Nucleic Acids Research, 2024, **52**, 2112–2129
https://doi.org/10.1093/nar/gkae103
Advance access publication date: 20 February 2024
Critical Reviews and Perspectives

The SMC5/6 complex: folding chromosomes back into shape when genomes take a break

Shamayita Roy[†], **Hemanta Adhikary**[†] **and Damien D'Amours**[*]

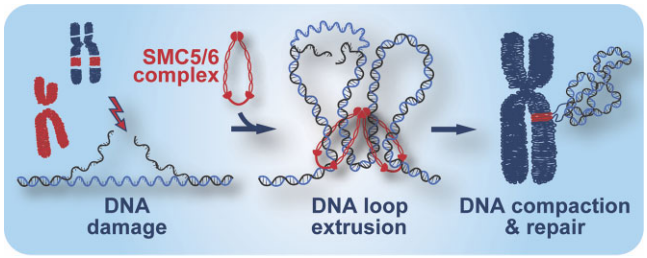
Ottawa Institute of Systems Biology, Department of Cellular and Molecular Medicine, University of Ottawa, Roger Guindon Hall, 451 Smyth Rd, Ottawa, ON K1H 8M5, Canada

[*]To whom correspondence should be addressed. Tel: +1 613 562 5700; Email: damien.damours@uottawa.ca
[†]The first two authors should be regarded as Joint First Authors.

Abstract

High-level folding of chromatin is a key determinant of the shape and functional state of chromosomes. During cell division, structural maintenance of chromosome (SMC) complexes such as condensin and cohesin ensure large-scale folding of chromatin into visible chromosomes. In contrast, the SMC5/6 complex plays more local and context-specific roles in the structural organization of interphase chromosomes with important implications for health and disease. Recent advances in single-molecule biophysics and cryo-electron microscopy revealed key insights into the architecture of the SMC5/6 complex and how interactions connecting the complex to chromatin components give rise to its unique repertoire of interphase functions. In this review, we provide an integrative view of the features that differentiates the SMC5/6 complex from other SMC enzymes and how these enable dramatic reorganization of DNA folding in space during DNA repair reactions and other genome transactions. Finally, we explore the mechanistic basis for the dynamic targeting of the SMC5/6 complex to damaged chromatin and its crucial role in human health.

Graphical abstract



Introduction: chromatin organization and SMC-family enzymes

The organization of chromatin in space is one of the key determinants of the functional state of genes and genomes. Several decades of research has revealed that open or accessible chromatin generally promotes DNA transactions while densely folded chromatin significantly limits enzyme mobility and access to DNA substrates (1,2). Eukaryotic organisms take advantage of the ability to finely tune the organization of chromatin in space to enhance the efficiency, fidelity, and regulation of vital cellular processes such as gene transcription, DNA repair and genome segregation.

Changes in chromatin state is a complex affair typically requiring multi-step processes that involve nucleosomes, post-translational modifications (PTMs) of histones, as well as chromatin remodeling enzymes (1,2). This paradigm represents the prevalent view of how chromatin organization is regulated in interphase cells. However, recent research has revealed that large-scale changes in genome organization can be imparted through completely different processes, often independently of canonical chromatin/histone modifying enzymes. One of these new processes is based on the unique capacity of a family of enzymes to create topological links that connect distant regions of chromosomes, thus generating DNA loops of varying sizes and complexity. The key enzymes responsible for this level of chromatin organization are the structural maintenance of chromosomes (SMC) family complexes (3). While these complexes have well-documented roles in mitosis, they also play crucial roles in interphase chromatin folding and regulation. What makes these enzymes interesting is their unique ability to regulate chromatin folding by themselves, independently of nucleosomes and most other enzymes typically associated with chromatin compaction processes (4). As such, SMC complexes represent a fascinating alternative to canonical chromatin modification pathways to regulate the repair and homeostasis of genes and genomes.

SMC family proteins are large ATPases known to play paramount roles in the spatial and temporal organization of

Received: November 13, 2023. **Revised:** January 28, 2024. **Editorial Decision:** January 30, 2024. **Accepted:** February 1, 2024

chromosomes in all domains of life. Eukaryotic genomes typically encode six evolutionarily conserved SMC proteins, while prokaryotes usually express a single SMC family member. To perform any of their designated roles, these SMC proteins need to assemble into large holoenzyme complexes in two distinct steps. First, SMC monomers associate as pairs with cognate partners (see example in Figure 1A) to create specific heterodimers: SMC1/SMC3, SMC2/SMC4 and SMC5/SMC6. Second, SMC heterodimers associate with additional non-SMC elements (NSEs) to generate three different and fully functional holoenzyme complexes: cohesin, condensin and the SMC5/6 complex. The relative positioning of NSE elements within SMC complexes together with multiple bivalent interactions among complex subunits (Figure 1) generates the prototypical ring-like architecture of SMC complexes and a functional identity that is unique to each holoenzyme (reviewed in (3,5,6)).

The SMC1 and SMC3 family members form the heterodimer at the core of the cohesin complex, and together with additional NSEs, Scc1/Mcd1/RAD21 and Scc3/STAG/SA, holds the replicated sister chromatids together. This process ensures error free segregation of chromatids during mitosis (7,8). Cohesin also participates in the establishment of complex chromatin structures, called topologically associating domains (TADs), to regulate gene expression and DNA replication during interphase (9–11). Likewise, SMC2 and SMC4 family members associate with CAP-D/G/H-family subunits (Ycg4/Cnd1, Ycg1/Cnd3 and Brn1/Cnd2 in yeast, respectively) to form the condensin holoenzyme, the main effector of chromosome condensation during mitosis (12).

The third and the most enigmatic SMC holoenzyme, the SMC5/6 complex, demonstrates a unique subunit composition and shape, thanks to an unusual number of NSE components and atypical positions of some of its subunits within the complex (13) (Figure 1). The non-SMC subunits of the SMC5/6 holoenzyme include Nse1–6 in yeasts and NSMCE1-4 in mammals (note that Nse2 is also known as Mms21 in budding yeast; (14–23)). Humans also co-express SMC5/6 complex localization factors 1 and 2 (SLF1 and SLF2), believed to be functional homologues of yeast Nse5 and Nse6 respectively, but it is unclear if these subunits are constitutive members of the human complex (Figure 1C) (24). Therefore, when it comes to non-SMC partners, the SMC5/6 complex has a significantly greater number of non-SMC subunits than cohesin or condensin complexes. For brevity and clarity, we will use below the 'NSE' nomenclature when discussing non-SMC subunits of the SMC5/6 complex, as well as the human notation convention for all proteins discussed hereafter.

In a broad sense, eukaryotic SMC complexes are large DNA binding/modulating ATPases that share several biochemical activities. Cohesin, condensin and the SMC5/6 complex have all been shown to form loops in DNA using an ATP hydrolysis-dependant loop extrusion activity (3,25–27). The mechanism of loop extrusion facilitated by the SMC5/6 complex is similar to the process catalysed by cohesin, which performs two-sided loop-extrusion as a dimeric complex. However, cohesin can also extrude DNA loops as a monomer similar to condensin (28). Thus, in addition to the structural similarities shared by all eukaryotic SMC complexes, DNA loop extrusion is considered one of the unifying features of this family of proteins. How this biochemical activity translates into effective maintenance of chromosome integrity is a topic of active investigation. An excellent discussion of loop extrusion models relevant to SMC complexes and their impact on chromatin structure can be found in several recent reviews (3,25–27,29,30). While it is evident that the SMC5/6 complex shares common features with its sister complexes, it is equally clear that it diverges from other family members in many ways, making it the most enigmatic of the three SMC complexes.

Distinctive features of the SMC5/6 complex

The impact of SMC family proteins on chromosomes is often perceived through the lens of cohesin and condensin's role as key effectors of global changes in chromosome morphology. However, the scope of SMC5/6 complex action is substantially different from that of canonical SMC complexes. The SMC5/6 holoenzyme acts at a more local and targeted level on chromosomes to promote DNA transactions essential for DNA repair and genome stability (Figure 2). Until recently, the exact nature of DNA transactions promoted by the SMC5/6 complex was not fully understood, but recent advances on the structural organization, motor activity and biochemistry of the SMC5/6 complex contributed crucial new insights into the specific role of this holoenzyme in DNA repair and replication. We review below distinct features of the SMC5/6 complex that differentiates it from canonical SMC complexes and how these promote the unique repertoire of functions of this holoenzyme (Figure 2). For a more detailed review of the common features of SMC complexes, we direct readers to several excellent reviews recently published on this topic (3,5,6).

The SMC5/6 complex is a post-translational modification enzyme

The SMC5/6 complex distinguishes itself from other SMC complexes by virtue of its unique role as a post-translational modification (PTM) enzyme. The PTM activities of the complex are mediated by two of its subunits, NSE1 and NSE2/MMS21 (Figure 1B), that contain RING-like domains known to stimulate ubiquitin and SUMO transfer on substrates, respectively (20,22,31,32). The best characterized of these two subunits, NSE2, is a major regulator of multiple functions in eukaryotes, as outlined in Table 1 (33). Notably, substrates of NSE2-mediated SUMOylation include the SMC5/6 complex itself (20,22,31,34–37) along with an impressive number of proteins implicated in genome organisation, DNA replication and DNA repair (Table 1).

How are SMC5/6-mediated SUMOylation reactions impacting genome stability? One of the primary functions of the SMC5/6 complex is to limit the formation of toxic recombination intermediates or help resolve them when formed at replication forks. In line with this, self-SUMOylation of the SMC5/6 complex by NSE2, especially in the coiled-coil domain of SMC5, is upregulated at sites of stalled replication forks (37). Also, NSE2-mediated SUMOylation of the yeast RecQ-family helicase SGS1 and its binding partners TOP3 and RMI1 (STR) allows the removal of recombination intermediates at DNA repair sites in a two-step process (38,39), specifically: self-SUMOylation of the SMC5/6 complex, an event that promotes STR loading at damaged sites, followed by additional SUMOylation events on SGS1 and TOP3 to increase the efficiency of the recombination function by STR. Similar patterns of SUMOylation are observed with the human Bloom syndrome helicase (BLM), a SGS1

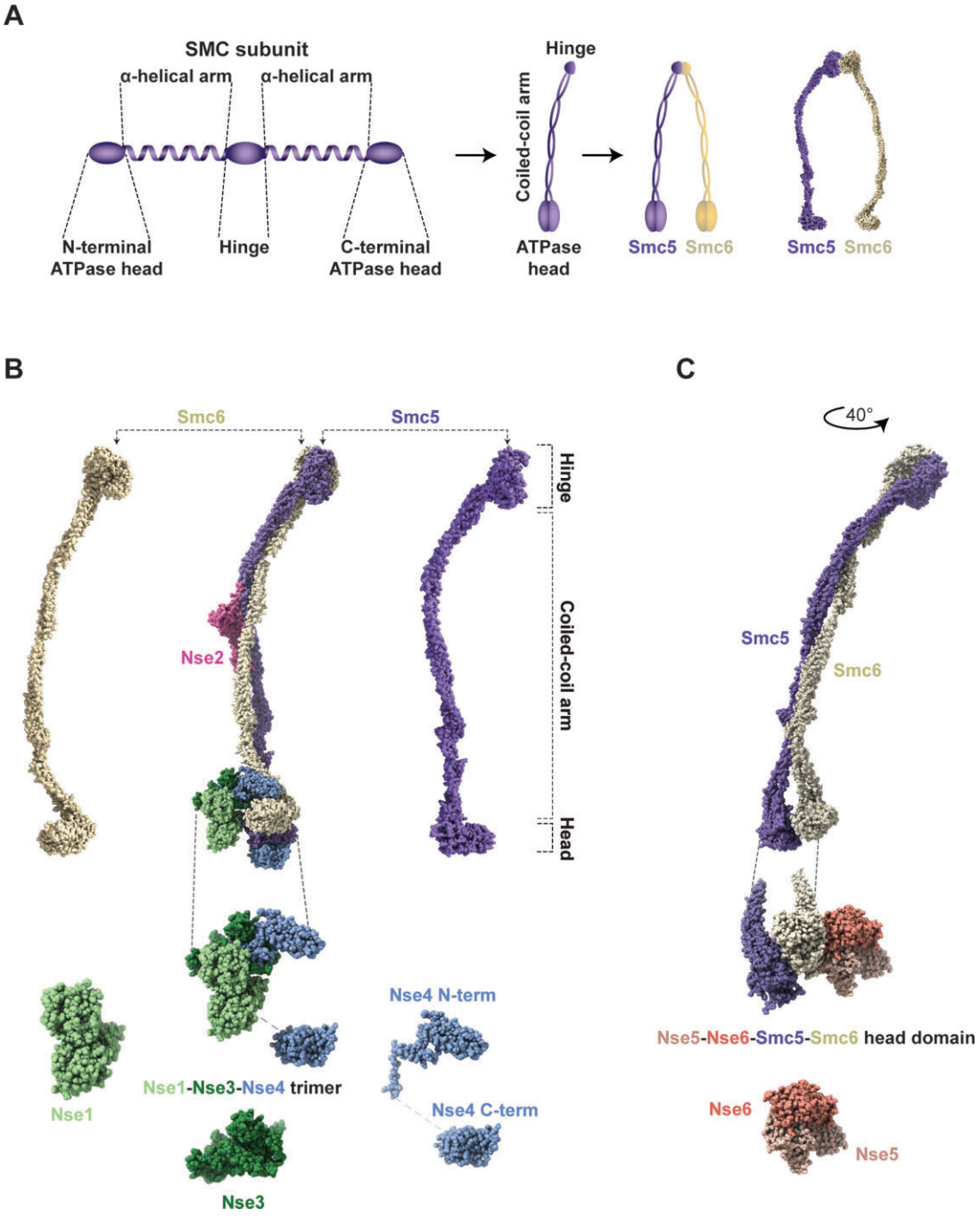


Figure 1. (**A**) Schematic representation of the functional domains, folding, and dimerization states of SMC5 and SMC6 proteins. Shown on the left is a cartoon representation of the domain organization of a typical SMC protein in its unfolded state. The middle section of the panel depicts the same SMC monomer in its mature state. Specifically, the newly synthesized SMC protein initially fold back on itself (in a manner analogous to a conventional hairpin) to create a functional coiled-coil arm and ATPase head domain. This allows the folded/mature protein to dimerize with another SMC family member using their respective hinge domains. The right side of the panel shows the SMC5/6 dimer in cartoon and structural representations. (**B**) Cryo-EM composite structure and molecular graphics representation of the SMC5/6 complex and its individual subunits (170) edited in UCFC ChimeraX. The hinge, coiled-coil arm and ATPase head domains are labelled on the right-hand side of the SMC5 structure. Individual subunits/subcomplexes are shown in different color schemes to facilitate visualization; SMC5, violet; SMC6, wheat/beige; NSE1, light green; NSE2, pink; NSE3, dark green; NSE4, blue; NSE5, peach; and NSE6, maroon (170). The model shown in this panel corresponds to the *Saccharomyces cerevisiae* enzyme and is derived from data of Hallett and collaborator (58), as deposited in the RCSB database (PDB: 7QCD). (**C**) Cryo-EM structure of the budding yeast SMC5/6 dimer and NSE5/NSE6 subcomplex interacting with the SMC5/SMC6-head neck region. The lower portion of the panel is derived from the SMC5/6–8mer complex structure of Li and colleagues (72), as deposited in the RCSB database (PDB: 8T8F).

