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The multi-functional Smc5/6 complex in genome protection and disease

Xiao P. Peng[1], **Xiaolan Zhao**[2,✉]

[1]Department of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

[2]Molecular Biology Program, Sloan Kettering Cancer Institute, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

Abstract

Structural maintenance of chromosomes (SMC) complexes are ubiquitous genome regulators with a wide range of functions. Among the three types of SMC complexes in eukaryotes, cohesin and condensin fold the genome into different domains and structures, while Smc5/6 plays direct roles in promoting chromosomal replication and repair and in restraining pathogenic viral extra-chromosomal DNA. The importance of Smc5/6 for growth, genotoxin resistance and host defense across species is highlighted by its involvement in disease prevention in plants and animals. Accelerated progress in recent years, including structural and single-molecule studies, has begun to provide greater insights into the mechanisms underlying Smc5/6 functions. Here we integrate a broad range of recent studies on Smc5/6 to identify emerging features of this unique SMC complex and to explain its diverse cellular functions and roles in disease pathogenesis. We also highlight many key areas requiring further investigation for achieving coherent views of Smc5/6-driven mechanisms.

Structural maintenance of chromosomes (SMC) complexes are primordial DNA modulators that are essential for genome maintenance in both prokaryotic and eukaryotic cells. Prokaryotic SMCs include members such as Smc-ScpAB and MukBEF, whereas the eukaryotic SMC complexes include cohesin, condensin and the Smc5/6 complex (Smc5/6). Each SMC complex is composed of a pair of SMC subunits and a set of non-SMC subunits. The coordination of DNA-binding and ATPase activities among these subunits underlies the SMC-based modulation of DNA, such as DNA loop extrusion[1]. As one of the eukaryotic SMCs, Smc5/6 directly regulates chromosomal replication and repair to prevent genome

✉**Correspondence and requests for materials** should be addressed to Xiaolan Zhao. zhaox1@mskcc.org.

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instability while limiting the functions of viral DNA[2]. Recent studies have shed light on the attributes that Smc5/6 shares with other SMCs and those it holds uniquely. In addition, new research in cell-based systems has contributed insight into the various roles played by Smc5/6. We aim to summarize these recent advances in our understanding of this versatile 'guardian of the genome', connecting new information with earlier observations. We refer readers to previous reviews for thorough surveys of earlier research that established Smc5/6 as a multi-functional genome maintenance factor.

Smc5/6 structure and biochemical activities

SMC proteins found in eukaryotic SMC complexes include Smc1 and 3 in cohesin, Smc2 and 4 in condensin, and Smc5 and 6 in Smc5/6. All six proteins adopt tripartite filamentous structures. Each of the SMC proteins folds back on itself at the middle 'hinge' region, enabling its N- and C-terminal ATPase domains to associate into a 'head' region and its two long coiled-coil domains to pair in an antiparallel fashion to form the 'arm' region that connects the hinge and head regions[1] (Fig. 1a). The two SMC subunits then associate with each other, forming the backbone of the SMC complex to which non-SMC subunits attach. A non-SMC subunit found in all SMC complexes is kleisin, which can bridge the head region of one SMC subunit with the head-proximal arm region (neck) of the other SMC subunit. Cohesin and condensin further contain large HEAT-repeat subunits, whereas Smc5/6 contains smaller non-SMC element (NSE) subunits (Fig. 1b). The complete set of yeast Smc5/6 subunits includes Smc5, Smc6 and Nse1–6, with Nse4 as the kleisin. Their homologs constitute the plant and animal Smc5/6 complexes.

Smc5/6 complex architecture

Recent studies examining the Smc5/6 architecture revealed that its arm configuration diverges from those of cohesin and condensin[3–7]. Cohesin and condensin arm regions bend sharply at the so-called elbow regions (arm-folding), bringing the hinge regions in contact with the head-proximal arm regions and head-bound non-SMC proteins[1] (Fig. 1a). In contrast, the arm regions of Smc5/6 show mild bending without folding back[3–6] (Fig. 1c). Recent studies suggest that arm-folding facilitates dynamic behaviors of cohesin on DNA, such as driving DNA loop extrusion[8–10], so the absence of this feature in Smc5/6 suggests possibly other means of enabling this reaction[11].

Lack of folding in the Smc5/6 arm regions correlates with their association to non-SMC subunits. The arm regions in cohesin and condensin are mostly free of non-SMC subunits and serve as flexible head-hinge linkers capable of folding back on themselves[1]. However, two thirds of the arm regions of Smc5/6 are occupied by NSEs[3–7], which in principle can reduce arm flexibility and/or sterically hinder arm-folding. Thus, the arm regions of Smc5/6 serve as protein–protein interaction platforms in addition to being head-hinge linkers. The NSE subunits tethered to Smc5/6's mid-arm regions were determined to be Nse2 and the Nse5–Nse6 subcomplex, while the remaining three NSE subunits (Nse1, Nse3 and Nse4) form a subcomplex that attaches to the Smc5 and Smc6 (Smc5–6) head and neck regions in both the budding yeast and human complexes[3,4,6,7,12,13] (Fig. 1b,c).

Conformational changes are common for SMCs during their ATP and DNA-binding cycles[1]. Indeed, changes in Smc5–6 interactions with NSE subunits upon DNA-binding, as well as rod- and ring-shaped configurations of Smc5/6, have been reported[4,12]. These data suggest a great need for structural examination to reveal the full dynamic range of Smc5/6 configurations under various physiologically relevant conditions.

Nse2 subunit and Nse5–6 subcomplex

Three NSEs in Smc5/6—namely Nse2 (aka Mms21, a protein-modification enzyme, discussed further below), Nse5 and Nse6—do not have counterparts in the other SMC complexes. Thus, this trio represents a defining feature of Smc5/6. Nse2 uses its N-terminal helix bundle to attach to the mid-arm of Smc5 (ref. 14) (Fig. 2a). Recent data showed Nse2 to be adjacent to a part of the Nse5–6 subcomplex, which also contacts the arm regions of Smc5/6[3,4,12] (Fig. 1b). Arm association by the trio is probably responsible for some of Smc5/6's unique architectural features and functions.

