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Review

The Yin and Yang of the MMS21–SMC5/6 SUMO ligase complex in homologous recombination

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ABSTRACT

Maintaining genomic stability is critical for the prevention of disease. Numerous DNA repair pathways help to maintain genomic stability by correcting potentially lethal or disease-causing lesions to our genomes. Mounting evidence suggests that the post-translational modification sumoylation plays an important regulatory role in several aspects of DNA repair. The E3 SUMO ligase MMS21/NSE2 has gained increasing attention for its function in homologous recombination (HR), an error-free DNA repair pathway that mediates repair of double-strand breaks (DSBs) using the sister chromatid as a repair template. MMS21/NSE2 is part of the SMC5/6 complex, which has been shown to facilitate DSB repair, collapsed replication fork restart, and telomere elongation by HR. Here, I review the function of the SMC5/6 complex and its associated MMS21/NSE2 SUMO ligase activity in homologous recombination.

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1. Introduction

The structural maintenance of chromosomes (SMC) family of proteins is essential for chromosomal architecture and organization [1]. There are six known eukaryotic SMC proteins, SMC1–6, which form three types of heterodimers [2]. The SMC1/3 heterodimer forms the cohesin complex that maintains sister chromatid cohesion during mitosis to ensure the proper segregation of sister chromatids [3]. Additionally, the SMC1/3 cohesin complex has been implicated in the repair of DNA double-strand breaks (DSBs) by homologous recombination (HR) [4]. The SMC2/4 heterodimer forms the condensin complex that promotes chromosome condensation during mitosis to allow segregation of sister chromatids to daughter cells during mitosis [5]. The SMC5/6 complex is intimately involved with several cellular processes involving HR, such as DNA damage repair, restart of collapsed replication forks, ribosomal DNA (rDNA) maintenance, and telomere elongation [6]. Much work has gone into identifying the components of the SMC5/6 complex and determining their role in DNA damage repair. The E3 SUMO ligase MMS21/NSE2 has emerged as a critical component of the SMC5/6 complex in facilitating HR [7]. In this review, I discuss the function of the SMC5/6 complex and its SUMO ligase activity in several HR-mediated cellular processes.

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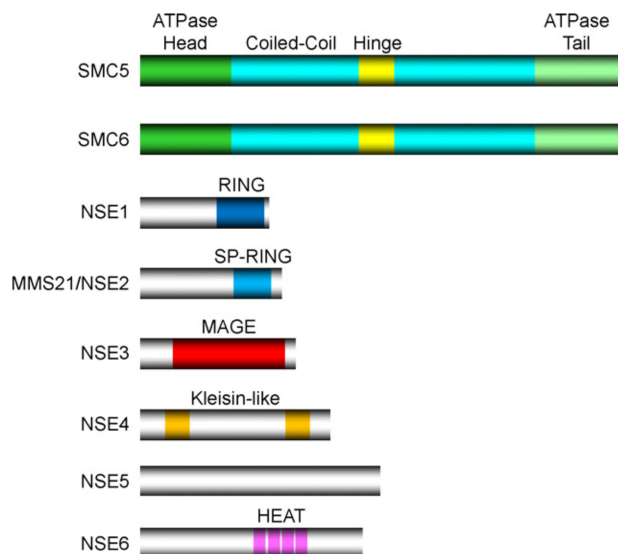

Fig. 1. Domain organization of the SMC5/6 complex proteins. SMC5 and SMC6 contain an N-terminal ATPase head domain with a Walker A motif and a C-terminal ATPase tail domain with a Walker B motif that are brought together by an intramolecular coiled-coil. The hinge domain mediates binding between SMC5 and SMC6. NSE1 contains a RING domain that is often associated with E3 ubiquitin ligase activity. MMS21/NSE2 contains a SP-RING domain that is often associated with E3 SUMO ligase activity. NSE3 contains a MAGE domain with unknown activity. Based on secondary structure prediction analysis, NSE4 contains helix-turn-helix and winged-helix motifs similar to other kleisins. NSE5 has no known domains. NSE6 contains several HEAT repeats.