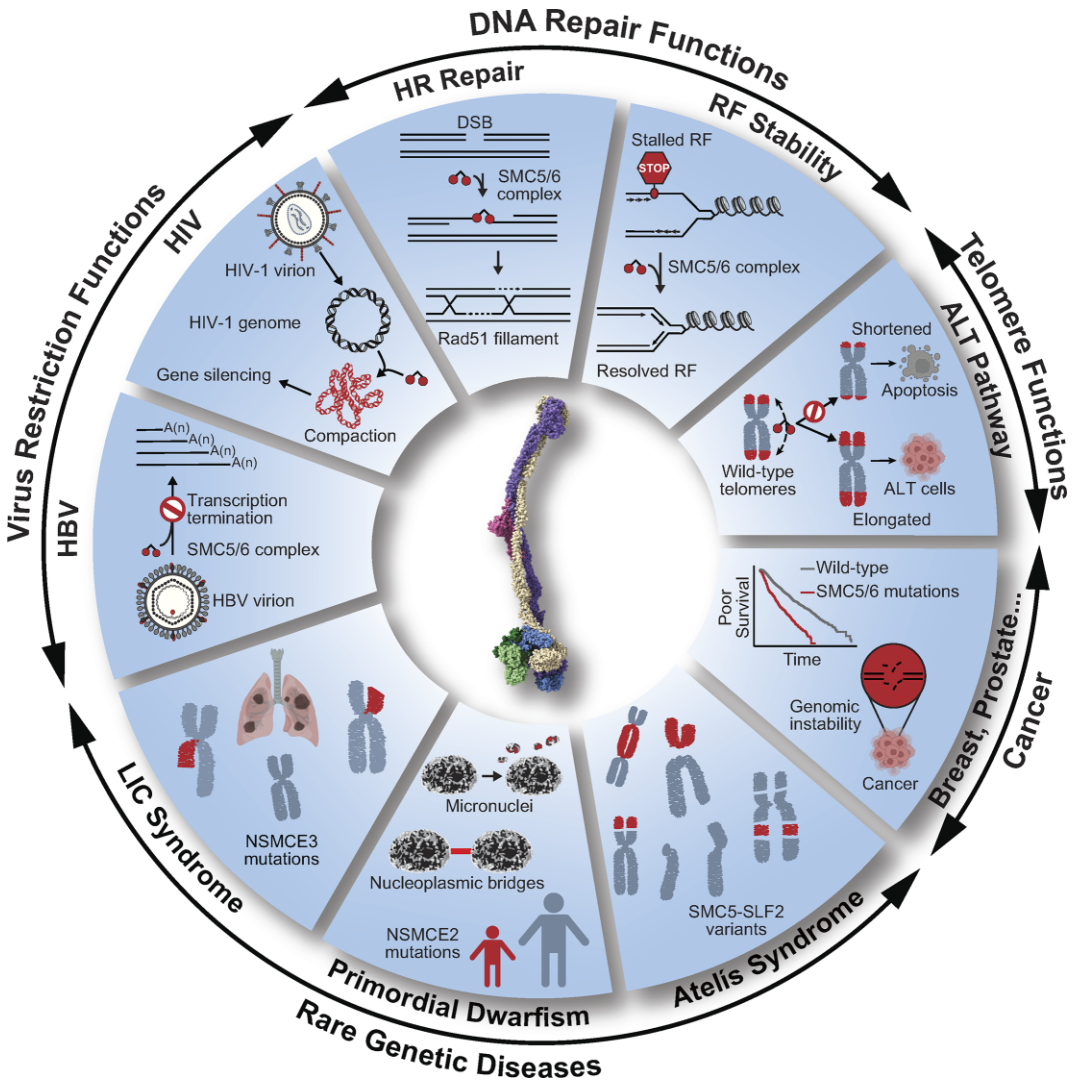


Figure 2. Landscape of the SMC5/6 complex functions and phenotypes. Each section of the figure depicts a distinct cellular function (DNA repair/telomere) and/or disease state associated with the SMC5/6 holoenzyme (viral infection, rare genetic diseases, and cancer). Abbreviations: HR, homologous recombination; RF, replication fork; ALT, alternative lengthening of telomeres; MV, mosaic variegated; LICS, lung disease immunodeficiency and chromosome breakage syndrome; HBV, hepatitis B virus; HIV, human immunodeficiency virus. See text for a detailed description of the molecular pathways and diseases presented in this figure.

homolog (40,41). Interestingly, binding of the SMC5/6 complex to collapsed forks or other damaged structures in chromosomes can promote NSE2-mediated SUMOylation of repair proteins, including Replication protein A (RPA) and RAD59 (42), as well as other SMC complex components (35,43,44). Altogether, proteins involved in DNA replication, DSB repair, rDNA maintenance and telomeric length regulation (Figure 2) have been shown to be direct targets of the SUMOylation activity of NSE2 (see Table 1 for an extensive list and ref. (24).

In contrast to the well-documented SUMO ligase activity of NSE2, much less is known regarding the ubiquitin ligase/E3 activity of NSE1. This protein forms a subcomplex with NSE3–4 proteins and associates with the ATPase head region of the SMC5/6 complex (21,45,46). Interestingly, NSE1 can promote the ubiquitination of NSE3 and NSE4, but how this impacts the SMC5/6 complex function is still under investigation (47,48). Advanced proteomics analysis has identified Rpa190, Rpl24b, Cdc73, Yhb1, Erg9, Cdc48 and Pol30/PCNA as putative targets for NSE1 for ubiquitination (49). This repertoire of substrates suggests roles beyond DNA repair for NSE1-mediated ubiquitination, notably in ribosome biogenesis and metabolism (Table 1) (49). One of the known functions of NSE1 E3 ligase activity is in RNA polymerase I/RPA190 ubiquitination and subsequent RNA pol I degradation. This process facilitates the SMC5/6-dependent separation of the ribosomal DNA locus in mitosis (49). Beyond this, little is known on the role of NSE1 in substrate ubiquitination, outlining a clear area for future expansion of research efforts.

The SMC5/6 complex is an unusual nucleic acid binding machine

One of the fundamental activities of all SMC complexes is DNA binding, but the DNA binding preference of the SMC5/6 holoenzyme is quite distinct from that of other SMC complexes. Specifically, components of the SMC5/6 complex demonstrate high affinity for structured DNA like Holliday junctions (HJ) and splayed Y structures, including

Table 1. Targets of the SMC5/6 complex SUMOylation, ubiquitination and interaction reactions

Targets	Biological pathways	References
SUMOylation by NSE2		
SMC5-SMC6	MPH1-mediated fork regression, DNA repair	(20,35,37,171)
NSE2-NSE3-NSE4	DNA damage response pathway	(20,31,34)
Cohesin (SMC1/SMC3/SCC1)	DSB repair/mitosis/rDNA maintenance	(43,44,172).
Condensin (SMC2/SMC4/BRN1/YCS4/YCG1)	rDNA maintenance	(35,172)
RPA-RNA pol1 and RAD59	Homologous recombination	(42,173)
STR (SGS1-TOP3-RMI1), BLM	Double Holliday junction removal	(38,39)
POL2, MCM6, RFA1,2,3	DNA replication	(42,86,171)
TRAX	DNA repair	(20)
YKU70	DSB repair by non-homologous end joining	(22)
RAD52	Recombinational repair in rDNA	(103)
Shelterin complex	Telomere length maintenance	(106)
FOB1	rDNA maintenance	(37)
Kinetochore proteins	Recombination at centromere	(174)
Zebularine induced MET1-DNA protein crosslink	Repair process in Arabidopsis	(175)
Ubiquitination by NSE1		
NSE3-NSE4	Replication stress	(47,48)
RNA polymerase I – RPA190	Ribosomal DNA biosynthesis	(49)
RPL24B, CDC73, YHB1, ERG9, CDC48 and POL30/PCNA	Ribosome biosynthesis and metabolism	(49)
MMS19	Cytosolic iron–sulfur cluster assembly pathway	(176)
Protein-protein interactions		
MPH1 helicase	Replication fork remodeling	(96)
STR (SGS1-TOP3-RMI1)	Resolution of recombination product, Holliday junctions	(39)
SRS2, MUS81-MMS4	Recombination intermediate formation and maturation, Holliday junctions	(39)
DNA topoisomerase II alpha (TOP2A)	Resolution of DSB-repair intermediates	(177)
POL2	Replication-S-phase checkpoint	(87)
GPS1 (G-protein pathway suppressor 1)	Signaling pathway	(178)
Telomeric proteins	Telomere maintenance	(105)
FANCD2-I	Fanconi-anaemia pathway, repair interstrand crosslink	(98)
Cohibin and CLIP	rDNA stability	(101)

ssDNA–dsDNA junctions and other types of recombination intermediates (Figure 3, Step 1) (50–55). The higher affinity of the holoenzyme for these substrates (compared to B-form DNA) suggests that the SMC5/6 complex can achieve differential association to chromosomes based on the nature of the DNA lesion/intermediate present at specific positions of the genome. Importantly, the higher abundance of conventional DNA compared to sites of DNA damage *in vivo* must be considered when assessing the impact of modest differences in DNA binding affinities. It is interesting to note that the human SMC5/6 complex shows a clear affinity towards short RNA-DNA hybrid structures that are common transcriptional intermediates known to stall replication fork progression (53). In addition, both human and yeast SMC5/6 complexes can bind and stabilize supercoiled/catenated DNA structures in an ATP dependent manner (53,56). The binding affinity of the SMC5/6 complex towards unconventional nucleic acid structures is a defining property of this complex, consistent with a function for the holoenzyme in the repair of DNA lesions formed at specific sites on chromosomes.

The SMC5/6 complex adopts a distinctive architecture

Recent advances in the structural characterization of SMC5/6 complex components provided exciting new insights into the global architecture of the holoenzyme and topology of its subunits (53,56–62). These studies reveal how the SMC5/6 complex uses distinctive structural features associated with its NSE subunits (Figure 1) to perform its unique repertoire of cellular functions.

Unsurprisingly, the SMC5 and SMC6 proteins at the core of the complex share a general architecture similar to that of other SMC family members, including long antiparallel coiled-coil arms connecting the hinges and ATPase head domains of each SMC subunit (Figure 1A) (53,63,64). Biochemical experiments with purified human and budding yeast SMC5/6 complexes have shown that SMC5 and SMC6 arms align in close proximity to each other throughout most of their lengths, adopting a rod-shaped architecture instead of the open-ring configuration that historically has been attributed to cohesin (Figure 1) (53,58,60,61). More recent small-angle X-ray scattering experiments show that the yeast cohesin complex can adopt —at least transiently— a rod-like structure with juxtaposed coiled-coils, suggesting that this arm configuration is a conserved feature of all SMC complexes (65). Importantly, the arm regions of cohesin and condensin are longer than those of the SMC5/6 complex (53,66) and bend sharply at so-called pseudo-elbow sites causing the hinge to contact the head-proximal coiled-coil or head-bound non-SMC proteins (26,66–70). In comparison, the arm region of the SMC5/6 holoenzyme bends slightly (Figure 1B) but does not appear to adopt a conventional elbow-bending configuration (53,59,61,71).

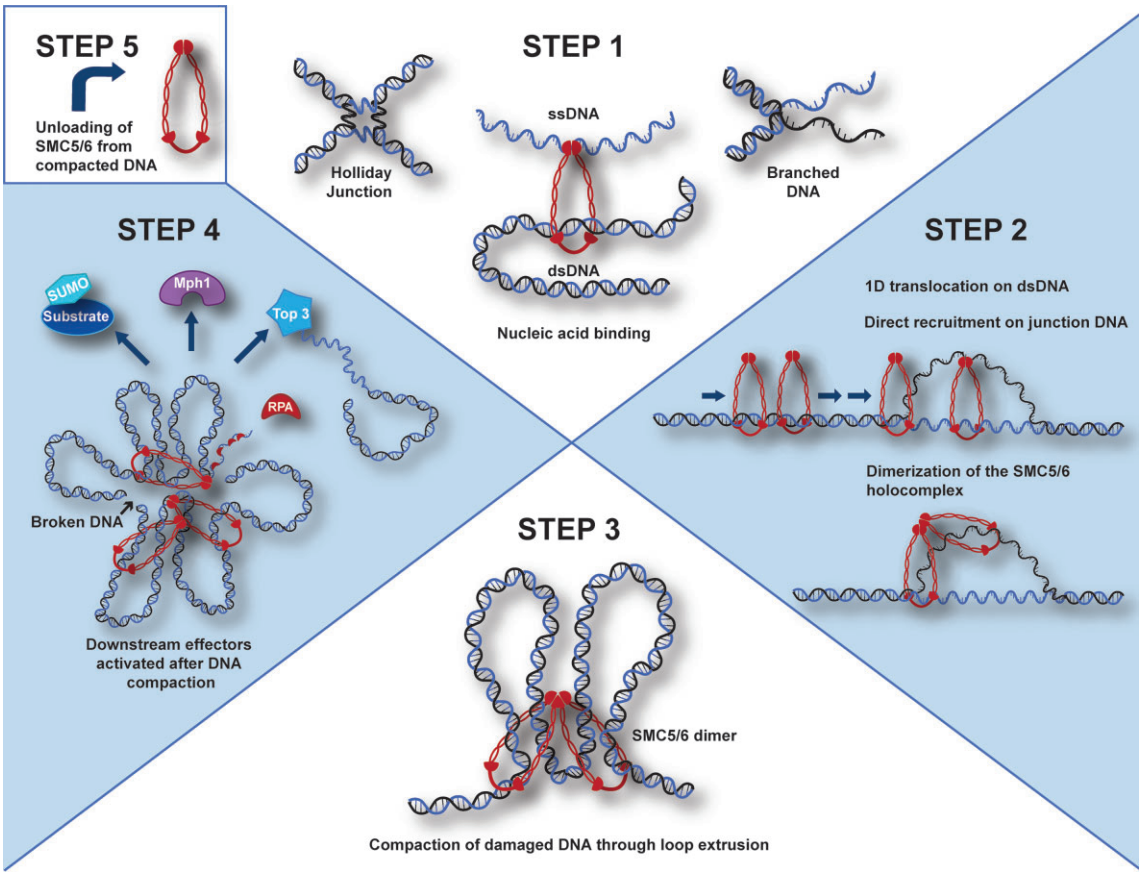


Figure 3. A 5-step model depicting the catalytic cycle of the SMC5/6 complex on chromosomal DNA substrates. Step 1 shows the differential nucleic acid binding patterns of the SMC5/6 complex. Examples of relevant types of nucleic acid substrates (ssDNA, dsDNA, Holliday junctions, and branched DNA structures) are shown. The schematic illustration of the SMC5/6 complex binding to DNA represents a generic association and no specific mode of DNA binding should be inferred from the illustration (see text for more details). Step 2 illustrates the movement dynamics of the complex along DNA. After its initial binding to DNA, the SMC5/6 complex translocates on double-stranded DNA to scan for a relevant substrate. The SMC5/6 complex is enriched at junction DNA where it undergoes dimerization. Note that while dimerization of the complex has been established on undamaged DNA (28) and appears likely at sites of DNA damage, it nevertheless remains to be formally demonstrated in the context of DNA lesions. Step 3 shows the nature of DNA compaction carried out by the SMC5/6 complex. DNA compaction likely occurs through loop extrusion mediated by the dimeric complex. Step 4 proposes downstream effects of the SMC5/6 complex after DNA compaction is completed. Among these effects, we envision that the compacted DNA creates a protective environment as well as an effective platform for the recruitment of downstream effectors for DNA repair. Step 5 depicts the unloading of the SMC5/6 complex from the compacted chromatin. See main text for a more detailed explanation of this hypothetical model.