Yeast Nse5 and Nse6 and their homologs in other organisms form stable subcomplexes, although their sequences are less conserved than those of other Smc5/6 subunits[15–18]. Crystal and cryo-EM structures of the budding yeast Nse5–6 subcomplex revealed enrichment of helices and coils in both proteins, which dimerize in a clamped hand configuration[3,4] (Fig. 2b). Studies of the human Nse5–6 orthologs SLF1-SLF2 and SLF2 in complex with SIMC1 (an SLF1 paralog) suggest Nse5–6-like dimeric core structures and association with Smc5–6; however, they have acquired domains to support additional roles[12,18]. Structures of Nse5–6 and their homologs show differences from the U-shaped structures adopted by the helix-rich HEAT-repeat subunits of cohesin and condensin, and the yeast Nse5–6 subunits also diverge from the HEAT-repeat cohesin and condensin subunits in their lack of DNA binding[3]. Thus, despite also being helix-rich, Nse5–6 and its human counterparts appear to be distinct in structure and function from the HEAT-repeat subunits of the other SMC complexes.

Instead of binding DNA, budding yeast Nse6 binds to another genome maintenance factor, Rtt107 (ref. 19) (Fig. 2c). Functional studies suggest that Nse6 binding to either Rtt107 or its fission yeast homolog Brc1 helps to recruit Smc5/6 to chromatin, probably at DNA replication and repair sites via Rtt107/Brc1-binding to DNA lesion marker γ-H2A[19–21]. High-resolution microscopy data further revealed a second Nse6-dependent but Brc1-independent pathway for Smc5/6-targeting to chromatin, suggesting multiple roles for Nse5–6 in the association of Smc5/6 with chromatin[22]. Currently, the basis for this second means of Smc5/6-targeting is unclear; however, Smc5/6's interactions with histone-modifying and chromatin-remodeling complexes implicate their involvement[23–25]. In addition, SLF1 has acquired two BRCT domains for association with the DNA repair protein Rad18, which helps recruit Smc5/6 to DNA damage sites[17]. Thus, the role that Nse5–6 plays in recruiting Smc5/6 to chromatin appears to be conserved, although the underlying mechanisms can vary among organisms. Interestingly, plant-specific mechanisms of Smc5/6 recruitment to damaged chromatin were reported to also involve RNAs and RNA-binding proteins[25,26].

Nse1–3–4 subcomplex

Like other kleisins, Nse4 can use its N-terminal helix-turn-helix (HTH) to bind to the neck region of one SMC (Smc6) and its C-terminal winged helix (WH) to associate with the head region of the other SMC (Smc5)[1]. Between these two Nse4 domains lies a less structured region that interacts with the WH-containing KITE proteins, Nse1 and Nse3[15,27,28]. Nse1 was shown to further acquire a RING domain that can support ubiquitin E3 activity, with Nse4 and RNA polymerase I among its reported targets[29–31]. Structures of the human, *Xenopus* and yeast Nse1–3, alone or with Nse4, are similar, supporting the conserved nature of the Nse1–3-4 subcomplex[13,31–33] (Fig. 2d). This subcomplex can undergo large positional changes when Smc5/6 engages with ATP and dsDNA: based on yeast Smc5/6 hexamer (lacking Nse5–6) structures, Nse1–3 appears to move from a position proximal to the Smc5-head in the absence of ligands to being sandwiched between the neck regions and contacting DNA[13,33] (Figs. 1c and 2e). Whether a similar positional change also occurs in the presence of Nse5–6 requires further study.

Smc5/6 complex activities

A key activity of SMCs is DNA-binding, and this is mediated by multiple parts of Smc5/6, including Nse1–3-4 and the head and hinge regions of Smc5–6 (ref. 2,32,34,35) (Fig. 1b). A cryo-EM structure of the ATP- and dsDNA-bound yeast Smc5/6 hexamer revealed that the complex forms a protein clamp to topologically entrap a single piece of dsDNA within its central channel[33] (Fig. 2e). Multiple positively charged residues from Nse3–4 and the Smc5–6 head regions line the inner surface of the channel to contact the DNA backbone, enabling nonspecific dsDNA trapping and possibly sliding[33]. This structure resembles those of the ATP- and dsDNA-bound forms of other SMCs, providing a basis for their dsDNA trapping abilities[1]. Recent single-molecule data suggest that Smc5/6 diffusion along dsDNA can be facilitated by its topological DNA trapping[11,36,37]. Smc5/6 also shows a remarkable ability to associate with ssDNA-dsDNA junctions, which are commonly found at stalled replication forks and DNA repair intermediates, and to stabilize both the junctions and adjacent replication protein A (RPA)-ssDNA filaments[36,37]. These activities suggest attractive mechanisms for Smc5/6-mediated regulation of genome functions. The ssDNA-binding ability observed for the hinge regions of Smc5 and Smc6 (ref. 34) (Fig. 2f) is expected to aid these activities, a notion worth testing in the future.

The ATP binding and hydrolysis activities of SMCs are linked to their dsDNA association. The yeast Smc5/6 dsDNA clamping structure formed with ATP-mediated head dimerization suggests that ATP binding favors dsDNA entrapment[33]. Conversely, dsDNA enhances the ATPase activity of Smc5/6, suggesting that dsDNA can be a factor promoting the ATPase cycle[4,5,38]. The ATPase and dsDNA-binding activities are also influenced by NSE subunits. While Nse1–3-4 positively affects both activities, Nse5–6 exhibits more complex effects: inhibition of ATPase activity and DNA loop extrusion, but promotion of dsDNA segment capture and entrapment[4,5,11,35,37,39]. The different effects of Nse5–6 will require further studies to clarify and reconcile with overall Smc5/6 dynamics. Studies will also be needed to understand how Smc5/6 associates with other forms of DNA and how this may be linked to its ATP binding and hydrolysis cycle.

Unlike other SMCs, Smc5/6 contains a highly conserved small ubiquitin-like modifier (SUMO) ligase (E3) subunit, Nse2[40–42]. Although Nse2 SUMO E3 activity is not essential, it is required for optimal fitness and coping with genotoxins, reflecting important Smc5/6 roles in DNA repair[40–42]. Nse2's substrates include Smc5/6 subunits and more than a dozen other proteins involved in genome functions (examples are given below), and its activity is regulated in multiple ways. A recent study revealed that Nse2 contains two SUMO-interacting (SIM) motifs that facilitate its interaction with the SUMO E2 Ubc9 to promote sumoylation in vitro[43] (Fig. 2g). In vivo, two SIMs found in Nse5 can boost Nse2-mediated Smc5 and Smc6 sumoylation[3]. Nse2-mediated sumoylation in yeasts can also be promoted by the conserved Esc2 and its homolog Rad60 via their ability to bind Ubc9[44,45]. Examination of the Nse2-Smc5 dimer suggested that its ATPase and DNA-binding activities directly support sumoylation, and this can be further tested in the context of the Smc5/6 holo-complex[46,47].

