al. 2003; Weitzer, et al. 2003), which is stimulated by interaction with the loader complex (Murayama, et al. 2014). However, protein fusion studies also suggest the interaction of the hinge domains needs to be opened to enable loading (Gruber, et al. 2006). Co-incident with DNA replication, the acetylation of Smc3 on N-terminal lysines (K106 and K107) by Eco1/Eso1 renders the complex resistant to the anti-cohesiveness effects of Pds5 and Wapl (Beckouet, et al. 2010; Ben-Shahar, et al. 2008; Toth, et al. 1999; Zhang, et al. 2008). Cohesin can then promote sister chromatid cohesion by embracing replicated sister chromatids. However, cohesin complexes are mobile, and can move along a chromosome, at least in part by the topological changes induced by transcription (Lengronne, et al. 2004).

As cells enter mitosis, cohesin is stripped off sister chromatids in a two-step process in most organisms studied. In prophase, the vast majority of cohesin comes off chromosome arms by a yet to be defined mechanism, though this is dependent on cohesin phosphorylation by the Polo and Aurora kinases (Hauf, et al. 2005; Sumara, et al. 2002; Waizenegger, et al. 2000).

At anaphase, the remaining cohesin at the centromeres is removed by proteolytic cleavage of the kleisin Scc1 by a protease known as separase (Hauf, et al. 2001; Uhlmann, et al. 1999). This enables the sister chromatids to separate, resetting the cohesin cycle.

In *S. cerevisiae*, all cohesin appears to be removed from chromosomes in a one-step mechanism by separase cleavage of Scc1 (Uhlmann, et al. 1999), and hence reloading is delayed as the new complexes are formed. Much of the data for the cohesin cycle above, however, comes from this organism, and this difference in cohesin regulation may explain observations in the fission yeast *Schizosaccharomyces pombe*, which uses the two-step mode of cohesin removal, that are not consistent with this model. For example, non-acetyltable *smc3* mutants are lethal in *S. cerevisiae*, but not in *S. pombe* (Feytout, et al. 2011). The same is true for cells lacking Pds5 (Hartman, et al. 2000; Panizza, et al. 2000; Vaur, et al. 2012; Wang, et al. 2002). Despite this, the Smc3 acetyltransferase gene *eso1* is essential in *S. pombe* (Tanaka, et al. 2001; Tanaka, et al. 2000), but Eso1 acetyltransferase activity, at least for the Smc3 K106 site, is not (Feytout, et al. 2011), suggesting this protein has at least one other function. Thus, while the cohesin cycle described above seems to explain events in *S. cerevisiae*, additional modes of cohesin regulation are likely to exist in other organisms.

Cohesin function

Genetic inactivation or depletion of cohesin results in sister chromatid cohesion defects leading to the premature separation of sister chromatids (Nasmyth 2011). Another phenotype conferred by cohesin dysfunction is failed repair of double-stranded DNA breaks (DSBs) by homologous recombination (HR) in the G2 phase of the cell cycle (Heidinger-Pauli, et al. 2008; Heidinger-Pauli, et al. 2009; Strom, et al. 2004; Strom, et al. 2005; Strom, et al. 2007; Unal, et al. 2007). Indeed, the first cohesin gene reported was *S. pombe rad21*, a gene from the original collection of radiation-sensitive mutants (Birkenbihl, et al. 1992; Birkenbihl, et al. 1995; Fortunato, et al. 1996). As HR-mediated repair uses the undamaged sister chromatid as a template for repair, an HR defect is likely due to a failure to establish cohesion coincident with replication.