2. Architecture of the SMC5/6 complex

The SMC5/6 complex is composed of two SMC family proteins, SMC5 and SMC6, as well as several non-SMC elements (NSE; Fig. 1) [8–17]. Although the primary sequences of SMC5 and SMC6 are more divergent than SMC1–4, these two proteins are speculated to form functional ATPases in an analogous manner, by intramolecular coiled-coil domains bringing together Walker A and B motifs (Fig. 2). SMC5 and SMC6 interact in their hinge region to form the core of the SMC5/6 complex [14]. The head domains of SMC5 and SMC6 are brought together by NSE4 [18]. Secondary structure analysis has predicted that NSE4 has helix-turn-helix and winged-helix folds similar to other kleisin family proteins, such as SCC1, CAP-H, and CAP-H2 [18]. In addition, humans encode a putative germ cell-specific NSE4 termed NSE4b/EID3 [19]. NSE4b/EID3 is approximately 50% identical to NSE4a and is expressed only in the testis [19,20]. Overexpression studies confirmed the ability of NSE4b/EID3 to bind to the SMC5/6 complex [19]. The function of this testis-specific NSE4 is still to be determined. Additional NSE proteins have been shown to bind to the SMC5/6 complex. These include NSE1, MMS21/NSE2 (herein referred to as MMS21 for simplicity), NSE3, NSE5, and NSE6 [8–16]. NSE1, NSE3, NSE4, NSE5, and NSE6 have all been shown to bind to the head domains of SMC5/6 in *in vitro* biochemical assays (Fig. 2) [18]. However, MMS21 does not bind to the head domains of SMC5/6, but rather to the coiled-coil region of SMC5 (Fig. 2) [14]. *In vitro* assays have supported the model that NSE1 binds to NSE3, and both NSE1 and NSE3 bind NSE4 (Fig. 2) [18,21].

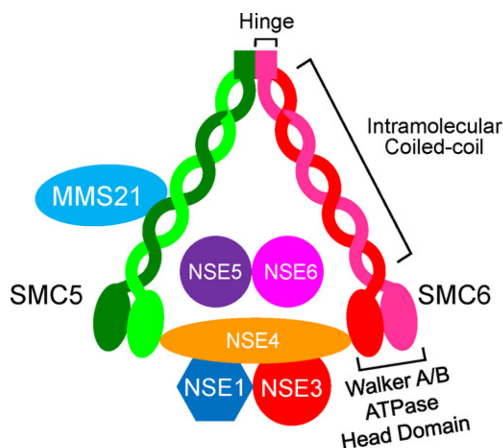

Fig. 2. Architecture of the SMC5/6 complex. The SMC5/6 complex is composed of the SMC5–SMC6 heterodimer and six non-SMC element (NSE) proteins. The putative ATPase head domains of SMC5 and SMC6 are linked together by the kleisin-like protein NSE4. NSE1 binds the MAGE domain protein NSE3. The NSE1–NSE3 complex interacts with NSE4. In yeast, NSE5 and NSE6 bind to the head domains of SMC5 and SMC6. The E3 SUMO ligase MMS21/NSE2 binds to the coiled-coil region of SMC5. The ring-shaped structure depicted is a hypothetical structure based on the SMC1/3 cohesin complex, as no detailed structural information for the SMC5/6 complex is known.