The central hinge regions of the SMC5 and SMC6 subunits are also diverged from the corresponding regions of canonical SMCs. X-ray crystal structure of fission yeast SMC5/6 proteins reveals that the hinge region forms a toroidal structure much akin to that of the canonical SMCs. However, the hinge region consists of an unusual 'molecular latch' in SMC5 and a functional 'hub' in SMC6, two conserved interfaces that are absent in cohesin and condensin SMC components. Introduction of mutations in these interfaces affects the functionality of the complex *in vivo* (52).

Whereas the SMC components of the SMC5/6 complex show interesting differences with other family members, it is clear that the distinctiveness of the SMC5/6 complex arises mostly from the NSE components of the holoenzyme. Early biochemical studies indicated that the NSE elements of the SMC5/6 complex form three subcomplexes; namely NSE1/NSE3/NSE4, NSE2/SMC5 and NSE5/NSE6 (16–18,21,45,46) (Figure 1B, C). Human NSE4 has also been found to exist in an alternate complex with E3 (TRIM31) and MAGE-A1 proteins (47). Overall, the positioning of NSE subcomplexes relative to the core SMC5-SMC6 heterodimer is highly conserved across the eukaryotic kingdom (3,5,6). The kleisin-family protein NSE4, through its N-terminal helix turn helix (HTH) domain, binds to SMC6 neck region and also connects to SMC5 ATPase head domain using its C-terminal winged helix (WH) region (Figure 1B)(45,72). Furthermore, kleisin-interacting tandem winged-helix element (KITE) proteins NSE1-NSE3 interact with NSE4 and bridge the entire SMC5–SMC6 head domain area (Figure 1B) (45,58,62). The NSE2/Mms21 subunit binds to the mid arm region of SMC5 —an unconventional position for NSE binding— where it can promote SUMO transfer on SMC5 (and other substrates; Table 1) (Figure 1B) using its RING-like domain (20,22,31).

The NSE5-NSE6 dimer, was initially believed to associate with the SMC5/SMC6 heterodimer via the SMC hinge region in early studies on the budding yeast enzyme (46). Some studies, however, indicate that the NSE5-NSE6 dimer binds to the SMC coiled-coil arm region closer to NSE2 and proximal to the ATPase head of the holoenzyme. The proximity of NSE2 and NSE5/6 on the SMC arms of the complex likely reflects a functional link connecting these proteins since it was

shown that NSE5/6 co-ordinates NSE2-mediated SMC5 and SMC6 SUMOylation (61). The most recent cryo-EM structure of the yeast SMC5/6 complex clearly indicates that the NSE5/6 subcomplex associates with the ATPase head domain and the adjacent coiled-coil neck region of SMC6 (Figure 1C). Interestingly, NSE6 competes with NSE4 in its binding to the neck region of SMC6 and thus regulates the ATPase activity of the complex (72). This situation has additional implications for DNA binding because the kleisin-SMC neck interaction is generally considered a gateway for DNA entry into the ring structure of all SMC complexes. In this context, alternative binding of the kleisin/NSE4 and NSE6 subunits to the SMC neck region of the SMC5/6 complex creates a process of 'gate switching' that is a unique functional feature of this complex (72). Together, the specialized topology and biochemical properties of NSE subunits within the SMC5/6 complex (Figure 1) enable the diverse and context-specific roles of this enzyme in the maintenance of genomic integrity. How this functional diversity is achieved is a key question addressed in the following section of the review.

Cellular functions of the SMC5/6 complex: a DNA repair factor with a twist

Genes encoding components of the SMC5/6 complex have first been identified in genetic screens for mutants affecting the resistance of yeast to DNA damaging agents, consistent with an important role for the complex in the DNA damage response (73–75). Whereas the involvement of the SMC5/6 complex in DNA repair cannot be overstated, it is equally important to recognize that the cellular roles of the holoenzyme exceed the realm of DNA repair as it also participates in biochemical transactions involving undamaged DNA during interphase and controls effective segregation of chromatin in mitosis. Hence, unlike canonical SMC complexes which have primary roles in the global organization of chromosomes at specific stages of the cell cycle, the SMC5/6 complex acts at a more local level to regulate or otherwise promote various DNA repair and non-repair transactions throughout the cell cycle (76,77) (Figure 2). As such, the primary roles of the SMC5/6 complex appear more diverse than those of canonical SMC complexes, which raises the question of whether the SMC5/6 complex acts in those multiple pathways using a unified/single mode of action or by taking advantage of selected activities residing within the complex to achieve functional diversity. We discuss these possibilities in detail in the next two sections of this review.

Roles in repair of damaged DNA by homologous recombination

One of the most important roles of the SMC5/6 complex *in vivo* is the promotion of DNA double strand break (DSB) repair by homologous recombination (HR; Figure 2) (75,76,78–80). The SMC5/6 complex facilitates recombination between sister chromatids by recruiting cohesin in the area surrounding damaged DNA, thus allowing it to hold sister chromatids in close proximity. During this process, the SMC5/6 complex SUMOylate the subunits of cohesin and promotes error-free progression of HR repair. In support of this notion, depletion of human NSE2 lead to a decrease in the loading of cohesin to DSB sites (80). Also, in presence of the defective SMC5/6 complex and cohesin, sister chromatids are misaligned prior to recombination, thereby restricting DNA strand invasion (77,80–82). Additionally, the SMC5/6 complex can act in conjunction with proteins like MUS81/MMS4 or STR/BTR (BLM-TOPIIIα-RMI1/2) in stimulating the resolution of complex recombination intermediates generated in the late stages of HR. As is the case for cohesin, the SUMO-ligase activity of the SMC5/6 complex plays a vital role in the SUMOylation of several proteins involved in the dissolution of HR intermediates (as discussed earlier; see full list in Table 1) (39,76,78,83).

Roles in the promotion of error-free DNA replication across the genome

Multiple lines of evidence support a conserved role for the SMC5/6 complex in the promotion of proper genome replication, both in the presence and absence of DNA damage (Figure 2). In buddying yeast, the SMC5/6 complex binds to chromatin at replication initiation sites in early S phase (84,85). The SMC5/6 complex is also directly involved in SUMO-based regulation of multiple important factors in the replisome, one of the most important among them being POL2, the catalytic subunit of DNA polymerase ε crucial for the initiation of DNA replication (86,87). Moreover, SMC5/6 works together with proteins such as STR to facilitate replication completion (39).

Interestingly, the SMC5/6 complex exhibits an important role in the maintenance of proper chromosome topology during DNA replication which allows faithful completion of replication and prevents chromosome fragmentation during mitosis. Indeed, the separation of the two parental strands during DNA replication creates positively supercoiled DNA ahead of advancing replication forks, a situation that can hinder replication fork progression if not addressed. The main players involved in the removal of replication-induced topological stress are topoisomerases TOP1 and TOP2 (88) along with the SMC5/6 complex itself (82). In yeast, the SMC5/6 complex associates with chromosome during S-phase in a length-dependent manner to decrease topological stress during replication especially when TOP2 activity is impaired (82). The impact of positive supercoils formed at replication forks can also be mitigated by rotating replication forks, which creates sister chromatids intertwinements (SCI) or catenation behind advancing forks. The SMC5/6 complex sequesters these SCIs behind the replication fork by facilitating fork rotation, a process that ultimately promotes normal replication progression (76,85,89,90). Hence, these observations point out to a conserved prerequisite for the SMC5/6 complex in successful completion of genome replication.

The SMC5/6 complex also promotes stabilization of replication forks at sites of DNA lesion to prevent fork collapse, mainly by limiting the formation and/or helping in the resolution of recombination intermediates at stalled replication forks (91). The SMC5/6 complex maintains the integrity of stalled replication forks by loading and SUMOylating RPA and RAD52 proteins, thus keeping forks in a recombination-competent configuration (42,91). Advancing replication fork can also bypass a DNA lesion in a HR-dependant template switch mechanism where SMC5/6 complex along with STR can participate in the resolution of recombination intermediates formed after the template switch (92–95).

In yeast, the SMC5/6 complex physically interacts with the MPH1 helicase, a homolog of Fanconi anemia protein M, in

a manner that prevents replication fork reversal by MPH1 (96,97). Genetic data in fission yeast also suggest that the NSE5/6 subunits, as a part of the SMC5/6 complex, play a crucial role in preventing the accumulation of Holliday junctions when replication forks encounter DNA lesions (18). Furthermore, mutagenesis data from a recent study reveals that the NSE5/6-SMC6 interaction promotes resolution of DNA repair intermediates but not replication termination (72). In higher eukaryotes, the SMC5/6 complex has been shown to associate with the FANCD2 and FANCI proteins of the Fanconi Anaemia pathway to promote recombination repair at stalled replication forks (98). Consistent with the observation that the SMC5/6 complex plays crucial roles during DNA replication, SMC5 depletion in mouse cells resulted in increased replication fork stalling that was associated with higher apoptosis in central cortical tissues along with reduced cortex sizes (99).

Promoting replication at hard-to-replicate genomic loci

Natural sites on chromosomes that act as replication barriers or that are otherwise difficult to replicate represent a unique type of challenge for genome stability, one whose effective resolution is especially dependent on SMC5/6 complex activity. This situation is well exemplified by the role of the SMC5/6 complex at replication fork barrier (RFB) sites in the ribosomal DNA (rDNA) array of yeast (34,100,101). Chromatin immunoprecipitation studies have shown that the SMC5/6 complex associates with chromosomal DNA across the genome, but is particularly enriched at the rDNA array on budding yeast chromosome XII during S phase (78,84,102). Because of its highly repetitive and heterochromatic nature, the rDNA array often experiences a higher frequency of defective replication relative to other regions of the genome. This is compounded by the unidirectional nature of DNA replication at this locus, which prevents the rescue of stalled replication forks by forks converging from the other side of the chromosome. As expected, rDNA replication delay and instability are prevalent in *smc5/6* mutants, reflecting the special contribution of the SMC5/6 complex to the maintenance of rDNA stability. Importantly, defects in rDNA replication in *smc5/6* mutants can cause subsequent chromosome non-disjunction and a higher frequency of Holliday junctions, thus impairing chromosomal segregation in mitosis (102–104).

Roles as a regulator of the DNA transactions in heterochromatin

Apart from the rDNA locus discussed above, localization analyses of the SMC5/6 complex have revealed that the complex is enriched within heterochromatic-like regions at telomeres (34,101,105). This enrichment appears to be functionally important for the maintenance of telomere length (Figure 2). In telomerase negative cancer cells, the NSE2 subunit SUMOylates multiple telomere binding proteins to promote telomeric HR and lengthening by alternative lengthening of telomeres (ALT) (Figure 2; Table 1) (106). In budding yeast, loss of the SMC5/6 complex activity in cells lacking telomerase enhances senescence via a HR independent pathway (107). Together, these studies suggest that the maintenance of telomeres by the SMC5/6 complex is evolutionary conserved. Furthermore, the SMC5/6 complex stabilizes telomeres through its involvement in the processing of recombination intermediates at chromosome ends (34,105).

Repair of DSB within heterochromatic regions of the genome is surprisingly different from that of DSBs in euchromatin. Late events of HR involving RAD51-mediated strand invasion occurs usually outside of the heterochromatin domain in Drosophila (108). Similarly in yeast, HR of DSBs in the heterochromatin-like rDNA requires re-localization of damaged DNA to extranucleolar sites before it can be repaired (103,109). HR is supressed at the rDNA locus through the SUMOylation of RAD52 by the SMC5/6 complex in yeast, an event that prevents RAD52 foci formation in the nucleolus (103). Importantly, this event supresses recombinational loss of rDNA repeats on chromosome XII, thus ensuring copy number stability at this locus (103). Likewise, in metazoans like flies and mice, the SMC5/6 complex and its SUMO-ligase activity are essential for recruiting and co-ordinating nuclear actin and myosin proteins to relocate heterochromatic DSBs to the nuclear periphery before strand invasion (110,111). This phenomenon allows error-free progression of HR repair within heterochromatin and limits ectopic recombination between repeated regions of the genome (77,110,111).

Despite substantial progress in recent years, the mechanistic basis for SMC5/6 complex-dependent movement of DNA damage across specific nuclear compartments is not fully understood. It is interesting to note that SMC5 and SMC6 both show significant affinity for microtubules (112) and microtubules can promote non-linear/directional motion of damaged chromatin in cells (113). It is therefore tempting to speculate that the SMC5/6 complex might act as a bridge connecting DNA damage to microtubules during DNA repair reactions. More work is required to shed light on this exciting, but putative mode of action.

Roles as a master silencer of episomal and viral DNA

Silencing of episomal DNA templates is an unexpected function specifically attributed to the SMC5/6 holoenzyme and does not appear to extend to other eukaryotic SMC complexes (Figure 2). Interestingly, the bacterial Wadjet defence system —where three Wadjet subunits form an SMC-family complex— recognizes and protects bacterial hosts from external plasmid transformation in an ATP hydrolysis dependant manner (114). Multiple recent studies have reported that the SMC5/6 complex can epigenetically silence extrachromosomal viral DNA, helping to defend cells against infections from hepatitis B virus (HBV), human immunodeficiency virus (HIV), herpes simplex virus and papillomavirus (115–119).