More recently, in higher eukaryotes, cohesin has been shown to function with the transcriptional regulator CTCF to act as transcriptional insulators through higher-order chromosome organization (Merkenschlager, et al. 2013; Parelho, et al. 2008; Rubio, et al. 2008; Wendt, et al. 2008). Such organization can also impact DNA replication (Guillou, et al. 2010; Hoang, et al. 2013; Terret, et al. 2009) and class switch recombination (Enervald, et al. 2013; Thomas-Claudepierre, et al. 2013). The DNA molecules that pass through the cohesin ring need not be adjacent sister chromatids, and so cohesin can loop DNA by bringing non-adjacent sequences together. Thus, cohesin could function in multiple ways to organize interphase chromatids in time and space. Indeed, the latter could also explain the DNA repair defects of cohesin mutants, spatially organizing regions for repair while insulating them from other events such as replication and transcription as repair proceeds.

In humans, cohesin mutations are associated with a number of severe developmental defects, collectively known as the cohesinopathies (Liu, et al. 2008). In addition, reduced expression

and mutations in the core cohesin genes and its regulators have been identified in a substantial percentage of tumors of diverse tissue origins (Kim, et al. 2013; Kon, et al. 2013; Rocquain, et al. 2010; Solomon, et al. 2013; Solomon, et al. 2011; Yoshida, et al. 2013), as well as ectopic expression of the meiosis-specific Rec8 subunit (Erenpreisa, et al. 2009). As gene targeting and RNA interference studies have shown cohesin genes to be essential for cell viability, the cohesin defects in these disease scenarios must either retain residual function, or exist on an altered genetic background that enables the tolerance of cohesin defects. If the latter is true in cancer, such alterations may provide opportunities that can be exploited for therapeutic benefit. Proof-of-principle has been demonstrated for this notion by the demonstration that cohesin mutations show synthetic lethality with PARP inhibition (Bailey, et al. 2014; O'Neil, et al. 2013).

Cohesin also plays critical roles in meiosis, where it regulates both homolog and sister chromatid separation, as well as facilitating meiotic recombination (Sakuno, et al. 2009). In this case, there is a meiosis-specific kleisin sub-unit, Rec8. In meiosis I, Rec8 is cleaved only on the chromosome arms, and the sister chromatids remain paired. Shugoshin and PP2A protect Rec8 from cleavage at kinetochores by counteracting phosphorylation by Casein Kinase I (Ishiguro, et al. 2010). This is relieved at the reductional anaphase of meiosis II, enabling the sisters to separate.

Cohesin's relative – Smc5/6

In the same *S. pombe* collection of radiation-sensitive mutants that included *rad21* was a gene denoted as *rad18* (Nasim, et al. 1975). Once cloned, it was clear that Rad18 was essential and similar to the Smc1 subunit of cohesin (Lehmann, et al. 1995), and has since been renamed Smc6. Paired with Smc5, this third heterodimer scaffolds the Smc5/6 complex. The lack of an acronym derived name from perceived function reflects the fact that a precise function for this complex has not been as evident as for cohesin and condensin, though one could argue that these names describe a subset of function for these complexes.

Smc5/6 has now been studied in a number of systems. As the original *smc6* mutant in *S. pombe* (*rad18-X*) possessed a DNA repair defect, most studies have focused on Smc5/6 function in recombination-mediated repair. However, most Smc5/6 genes are essential for cell viability, though HR genes are not, and the terminal phenotype of null alleles is mitotic failure (Harvey, et al. 2004; Verkade, et al. 1999). Thus, it is possible that by selecting repair-defective alleles of Smc5/6 genes, those studying this complex have perhaps biased attempts to decipher cellular function, where failed DNA repair may derive from a more fundamental defect in chromosome organization. Notably, repair defects have also been described in cohesin and condensin mutants (Aono, et al. 2002; Lehmann 2005).