Interestingly, several of these NSE proteins have potentially intriguing activities. MMS21 contains a modified RING (really interesting new gene) domain known as a SP-RING (SIZ/PIAS-RING) domain, which is associated with E3 SUMO (small ubiquitin-like modifier) ligase activity (Fig. 1) [10,12]. SUMO is a small protein that is covalently attached to lysine residues on protein substrates by a multi-step enzymatic cascade involving SUMO E1 (AOS1/UBA2), E2 (UBC9), and E3 enzymes (reviewed in [22]). Although SUMO is conjugated to substrates in a similar manner to ubiquitin, it does not generally target proteins for proteasome-dependent degradation [22]. Instead, it can have wide-ranging, protein-specific effects, such as alteration of protein–protein interactions, protein subcellular localization, or protein stability [23]. MMS21 exhibits E3 SUMO ligase activity, and has been shown to target several proteins for sumoylation in various organisms (Table 1) [16,24,25]. Intriguingly, the SMC5/6 complex component NSE1 contains a RING domain that is often found in E3 ubiquitin ligases (Fig. 1) [8,10]. However, a recent report has suggested that NSE1 does not display E3 ubiquitin ligase activity *in vitro* [21]. The authors instead propose that the RING domain of NSE1 functions as a structural element to form the trimeric sub-complex of NSE1, NSE3, and NSE4 (Fig. 2) [21].

In addition to MMS21 and NSE1, several other proteins have been identified as components of the SMC5/6 complex. NSE3 contains a MAGE (melanoma-associated antigen gene) domain of unknown function (Fig. 1) [12]. In fission yeast, a dimeric complex of NSE5 and NSE6 has been reported to bind the head domains of SMC5 and SMC6 at a distinct site from NSE1/NSE3/NSE4 (Fig. 2) [13,18]. NSE5 contains no known domains, whereas NSE6 contains HEAT domain repeats (Fig. 1) [13,18]. To date, no human homologues of NSE5 and NSE6 have been reported. NSE5 and NSE6

Table 1
MMS21 sumoylation substrates.

Substrate	Organism	Substrate function
KU70	Budding yeast [16]	Non-homologous end-joining
MMS21	Human [25]; fission yeast [24]; budding yeast [16]	Homologous recombination
NSE3	Fission yeast [24]	Homologous recombination
NSE4	Fission yeast [33]	Homologous recombination
RAP1	Human [38]	Telomere maintenance
SA2	Human [37]	Sister chromatid cohesion
SCC1	Human [37]	Sister chromatid cohesion
SMC5	Budding yeast [16]	Homologous recombination
SMC6	Human [25]; fission yeast [24]	Homologous recombination
TIN2	Human [38]	Telomere maintenance
TRAX	Human [25]	Unknown
TRF1	Human [38]	Telomere maintenance
TRF2	Human [38]	Telomere maintenance

may function as regulators of the SMC5/6 complex, because unlike the other SMC5/6 complex components, NSE5 and NSE6 are not essential genes in fission yeast [13]. The fission yeast SMC5/6 complex has also been shown to associate with several other proteins that are not thought to be core components of the SMC5/6 complex. These include RAD60/NIP45 and RAD62 [11,26]. Interestingly, RAD60/NIP45 contains two SUMO-like domains that are important for RAD60/NIP45-mediated replication stress response [27,28]. The function of these SMC5/6 complex associated proteins is currently unknown.

3. The SUMO ligase MMS21/NSE2

Sumoylation has become a prominent post-translational modification regulating the repair of DNA lesions [29,30]. The SMC5/6 complex SUMO ligase MMS21 was originally identified in a genetic screen for methyl methanesulfonate (MMS)-sensitive mutants in budding yeast [31,32]. It remained relatively obscure until 2005 when studies in budding yeast, fission yeast, and human cells identified MMS21 as a component of the SMC5/6 complex [16,24,25]. Based on primary sequence homology, MMS21 contains a SP-RING domain common to other E3 SUMO ligases [16,24,25]. Similarly to other E3 SUMO ligases, MMS21 undergoes auto-sumoylation *in vitro* and in cells [16,24,25]. Like SMC5, SMC6, NSE1, NSE3, and NSE4, MMS21 is an essential gene in fission and budding yeast [16,24]. Intriguingly, budding and fission yeast in which the SP-RING domain of MMS21 is mutated to abolish its SUMO ligase activity are viable, but remain hypersensitive to DNA damaging agents [16,24]. These results suggest that the SUMO ligase activity of MMS21 is not required for its essential function, but is required for a proper DNA damage response. It is unclear what may be the essential function of MMS21 that does not require its SUMO ligase activity.