In summary, recent studies have revealed that Smc5/6 resembles the other SMC complexes in its adoption of a filamentous structure and possession of DNA loop extrusion and entrapment as well as ATPase activities. However, Smc5/6 exhibits distinct arm features and has additional activities. The trio of Nse2 and Nse5–6 seems to convert a canonical DNA-organizing SMC to a specialized SMC targeted to sites of DNA replication, repair and damage response (3R) and capable of sumoylating 3R proteins. Such a specialized 3R-SMC probably benefits from its ability to bind ds-ssDNA junctions and stabilize protein-coated ssDNA. Discovery of these attributes helps us begin to comprehend the mechanisms of Smc5/6 actions. Future studies are required to elucidate Smc5/6 structural dynamics and how the activities of its different subunits are integrated.

Smc5/6 in recombinational repair

Genetic studies of Smc5/6 mutants in different organisms have unveiled its multifaceted roles in regulating homologous recombination (HR), a process key to the repair of DNA breaks and gaps arising during mitotic and meiotic cell cycles. Some aspects of these roles appear to be conserved, whereas others may be tailored to the needs of specific organisms.

Smc5/6 regulates HR intermediates in mitotic cells

Early genetic studies found that the DNA damage sensitivities or growth defects of Smc5/6 mutants can be suppressed by removing upstream HR proteins such as the recombinase Rad51[2]. A plausible interpretation is that Smc5/6 limits and/or removes toxic HR intermediates, and their accumulation has indeed been detected in yeast Smc5/6 mutants. This function partially requires Nse2 SUMO ligase activity, and relevant sumoylation targets include the Sgs1–Top3–Rmi1 (STR) Holliday junction (HJ) dissolution complex in yeast[48,49] (Fig. 3a). STR and its mammalian counterpart BTR (BLM-TopoIIIα-RMI1/2) process HJ structures into non-crossover repair products. Smc5/6 associates with and sumoylates STR, facilitating STR accumulation at DNA repair foci[48,49]. Human Smc5/6 is also required to sumoylate the Sgs1 homolog BLM (Bloom syndrome helicase) and to promote BLM accumulation at repair foci, suggesting a conserved Smc5/6 role in regulating these orthologous helicases[50] (Fig. 3a). Furthermore, Nse2-haploinsufficient mice

show increased sister chromatid exchange levels, a defect also seen with impaired BLM function[51]. Conditional Nse2 ablation in adult mice phenocopies some features of BLM null mice, including premature aging, growth retardation, and abnormalities of fat distribution and pigmentation[51]. Collectively, these data support conserved interactions between Smc5/6 and BLM/Sgs1. However, it is worth noting that Smc5/6 and BLM or Sgs1 also have non-overlapping functions; thus, their loss-of-function (LOF) mutants also show distinct phenotypes and lack of epistasis[50–52].

In addition to its roles in resolving HR intermediates, Smc5/6 helps limit their generation, especially when they arise from replication fork reversal. This idea is supported by observations that yeast Smc5/6 binds to the DNA helicase Mph1 to limit Mph1-mediated fork reversal and toxic HR intermediate production[53,54] (Fig. 3b). Smc5/6 also influences HR via other means, such as by regulating cohesin sumoylation in yeast and human cells to impact sister chromatid recombination[2] (Fig. 3a). In higher eukaryotes, Smc5/6 associates with the FANCD2 and FANCI proteins and shows epistasis with FANCC in sensitivity to inter-strand crosslinking agents[55] (Fig. 3a). Further examination of the interplay between Smc5/6 and Fanconi anemia (FA) proteins can shed more light on how they collaborate in DNA repair. In summary, although Smc5/6 appears to mainly control cellular levels of HR intermediates in yeasts, it takes on broader repair roles in higher eukaryotes.

Smc5/6 regulates HR and other processes during meiosis

Smc5/6 is required for meiotic progression via HR-dependent and HR-independent roles. Like the mitotic situation, Smc5/6 defects increase levels of meiotic HR intermediates in yeast[2]. This was attributed to its regulation of both the STR- and Mus81/Mms4 nuclease-mediated HJ cleavage pathways (Fig. 3a), restraining the formation of inappropriate meiotic HR intermediates and regulating their spacing. Smc5/6 is also found along chromosome arms in a similar distribution as cohesin, and cohesin depletion reduces Smc5/6 enrichment on DNA, suggesting a functional link between the two SMC complexes[56].

A requirement for Smc5/6 in the completion of meiotic HR has also been reported in *Caenorhabditis elegans*[57,58]. For example, *CeSMC5/6* deficiency leads to accumulation of inter-chromosomal bridges that can be rescued by removing the upstream HR factor BRCA1[57]. However, the mammalian situation is more complex, as Smc5/6 appears to both prevent aberrant HR processes and help generate segregation-competent bivalents[59,60]. The mechanisms underlying these effects await further elucidation, but the conserved role of Smc5/6 in controlling HR intermediate accumulation can partly explain these effects.

Spatial control of heterochromatin break repair by Smc5/6

When heterochromatin suffers breaks, the broken DNA is often translocated outside this chromatin domain for repair. Smc5/6 plays important roles in this movement. Following early observations in yeast, a role for Smc5/6 in targeting heterochromatic breaks to the nuclear envelope in *Drosophila* and human cells was discovered and found to involve collaboration with myosin and nuclear actin[2,61] (Fig. 3a).

Smc5/6 also plays a role in the maintenance of telomeric heterochromatin. This has been best studied in the context of alternative lengthening of telomeres (ALT), a

recombination-dependent and telomerase-independent mechanism to maintain telomeres in cancer cells. Smc5/6 co-localizes with ALT-associated promyelocytic leukemia bodies (APBs) and is required for telomere clustering as well as recombination-mediated telomere lengthening[2,62,63]. Some of these functions rely on Nse2-mediated sumoylation of telomere-binding proteins and the Rad51AP1 protein, which are important for ALT (Fig. 3a). Currently, it is unclear whether additional Smc5/6 activities besides sumoylation are required to modulate telomere localization and function.

In summary, studies in model organisms and human cells have uncovered the multi-functional nature of Smc5/6 in HR regulation (Fig. 3a). One important and conserved role is the suppression and elimination of HR intermediates. Depending on the organism, cell-cycle context and chromatin state, Smc5/6 also carries out additional roles during HR repair, such as regulating events further upstream or aiding relocation to more favorable environments for repair. How Smc5/6 executes such a wide range of functions during HR is a fascinating question that requires more detailed examination.