The SMC5/6 complex restricts HBV by entrapment of the viral episome DNA inside the complex (Figure 2). This is followed by recruitment of the episomal DNA-protein complex to promyelocytic leukemia nuclear bodies (PML-NB) for subsequent transcriptional repression of viral DNA by NSE2 (120,121). The recruitment step depends on SIMC1, a paralog of SLF1 that forms a NSE5/NSE6-like complex with SLF2 and behave similarly to its yeast counterpart in the localization of the SMC5/6 complex to specific regions of chromosomes. The virus silencing function of the SMC5/6 complex is not limited to HBV since it can also silence HIV1 DNA provirus before integration into the host cell chromosomes by inhibitory epigenetic modification (Figure 2). This process is mediated mainly by NSE2-dependent SUMOylation

(119,122). Furthermore, the SMC5/6 complex appears to act as a global guardian against virus infection because several recent studies reported similar virus-restriction roles of the SMC5/6 complex in epigenetic silencing and/or inhibition of DNA replication of Epstein–Barr virus (EBV) (123), Kaposi's sarcoma herpesvirus (KSHV) (118), human papilloma virus (HPV) (117), and herpes simplex virus 1 (124).

The role of the SMC5/6 complex in episome silencing appear to exceed the domain of viral genomes since a recent study has revealed a potential role for the budding yeast SMC5/6 complex —specifically the NSE2 subunit— in restricting the parasitic 2μ plasmid (125). It will be interesting for future studies to define the full repertoire of episomes targeted by the SMC5/6 complex *in vivo* and whether it achieves silencing of extrachromosomal DNA by a universal mechanism applicable to all episome types across the eukaryotic kingdom.

The mode of action of the SMC5/6 complex: recognition of DNA substrates and subsequent activation

The involvement of the SMC5/6 complex in wide range of cellular functions implies its activity must be precisely controlled in different chromosomal contexts. How context-specific activation of the SMC5/6 complex is achieved is unclear, but the consequences of allowing unrestricted activation of the SMC5/6 complex are likely to be very toxic, highlighting the crucial importance of this issue for cell homeostasis. We discuss in the next section how the SMC5/6 complex precisely and dynamically recognize its substrates *in vivo* and what impact it has on chromosomal DNA once the holoenzyme is activated.

Basal DNA binding activity of the SMC5/6 complex: who does what?

The SMC5/6 complex associates with DNA via at least three general types of intrinsic DNA binding activities which are not mutually exclusive and often rely on each other for the execution of specific cellular function. The different modes of binding include topological DNA entrapment by the SMC5/6-NSE1/3/4 ring (56,126,127), conventional/direct (usually electrostatic) DNA binding, and a specific form of DNA loop entrapment (usually called pseudo-topological entrapment) mediated by selective subunits/domains within the complex (50,56,127–129). The DNA binding domains of the SMC5/6 complex can mediate direct interaction with diverse types of DNA repair and recombination intermediates as evidenced from biochemical analyses (50,51,128,129) as well as from *in vivo* experiments (51,130).

The core components of the SMC5/6 complex, the SMC5-SMC6 dimer, has its independent DNA binding properties apart from the holocomplex. For instance, structural and biochemical assays show that the hSMC5–SMC6 heterodimer has higher binding affinity towards ssDNA compared to dsDNA (53). In-depth DNA binding assays with a series of functional domain fragments revealed that SMC5 and SMC6 monomers can interact with their DNA substrates via two distinct DNA-binding domains on each SMC molecule, one located in the hinge domain and adjacent coiled-coil sequences, and the second one in the ATPase head domain of the protein (50,52). Interestingly, formation of the SMC5–SMC6 heterodimer (Figure 1A) improves affinity for dsDNA substrates relative to that observed with either of the SMC components in their monomeric forms (50). Overall, these observations indicate that the basal DNA binding activity of the SMC5/6 complex comes at least in part from the intrinsic ability of SMC5 and SMC6 monomers to associate with nucleic acid substates (128,129).

Association of the SMC5/6 holoenzyme to chromosomal DNA is also regulated by the NSE subunits of the complex. Additionally, the NSE1/NSE3/NSE4 trimer (Figure 1B) demonstrates intrinsic DNA binding activity with higher affinity towards short dsDNA than ssDNA, an activity mainly attributed to the NSE3 subunit (51). The cryo-EM structure of DNA-bound yeast Smc5/6 complex reveals that the NSE1/NSE3/NSE4 subcomplex connects the SMC5/6 ATPase-head domain and locks the SMC5/6 complex encircling the DNA double helix by forming a clamp (62). Interestingly, dsDNA entrapment into the ring structure made of SMC5/6-NSE1/3/4 (i.e. clamp formation) requires ATP hydrolysis, pointing towards a contribution of the ATPase head domains of SMC subunits in this process (51,54,58,59,71,130,131). During DNA clamp formation, the complex configuration created when the SMC5-SMC6 heterodimer associates with NSE3-NSE4 gives rise to a positively charged central tunnel that stabilizes the negatively charged DNA backbone (62). Using cryo-EM approach, it was possible to generate a detailed view of the critical DNA binding patches/residues in each of the complex subunit that guide DNA binding and/or clamp formation (62). Consistent with this view, alterations of individual domains/subunits are met with varied effects on growth and cellular response to DNA damage (62).

The NSE5–NSE6 subcomplex is another important contributor to the DNA binding activity of the SMC5/6 holoenzyme. Recent studies have revealed that the use of alternative subunits as binding partners for the SMC6 neck region (i.e. NSE4 or NSE6) can potentially gate or otherwise influence DNA entry into the SMC5/6 complex (72). The switch from NSE4 to NSE6 as binding partner for SMC6 neck region also modulates the engagement of the SMC6 head domain with that of SMC5 head domain, ultimately regulating the ATPase activity of the complex (72).

Overall, the ATP hydrolysis cycle involves three main structural conformation of the SMC5/6 complex. First, the 'ATP-engaged state' that includes two ATP molecules 'sandwiched' between the residues of the SMC5-SMC6 head domains maintains adjacent SMC arms at a distance, thus opening the SMC compartment. Second, the 'juxtaposed state' where the heads are disengaged promotes co-alignment of SMC5 and SMC6 arms, yielding a closed SMC compartment. And finally, the 'inhibited state' where NSE5/6 interacts with the arm and heads of the SMC5/6 complex prevents head engagement and ATP hydrolysis (127). This inhibition is counteracted by the binding of a suitable DNA substrate, an event that promotes more stable DNA clamping by the SMC5/6 complex (62). Overall, the NSE5/6 complex interacts with the SMC5/6 head and arm domains through both stable and dynamic interactions to allow intricate regulation of ATP hydrolysis, DNA segment capture and DNA entrapment/clamping.

Apart from the above-mentioned configurations, another model proposes a pseudo-topological entrapment of DNA in two SMC sub-compartments The SMC5-SMC6 head engagement creates an SMC ('S') and a kleisin ('K') compartment

(60,127). Additionally, cryo-EM structure revealed that the NSE3 subunit may link the SMC5 and the SMC6 coiled-coil to separate the S compartment into two halves, the upper and the lower SMC compartments (62). Most up to date cystine crosslinking experiments show that a DNA segment is often effectively held as a loop with (at least) one dsDNA passing through the lower S compartment and another one (or more) through the upper S compartment. This mode of binding is referred to as DNA segment capture and the conformation taken by the SMC5/6 complex during this capture is an important intermediate that connects initial DNA loading to subsequent biochemical activities of the SMC5/6 complex (72,127).

The contribution of several subunits of the SMC5/6 complex to its DNA binding activity combined with the intricate pattern of association of these subunits to chromosomal DNA have important implications for the mechanism of action of the enzyme. During DNA loop extrusion, the multiple DNA binding sites within the SMC5/6 complex might trigger the reeling of DNA while simultaneously holding onto both arms of the forming loop. Also, SMC complexes do not necessarily entrap DNA topologically during the extrusion process (72). It is therefore possible that in the execution of different cellular functions, the SMC5/6 complex might use different modes of DNA interaction to create DNA loops in the genome (72,127). Indeed, the array of different DNA binding sites within the complex and conformational changes associated with DNA binding are likely to support different loop extrusion models, as observed with cohesin and condensin (25). Taken together, these observations indicate that the SMC5/6 complex uses multiple distinct DNA binding domains/modes to affect functional changes in chromatin/DNA configuration.

Context matters: when and how the SMC5/6 complex binds to chromosomes *in vivo*?

As previously discussed, binding of the SMC5/6 complex to damaged DNA does not appear to impact chromatin at the level of entire chromosomes because genome-level condensation or cohesion are not observed as a direct consequence of SMC5/6 holoenzyme activation. How the complex achieves selectivity in DNA substrate binding and modification *in vivo* is a highly relevant issue for the mode of action of the SMC5/6 holoenzyme. This is accomplished through context-specific activation during DNA transactions combined with a mechanism of proactive inhibition of the complex in non-targeted regions of the genome.

We now know that several proteins play a concerted role to facilitate the loading of the SMC5/6 complex at specific sites of repair during the DNA damage response. For example, fission yeast BRCT domain-containing protein BRC1, and its buddying yeast homologue RTT107 are important recruiters of the complex to chromosomes. Non-SMC elements such as NSE5 and NSE6 also perform an important role in the loading of the SMC5/6 complex on chromatin *in vivo* (57,60,61,130,132,133). In yeast, BRC1/RTT107 recruits the SMC5/6 complex to lesioned/γH2A-covered DNA via the NSE5-NSE6 sub-complex (130,132,134). Similarly, human SLF1 (NSE5-like) and SLF2 (NSE6-like) act as a SMC5/6 complex loader through RAD18-mediated binding to damaged DNA sites (24). In budding yeast, the NSE5/6 complex also promote loading of the SMC5/6 complex at stalled replication forks (135). Interestingly, during viral infection a paralog of SLF1, SIMC1, forms a complex with SLF2 to localize the SMC5/6 complex to virus DNA to restrict virus DNA replication (120), although it is not clear yet whether SIMC1 has roles in other cellular contexts. The NSE5/6-complex is not only essential for directly loading the complex at sites of DNA damage, but also for the stabilization of its binding to dsDNA when the complex bind nucleic acids initially by itself (130).

Chromatin marks such as histone modifications can also act as a cue for the recruitment of the SMC5/6 complex to specific chromosomal regions. For instance, the recruitment of BRCA1 —an important HR promoting factor— requires the recognition of histone H4 unmethylated at lysine 20 (K20me0) through the ankyrin repeat domain of BRCA1-associated RING domain protein 1 (BARD1). The SLF1/SLF2 complex can also specifically recognize and bind histone H4-K20me0 (136). Hence, this provides an intriguing putative mechanism for the selective recruitment of the SMC5/6 complex to newly replicated DNA, an event that may be important for the promotion of HR. Consistent with this observation, the ankyrin repeat domain (ARD) responsible for histone H4-K20me0 recognition of BARD1 is conserved in SLF1 and several other proteins involved in DNA repair and replication, such as TONSL (Tonsoku-like protein) (136). Whether this ARD-dependent recruitment mechanism is operational across the genome for the SMC5/6 complex is unclear. Interestingly, it has been established that enrichment of the SMC5/6 complex at undamaged regions of the genome, such as centromere and intergenic regions, requires the cohesin loading factor SCC2, which indicates that alternative modes of recruitment likely operate in the absence of DNA damage (84).

Once the SMC5/6 complex is recruited to genomic DNA by chromatin loaders, the first level of regulation of DNA-binding occurs at the level of ATP binding and hydrolysis by the holoenzyme. The SMC5/6 complex interacts with ssDNA in an ATP-independent manner and binding to dsDNA occurs through predominantly ATP-dependent mechanisms especially during clamp formation (55,62,126). The ATP-dependent remodelling of the SMC5/6 complex and subsequent binding to DNA also promotes the SUMO-E3 ligase activity of NSE2 (83,137). The NSE2 subunit lacks DNA binding domains, but its DNA repair functions require stable docking onto the SMC5 protein (137). Specifically, an electrostatic interaction between DNA and a positively charged patch in the ARM domain of SMC5 activates the E3-SUMO-ligase activity of NSE2, which further impacts the loading of the complex on chromatin by self-SUMOylation of the core SMC proteins (61,137). Furthermore, NSE5/6-mediated loading of the SMC5/6 complex on DNA can promote the SUMO-ligase activity of NSE2 (61,134).

Recent evidence suggests the existence of additional layers of functional interplay connecting the various subunits and substrates of the SMC5/6 complex. For instance, we now know that the proximity of NSE2 (in addition to NSE5 and NSE6 subunits) to the ATPase head domain of the SMC components (61,72,127) can potentially regulate DNA entry into the ring structure of the complex (28). Also interestingly, the NSE5/6 subcomplex compete with high amounts of dsDNA structures to negatively modulate the binding efficiency of the complex to DNA duplex structures (57), thus showing both negative and positive impacts on the DNA binding activity of the SMC5/6 complex. This dual impact of NSE5/6 may provide a regulatory mechanism to limit SMC5/6 complex activity to specific regions of chromosomes and allows the complex

to adapt to diverse requirements of its ATPase activity during DNA replication and repair actions.

What happens after the SMC5/6 complex binds its DNA substrates *in vivo*?

Recent biochemical and biophysical advances on the mechanism of the SMC5/6 complex suggest a multi-step catalytic cycle on DNA substrates. One of the earliest steps likely involves SMC5/6 complex translocation along dsDNA by one-dimensional diffusion either as monomeric or oligomeric complex (28,55). Its dynamic interaction with chromatin increases scanning for probable lesions on DNA molecules, especially enriched at ssDNA-dsDNA interfaces (53–56). Sliding on dsDNA may not be a pre-requisite for binding to ssDNA-dsDNA junction site as the complex can also bind this type of DNA structures directly (Figure 3) (54).

Translocation on DNA is subsequently accompanied by local DNA compaction at sites of DNA lesion, likely involving dimerization of the holoenzyme (55) (Figure 3). DNA compaction by the SMC5/6 complex uses the energy of ATP hydrolysis and proceeds through loop extrusion of chromatin (28) (Figure 3), as seen with other SMC proteins. The complex performs symmetrical DNA loop extrusion in a dimer configuration very similar to that of cohesin. The loop-extrusion based DNA compaction activity of the SMC5/6 complex is consistent with its originally assigned role as an intermolecular DNA linker. This is because during compaction, the SMC5/6 complex can link and load multiple molecules of DNA in trans while performing its cellular functions (55,126). Recently, the loop extrusion activity of SMC5/6 complex has also been observed during transcription. Single-molecule imaging and Hi-C analyses show that SMC5/6 complex dimers can recognize and link chromosomal regions containing positively supercoiled DNA especially during transcription, and initiate loop extrusion to fold the supercoiled DNA into large plectonemic loops for three-dimensional organization of chromosomes (138,139).