So far only a relatively small number of sumoylation substrates have been identified for MMS21 (Table 1 and discussed herein). Several components of the SMC5/6 complex are targets of MMS21, including MMS21 itself, SMC5, SMC6, NSE3, and NSE4 [16,24,25,33]. Of these substrates, only the sumoylation of SMC6 and NSE4 has been shown to be upregulated upon MMS-induced DNA damage [24,33]. Notably, NSE4 sumoylation is not induced by other DNA damaging agents, such as hydroxyurea (HU) and gamma-irradiation (IR) [33]. The SUMO attachment sites on these SMC5/6 complex components have yet to be identified; therefore the functional significance of sumoylation is unknown. Unlike other E3 SUMO ligases, such as the PIAS family, RANBP2, and PC2, sumoylation induced by MMS21 does not typically result in increased mono- or di-sumoylation [24,25,34–39]. Instead, MMS21-induced sumoylation in cells often results in high molecular weight smears, indicating poly-sumoylation and/or multiple sumoylation attachment sites [24,25,37,38]. It remains to be determined whether this difference between MMS21 and other E3 SUMO ligases is functionally relevant and if other components in the SMC5/6 complex contribute to this difference.

4. Localization of the SMC5/6 complex on chromatin

The SMC5/6 complex is associated with chromatin in budding and fission yeast, *Xenopus laevis* egg extracts, and human cells [15,33,40–42]. Loading of the SMC5/6 complex onto chromatin is likely coupled with replication [33,41,42]. Detailed analysis of the genome-wide localization of the SMC5/6 complex in budding and fission yeast has been performed using chromatin immunoprecipitation (ChIP) followed by hybridization to DNA tiling arrays (ChIP-on-chip) [33,41]. These studies have found several intriguing commonalities and differences between the localization of the SMC5/6 complex in budding and fission yeast. First, the SMC5/6 complex appears to localize throughout chromosomes in fission yeast, whereas it is enriched at intergenic regions in budding yeast [33,41]. Additionally, in budding yeast the relative abundance (binding sites per kilobase of DNA) of the SMC5/6 complex is significantly increased as chromosome size increases [41]. Second, the SMC5/6 complex is localized to centromeres and is required for proper centromere separation during anaphase in both budding and fission yeast [33,41]. However, in budding yeast the complex showed maximal occupancy at centromeres in G2/M-phase of the cell cycle, whereas in fission yeast maximal occupancy occurred in S-phase during replication [33,41]. One potential reason for this discrepancy is the lack of centromeric heterochromatin in budding yeast as compared to fission yeast [43]. Consistent with this hypothesis, centromeric localization of the SMC5/6 complex is abolished in fission yeast defective for the H3K9 methyltransferase CLR4 or the chromodomain-containing protein SWI6, which are both required for heterochromatin establishment [21,44]. Third, the SMC5/6 complex is enriched at rDNA repeats in both budding and fission yeast [41,44,45]. It has been hypothesized that the SMC5/6 complex is enriched at rDNA due to the difficulty in replicating this repetitive region (see below for detailed discussion) [46]. In support of this hypothesis, treatment of fission yeast with HU, which induces replication stress and S-phase arrest, results in increased localization of the SMC5/6 complex to rDNA [33]. However, in budding yeast HU treatment diminishes the localization of the SMC5/6 complex to rDNA [41]. The reason for this difference is unclear, but is consistent with the fact that the complex shows maximal centromere localization in G2/M-phase in budding yeast and S-phase in fission yeast [33,41] Fourth, it was reported in fission yeast, but not budding yeast, that the SMC5/6 complex is enriched at all tDNAs in a TFIIIC and transcription-dependent manner [33]. This localization is similar to that of the related SMC2/4 condensin complex, which is localized to tDNAs in budding and fission yeast [47]. The enrichment of the SMC5/6 complex at tDNAs probably reflects the role of HR in repairing abundant replication fork stalls created by the collision of RNA polymerase III transcriptional and DNA replication fork machineries [48,49]. Finally, the SMC5/6 complex localizes to the telomeric region of chromosome ends in both budding and fission yeast [33,41,45]. In fission yeast, this localization is significantly enhanced by MMS treatment and is dependent on the SUMO ligase activity of MMS21 (see below for detailed discussion) [33]. These studies suggest that the SMC5/6 complex is generally enriched at genomic loci prone to replication fork stalling and collapse.