The proposed overall structure of Smc5/6 is reminiscent to cohesin, though with more non-SMC subunits (Fig 1B). In addition to the Smc5/6 heterodimer, there is the kleisin Nse4 that closes the Smc5/6 ring, which is in turn bound to two other proteins, Nse1 and Nse3 (Morikawa, et al. 2004; Palecek, et al. 2006; Pebernard, et al. 2004; Sergeant, et al. 2005). Nse1 contains a variant RING (vRING) domain (Fujioka, et al. 2002; Harvey, et al. 2004) and Nse3 is a member of the large family of the Melanoma-Associated Antigen (MAGE)

domain proteins (Taylor, et al. 2008). Nse1 has been proposed to possess an E3 ubiquitin ligase activity that is stimulated by Nse3 (Doyle, et al. 2010). The essential Nse2 subunit, which associates with Smc5, is an E3 SUMO ligase (Andrews, et al. 2005; Zhao, et al. 2005). In *S. pombe*, the activity of this enzyme is required for survival of replication stress, but not for post-replicative DNA repair (Tapia-Alveal, et al. 2011). Finally, there are two HEAT-repeat containing proteins, Nse5 and Nse6, which are not essential for cell viability in *S. pombe*, but are required for resistance to DNA damage (Pebernard, et al. 2006; Zhao, et al. 2005).

As with cohesin, Smc5/6 is associated with chromosomes throughout interphase (Lindroos, et al. 2006; Pebernard, et al. 2008; Tapia-Alveal, et al. 2011). In *S. cerevisiae*, Smc5/6 loading is dependent on the cohesin loader Scc2 (Lindroos, et al. 2006), but whether this is a direct effect on Smc5/6, or an indirect effect via defective cohesin loading is not known. Both are possibilities, especially as the localization of cohesin and Smc5/6 along chromosomes is significantly overlapping, and both complexes are enriched at sites of DNA damage and replication stress. Like cohesin, Smc5/6 temporarily leaves mitotic chromosomes (Gallego-Paez, et al. 2014; Gomez, et al. 2013; Taylor, et al. 2008). However, this occurs without detectable cleavage of the kleisin Nse4 (Palecek, et al. 2006), and the mechanism of Smc5/6 unloading is yet to be determined.

Smc5/6 is also crucial for meiosis, where it has been shown to function in the regulation and resolution of meiotic recombination (Copsey, et al. 2013; Lilienthal, et al. 2013; Pebernard, et al. 2004; Verver, et al. 2013; Xaver, et al. 2013). To date, there are no meiosis-specific sub-units or post-translational modifications to Smc5/6 during meiosis, but this seems likely as the localization of the complex is dynamic as meiosis proceeds (Gomez, et al. 2013).

What does Smc5/6 actually do?

As mentioned above, most studies of Smc5/6 function have focused on DNA damage responses. However, this has the significant caveat that many of these studies have been led by the isolation of damage-sensitive hypomorphic mutants, and it is possible (if not likely) that screens for alleles that conferred defects in mitosis, DNA replication or even transcriptional regulation might have taken the field in an entirely different direction.

Initially, through the analysis of *smc6* alleles in *S. pombe*, it was concluded (by we and others) that Smc6 (and hence the complex) is required for recombinational repair, and to maintain DNA damage induced checkpoint arrest (Lehmann, et al. 1995; Verkade, et al. 1999). However, a more accurate description of the observations, with the benefit of hindsight, is that *smc6* hypomorphs showed defects in these processes.

The remaining genes for Smc5/6 subunits were identified by protein association studies and the advent of complete genome sequences. Again, to study these essential genes, damage-sensitive hypomorphs or dominant-negative mutants were employed, and from these studies, a detailed description of repair defects has emerged, primarily from studies with DNA damage induced during DNA replication with alkylating agents and dNTP depletion, with and without replication fork collapse. From 2D gel analyses of *S. pombe smc6* mutants, both “early” and “late” recombination defects have been uncovered. At stably

stalled replication forks, *smc6-74* mutants are defective in the recruitment of the recombination initiator Rad52 (Irmisch, et al. 2009). This is without other recombination proteins, and may rather utilize the strand-annealing activity of Rad52, instead of its Rad51-loading capacity. This defect is not seen in the original *smc6-X* mutant, though is not specific to the *smc6-74* allele (our unpublished observations). When the replicative polymerase and its associated factors are lost from stalled replication forks (fork collapse), their restart is dependent on HR. This initiates normally in *smc6-74* and *smc6-X* mutants, but arrests at a point after joint molecule formation, suggesting a defect in resolution (Ampatzidou, et al. 2006).