Smc5/6 promotes genome replication

Multiple lines of evidence suggest a conserved requirement for Smc5/6 in genome replication. Deeper insights into the replication functions of Smc5/6 have recently been provided from studies in yeast systems. Mammalian studies further suggest the possibility of cell-type specific functions for Smc5/6 depending on the systems used. The multiple roles played by Smc5/6 in genome replication can partly account for its requirement in curbing chromosomal rearrangements[2,64].

Smc5/6 facilitates the duplication of rDNA and non-rDNA regions in yeast

Degron-based acute Smc5/6 depletion in yeast suggests that Smc5/6 facilitates replication at the replication fork barrier (RFB) sites of the rDNA locus[65]. Smc5/6 may maintain stability of arrested forks in this repetitive region by preventing fork reversal catalyzed by the Mph1 DNA helicase[65] (Fig. 3b). Additional studies suggest that Smc5/6 also affects rDNA stability via other means, including interactions with the cohibin and CLIP complexes that tether rDNA to the nuclear envelope and regulation of RNA polymerase I (refs. 29,66) (Fig. 3b). As rDNA serves as a nexus for multiple essential cellular events and affects replicative lifespan, Smc5/6's rDNA-related roles potentially exert a profound effect on organismal physiology, as discussed in later sections.

Accumulating evidence in yeasts suggest roles for Smc5/6 in replicating non-rDNA regions[2]. More recently, EM studies revealed increased levels of reversed replication forks, hemicatenanes and dHJs in Smc5/6 mutants, resembling *sgs1* and *top3* mutants[67]. Both Smc5/6 and STR are present at replication termination regions, and *smc6* mutants reduce Top3 localization at these sites[67] (Fig. 3b). As Smc5/6 interacts with STR and favors Top3-Rmi1 localization to DNA repair foci[48], there appears to be a convergent function for Smc5/6 and STR in both HR repair and replication termination. Future evaluation of this model can shed light on fundamental principles involved in replication and HR intermediate processing.

SUMO-dependent and SUMO-independent roles of Smc5/6 in replication progression

Smc5/6-based regulation of DNA replication partially requires its sumoylation activity (Fig. 3c). In yeast, Smc5/6 aids sumoylation of the ssDNA binding complex RPA, enabling collapsed replication forks to be targeted to nuclear pore complexes for repair[68,69]. Sumoylation of Smc5 itself has also been implicated in fork regulation[70]. In addition, Smc5/6 sumoylates the leading-strand polymerase subunit Pol2 during S phase[71,72]. Smc5/6 also regulates replication in SUMO-independent ways. For example, Smc5/6's interaction with topoisomerase II has been implicated in helping the replicative machinery manage topological stress[38].

Smc5/6 also affects genome replication in mammals, but the underlying mechanisms are less clear. Knocking down Smc5/6 impaired replication in cell lines, causing accumulation of ultra-fine DNA bridges and Rad51 foci at newly synthesized sites[73–75] (Fig. 4a). Acute Smc5/6 loss in human cells also prominently affects rDNA replication and/or repair, echoing yeast data and suggesting a conserved role for Smc5/6 in rDNA integrity[76]. Smc5 depletion in mouse cells is associated with increased replication fork stalling, which partly explains the increased apoptosis seen in central cortical tissues along with reduced cortex sizes[77]. Although these studies all point to a role for Smc5/6 in genome replication, some different findings have been reported in different systems and may be attributable to tissue- and cell-type-specific differences in the requirement for Smc5/6, an important topic to be explored in the future.

In summary, recent progress has revealed important roles for Smc5/6 in genome replication, wherein it regulates multiple replication factors, affects repetitive and non-repetitive regions, and uses SUMO and non-SUMO-based strategies. An outstanding question is whether these functions are linked by a common underlying mechanism that could also explain Smc5/6's multifaceted roles in other processes. Addressing this question will help us begin to generate a coherent model for Smc5/6-based regulation of the genome.

Plant Smc5/6 contributes to DNA repair, checkpoint, immunity and reproduction

Studies in plants suggest that Smc5/6 plays multiple roles during the mitotic and meiotic cell cycles. Some of these roles recapitulate those seen in yeast and animals, such as HR-mediated DNA repair. Others appear to be plant-specific, such as coping with plant pathogens and ploidy changes, with important agricultural implications. Plant-specific roles were recently reviewed[78,79], and are thus not elaborated here. Rather, we focus on recent studies that have provided mechanistic insight into a subset of Smc5/6 functions in plants.

Smc5/6 affects HR repair and checkpoint functions

Examination of Smc5/6 subunit mutants, particularly those of Nse2 and the plant Nse6 counterpart SNI1, suggests that the complex helps the development of multiple plant tissues[78]. Mutant phenotypes feature increased DNA lesions combined with failed DNA damage checkpoint activation[80]. Removing upstream HR proteins such as RAD51 and BRCA2 can mitigate these defects, implying that Smc5/6 may help complete HR repair, as

seen in yeast and animals[78]. Unique to plant systems is the additional control exerted by Smc5/6 on the E2F-mediated G1/S checkpoint[80,81]. Specifically, NSE2 and SNI1 inhibit the E2F transcriptional activator required for cell-cycle progression, and loss of such inhibition permits inappropriate cell-cycle progression in the presence of unresolved damage. Of note, these defects in plant *sni1* and *nse2* mutants echo observations made in yeast—wherein *smc5/6* mutant cells continue to progress through the cell cycle despite accumulation of DNA lesions, leading to cell death[2]. The mechanism by which Smc5/6 affects genome stress checkpoints in yeast remains to be elucidated but has recently been linked to the DNA helicase Sgs1's homolog Rqh1 in fission yeast[82]. A direct role for Smc5/6 in regulating cell-cycle progression factors has yet to be reported for other systems, suggesting that this role may have evolved in certain organisms to accommodate the unique properties of their cell cycles and stress response.

Smc5/6 in plant innate immune response

The plant DNA damage response is closely linked to immunity, as infection-associated DNA damage is generated as a consequence of plant responses to pathogens. A main activator of this response is NPR1, which upregulates pathogenesis-related genes. Interestingly, SNI1 loss suppresses the immune defects of *npr1* mutants by increasing transcription of NPR1-dependent genes[78]. Mutations in *sni1* alone are associated with dysregulated immunity and DNA lesion accumulation as well[83]. Whether SNI1 and Smc5/6 play a direct role in plant immune responses or trigger such responses indirectly by altering levels of nucleic-acid intermediates remains to be clarified. It is interesting to note that other HR proteins, such as RAD51 and BRCA2, have also been implicated in the plant immune response and, at least in some cases, may act by turning on gene expression[78]. Consequently, the increased pathogenesis-related gene expression seen in *sni1* mutants can be suppressed by mutating either RAD51 or BRCA2.