5. The SMC5/6 complex promotes DSB repair

Components of the SMC5/6 complex were originally identified in a 1970s genetic screen for radiation sensitive mutations in fission yeast [50]. Hypomorphic alleles of any of its components result in hypersensitivity to a broad spectrum of DNA damaging agents, such as ionizing radiation, ultraviolet radiation, methylmethane sulfonate (MMS), mitomycin C, and HU [8,10,11,51,52]. In fission yeast, SMC6 is required for IR-induced DSB repair [53]. Inhibition of the SMC5/6 complex does not enhance the sensitivity of hypomorphic *rad51* alleles in fission yeast, suggesting that the SMC5/6 complex functions in HR-mediated DNA damage repair [10,12,51,54]. Inhibition of the SMC5/6 complex in fission yeast, plants, and humans results in sister chromatid HR defects [37,55,56]. Additionally, hypomorphic alleles of the SMC5/6 complex in budding yeast display an increased number of translocation class, gross chromosomal rearrangements, suggesting that the complex is required for genome maintenance and stability [55,57]. Similar results have been observed in budding yeast with defective MMS21 SUMO ligase activity, suggesting that the SUMO ligase activity is required for genome maintenance and stability in budding yeast [57]. The relevant sumoylation targets of MMS21 for this activity are unknown.

The role of the SMC5/6 complex in HR is further supported by studies in yeast and human cells using chromatin immunoprecipitation. The SMC5/6 complex has been shown to be recruited to HO-induced DSBs in budding yeast and I-SceI-induced DSBs in humans [37,41,55]. Consistent with its function in HR, the complex does not localize to HO-induced DSBs in budding yeast cells that cannot utilize HR to repair DSBs due to the absence of sister chromatids (i.e. in the G1-phase of the cell cycle) [41,55]. Additionally, human cells depleted of the SMC5/6 complex by RNA interference exhibit decreased DNA repair capacity in G2/M-phase, but not in G1-phase [37]. The mechanisms controlling the localization of the SMC5/6 complex to DSBs are not precisely known. In budding yeast, localization of the SMC5/6 complex to HO-induced DSBs requires MRE11, a component of the MRE11/RAD50/NBS1 complex that is essential in recognizing and defining DSBs [41].

The mechanisms by which the SMC5/6 complex facilitates HR-mediated DNA repair at DSBs are not entirely known. In undamaged budding yeast, the SMC5/6 complex localization partially overlaps with the chromosomal localization of the SMC1/3 cohesin complex [41]. Interestingly, the localization of the SMC5/6 complex is altered in budding yeast defective for the cohesin subunit SCC1, and its chromatin loader SCC2 [41]. These results suggest the potential for crosstalk between the SMC5/6 and SMC1/3 complexes. One possible function of the SMC5/6 complex in HR is to facilitate sister chromatid cohesion at DSBs to allow proper RAD51-mediated strand invasion and exchange (Fig. 3). The importance of the SMC5/6 complex in holding sister chromatids in close proximity in response to DNA damage has been shown in budding yeast, where the SMC5/6 complex is required for generation of global cohesion between sister chromatids after DNA damage (Fig. 3) [58]. One mechanism by which the SMC5/6 complex could promote sister chromatid cohesion after DNA damage is through recruitment and/or maintenance of cohesin at DSBs (Fig. 3A). In human cells, but potentially not in budding yeast, depletion of the SMC5/6 complex results in diminished localization of the SMC1/3 cohesin complex to I-SceI-induced DSBs [37,58]. Interestingly, MMS21 stimulates the sumoylation of two cohesin complex components, SCC1 and SA2 [37]. Whether these sumoylation events are required for recruitment and/or maintenance of cohesin at DSBs is currently under investigation. In support of a specific role of the SMC5/6 complex in sister chromatid HR, the human SMC5/6 complex is not required for RAD51-mediated episomal HR [37]. Therefore, the repair of DSBs by sister chromatid HR may require the crosstalk between the related SMC1/3 cohesin and SMC5/6 complexes.