As the SMC5/6 complex engages in loop extrusion, its different subunits play distinct and sometimes antagonistic contributions to the overall activity of the enzyme. For instance, the NSE2 subunit is essential for the loop extrusion activity of the SMC5/6 complex, which is a unique property of this enzyme as other SMC complexes do not require an additional subunit to be bound to the coiled-coil arms of SMC proteins to allow loop extrusion. In contrast, the NSE5/6 subunits may act as negative regulators of loop extrusion by the SMC5/6 complex since they seem to reduce loop initiation rate and loop persistence. The NSE5/6 subcomplex can also prevent the dimerization of the holoenzyme, which is essential for loop extrusion (28). Interestingly, the NSE5/6 subcomplex does not affect loop extrusion dynamics after loop initiation has occurred. At first glance, NSE5/6 inhibiting loop extrusion might seem conflicting with the fact that this subcomplex is also a primary loader of the SMC5/6 holoenzyme on chromatin, a precondition for topological loading of DNA into the complex (60,127). However, SMC5/6 complex-mediated loop extrusion is known to occur independently of topological entrapment of DNA. The SMC5/6 complex can extrude loops in the absence of NSE5/6 but cannot topologically bind to DNA without NSE5/6 (28).

Overall, the catalytic cycle of the SMC5/6 complex will result in local DNA/chromatin compaction and looping at specific sites of the genome. We hypothesize that this localised compaction of chromatin will create a phenomenon of steric hindrance that diminishes DNA mobility in the vicinity of repair sites, likely shielding fragile DNA intermediates from the action of undesirable modifying enzymes and acting as a cue for the loading of specific DNA repair enzymes (Figure 3). Such local restriction of DNA mobility is expected to be transient and not extend beyond the initial recruitment of early DNA repair effectors, consistent with the *in vivo* observed decrease in the mobility of newly-formed DNA lesions followed by a distinct increase at later stages of the DNA repair process (140–143). Finally, we anticipate that DNA looping by the SMC5/6 complex will have other impacts on chromatin dynamics. For instance, based on what is observed for cohesin, SMC5/6 complex-mediated chromosome looping might facilitate DNA repair by controlled distribution of the histone phosphorylation mark γH2AX (144).

Completing the catalytic cycle: releasing the SMC5/6 complex from compacted DNA

Similar to the loading of the SMC5/6 complex on damaged DNA, its effective unloading from densely compacted chromatin is critically important to enable effective turnover of the holoenzyme *in vivo* (Figure 3), as previously observed with condensin and other chromatin-binding enzymes (145–148). Previous studies suggest that the unloading of the SMC5/6 complex in late mitosis is important for effective chromosome segregation since misregulation of the SMC5/6 complex can lead to the formation of anaphase bridges (149,150). Despite extensive knowledge of the mechanisms underpinning SMC5/6 complex loading onto chromatids, very little is known about the mechanism responsible for unloading the holoenzyme from chromosomal substrates once its catalytic cycle is completed.

What mechanism might be responsible for SMC5/6 complex release from compacted chromatin? The unloading of the SMC5/6 complex might be similar to its sister SMC complexes since they share some structural and functional features. In the case of condensin, a chromatin rescue factor known as the CDC48 segregase (together with its ubiquitin-adaptor complex UFD1-NPL4) is recruited onto condensin and extracts the trapped enzyme from its compacted product (145). Whether the Cdc48 segregase or a similar enzyme facilitates unloading of the SMC5/6 complex from compacted chromatin remains to be established. However, it is noteworthy that CDC48 has been identified as a target for ubiquitination by the NSE1 subunit of the SMC5/6 complex (49), suggesting a likely functional link between these two proteins.

More direct/intrinsic mechanisms for the release of the SMC5/6 complex from chromatin may be involved in its catalytic cycle. For instance, it is possible that the ATPase activities of the complex might generate sufficient mechanical force to promote its release from compacted chromatin (131). Finally, it is conceivable that compacted DNA itself might trigger the dissociation of some subunits from the SMC5/6 complex, thus enabling a reduction in its affinity for chromosomal DNA or otherwise opening the ring structure necessary for topological entrapment (50,53). Consistent with this view, analysis of nuclear extracts by size exclusion chromatography reveals that the interaction connecting SMC5 and SMC6 is weakened or lost specifically during mitosis in human cells (151), suggesting a possible disassembly of the complex

under conditions of generalized chromatin compaction. While the unloading of cohesin and condensin from genomic DNA is often regulated by post-translational modification (PTM), it is important to recognize that non-PTM mechanisms can also promote chromatin unloading of these complexes (145,152). Similar mechanisms might also contribute to the release of the SMC5/6 complex from its genomic substrates.

Taken together, extensive progress in deciphering the structure and function of the SMC5/6 complex suggests a 5-step model to explain the catalytic cycle of the holoenzyme on DNA and how it stimulates DNA repair. Chronologically, the first step involves a basal association of the SMC5/6 complex to undamaged DNA using both ATP-dependent and independent mechanisms (step 1; Figure 3). This is followed by one-dimensional translocation of the complex on dsDNA, which leads to dimerization of the holoenzyme at sites of DNA damage (step 2; Figure 3). Subsequently, the complex would engage in DNA loop extrusion leading to chromatin compaction (step 3; Figure 3). We propose that chromatin compaction at sites of DNA damage will stabilize and protect fragile DNA repair intermediates. Either concomitantly or subsequently with the previous step, the SMC5/6 holoenzyme would recruit and activate DNA repair factors at sites of DNA damage through its post-translational modification activities, thereby promoting downstream steps in the DNA repair process (step 4; Figure 3). Completion of repair would finally lead to chromosome unloading of the SMC5/6 holoenzyme, which would then be free to initiate a new catalytic cycle (step 5; Figure 3).

What happens when the SMC5/6 complex fails?

Rare genetic disorders

Systematic identification of *de novo* and inherited mutations in subunits of the SMC5/6 complex have revealed its causative role in three rare genetic diseases. Notably, bi-allelic missense mutations in the human *NSMCE3* gene (encoding NSE3) have been associated with a fatal genetic disorder named lung disease immunodeficiency and chromosome breakage syndrome (LICS) (153). NSE3 mutations disrupt the inter-subunit interactions within the complex ultimately leading to faulty HR, sensitivity to DNA replication stress, chromosomal rearrangements, and micronuclei formation (153) (Figure 2).

Another report identified compound heterozygous frameshift mutations in the *NSMCE2* gene leading to reduced NSE2 protein abundance in patients with primordial dwarfism, extreme insulin resistance and gonadal failure (154). Alterations in *NSMCE2* correlated with increased frequency of micronuclei, nucleoplasmic bridge formation, and DNA replication delay in patients (154) (Figure 2). Patients carrying mutations in the genes encoding SMC5 and its targeting factor SLF2 experience multiple mitotic abnormalities that give rise to a prognostic genome instability phenotype consisting of segmented, dicentric and rail-road chromosomes associated with mosaic variegated hyperploidy (MVH), a disorder known as the Atelís syndrome (155) (Figure 2).

The types of cellular phenotypes observed in the three syndromes listed above are fully consistent with the known functions of the SMC5/6 complex in DNA replication and repair processes. We anticipate that the hypomorphic mutations affecting other subunits of the complex are likely to occur at a low frequency in humans and would give rise to similar but still undiscovered inherited disorders in patients.

Cancer

Mutations in DNA repair enzymes have historically been associated with increased rates of cancer formation in humans (156–159). Consistent with this view, studies have reported the presence of gene alterations affecting SMC5/6 complex components in a number of human cancers, including: hepatocellular carcinoma (160,161), sarcoma (162), breast cancer (163), colorectal cancer (164) and brain metastasis (165) (Figure 2). Whether those alterations act as passenger mutations or driver of cancer formation remained unknown until recently. Experiments using a murine model of cancer have revealed that haplo-insufficiency of *NSMCE2* is associated with tumor formation and overall poor survival (166), supporting the notion that loss of SMC5/6 complex function can promote oncogenesis.

A very recent phenogenomic analysis of cancer genomes suggests a direct link connecting SMC5/6 complex mutations to cancer development (167). This study evaluated the presence, frequency and nature of SMC5/6 gene alterations across a vast range of tumor types in a dataset comprising ∼65 000 cancer genomes. This systematic analysis revealed that components of the human SMC5/6 complex are frequently altered in several types of cancers, including breast, prostate and ovarian cancers. Patients carrying genetic alterations in the subunits of the SMC5/6 complex experience strong phenotypic effects involving changes in genome ploidy and reduced overall survival compared to patients carrying a wild-type SMC5/6 complex (Figure 2).

In closing, we note that virus infections associated with degradation and/or inactivation of the SMC5/6 complex might also lead to tumorigenic conditions. This notion finds support in human cases of hepatitis B virus (HBV) infection where the viral regulatory protein X (HBx) induces the degradation of the SMC5/6 complex (115), leading to a reduced DNA repair capacity and accumulation of DNA damage in liver cells. Not surprisingly, this situation is associated with increased tumorigenesis in HBV-associated hepatocellular carcinoma (168,169).

Conclusion

Taken together, research over the last few years has revealed that the SMC5/6 complex promotes the repair of eukaryotic genomes using a sequence of multimodal interactions that reconfigures chromosomal DNA in space. This mode of action is distinct from canonical pathways of chromatin modification that act at the level of histones, PTMs and chromatin remodeling enzymes. Recent structural and biophysical studies have provided a clear outline of the steps involved in the catalytic cycle of the SMC5/6 complex, but it will be important for future research to focus on revealing how the reorganization of chromatin through looping and compaction can specifically enhance DNA repair reactions as well as other genome transactions. Given the multifarious impact of the SMC5/6 complex on genome stability, future research on this topic is likely to provide remarkable mechanistic advances on DNA repair pathways and their health-related impacts in oncology, virology and aging.

Data availability

No new data were generated or analysed in support of this research.

Acknowledgements

We apologize to our colleagues for not being able to cite references more expansively due to space constraints. We thank members of the D'Amours laboratory for their comments on the manuscript.

Funding

Work in D.D.'s laboratory is supported by a grant from Canadian Institutes of Health Research [FDN-167265]; Canada Research Chair in Chromatin Dynamics & Genome Architecture [CRC-2017-00064]. Funding for open access charge: CIHR.

Conflict of interest statement

None declared.

References

1. Bell,O., Tiwari,V.K., Thomä,N.H. and Schübeler,D. (2011) Determinants and dynamics of genome accessibility. *Nat. Rev. Genet.*, **12**, 554–564.
2. Shi,X., Zhai,Z., Chen,Y., Li,J. and Nordenskiöld,L. (2022) Recent advances in investigating functional dynamics of chromatin. *Front. Genet.*, **13**, 870640.
3. Kim,E., Barth,R. and Dekker,C. (2023) Looping the genome with SMC complexes. *Annu. Rev. Biochem.*, **92**, 15–41.
4. Shintomi,K., Inoue,F., Watanabe,H., Ohsumi,K., Ohsugi,M. and Hirano,T. (2017) Mitotic chromosome assembly despite nucleosome depletion in Xenopus egg extracts. *Science*, **356**, 1284–1287.
5. Bürmann,F. and Löwe,J. (2023) Structural biology of SMC complexes across the tree of life. *Curr. Opin. Struct. Biol.*, **80**, 102598.
6. Hoencamp,C. and Rowland,B.D. (2023) Genome control by SMC complexes. *Nat. Rev. Mol. Cell Biol.***24**, 633–650.
7. Guacci,V., Koshland,D. and Strunnikov,A. (1997) A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. *Cell*, **91**, 47–57.
8. Michaelis,C., Ciosk,R. and Nasmyth,K. (1997) Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell*, **91**, 35–45.
9. Wutz,G., Várnai,C., Nagasaka,K., Cisneros,D.A., Stocsits,R.R., Tang,W., Schoenfelder,S., Jessberger,G., Muhar,M., Hossain,M.J., *et al.* (2017) Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins. *EMBO J.*, **36**, 3573–3599.
10. Hansen,A.S., Cattoglio,C., Darzacq,X. and Tjian,R. (2018) Recent evidence that TADs and chromatin loops are dynamic structures. *Nucleus*, **9**, 20–32.
11. Emerson,D.J., Zhao,P.A., Cook,A.L., Barnett,R.J., Klein,K.N., Saulebekova,D., Ge,C., Zhou,L., Simandi,Z., Minsk,M.K., *et al.* (2022) Cohesin-mediated loop anchors confine the locations of human replication origins. *Nature*, **606**, 812–819.
12. Hirano,T., Kobayashi,R. and Hirano,M. (1997) Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E and a Xenopus homolog of the Drosophila Barren protein. *Cell*, **89**, 511–521.
13. Peng,X.P. and Zhao,X. (2023) The multi-functional Smc5/6 complex in genome protection and disease. *Nat. Struct. Mol. Biol.*, **30**, 724–734.
14. Fujioka,Y., Kimata,Y., Nomaguchi,K., Watanabe,K. and Kohno,K. (2002) Identification of a novel non-structural maintenance of chromosomes (SMC) component of the SMC5-SMC6 complex involved in DNA repair. *J. Biol. Chem.*, **277**, 21585–21591.
15. Hazbun,T.R., Malmström,L., Anderson,S., Graczyk,B.J., Fox,B., Riffle,M., Sundin,B.A., Aranda,J.D., McDonald,W.H., Chiu,C.-H., *et al.* (2003) Assigning function to yeast proteins by integration of technologies. *Mol. Cell*, **12**, 1353–1365.
16. McDonald,W.H., Pavlova,Y., Yates,J.R. and Boddy,M.N. (2003) Novel essential DNA repair proteins Nse1 and Nse2 are subunits of the fission yeast Smc5-Smc6 complex. *J. Biol. Chem.*, **278**, 45460–45467.
17. Pebernard,S., McDonald,W.H., Pavlova,Y., Yates,J.R. and Boddy,M.N. (2004) Nse1, Nse2, and a novel subunit of the Smc5-Smc6 complex, Nse3, play a crucial role in meiosis. *MBoC*, **15**, 4866–4876.
18. Pebernard,S., Wohlschlegel,J., McDonald,W.H., Yates,J.R. and Boddy,M.N. (2006) The Nse5-Nse6 dimer mediates DNA repair roles of the Smc5-Smc6 complex. *Mol. Cell. Biol.*, **26**, 1617–1630.
19. Hu,B., Liao,C., Millson,S.H., Mollapour,M., Prodromou,C., Pearl,L.H., Piper,P.W. and Panaretou,B. (2005) Qri2/Nse4, a component of the essential Smc5/6 DNA repair complex. *Mol. Microbiol.*, **55**, 1735–1750.
20. Potts,P.R. and Yu,H. (2005) Human MMS21/NSE2 is a SUMO ligase required for DNA repair. *Mol. Cell. Biol.*, **25**, 7021–7032.
21. Sergeant,J., Taylor,E., Palecek,J., Fousteri,M., Andrews,E.A., Sweeney,S., Shinagawa,H., Watts,F.Z. and Lehmann,A.R. (2005) Composition and architecture of the *Schizosaccharomyces pombe* Rad18 (Smc5-6) complex. *Mol. Cell. Biol.*, **25**, 172–184.
22. Zhao,X. and Blobel,G. (2005) A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 4777–4782.
23. Taylor,E.M., Copsey,A.C., Hudson,J.J.R., Vidot,S. and Lehmann,A.R. (2008) Identification of the proteins, including MAGEG1, that make up the Human SMC5-6 protein complex. *Mol. Cell. Biol.*, **28**, 1197–1206.
24. Räschle,M., Smeenk,G., Hansen,R.K., Temu,T., Oka,Y., Hein,M.Y., Nagaraj,N., Long,D.T., Walter,J.C., Hofmann,K., *et al.* (2015) DNA repair. Proteomics reveals dynamic assembly of repair complexes during bypass of DNA cross-links. *Science*, **348**, 1253671.
25. Davidson,I.F. and Peters,J.-M. (2021) Genome folding through loop extrusion by SMC complexes. *Nat. Rev. Mol. Cell Biol.*, **22**, 445–464.
26. Davidson,I.F., Bauer,B., Goetz,D., Tang,W., Wutz,G. and Peters,J.-M. (2019) DNA loop extrusion by human cohesin. *Science*, **366**, 1338–1345.
27. Moon,K.-W. and Ryu,J.-K. (2023) Current working models of SMC-driven DNA-loop extrusion. *Biochem. Soc. Trans.*, **51**, 1801–1810.
28. Pradhan,B., Kanno,T., Umeda Igarashi,M., Loke,M.S., Baaske,M.D., Wong,J.S.K., Jeppsson,K., Björkegren,C. and Kim,E. (2023) The Smc5/6 complex is a DNA loop-extruding motor. *Nature*, **616**, 843–848.
29. Higashi,T.L. and Uhlmann,F. (2022) SMC complexes: lifting the lid on loop extrusion. *Curr. Opin. Cell Biol.*, **74**, 13–22.
30. Barth,R., Pradhan,B., Kim,E., Davidson,I.F., van der Torre,J., Peters,J.-M. and Dekker,C. (2023) Testing pseudotopological and nontopological models for SMC-driven DNA loop extrusion against roadblock-traversal experiments. *Sci. Rep.*, **13**, 8100.
31. Andrews,E.A., Palecek,J., Sergeant,J., Taylor,E., Lehmann,A.R. and Watts,F.Z. (2005) Nse2, a component of the Smc5-6 complex, is a SUMO ligase required for the response to DNA damage. *Mol. Cell. Biol.*, **25**, 185–196.