Smc5/6 in plant reproduction

Smc5/6 is also key for meiosis and gamete formation, and thus crop fertility, as recently reviewed[79]. An Smc5/6 role early in the process appears to be inhibition of meiotic recombinase DMC1[84], although it remains unclear whether this also occurs in other species. A downstream role involves regulation of crossover frequency and distribution[85]. In addition, Smc5/6 affects plant gametophytic ploidy via HR-dependent and HR-independent means, the latter of which may involve its spindle and kinetochore functions[86]. Interestingly, Smc5/6 also mediates sumoylation of a subset of yeast kinetochore proteins and localizes to spindle poles in mouse embryonic stem cells[74,87]. It remains to be seen if plant Smc5/6 shares conserved spindle and kinetochore functions with other organisms or invokes its own unique mechanisms.

In summary, studies of Smc5/6 in plants have shed light on its evolutionarily conserved and plant-specific functions. As we move on to thinking about potentially similar paradigms in human cells, it is intriguing to consider that some of the latter functions, such as suppression of immunity-related gene expression, might arise from adaptation of the former functions to address organism-specific challenges.

SMC5/6 in human disease

In view of its importance for multiple aspects of genome regulation, it is not surprising that SMC5/6 subunits have been found mutated in several genome instability syndromes sharing phenotypes with diverse Smc5/6 LOF models. Recent studies have also identified SMC5/6 as a host restriction factor against a variety of viruses, many harboring oncogenic potential, while emerging tumor suppressive role(s) for the complex are also suggested by recurrent somatic mutations in cancers.

SMC5/6 and genome instability syndromes

Individuals bearing germline SMC5/6 mutations reported thus far show diverse features that clinically and molecularly overlap those of other genome instability syndromes (Fig. 4a,b). Compound heterozygous *NSMCE2* (Nse2) mutations leading to severe loss of the protein and its sumoylation activity, with mild destabilization of SMC5–6, were identified in patients with primordial dwarfism, microcephaly, developmental delays, and metabolic and endocrine abnormalities[88]. Patient-derived cells showed impaired S phase progression and recovery from replication stress, suggesting that the multi-organ phenotypes seen may partially stem from abnormalities in cellular proliferation across lineages. Defective recovery from replication stress was also reported for cells from children with biallelic germline *NSMCE3* (Nse3) mutations presenting with adaptive immunodeficiency and early demise from rapidly progressive lung disease[89]. Phenotypic differences from NSMCE2-deficient individuals may be explained by the stronger impact of *NSMCE3* mutations on overall SMC5/6 stability and functions, distinctions between SUMO-dependent and SUMO-independent functions, and/or early mortality limiting appreciation of later-onset phenotypes.

Most recently, individuals with *SMC5* or *SLF2* (Nse6 ortholog) mutations were described with growth, cardiovascular and endocrine findings similar to those of NSMCE2-deficient individuals, as well as skin, immune and hematopoietic findings similar to those of NSMCE3-deficient individuals[90]. Cells derived from these patients with ‘Atelis syndrome’ also showed mosaic variegated hyperploidy. This was partly attributed to loss of sister chromatid cohesion, along with increased spontaneous replication fork stalling from impaired replication through DNA secondary structures.

The clinical and cellular features of SMC5/6-deficient individuals described thus far resemble those arising from mutations in the BTR complex[91–93] and replisome proteins, such as the leading-strand polymerase POLE subunits[94], FA[95] and ribosomopathies[96], the last echoing Smc5/6’s conserved roles in rDNA stability (Fig. 4b). The phenotypic overlap also supports the conserved genetic and functional interactions that Smc5/6 has with these complexes, as described above.

SMC5/6 as a viral restriction factor

Mammalian Smc5/6 functions have recently expanded from regulating host genomes to control of viral DNA. Human SMC5/6 suppresses transcription and replication of unintegrated hepatitis B virus (HBV) and other viral pathogens, including human

immunodeficiency virus (HIV), human papillomavirus (HPV), and herpesviruses[97] (Table 1). Ubiquitin-proteasome pathways play key conserved roles in virus– SMC5/6 antagonism. For example, viral proteins such as HBx hijack host ubiquitin ligases to target SMC5/6 for degradation, while host ubiquitin ligases like TRIM21 degrade HBx and rescue SMC5/6[97–99]. HIV-1 hijacks CRL4 host ubiquitin ligase using its accessory protein Vpr to remove SMC5/6-dependent inhibition of extra-chromosomal lentiviral DNA, yet the Epstein–Barr virus (EBV) BNRF1 protein and Kaposi's sarcoma-associated herpesvirus (KSHV) RTA ubiquitin ligase target SMC5/6-dependent inhibition of EBV and KSHV lytic replication and expression, respectively[97,100–102].

SMC5/6 preferentially localizes to viral episomes rather than integrated viral DNA, providing a first line of defense against viral propagation after viral entry. We highlight several mechanisms by which SMC5/6 could restrict viral DNA. SMC5/6 suppresses HIV-1 and KSHV transcription partly via compaction of unintegrated viral DNA and sumoylation of HIV-1 proviral chromatin, but is thought to recruit HBV DNA to SUMO-rich ND10 or promyelocytic leukemia bodies to engender silencing[97,100,102–104]. Yet another mechanism entails direct competition—in the case of HPV, between SMC5/6 and viral regulatory protein E2 for binding to the viral E1 protein required for viral replication and transcription[105]. We anticipate that additional mechanisms employed by Smc5/6 to achieve viral restriction may yet emerge for viruses with other architectures and life cycles.

The recruitment of SMC5/6 to viral episomes also involves virus-specific strategies. Its SLF2 subunit targets the complex to unintegrated lentiviruses, while the Nse1-like host ubiquitin ligase PJA1 promotes association of SMC5/6 with the HSV episome[100,106]. This suggests an interesting paradigm whereby paralogs of canonical Smc5/6 subunits are swapped in to redirect the complex's activities from endogenous genome maintenance to other cell type- or species-specific genome protective functions such as viral defense. Indeed, although Nse5 ortholog SLF1 recruits SMC5/6 to sites of DNA damage, its paralog SIMC1 recruits the complex to polyomavirus replication centers[18]. Further investigation is required to understand whether and how distinct host protein intermediaries target Smc5/6 to different viruses.