Despite these observations, and the enrichment of Smc5/6 at sites of DNA damage and replication stress, little more is known as to how Smc5/6 functions in recombination. Early reports based on siRNA experiments that concluded Smc5/6 was responsible for cohesin recruitment (Potts, et al. 2006) were shown to be an artifact (Wu, et al. 2012), and regardless, how cohesin facilitates repair is not really known. In *S. pombe*, Smc5/6 is more abundant at Pol III genes (Pebernard, et al. 2008), many of which are centromere adjacent, and at telomeres. These are both regions that are difficult to replicate and where recombination is repressed, leading to the notion that coordinating these events is an important part of Smc5/6 function (Murray, et al. 2008). Similarly, in *Drosophila*, Smc5/6 is enriched in heterochromatin, and depletion of Smc5/6 has been shown to result in aberrant recombination in heterochromatin (Chiolo, et al. 2011). However, in such studies it is difficult to distinguish between a particular mutant allowing defective recombination to occur, and a mutant that is defective in completing recombination that would normally have proceeded swiftly and without physiological consequence.

A study from our laboratory has further complicated matters by showing that Smc5/6 is not formally *required* for DNA repair (Tapia-Alveal, et al. 2011). Smc5/6 is enriched at sites of DNA damage, and Smc5/6 mutants are defective in repairing these lesions. However, the enrichment of Smc5/6 at lesions is dependent on the vRING domain of Nse1. Conservative cysteine-to-serine mutations in Nse1's vRING that prevent Smc5/6 enrichment at lesions do not confer a repair defect. Instead, this lack of recruitment actually suppresses the repair defects of other Smc5/6 mutants by preventing mutant complexes from being recruited to lesions. In this case, the post-replication repair pathway becomes critical for resistance to DNA damaging agents, a recurring phenomenon in situations where Smc5/6 dysfunction is bypassed.

Note that this experiment is not the same as the analysis of null mutants or siRNA experiments, because the same vRING mutant *nse1* alleles discussed above do not affect the association of Smc5/6 with chromosomes in the absence of ectopic DNA damage, and mitotic defects in the nulls (see below) are greatly exacerbated by DNA damage. Nevertheless, rather than Smc5/6 being absolutely required for DNA repair, it is the presence of defective Smc5/6 complexes that causes the repair defect.

Coming together in mitosis to allow sisters to come apart

Although cohesin functions on chromosomes throughout interphase, the terminal effects of lacking cohesin function manifests in mitosis with premature sister chromatid separation interfering with sister chromatid segregation. Otherwise unperturbed cells carrying null alleles of the essential Smc5/6 genes in *S. pombe* also die in lethal mitoses, where sister chromatid separation fails leading to the characteristic “cut” phenotype. The same phenotype is observed when the hypomorphic Smc5/6 alleles are exposed to DNA damage or replication stress. Further, these alleles are synthetically lethal with a loss-of-function allele of the topoisomerase II gene *top2* (*top2-191*), and again these cells die in mitosis (Harvey, et al. 2004; Outwin, et al. 2009; Verkade, et al. 1999). In *S. cerevisiae*, Smc5/6 has also been shown to functionally interact with topoisomerases to regulate the topology of the longer chromosomes (Carter, et al. 2012; Kegel, et al. 2011), and as these are actually shorter than most eukaryotic chromosomes, this may represent a general function for the complex. Related to this, a recent study in human cells showed severe disruption to mitotic chromosome structure and segregation following Smc5 or Smc6 depletion by siRNAs. Interestingly, these aberrant mitoses were also accompanied by abnormal distribution of both topol1 α and condensin (Gallego-Paez, et al. 2014). While genetic interactions with condensin mutants, or mitotic condensation defects have not been observed in the *S. pombe* Smc5/6 mutants, the available alleles may not be appropriate, and the morphological condensation of yeast chromosome is not as evident as with human chromosomes, making this interaction more difficult to dissect. For both cohesin and Smc5/6 dysfunction leading to mitotic failure, it is entirely possible (if not likely) that the primary defect occurred earlier in the cell cycle, especially during DNA replication where cohesion is established and Smc5/6-dependent rescue of replication forks occurs. The mitotic defects would then manifest as inappropriately structured chromosomes attempt mitosis.