32. Doyle,J.M., Gao,J., Wang,J., Yang,M. and Potts,P.R. (2010) MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. *Mol. Cell*, **39**, 963–974.
33. Solé-Soler,R. and Torres-Rosell,J. (2020) Smc5/6, an atypical SMC complex with two RING-type subunits. *Biochem. Soc. Trans.*, **48**, 2159–2171.
34. Pebernard,S., Schaffer,L., Campbell,D., Head,S.R. and Boddy,M.N. (2008) Localization of Smc5/6 to centromeres and telomeres requires heterochromatin and SUMO, respectively. *EMBO J.*, **27**, 3011–3023.
35. Albuquerque,C.P., Wang,G., Lee,N.S., Kolodner,R.D., Putnam,C.D. and Zhou,H. (2013) Distinct SUMO ligases cooperate with Esc2 and Slx5 to suppress duplication-mediated genome rearrangements. *PLoS Genet.*, **9**, e1003670.
36. Bermúdez-López,M., Pociño-Merino,I., Sánchez,H., Bueno,A., Guasch,C., Almedawar,S., Bru-Virgili,S., Garí,E., Wyman,C., Reverter,D., *et al.* (2015) ATPase-dependent control of the Mms21 SUMO ligase during DNA repair. *PLoS Biol.*, **13**, e1002089.
37. Zapatka,M., Pociño-Merino,I., Heluani-Gahete,H., Bermúdez-López,M., Tarrés,M., Ibars,E., Solé-Soler,R., Gutiérrez-Escribano,P., Apostolova,S., Casas,C., *et al.* (2019) Sumoylation of Smc5 promotes error-free bypass at damaged replication forks. *Cell Rep.*, **29**, 3160–3172.
38. Li,S., Mutchler,A., Zhu,X., So,S., Epps,J., Guan,D., Zhao,X. and Xue,X. (2022) Multifaceted regulation of the sumoylation of the Sgs1 DNA helicase. *J. Biol. Chem.*, **298**, 102092.
39. Agashe,S., Joseph,C.R., Reyes,T.A.C., Menolfi,D., Giannattasio,M., Waizenegger,A., Szakal,B. and Branzei,D. (2021) Smc5/6 functions with Sgs1-Top3-Rmi1 to complete chromosome replication at natural pause sites. *Nat. Commun.*, **12**, 2111.
40. Bermúdez-López,M., Villoria,M.T., Esteras,M., Jarmuz,A., Torres-Rosell,J., Clemente-Blanco,A. and Aragon,L. (2016) Sgs1's roles in DNA end resection, HJ dissolution, and crossover suppression require a two-step SUMO regulation dependent on Smc5/6. *Genes Dev.*, **30**, 1339–1356.
41. Bonner,J.N., Choi,K., Xue,X., Torres,N.P., Szakal,B., Wei,L., Wan,B., Arter,M., Matos,J., Sung,P., *et al.* (2016) Smc5/6 Mediated sumoylation of the Sgs1-Top3-Rmi1 complex promotes removal of recombination intermediates. *Cell Rep.*, **16**, 368–378.
42. Whalen,J.M., Dhingra,N., Wei,L., Zhao,X. and Freudenreich,C.H. (2020) Relocation of collapsed forks to the nuclear pore complex depends on sumoylation of DNA repair proteins and permits Rad51 Association. *Cell Rep.*, **31**, 107635.
43. McAleenan,A., Cordon-Preciado,V., Clemente-Blanco,A., Liu,I.-C., Sen,N., Leonard,J., Jarmuz,A. and Aragón,L. (2012) SUMOylation of the α-kleisin subunit of cohesin is required for DNA damage-induced cohesion. *Curr. Biol.*, **22**, 1564–1575.
44. Wu,N., Kong,X., Ji,Z., Zeng,W., Potts,P.R., Yokomori,K. and Yu,H. (2012) Scc1 sumoylation by Mms21 promotes sister chromatid recombination through counteracting Wapl. *Genes Dev.*, **26**, 1473–1485.
45. Palecek,J., Vidot,S., Feng,M., Doherty,A.J. and Lehmann,A.R. (2006) The Smc5-Smc6 DNA repair complex bridging of the Smc5-Smc6 heads by the kleisin, Nse4, and non-kleisin subunits. *J. Biol. Chem.*, **281**, 36952–36959.
46. Duan,X., Yang,Y., Chen,Y.-H., Arenz,J., Rangi,G.K., Zhao,X. and Ye,H. (2009) Architecture of the Smc5/6 complex of saccharomyces cerevisiae reveals a unique interaction between the Nse5-6 subcomplex and the hinge regions of Smc5 and Smc6. *J. Biol. Chem.*, **284**, 8507–8515.
47. Kozakova,L., Vondrova,L., Stejskal,K., Charalabous,P., Kolesar,P., Lehmann,A.R., Uldrijan,S., Sanderson,C.M., Zdrahal,Z. and Palecek,J.J. (2015) The melanoma-associated antigen 1 (MAGEA1) protein stimulates the E3 ubiquitin-ligase activity of TRIM31 within a TRIM31-MAGEA1-NSE4 complex. *Cell Cycle*, **14**, 920–930.
48. Kolesar,P., Stejskal,K., Potesil,D., Murray,J.M. and Palecek,J.J. (2022) Role of Nse1 subunit of SMC5/6 complex as a ubiquitin ligase. *Cells*, **11**, 165.
49. Ibars,E., Codina-Fabra,J., Bellí,G., Casas,C., Tarrés,M., Solé-Soler,R., Lorite,N.P., Ximénez-Embún,P., Muñoz,J., Colomina,N., *et al.* (2023) Ubiquitin proteomics identifies RNA polymerase I as a target of the Smc5/6 complex. *Cell Rep.*, **42**, 112463.
50. Roy,M.-A., Dhanaraman,T. and D'Amours,D. (2015) The Smc5-Smc6 heterodimer associates with DNA through several independent binding domains. *Sci. Rep.*, **5**, 9797.
51. Zabrady,K., Adamus,M., Vondrova,L., Liao,C., Skoupilova,H., Novakova,M., Jurcisinova,L., Alt,A., Oliver,A.W., Lehmann,A.R., *et al.* (2016) Chromatin association of the SMC5/6 complex is dependent on binding of its NSE3 subunit to DNA. *Nucleic Acids Res.*, **44**, 1064–1079.
52. Alt,A., Dang,H.Q., Wells,O.S., Polo,L.M., Smith,M.A., McGregor,G.A., Welte,T., Lehmann,A.R., Pearl,L.H., Murray,J.M., *et al.* (2017) Specialized interfaces of Smc5/6 control hinge stability and DNA association. *Nat. Commun.*, **8**, 14011.
53. Serrano,D., Cordero,G., Kawamura,R., Sverzhinsky,A., Sarker,M., Roy,S., Malo,C., Pascal,J.M., Marko,J.F. and D'Amours,D. (2020) The Smc5/6 core complex is a structure-specific DNA binding and compacting machine. *Mol. Cell*, **80**, 1025–1038.
54. Chang,J.T.-H., Li,S., Beckwitt,E.C., Than,T., Haluska,C., Chandanani,J., O'Donnell,M.E., Zhao,X. and Liu,S. (2022) Smc5/6's multifaceted DNA binding capacities stabilize branched DNA structures. *Nat. Commun.*, **13**, 7179.
55. Tanasie,N.-L., Gutiérrez-Escribano,P., Jaklin,S., Aragon,L. and Stigler,J. (2022) Stabilization of DNA fork junctions by Smc5/6 complexes revealed by single-molecule imaging. *Cell Rep.*, **41**, 111778.
56. Gutierrez-Escribano,P., Hormeño,S., Madariaga-Marcos,J., Solé-Soler,R., O'Reilly,F.J., Morris,K., Aicart-Ramos,C., Aramayo,R., Montoya,A., Kramer,H., *et al.* (2020) Purified Smc5/6 complex exhibits DNA substrate recognition and compaction. *Mol. Cell*, **80**, 1039–1054.
57. Hallett,S.T., Schellenberger,P., Zhou,L., Beuron,F., Morris,E., Murray,J.M. and Oliver,A.W. (2021) Nse5/6 is a negative regulator of the ATPase activity of the Smc5/6 complex. *Nucleic Acids Res.*, **49**, 4534–4549.
58. Hallett,S.T., Campbell Harry,I., Schellenberger,P., Zhou,L., Cronin,N.B., Baxter,J., Etheridge,T.J., Murray,J.M. and Oliver,A.W. (2022) Cryo-EM structure of the Smc5/6 holo-complex. *Nucleic Acids Res.*, **50**, 9505–9520.
59. Jo,A., Li,S., Shin,J.W., Zhao,X. and Cho,Y. (2021) Structure basis for shaping the Nse4 protein by the Nse1 and Nse3 dimer within the Smc5/6 complex. *J. Mol. Biol.*, **433**, 166910.
60. Taschner,M., Basquin,J., Steigenberger,B., Schäfer,I.B., Soh,Y.-M., Basquin,C., Lorentzen,E., Räschle,M., Scheltema,R.A. and Gruber,S. (2021) Nse5/6 inhibits the Smc5/6 ATPase and modulates DNA substrate binding. *EMBO J.*, **40**, e107807.
61. Yu,Y., Li,S., Ser,Z., Sanyal,T., Choi,K., Wan,B., Kuang,H., Sali,A., Kentsis,A., Patel,D.J., *et al.* (2021) Integrative analysis reveals unique structural and functional features of the Smc5/6 complex. *Proc. Natl. Acad. Sci. U.S.A.*, **118**, e2026844118.
62. Yu,Y., Li,S., Ser,Z., Kuang,H., Than,T., Guan,D., Zhao,X. and Patel,D.J. (2022) Cryo-EM structure of DNA-bound Smc5/6 reveals DNA clamping enabled by multi-subunit conformational changes. *Proc. Natl. Acad. Sci. U.S.A.*, **119**, e2202799119.
63. Anderson,D.E., Losada,A., Erickson,H.P. and Hirano,T. (2002) Condensin and cohesin display different arm conformations with characteristic hinge angles. *J. Cell Biol.*, **156**, 419–424.
64. Haering,C.H., Löwe,J., Hochwagen,A. and Nasmyth,K. (2002) Molecular architecture of SMC proteins and the yeast cohesin complex. *Mol. Cell*, **9**, 773–788.
65. Soh,Y.-M., Bürmann,F., Shin,H.-C., Oda,T., Jin,K.S., Toseland,C.P., Kim,C., Lee,H., Kim,S.J., Kong,M.-S., *et al.* (2015)