SMC5/6 in cancer

Somatic SMC5/6 mutations have been seen in many cancers, although the 'driver' versus 'passenger' status of these changes remains unexplored[107]. Malignancy has rarely been reported in SMC5/6-deficient individuals thus far, although the potential for cancer predisposition is difficult to assess given disease rarity and early mortality from other complications[88–90]. Many of the viruses targeted by SMC5/6 are also well-known for their oncogenic potential, and SMC5/6 degradation by HBx can cause DNA break accumulation in HBV-infected liver cells, potentially predisposing to tumorigenesis[108]. Thus, SMC5/6 may be important for averting malignancy through dual roles in restricting viral infection and maintaining host genome stability.

Conclusion

The past few years have witnessed a rapid expansion in Smc5/6 biology, from structures and activities to roles in human disease. As some puzzles are solved, additional mysteries arise, some of which we have explored in the above sections. For example, there remains a need to understand how the myriad Smc5/6 functions are supported by its individual subunits, subcomplexes and their various activities. Studies across diverse systems have begun to reconcile highly conserved Smc5/6 functions, such as regulating HR intermediates, but mechanistic details remain outstanding. Our understanding of how Smc5/6 engages with and manipulates different types of DNA structure remain limited, making it difficult to propose a coherent molecular model for how the complex achieves specific functional outcomes. Moreover, when considering Smc5/6 in the evolutionary context of its many roles across species, diverse partner proteins, and divergence from other SMC complexes, we may need to embrace plurality of context-specific mechanisms as a central paradigm of Smc5/6 function. Addressing these issues with additional studies will substantially propel Smc5/6 biology forward towards a better molecular appreciation of its diverse roles.

Further inquiry is also needed to explain Smc5/6-associated pathophysiology at cellular and organismal levels. Detailed studies of how Smc5/6 interacts with other genome maintenance pathways across cell types and species will be informative. Although genome protection appears to be a theme unifying Smc5/6 functions across organisms, we know little about how Smc5/6 evolved from its host genome maintenance role to defending against foreign genetic material. This ability to adapt anciently conserved functions to increasingly complex multicellular needs may prove a general evolutionary paradigm. Finally, elucidation of the Smc5/6-dependent mechanisms leading to human disease prevention will inform potentially novel strategies for immunomodulation and treating malignancy.

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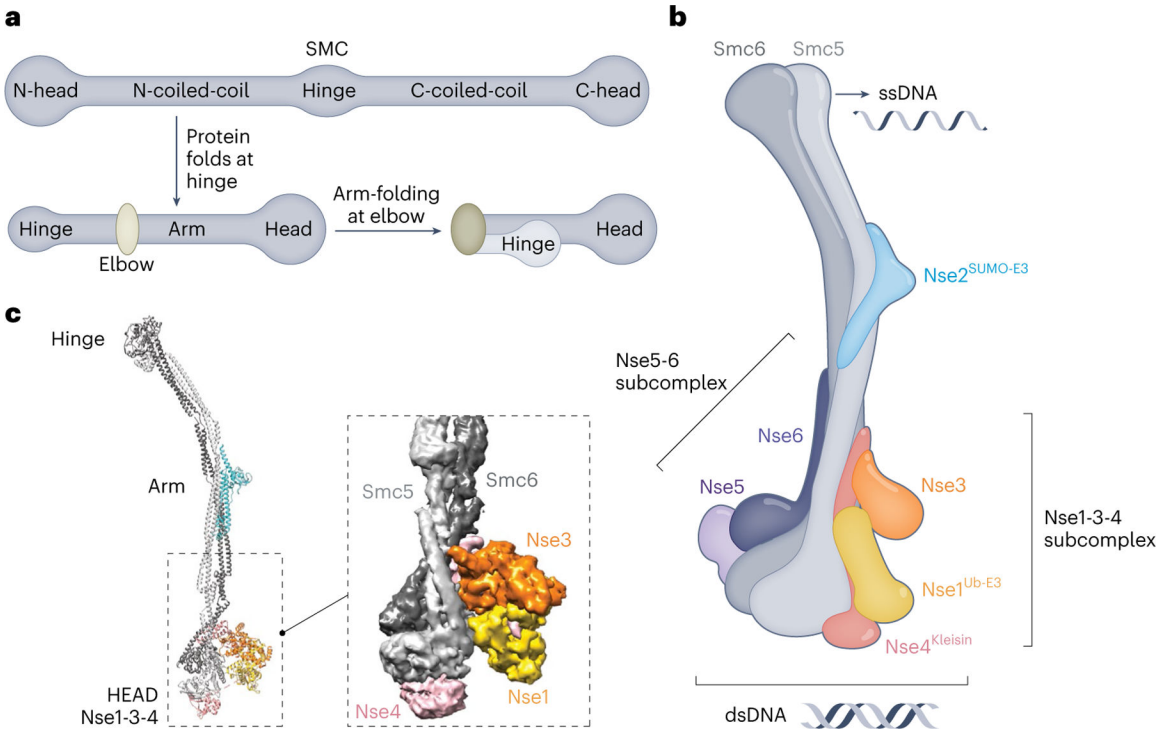

Fig. 1 |. Architecture of the Smc5/6 complex.

a, Diagrams showing the SMC protein domains and folding mechanisms. See main text for details. **b**, Diagrams showing a model of the budding yeast Smc5/6 architecture and subunit activities. See main text for details. Subunit colors are used consistently in all panels: Smc5, light gray; Smc6, dark gray; Nse1, gold; Nse2, cyan; Nse3, orange; Nse4, pink; Nse5, dark purple; Nse6, dark blue. As detailed in the text, Nse2 and Nse1 exhibit SUMO and ubiquitin (Ub) E3 activities, respectively, while Nse4 is the kleisin subunit. **c**, A medium-resolution cryo-EM structure of the budding yeast Smc5/6 hexamer complex, which contains Smc5–6 and Nse1–4, but lacks Nse5–6. Panel **c** adapted under a Creative Commons license CC BY 4.0 from Hallett et al.[13].