It is during chromosome segregation that crosstalk between cohesin and Smc5/6 is observed. Studies in our laboratory have shown that the failed mitoses of Smc5/6 mutants in *S. pombe* are associated with a failure to strip cohesin from chromosome arms (Outwin, et al. 2009). That is, the first step of cohesin removal does not occur, but cohesin is removed from the kinetochores. Two observations suggest that cohesin retention is causal in the mitotic failure. First, overexpression of the protease separase, which cleaves the kleisin subunit of cohesin, removes the arm cohesin complexes and suppresses the mitotic defects (Outwin, et al. 2009). Second, in a screen for suppressors of the mitotic defects of *smc6* mutants, we found that loss of the histone variant H2A.Z lowers arm cohesin levels by up to 50%, and this too allows mitosis to proceed (Tapia-Alveal, et al. 2014). Importantly, in both scenarios the DNA damage and/or replication stress sensitivity of *smc6* mutants is also suppressed, showing that the sensitivity is largely due to the mitotic defects.

The finding that H2A.Z affects cohesin dynamics is reminiscent of the finding that both H2A and H2A.Z act as chromosomal receptors for condensin (Tada, et al. 2011). In the case of cohesin, it is specifically the hyper-acetylated form of H2A.Z that promotes cohesin binding and/or retention. These observations open the real possibility that the histone code determines SMC complex dynamics, which is perhaps not surprising but nevertheless another important function for these epigenetic modifications.

Similar cohesin retention defects on chromosome arms have also been described in *S. cerevisiae smc5* and *nse4* mutants during meiosis (Copsey, et al. 2013). Given the differences in the cohesin cycle between the yeasts, it is not clear at this point whether the mechanism behind the cohesin retention is the same in these scenarios.

SUMOylation as a messenger between Smc5/6 and cohesin

Nse2/Mms21 is an E3 SUMO ligase that is essential for DNA damage resistance (Lee, et al. 2006). Using mutants in the yeasts and RNA interference in mammalian cells, Nse2 homologs have been implicated in the SUMOylation of a number of proteins involved in DNA repair and recombination (Albuquerque, et al. 2013; Branzei, et al. 2006; Zhao, et al. 2005), including the ALT pathway of telomere lengthening (Potts, et al. 2007). However, some of these RNAi studies may need to be confirmed, together with an Smc5/6-independent role for Nse2, as off target effects are evident for the Nse2-based reagents (Wu, et al. 2012). Nevertheless, studies in *S. cerevisiae* and human cells suggest that Nse2 may regulate cohesin through the SUMOylation of the kleisin subunit Scc1. Two studies in *S. cerevisiae* showed Mms21-dependent SUMOylation of Scc1. In one studying, Scc1 was shown to be SUMOylated following DNA damage, and that this enforces damage-induced cohesion (McAleenan, et al. 2012). In addition, Almedawar and colleagues showed that Scc1 SUMOylation occurs when cohesion is established, and by fusing a SUMO peptidase domain to Scc1, that a lack of SUMOylation is lethal due to cohesion defects (Almedawar, et al. 2012). Working in human cells, Wu *et al.* showed Nse2-dependent SUMOylation of Scc1 following DNA damage, and that a non-SUMOylatable mutant with 15 lysine mutated to alanine was defective in sister chromatid recombination. They found that this could be rescued by depleting Wapl, and suggested therefore that Scc1 SUMOylation promotes recombination by counteracting Wapl-mediated regulation of cohesin (Wu, et al. 2012). Whether SUMOylation of Scc1 is related to the cohesin retention defects of yeast Smc5/6 mutants described above is unclear at this point. However, it is notable that these defects can be induced by DNA damage in G2, and at least in *S. pombe*, ligase-dead *nse2* mutants are only sensitive to DNA damage during DNA replication (Tapia-Alveal, et al. 2011). Nevertheless, these observations further demonstrate crosstalk between cohesin and Smc5/6.