Molecular basis for SMC rod formation and its dissolution upon DNA binding. *Mol. Cell*, **57**, 290–303.
66. Bürmann,F., Lee,B.-G., Than,T., Sinn,L., O'Reilly,F.J., Yatskevich,S., Rappsilber,J., Hu,B., Nasmyth,K. and Löwe,J. (2019) A folded conformation of MukBEF and cohesin. *Nat. Struct. Mol. Biol.*, **26**, 227–236.
67. Lee,B.-G., Merkel,F., Allegretti,M., Hassler,M., Cawood,C., Lecomte,L., O'Reilly,F.J., Sinn,L.R., Gutierrez-Escribano,P., Kschonsak,M., *et al.* (2020) Cryo-EM structures of holo condensin reveal a subunit flip-flop mechanism. *Nat. Struct. Mol. Biol.*, **27**, 743–751.
68. Kong,M., Cutts,E.E., Pan,D., Beuron,F., Kaliyappan,T., Xue,C., Morris,E.P., Musacchio,A., Vannini,A. and Greene,E.C. (2020) Human condensin I and II drive extensive ATP-dependent compaction of nucleosome-bound DNA. *Mol. Cell*, **79**, 99–114.
69. Ganji,M., Shaltiel,I.A., Bisht,S., Kim,E., Kalichava,A., Haering,C.H. and Dekker,C. (2018) Real-time imaging of DNA loop extrusion by condensin. *Science*, **360**, 102–105.
70. Shaltiel,I.A., Datta,S., Lecomte,L., Hassler,M., Kschonsak,M., Bravo,S., Stober,C., Ormanns,J., Eustermann,S. and Haering,C.H. (2022) A hold-and-feed mechanism drives directional DNA loop extrusion by condensin. *Science*, **376**, 1087–1094.
71. Adamus,M., Lelkes,E., Potesil,D., Ganji,S.R., Kolesar,P., Zabrady,K., Zdrahal,Z. and Palecek,J.J. (2020) Molecular insights into the architecture of the Human SMC5/6 complex. *J. Mol. Biol.*, **432**, 3820–3837.
72. Li,S., Yu,Y., Zheng,J., Miller-Browne,V., Ser,Z., Kuang,H., Patel,D.J. and Zhao,X. (2023) Molecular basis for Nse5-6 mediated regulation of Smc5/6 functions. *Proc. Natl. Acad. Sci. U.S.A.*, **120**, e2310924120.
73. Nasim,A. and Smith,B.P. (1975) Genetic control of radiation sensitivity in *Schizosaccharomyces pombe*. *Genetics*, **79**, 573–582.
74. Prakash,L. and Prakash,S. (1977) Isolation and characterization of MMS-sensitive mutants of *Saccharomyces cerevisiae*. *Genetics*, **86**, 33–55.
75. Lehmann,A.R., Walicka,M., Griffiths,D.J., Murray,J.M., Watts,F.Z., McCready,S. and Carr,A.M. (1995) The rad18 gene of Schizosaccharomyces pombe defines a new subgroup of the SMC superfamily involved in DNA repair. *Mol. Cell. Biol.*, **15**, 7067–7080.
76. Kegel,A. and Sjögren,C. (2010) The Smc5/6 complex: more than repair?*Cold Spring Harb Symp Quant Biol*, **75**, 179–187.
77. Palecek,J.J. (2018) SMC5/6: multifunctional player in replication. *Genes (Basel)*, **10**, 7.
78. Ampatzidou,E., Irmisch,A., O'Connell,M.J. and Murray,J.M. (2006) Smc5/6 Is required for repair at collapsed replication forks. *Mol. Cell. Biol.*, **26**, 9387–9401.
79. Cost,G.J. and Cozzarelli,N.R. (2006) Smc5p Promotes faithful chromosome transmission and DNA repair in *saccharomyces cerevisiae*. *Genetics*, **172**, 2185–2200.
80. Potts,P.R., Porteus,M.H. and Yu,H. (2006) Human SMC5/6 complex promotes sister chromatid homologous recombination by recruiting the SMC1/3 cohesin complex to double-strand breaks. *EMBO J.*, **25**, 3377–3388.
81. Ström,L. and Sjögren,C. (2007) Chromosome segregation and double-strand break repair — a complex connection. *Curr. Opin. Cell Biol.*, **19**, 344–349.
82. Jeppsson,K., Carlborg,K.K., Nakato,R., Berta,D.G., Lilienthal,I., Kanno,T., Lindqvist,A., Brink,M.C., Dantuma,N.P., Katou,Y., *et al.* (2014) The chromosomal association of the Smc5/6 complex depends on cohesion and predicts the level of sister chromatid entanglement. *PLoS Genet.*, **10**, e1004680.
83. Bermudez-Lopez,M., Ceschia,A., de Piccoli,G., Colomina,N., Pasero,P., Aragon,L. and Torres-Rosell,J. (2010) The Smc5/6 complex is required for dissolution of DNA-mediated sister chromatid linkages. *Nucleic Acids Res.*, **38**, 6502–6512.
84. Betts Lindroos,H., Ström,L., Itoh,T., Katou,Y., Shirahige,K. and Sjögren,C. (2006) Chromosomal association of the Smc5/6 complex reveals that it functions in differently regulated pathways. *Mol. Cell*, **22**, 755–767.
85. Kegel,A., Betts-Lindroos,H., Kanno,T., Jeppsson,K., Ström,L., Katou,Y., Itoh,T., Shirahige,K. and Sjögren,C. (2011) Chromosome length influences replication-induced topological stress. *Nature*, **471**, 392–396.
86. Meng,X., Wei,L., Peng,X.P. and Zhao,X. (2019) Sumoylation of the DNA polymerase ε by the Smc5/6 complex contributes to DNA replication. *PLoS Genet.*, **15**, e1008426.
87. Winczura,A., Appanah,R., Tatham,M.H., Hay,R.T. and Piccoli,G.D. (2019) The S phase checkpoint promotes the Smc5/6 complex dependent SUMOylation of Pol2, the catalytic subunit of DNA polymerase ε. *PLoS Genet.*, **15**, e1008427.
88. Bermejo,R., Doksani,Y., Capra,T., Katou,Y.-M., Tanaka,H., Shirahige,K. and Foiani,M. (2007) Top1- and Top2-mediated topological transitions at replication forks ensure fork progression and stability and prevent DNA damage checkpoint activation. *Genes Dev.*, **21**, 1921–1936.
89. Postow,L., Crisona,N.J., Peter,B.J., Hardy,C.D. and Cozzarelli,N.R. (2001) Topological challenges to DNA replication: conformations at the fork. *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 8219–8226.
90. Wang,J.C. (2002) Cellular roles of DNA topoisomerases: a molecular perspective. *Nat. Rev. Mol. Cell Biol.*, **3**, 430.
91. Irmisch,A., Ampatzidou,E., Mizuno,K., O'Connell,M.J. and Murray,J.M. (2009) Smc5/6 maintains stalled replication forks in a recombination-competent conformation. *EMBO J.*, **28**, 144–155.
92. Branzei,D., Vanoli,F. and Foiani,M. (2008) SUMOylation regulates Rad18-mediated template switch. *Nature*, **456**, 915–920.
93. Sollier,J., Driscoll,R., Castellucci,F., Foiani,M., Jackson,S.P. and Branzei,D. (2009) The saccharomyces cerevisiae Esc2 and Smc5-6 proteins promote sister chromatid junction-mediated intra-S repair. *MBoC*, **20**, 1671–1682.
94. Choi,K., Szakal,B., Chen,Y.-H., Branzei,D. and Zhao,X. (2010) The Smc5/6 complex and Esc2 influence multiple replication-associated recombination processes in *saccharomyces cerevisiae*. *MBoC*, **21**, 2306–2314.
95. Menolfi,D., Delamarre,A., Lengronne,A., Pasero,P. and Branzei,D. (2015) Essential roles of the Smc5/6 complex in replication through natural pausing sites and endogenous DNA damage tolerance. *Mol. Cell*, **60**, 835–846.
96. Chen,Y.-H., Choi,K., Szakal,B., Arenz,J., Duan,X., Ye,H., Branzei,D. and Zhao,X. (2009) Interplay between the Smc5/6 complex and the Mph1 helicase in recombinational repair. *Proc. Natl Acad. Sci. U.S.A.*, **106**, 21252–21257.
97. Lafuente-Barquero,J., Luke-Glaser,S., Graf,M., Silva,S., Gómez-González,B., Lockhart,A., Lisby,M., Aguilera,A. and Luke,B. (2017) The Smc5/6 complex regulates the yeast Mph1 helicase at RNA-DNA hybrid-mediated DNA damage. *PLoS Genet.*, **13**, e1007136.
98. Rossi,F., Helbling-Leclerc,A., Kawasumi,R., Jegadesan,N.K., Xu,X., Devulder,P., Abe,T., Takata,M., Xu,D., Rosselli,F., *et al.* (2020) SMC5/6 acts jointly with Fanconi anemia factors to support DNA repair and genome stability. *EMBO Rep.*, **21**, e48222.
99. Atkins,A., Xu,M.J., Li,M., Rogers,N.P., Pryzhkova,M.V. and Jordan,P.W. (2020) SMC5/6 is required for replication fork stability and faithful chromosome segregation during neurogenesis. *eLife*, **9**, e61171.
100. Peng,X.P., Lim,S., Li,S., Marjavaara,L., Chabes,A. and Zhao,X. (2018) Acute Smc5/6 depletion reveals its primary role in rDNA replication by restraining recombination at fork pausing sites. *PLoS Genet.*, **14**, e1007129.
101. Moradi-Fard,S., Mojumdar,A., Chan,M., Harkness,T.A.A. and Cobb,J.A. (2021) Smc5/6 in the rDNA modulates lifespan independently of Fob1. *Aging Cell*, **20**, e13373.

102. Torres-Rosell,J., Machín,F., Farmer,S., Jarmuz,A., Eydmann,T., Dalgaard,J.Z. and Aragón,L. (2005) SMC5 and SMC6 genes are required for the segregation of repetitive chromosome regions. *Nat. Cell Biol.*, **7**, 412–419.
103. Torres-Rosell,J., Sunjevaric,I., De Piccoli,G., Sacher,M., Eckert-Boulet,N., Reid,R., Jentsch,S., Rothstein,R., Aragón,L. and Lisby,M. (2007) The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. *Nat. Cell Biol.*, **9**, 923–931.
104. Murray,J.M. and Carr,A.M. (2008) Smc5/6: a link between DNA repair and unidirectional replication?*Nat. Rev. Mol. Cell Biol.*, **9**, 177–182.
105. Moradi-Fard,S., Sarthi,J., Tittel-Elmer,M., Lalonde,M., Cusanelli,E., Chartrand,P. and Cobb,J.A. (2016) Smc5/6 Is a telomere-associated complex that regulates Sir4 binding and TPE. *PLoS Genet.*, **12**, e1006268.
106. Potts,P.R. and Yu,H. (2007) The SMC5/6 complex maintains telomere length in ALT cancer cells through SUMOylation of telomere-binding proteins. *Nat. Struct. Mol. Biol.*, **14**, 581.
107. Noël,J.-F. and Wellinger,R.J. (2011) Abrupt telomere losses and reduced end-resection can explain accelerated senescence of Smc5/6 mutants lacking telomerase. *DNA Repair (Amst.)*, **10**, 271–282.
108. Chiolo,I., Minoda,A., Colmenares,S.U., Polyzos,A., Costes,S.V. and Karpen,G.H. (2011) Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. *Cell*, **144**, 732–744.
109. Horigome,C., Bustard,D.E., Marcomini,I., Delgoshaie,N., Tsai-Pflugfelder,M., Cobb,J.A. and Gasser,S.M. (2016) PolySUMOylation by Siz2 and Mms21 triggers relocation of DNA breaks to nuclear pores through the Slx5/Slx8 STUbL. *Genes Dev.*, **30**, 931–945.
110. Ryu,T., Spatola,B., Delabaere,L., Bowlin,K., Hopp,H., Kunitake,R., Karpen,G.H. and Chiolo,I. (2015) Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. *Nat. Cell Biol.*, **17**, 1401–1411.
111. Caridi,C.P., D'Agostino,C., Ryu,T., Zapotoczny,G., Delabaere,L., Li,X., Khodaverdian,V.Y., Amaral,N., Lin,E., Rau,A.R., *et al.* (2018) Nuclear F-actin and myosins drive relocalization of heterochromatic breaks. *Nature*, **559**, 54–60.
112. Laflamme,G., Tremblay-Boudreault,T., Roy,M.-A., Andersen,P., Bonneil,É., Atchia,K., Thibault,P., D'Amours,D. and Kwok,B.H. (2014) Structural maintenance of chromosome (SMC) proteins link microtubule stability to genome integrity. *J. Biol. Chem.*, **289**, 27418–27431.
113. Oshidari,R., Strecker,J., Chung,D.K.C., Abraham,K.J., Chan,J.N.Y., Damaren,C.J. and Mekhail,K. (2018) Nuclear microtubule filaments mediate non-linear directional motion of chromatin and promote DNA repair. *Nat. Commun.*, **9**, 2567.
114. Deep,A., Gu,Y., Gao,Y.-Q., Ego,K.M., Herzik,M.A., Zhou,H. and Corbett,K.D. (2022) The SMC-family Wadjet complex protects bacteria from plasmid transformation by recognition and cleavage of closed-circular DNA. *Mol. Cell*, **82**, 4145–4159.
115. Decorsière,A., Mueller,H., van Breugel,P.C., Abdul,F., Gerossier,L., Beran,R.K., Livingston,C.M., Niu,C., Fletcher,S.P., Hantz,O., *et al.* (2016) Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. *Nature*, **531**, 386–389.
116. Bentley,P., Tan,M.J.A., McBride,A.A., White,E.A. and Howley,P.M. (2018) The SMC5/6 complex interacts with the Papillomavirus E2 protein and influences maintenance of viral episomal DNA. *J. Virol.*, **92**, e00356-18.
117. Gibson,R.T. and Androphy,E.J. (2020) The SMC5/6 complex represses the replicative program of high-risk Human papillomavirus type 31. *Pathogens*, **9**, 786.
118. Han,C., Zhang,D., Gui,C., Huang,L., Chang,S., Dong,L., Bai,L., Wu,S. and Lan,K. (2022) KSHV RTA antagonizes SMC5/6 complex-induced viral chromatin compaction by hijacking the ubiquitin-proteasome system. *PLoS Pathog.*, **18**, e1010744.
119. Irwan,I.D., Bogerd,H.P. and Cullen,B.R. (2022) Epigenetic silencing by the SMC5/6 complex mediates HIV-1 latency. *Nat. Microbiol.*, **7**, 2101–2113.
120. Oravcová,M., Nie,M., Zilio,N., Maeda,S., Jami-Alahmadi,Y., Lazzerini-Denchi,E., Wohlschlegel,J.A., Ulrich,H.D., Otomo,T. and Boddy,M.N. (2022) The Nse5/6-like SIMC1-SLF2 complex localizes SMC5/6 to viral replication centers. *eLife*, **11**, e79676.
121. Abdul,F., Diman,A., Baechler,B., Ramakrishnan,D., Kornyeyev,D., Beran,R.K., Fletcher,S.P. and Strubin,M. (2022) Smc5/6 silences episomal transcription by a three-step function. *Nat. Struct. Mol. Biol.*, **29**, 922–931.
122. Dupont,L., Bloor,S., Williamson,J.C., Cuesta,S.M., Shah,R., Teixeira-Silva,A., Naamati,A., Greenwood,E.J.D., Sarafianos,S.G., Matheson,N.J., *et al.* (2021) The SMC5/6 complex compacts and silences unintegrated HIV-1 DNA and is antagonized by Vpr. *Cell Host Microbe*, **29**, 792–805.
123. Yiu,S.P.T., Guo,R., Zerbe,C., Weekes,M.P. and Gewurz,B.E. (2022) Epstein-Barr virus BNRF1 destabilizes SMC5/6 cohesin complexes to evade its restriction of replication compartments. *Cell Rep.*, **38**, 110411.
124. Xu,W., Ma,C., Zhang,Q., Zhao,R., Hu,D., Zhang,X., Chen,J., Liu,F., Wu,K., Liu,Y., *et al.* (2018) PJA1 Coordinates with the SMC5/6 complex to restrict DNA viruses and episomal genes in an interferon-independent manner. *J. Virol.*, **92**, e00825–e18.
125. Hays,M., Young,J.M., Levan,P.F. and Malik,H.S. (2020) A natural variant of the essential host gene MMS21 restricts the parasitic 2-micron plasmid in Saccharomyces cerevisiae. *eLife*, **9**, e62337.
126. Kanno,T., Berta,D.G. and Sjögren,C. (2015) The Smc5/6 complex is an ATP-dependent intermolecular DNA linker. *Cell Rep.*, **12**, 1471–1482.
127. Taschner,M. and Gruber,S. (2023) DNA segment capture by Smc5/6 holocomplexes. *Nat. Struct. Mol. Biol.*, **30**, 619–628.
128. Roy,M.-A. and D'Amours,D. (2011) DNA-binding properties of Smc6, a core component of the Smc5-6 DNA repair complex. *Biochem. Biophys. Res. Commun.*, **416**, 80–85.
129. Roy,M.-A., Siddiqui,N. and D'Amours,D. (2011) Dynamic and selective DNA-binding activity of Smc5, a core component of the Smc5-Smc6 complex. *Cell Cycle*, **10**, 690–700.
130. Etheridge,T.J., Villahermosa,D., Campillo-Funollet,E., Herbert,A.D., Irmisch,A., Watson,A.T., Dang,H.Q., Osborne,M.A., Oliver,A.W., Carr,A.M., *et al.* (2021) Live-cell single-molecule tracking highlights requirements for stable Smc5/6 chromatin association in vivo. *eLife*, **10**, e68579.
131. Vondrova,L., Kolesar,P., Adamus,M., Nociar,M., Oliver,A.W. and Palecek,J.J. (2020) A role of the Nse4 kleisin and Nse1/Nse3 KITE subunits in the ATPase cycle of SMC5/6. *Sci. Rep.*, **10**, 9694.
132. Leung,G.P., Lee,L., Schmidt,T.I., Shirahige,K. and Kobor,M.S. (2011) Rtt107 is required for recruitment of the SMC5/6 complex to DNA double strand breaks. *J. Biol. Chem.*, **286**, 26250–26257.
133. Oravcová,M., Gadaleta,M.C., Nie,M., Reubens,M.C., Limbo,O., Russell,P. and Boddy,M.N. (2019) Brc1 Promotes the focal accumulation and SUMO ligase activity of Smc5-Smc6 during replication stress. *Mol. Cell. Biol.*, **39**, e00271-18.
134. Oravcová,M. and Boddy,M.N. (2019) Recruitment, loading, and activation of the Smc5-Smc6 SUMO ligase. *Curr. Genet.*, **65**, 669–676.
135. Bustard,D.E., Menolfi,D., Jeppsson,K., Ball,L.G., Dewey,S.C., Shirahige,K., Sjögren,C., Branzei,D. and Cobb,J.A. (2012) During replication stress, non-smc element 5 (Nse5) is required for Smc5/6 protein complex functionality at stalled forks. *J. Biol. Chem.*, **287**, 11374–11383.
136. Nakamura,K., Saredi,G., Becker,J.R., Foster,B.M., Nguyen,N.V., Beyer,T.E., Cesa,L.C., Faull,P.A., Lukauskas,S., Frimurer,T., *et al.* (2019) H4K20me0 recognition by BRCA1–BARD1 directs homologous recombination to sister chromatids. *Nat. Cell Biol.*, **21**, 311–318.