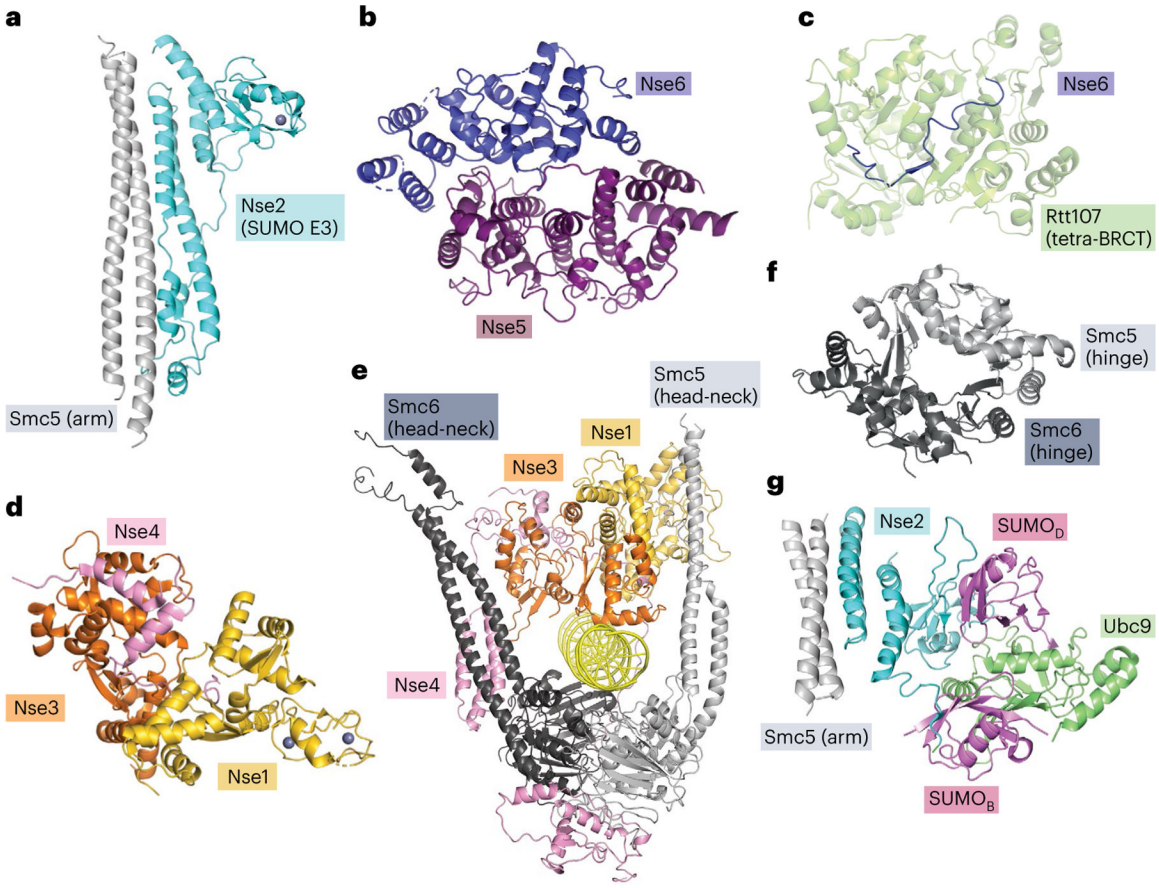

Fig. 2 |. Structures of different subunits and subcomplexes of the Smc5/6 complex.

a, Crystal structure of the budding yeast SUMO E3 subunit Nse2 (cyan) bound to a region of the Smc5 arm (light gray) (PDB 3HTK). Spheres indicate zinc ions in the SUMO E3 domain. **b**, Cryo-EM structure of the budding yeast Nse5–6 subcomplex (PDB 7SDE), showing the Nse5 (dark purple) and Nse6 (dark blue) dimerization domains. **c**, Crystal structure of the Rtt107 tetra-BRCT domain (green, transparent) bound to the Rtt107 interaction motif (RIM, dark blue) of Nse6 (PDB 6J0W). **d**, Crystal structure of the *Xenopus* Nse1–3-4 complex, with Nse1 shown in gold, Nse3 orange and Nse4 pink (PDB 7DG2). Spheres indicate zinc ions incorporated in the Nse1 ubiquitin E3 domain. **e**, Cryo-EM structure of the budding yeast Smc5/6 hexamer bound to ATP and dsDNA (yellow) (PDB 7TVE). Colors of proteins are as in Fig. 1b,c. **f**, Crystal structure of the hinge regions of fission yeast Smc5–6 (PDB 5MG8). **g**, Crystal structure of the Nse2 SUMO E3 subunit (cyan) bound to the Smc5 arm (light gray) and SUMO E2 Ubc9 (light green). Ubc9 is conjugated with a donor SUMO ($SUMO_D$, purple) and binds to a backside SUMO ($SUMO_B$, purple) (PDB 7P47). Figures adapted with permission from: **a**, Duan et al.[14], Elsevier; **b**, Yu et al.[3], PNAS; **c**, Wan et al.[19], Elsevier; **d**, Jo et al.[32], Elsevier; **e**, Yu et al.[33], PNAS; **f**, Alt et al.[34], Springer Nature; **g**, Varejao et al.[43], Springer Nature.

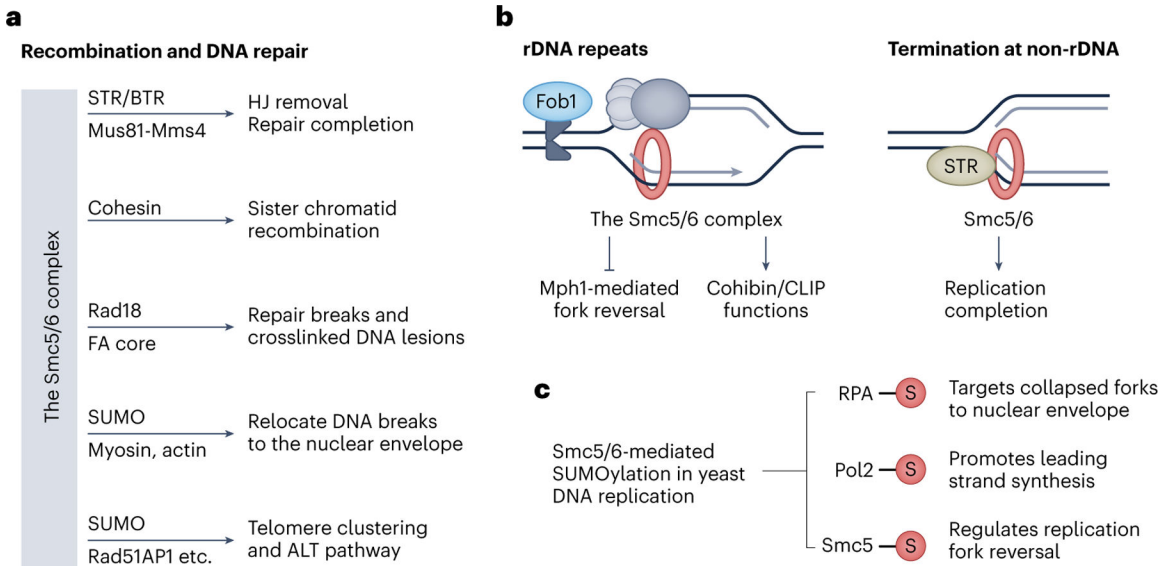

Fig. 3 |. Multifaceted roles of Smc5/6 in recombinational repair and DNA replication.