6. The SMC5/6 complex promotes the restart of collapsed replication forks

In addition to its role in repairing IR-induced or endonuclease-induced DSBs, the SMC5/6 complex has been shown to be important for repair of collapsed replication forks. During DNA replication, replication forks stall at sites of DNA damage [59]. If the replication fork cannot proceed, it will eventually collapse, potentially generating a DSB [60]. Previous studies suggest that one mechanism by which collapsed replication forks can be restarted is through HR [61]. Hypomorphic alleles of multiple components of the SMC5/6 complex in fission yeast are hypersensitive to replication arrest [12,13,40,53,62]. However, inhibition of HR by deletion of *RAD51* can partially rescue these defects [40,62]. These results suggest that the SMC5/6 complex may have a role downstream of RAD51, or alternatively, that the SMC5/6 complex may be required for proper RAD51-dependent strand invasion and exchange (Fig. 3) [46]. Consistent with this idea, budding yeast defective in the SUMO ligase activity of MMS21 show an increased number of collapsed replication forks after MMS treatment [63]. The relevant targets of MMS21-induced sumoylation that prevent replication fork collapse or promote replication fork restart are not known. In addition, overexpression of the bacterial resolvase RusA rescues the hypersensitization of *nse5* and *nse6* mutants to replication arrest [13], and overexpression of the BRCT domain-containing protein BRC1