137. Varejão,N., Ibars,E., Lascorz,J., Colomina,N., Torres-Rosell,J. and Reverter,D. (2018) DNA activates the Nse2/Mms21 SUMO E3 ligase in the Smc5/6 complex. *EMBO J.*, **37**, e98306.
138. Jeppsson,K., Pradhan,B., Sutani,T., Sakata,T., Igarashi,M.U., Berta,D.G., Kanno,T., Nakato,R., Shirahige,K., Kim,E., *et al.* (2024) Loop-extruding Smc5/6 organizes transcription-induced positive DNA supercoils. *Mol. Cell* , https://doi.org/10.1016/j.molcel.2024.01.005.
139. Diman,A., Panis,G., Castrogiovanni,C., Prados,J., Baechler,B. and Strubin,M. (2023) Human Smc5/6 recognises transcription-generated positive DNA supercoils. bioRxiv doi: https://doi.org/10.1101/2023.05.04.539344, 04 May 2023, preprint: not peer reviewed.
140. Smith,M.J., Bryant,E.E. and Rothstein,R. (2018) Increased chromosomal mobility after DNA damage is controlled by interactions between the recombination machinery and the checkpoint. *Genes Dev.*, **32**, 1242–1251.
141. Smith,M.J., Bryant,E.E., Joseph,F.J. and Rothstein,R. (2019) DNA damage triggers increased mobility of chromosomes in G1-phase cells. *Mol. Biol. Cell*, **30**, 2620–2625.
142. Hauer,M.H. and Gasser,S.M. (2017) Chromatin and nucleosome dynamics in DNA damage and repair. *Genes Dev.*, **31**, 2204–2221.
143. García Fernández,F. and Fabre,E. (2022) The dynamic behavior of chromatin in response to DNA double-strand breaks. *Genes (Basel)*, **13**, 215.
144. Arnould,C., Rocher,V., Finoux,A.-L., Clouaire,T., Li,K., Zhou,F., Caron,P., Mangeot,P..E., Ricci,E.P., Mourad,R., *et al.* (2021) Loop extrusion as a mechanism for DNA damage repair foci formation. *Nature*, **590**, 660–665.
145. Thattikota,Y., Tollis,S., Palou,R., Vinet,J., Tyers,M. and D'Amours,D. (2018) Cdc48/VCP promotes chromosome morphogenesis by releasing condensin from self-entrapment in chromatin. *Mol. Cell*, **69**, 664–676.
146. Chen,D., Dundr,M., Wang,C., Leung,A., Lamond,A., Misteli,T. and Huang,S. (2004) Condensed mitotic chromatin is accessible to transcription factors and chromatin structural proteins. *J. Cell Biol.*, **168**, 41–54.
147. Cheutin,T., McNairn,A.J., Jenuwein,T., Gilbert,D.M., Singh,P.B. and Misteli,T. (2003) Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science*, **299**, 721–725.
148. Martin,R.M. and Cardoso,M.C. (2010) Chromatin condensation modulates access and binding of nuclear proteins. *FASEB J.*, **24**, 1066–1072.
149. Gallego-Paez,L.M., Tanaka,H., Bando,M., Takahashi,M., Nozaki,N., Nakato,R., Shirahige,K. and Hirota,T. (2014) Smc5/6-mediated regulation of replication progression contributes to chromosome assembly during mitosis in human cells. *Mol. Biol. Cell*, **25**, 302–317.
150. Gomez,R., Jordan,P.W., Viera,A., Alsheimer,M., Fukuda,T., Jessberger,R., Llano,E., Pendas,A.M., Handel,M.A. and Suja,J.A. (2013) Dynamic localization of SMC5/6 complex proteins during mammalian meiosis and mitosis suggests functions in distinct chromosome processes. *J. Cell Sci.*, **126**, 4239–4252.
151. Behlke-Steinert,S., Touat-Todeschini,L., Skoufias,D.A. and Margolis,R.L. (2009) SMC5 and MMS21 are required for chromosome cohesion and mitotic progression. *Cell Cycle*, **8**, 2211–2218.
152. Murayama,Y. and Uhlmann,F. (2015) DNA entry into and exit out of the cohesin ring by an interlocking gate mechanism. *Cell*, **163**, 1628–1640.
153. van der Crabben,S.N., Hennus,M.P., McGregor,G.A., Ritter,D.I., Nagamani,S.C.S., Wells,O.S., Harakalova,M., Chinn,I.K., Alt,A., Vondrova,L., *et al.* (2016) Destabilized SMC5/6 complex leads to chromosome breakage syndrome with severe lung disease. *J. Clin. Invest.*, **126**, 2881–2892.
154. Payne,F., Colnaghi,R., Rocha,N., Seth,A., Harris,J., Carpenter,G., Bottomley,W.E., Wheeler,E., Wong,S., Saudek,V., *et al.* (2014) Hypomorphism in human NSMCE2 linked to primordial dwarfism and insulin resistance. *J. Clin. Invest.*, **124**, 4028–4038.
155. Grange,L.J., Reynolds,J.J., Ullah,F., Isidor,B., Shearer,R.F., Latypova,X., Baxley,R.M., Oliver,A.W., Ganesh,A., Cooke,S.L., *et al.* (2022) Pathogenic variants in SLF2 and SMC5 cause segmented chromosomes and mosaic variegated hyperploidy. *Nat. Commun.*, **13**, 6664.
156. Jeggo,P.A., Pearl,L.H. and Carr,A.M. (2016) DNA repair, genome stability and cancer: a historical perspective. *Nat. Rev. Cancer*, **16**, 35–42.
157. Perera,D., Poulos,R.C., Shah,A., Beck,D., Pimanda,J.E. and Wong,J.W.H. (2016) Differential DNA repair underlies mutation hotspots at active promoters in cancer genomes. *Nature*, **532**, 259–263.
158. Helleday,T., Eshtad,S. and Nik-Zainal,S. (2014) Mechanisms underlying mutational signatures in human cancers. *Nat. Rev. Genet.*, **15**, 585–598.
159. Romero-Laorden,N. and Castro,E. (2017) Inherited mutations in DNA repair genes and cancer risk. *Curr. Probl. Cancer.*, **41**, 251–264.
160. Nie,H., Wang,Y., Yang,X., Liao,Z., He,X., Zhou,J. and Ou,C. (2021) Clinical significance and integrative analysis of the SMC Family in hepatocellular carcinoma. *Front. Med. (Lausanne)*, **8**, 727965.
161. Yang,H., Gao,S., Chen,J. and Lou,W. (2020) UBE2I promotes metastasis and correlates with poor prognosis in hepatocellular carcinoma. *Cancer Cell Int.*, **20**, 234.
162. Zhou,J., Wu,G., Tong,Z., Sun,J., Su,J., Cao,Z., Luo,Y. and Wang,W. (2020) Prognostic relevance of SMC family gene expression in human sarcoma. *Aging (Albany NY)*, **13**, 1473–1487.
163. Di Benedetto,C., Oh,J., Choudhery,Z., Shi,W., Valdes,G. and Betancur,P. (2022) NSMCE2, a novel super-enhancer-regulated gene, is linked to poor prognosis and therapy resistance in breast cancer. *BMC Cancer*, **22**, 1056.
164. Venegas,A.B., Natsume,T., Kanemaki,M. and Hickson,I.D. (2020) Inducible degradation of the Human SMC5/6 complex reveals an essential role only during interphase. *Cell Rep.*, **31**, 107533.
165. Saunus,J.M., Quinn,M.C.J., Patch,A.-M., Pearson,J.V., Bailey,P.J., Nones,K., McCart Reed,A.E., Miller,D., Wilson,P.J., Al-Ejeh,F., *et al.* (2015) Integrated genomic and transcriptomic analysis of human brain metastases identifies alterations of potential clinical significance. *J. Pathol.*, **237**, 363–378.
166. Jacome,A., Gutierrez-Martinez,P., Schiavoni,F., Tenaglia,E., Martinez,P., Rodríguez-Acebes,S., Lecona,E., Murga,M., Méndez,J., Blasco,M.A., *et al.* (2015) NSMCE2 suppresses cancer and aging in mice independently of its SUMO ligase activity. *EMBO J.*, **34**, 2604–2619.
167. Roy,S., Zaker,A., Mer,A. and D'Amours,D. (2023) Large-scale phenogenomic analysis of human cancers uncovers frequent alterations affecting SMC5/6 complex components in breast cancer. *NAR Cancer*, **5**, zcad047.
168. Funato,K., Otsuka,M., Sekiba,K., Miyakawa,Y., Seimiya,T., Shibata,C., Kishikawa,T. and Fujishiro,M. (2022) Hepatitis B virus-associated hepatocellular carcinoma with Smc5/6 complex deficiency is susceptible to PARP inhibitors. *Biochem. Biophys. Res. Commun.*, **607**, 89–95.
169. Sekiba,K., Otsuka,M., Funato,K., Miyakawa,Y., Tanaka,E., Seimiya,T., Yamagami,M., Tsutsumi,T., Okushin,K., Miyakawa,K., *et al.* (2022) HBx-induced degradation of Smc5/6 complex impairs homologous recombination-mediated repair of damaged DNA. *J. Hepatol.*, **76**, 53–62.
170. Pettersen,E.F., Goddard,T.D., Huang,C.C., Meng,E.C., Couch,G.S., Croll,T.I., Morris,J.H. and Ferrin,T.E. (2021) UCSF ChimeraX: structure visualization for researchers, educators, and developers. *Protein Sci.*, **30**, 70–82.
171. Hang,L.E., Peng,J., Tan,W., Szakal,B., Menolfi,D., Sheng,Z., Lobachev,K., Branzei,D., Feng,W. and Zhao,X. (2015) Rtt107 Is a

multi-functional scaffold supporting replication progression with partner SUMO and Ubiquitin ligases. *Mol. Cell*, **60**, 268–279.
172. Yuen,K.C. and Gerton,J.L. (2018) Taking cohesin and condensin in context. *PLoS Genet*., **14**, e1007118.
173. Heideker,J., Prudden,J., Perry,J.J.P., Tainer,J.A. and Boddy,M.N. (2011) SUMO-targeted ubiquitin ligase, Rad60, and Nse2 SUMO ligase suppress spontaneous Top1–Mediated DNA damage and genome instability. *PLoS Genet*., **7**, e1001320.
174. Yong-Gonzales,V., Hang,L.E., Castellucci,F., Branzei,D. and Zhao,X. (2012) The Smc5-Smc6 complex regulates recombination at centromeric regions and affects kinetochore protein sumoylation during normal growth. *PLoS One*, **7**, e51540.
175. Dvořák Tomaštíková,E., Prochazkova,K., Yang,F., Jemelkova,J., Finke,A., Dorn,A., Said,M., Puchta,H. and Pecinka,A. (2023) SMC5/6 complex-mediated SUMOylation stimulates DNA-protein cross-link repair in Arabidopsis. *Plant Cell*, **35**, 1532–1547.
176. Weon,J.L., Yang,S.W. and Potts,P.R. (2018) Cytosolic iron-sulfur assembly is evolutionarily tuned by a cancer-amplified ubiquitin ligase. *Mol. Cell*, **69**, 113–125.
177. Verver,D.E., Zheng,Y., Speijer,D., Hoebe,R., Dekker,H.L., Repping,S., Stap,J. and Hamer,G. (2016) Non-SMC element 2 (NSMCE2) of the SMC5/6 complex helps to resolve topological stress. *Int. J. Mol. Sci*., **17**, 1782.
178. Horváth,A., Rona,G., Pagano,M. and Jordan,P.W. (2020) Interaction between NSMCE4A and GPS1 links the SMC5/6 complex to the COP9 signalosome. *BMC Mol. Cell Biol*., **21**, 36.

Received: November 13, 2023. **Revised:** January 28, 2024. **Editorial Decision:** January 30, 2024. **Accepted:** February 1, 2024