Why do cells have both cohesin and Smc5/6?

With their similar architecture and chromosomal localization, the evolutionary retention of these related though distinct SMC complexes, both essential for cell viability, remains somewhat of a conundrum. Are these completely functionally distinct complexes? Did one evolve from the other? Do they use different mechanisms to achieve common tasks? Do mutations in Smc5/6 contribute to human disease? So far, the field is only beginning to address these issues, though comparing and contrasting the function(s) of Smc5/6 and cohesin seems a likely way to make progress in how they control chromosomal dynamics.

If the central role of cohesin is to embrace sister chromatids and/or loop regions on a single chromatid, why cannot Smc5/6 carry out this function if it forms the same shaped structure to compensate for cohesin dysfunction? Smc5/6 does contain more non-SMC subunits than cohesin, which could provide specific functions that cohesin cannot perform. The (potential)

Ubiquitin and SUMO ligase activities of Nse1 and Nse2 are obvious distinctions from cohesin, but ligase-dead RING domain mutants in *nse1* and *nse2* are viable (Andrews, et al. 2005; Pebernard, et al. 2008; Tapia-Alveal, et al. 2011), suggesting it is still the core scaffold that is critical for Smc5/6 to maintain cell viability.

One hint that cohesin and Smc5/6 may have overlapping functions comes from the observation that most combinations of hypomorphic alleles of cohesin and Smc5/6 genes in *S. pombe* are either synthetic lethal or confer severe mitotic defects (Tapia-Alveal, et al. 2014; Tapia-Alveal, et al. 2011; Verkade, et al. 1999). At least in the case of cohesin, the cyclic nature of its association with chromosomes means that a delicate balance of cohesin dynamics is critical; either too much or too little sister chromatid cohesion is incompatible with accurate chromosome segregation. Therefore, it is also possible that rather than overlapping functions, Smc5/6 may correct the consequences of cohesin dysfunction and vice-versa.

The road ahead

It is notable that the earliest studies of what turned out to be cohesin, the *S. pombe rad21* gene, focused on apparent roles in DSB repair. However, direct searches for sister chromatid cohesion genes in the yeasts led to a reassessment of this, together with elegant cell biological studies in vertebrates. Because of the phenotypes of the founding member of Smc5/6, *S. pombe rad18/smc6*, the field has similarly focused on DNA repair roles for this SMC complex, which no doubt are critically important though part of a repertoire of function. To move forward in deciphering Smc5/6 function, it is possible that “going back to the drawing board” might be the best way forward. That is, while data coming from the DNA repair studies is important and should be kept in mind, new studies taking unbiased approaches are clearly required. Among other approaches, one could imagine that new genetic screens for Smc5/6 modifiers, *in vitro* reconstitution experiments, genome architecture studies such as Hi-C, or sophisticated imaging studies in live cells, could provide the key findings to bring our understanding of Smc5/6 function up to that of cohesin.

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A. Cohesin

B. Smc5/6

Figure 1. Molecular architecture of the cohesin and Smc5/6 complexes
(A) The cohesin complex and its regulators. **(B)** The Smc5/6 complex. See text for details.