a, The multiple roles played by Smc5/6 during recombinational repair. See main text for details. **b**, Smc5/6's effects on DNA replication based on studies in budding yeast. Left: Smc5/6 has at least two roles in helping rDNA replication and stability: (i) negative regulation of Mph1-mediated replication fork reversal at replication fork pausing sites marked by the Fob1 protein and (ii) collaboration with the Cohibin and CLIP complexes. Right: Smc5/6 cooperates with the STR complex for proper replication termination. **c**, Examples of SUMO-based regulation of DNA replication by the yeast Smc5/6 complex. Smc5/6 promotes sumoylation of the ssDNA binding complex RPA, the leading-strand DNA polymerase subunit Pol2, and Smc5 itself. The consequence of each modification is indicated and explained in the text.

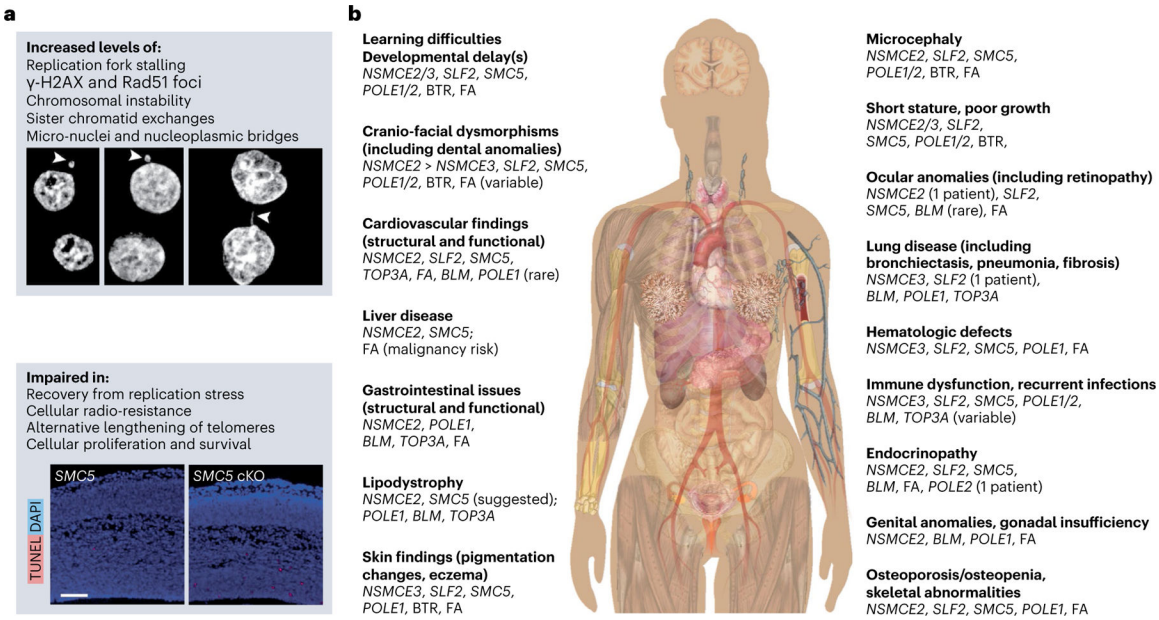

Fig. 4 |. Summary of phenotypes caused by defective Smc5/6 in mammalian systems.

a, Cellular defects reported for SMC5/6 LOF in mammalian systems. SMC5/6 deficiencies lead to increased levels of replication stress markers and abnormal chromosome structures[89] (arrows) (top), as well as impairments in growth, telomere maintenance, and survival of replication stress and radiation (bottom)[51,63,73,74,76,77,88–90]. **b**, Clinical phenotypes of SMC5/6-deficient individuals overlap those of other genome instability syndromes caused by mutations in genes encoding BTR and FA proteins and leading-strand DNA polymerases (*POLE1* and *POLE2*). Individual features are shown for germline mutations in *NSMCE2*[88], *NSMCE3*[89], *SMC5*[90] and *SLF2*[90] and other genes whose functional interactions with Smc5/6 are discussed in the text, including BTR genes (*BLM*[92], *TOP3A*[91], *RMI1/2*[93], *POLE1/2*[94]) and FA genes[95]. Images in **a** reproduced under a Creative Commons license CC BY 4.0 from Payne et al.[88] (top) and Atkins et al.[77] (bottom). Image in **b** adapted from Wikimedia (https://commons.wikimedia.org/wiki/Human_body_diagrams).

Table 1 |

Reported roles of SMC5/6 in regulating pathogenic viruses

Virus	Smc5/6 effects	Viral antagonist
Hepatitis B virus (HBV)[98,99]	Reduces gene expression and replication of unintegrated viral genomes	HBx hijacks host Cullin-4 ubiquitin E3 to target SMC5/6 for degradation
Human immunodeficiency virus (HIV)[100]	Compacts and silences unintegrated viral DNA to restrict viral gene expression	Vpr collaborates with host Cullin-4 ubiquitin E3 to degrade SLF2
Herpes simplex virus 1 (HSV-1)[106]	Represses expression from extra-chromosomal viral DNA	Not identified
Kaposi's sarcoma-associated herpesvirus (KSHV)[102]	Reduces viral gene expression and replication; compacts and represses viral chromatin	RTA ubiquitin E3 degrades SMC5/6
Epstein–Barr virus (EBV)[101]	Interferes with viral replication compartment formation and encapsidation	BNRF1 targets SMC5/6 for Cullin-7 ubiquitin E3-mediated degradation
Human papillomavirus (HPV)[105,109]	Interacts with viral E2 and affects levels of viral episomal DNA in host cells	Not identified
Polyomavirus – JC virus (JCV)[110] and SV40[18]	Interacts with oncogenic JCV LT-Ag; targets polyomavirus replication centers	Not identified

Additional references and details are provided in a review by Irwan and Cullen[97].