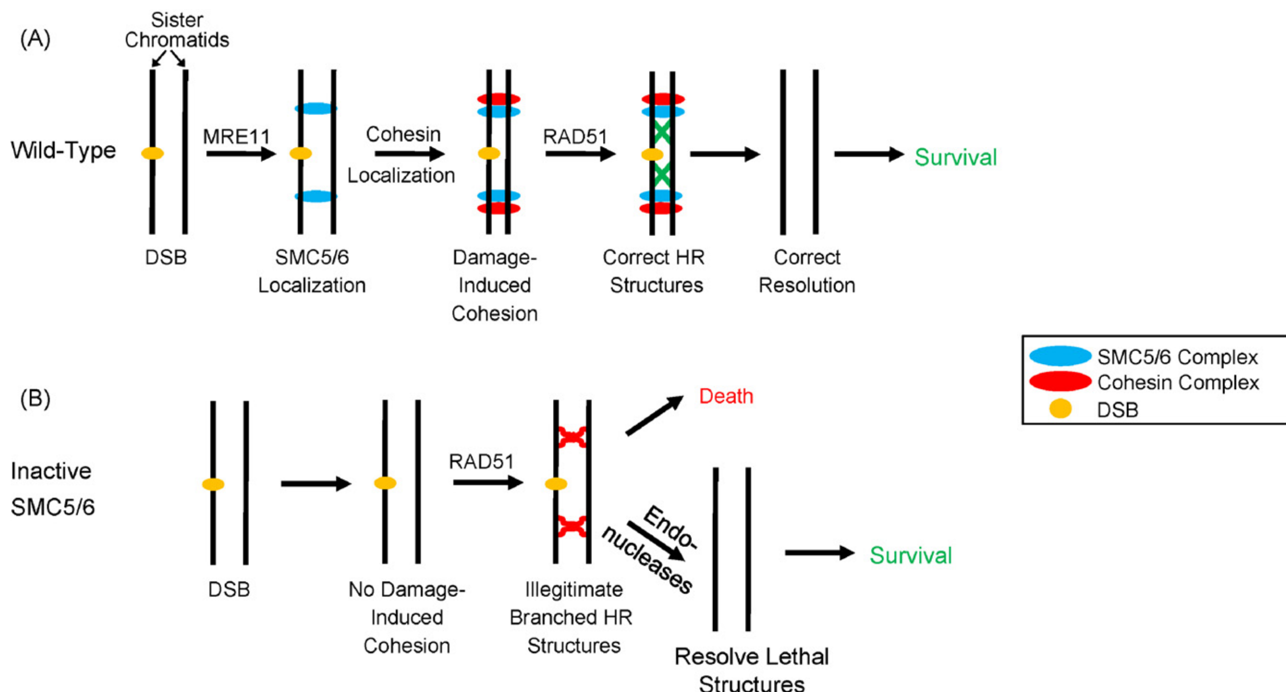

Fig. 3. Model for the function of the SMC5/6 complex in homologous recombination. (A) The SMC5/6 complex localizes to DSBs in an MRE11-dependent manner. The SMC5/6 complex facilitates the localization of the SMC1/3 cohesin complex and establishes sister chromatid cohesion at DSBs. As a result, RAD51 strand invasion and exchange occur correctly to repair DSBs. (B) In the absence of the SMC5/6 complex, the SMC1/3 cohesin complex does not localize to DSBs, and no sister chromatid cohesion is generated. In addition or as a consequence, illegitimate branched DNA structures accumulate in a RAD51-dependent manner. These structures result in cell death due to chromosomal missegregation. *smc6* hypomorphic yeast cells can be rescued by the activation of structure-specific endonucleases to resolve lethal HR intermediates.

rescues fission yeast *smc6-74* alleles through the structure-specific endonucleases SLX1/4 and MUS81/EME1 (Fig. 3B) [64,65]. Finally, the SMC5/6 complex localizes to collapsed replication forks in budding yeast [41]. Therefore, it is likely that the complex has a poorly defined role in preventing the accumulation of lethal recombination structures. It is unknown whether this function of the SMC5/6 complex is separate from its role in establishing cohesion at DSBs.

7. The SMC5/6 complex maintains rDNA integrity

Replication fork collapse can also occur when DNA replication and transcriptional machineries collide [66]. Because these collapsed DNA replication forks are restarted by HR, they are particularly dangerous in repetitive DNA elements, such as rDNA, that can be expanded and contracted by unequal sister chromatid HR [67]. To decrease the frequency of replication fork collapse, rDNA is replicated unidirectionally to decrease the chances of collisions between transcriptional and replication machineries [68]. Interestingly, the SMC5/6 complex localizes to rDNA, and mutation of the SMC5/6 complex results in defective rDNA segregation during mitosis [41,45]. In addition, the SMC5/6 complex localizes to nucleoli, the nuclear structures containing rDNA, and nucleolar localization increases upon replication stress [40,45]. Depletion of SMC5 or inhibition of MMS21 SUMO ligase activity results in budding yeast with fragmented and irregular shaped nucleoli [16,69]. The relevant sumoylation targets of MMS21 for maintaining proper nucleolar structure are unknown. Consistent with a role of the SMC5/6 complex in facilitating collapsed replication fork restart in rDNA, *smc5/6* mutants display increased rDNA instability and increased number of HR protein foci in nucleoli [69,70]. These results suggest that the SMC5/6 complex is required for preventing or repairing collapsed replication forks in rDNA. However, the SMC5/6 complex has an essential function outside of the nucleolus, because the budding yeast temperature sensitive allele *smc6-9* cannot be rescued by replacing the rDNA array with a plasmid expressing the ribosomal genes [45]. Therefore, rDNA appears to be especially sensitive to the loss of the SMC5/6 complex due to the increased tendency for replication fork collapses resulting from its repetitive nature.

8. The SMC5/6 complex and telomeres

In addition to rDNA, telomeres are a major repetitive component of the genome. Telomeres are proteinaceous, repetitive DNA elements, involving the repeat sequence TTAGGG in humans, which comprise 5–15 kilobases at the ends of chromosomes [71]. They form loops, known as T-loops, which prevent the ends of chromosomes from being recognized as DSBs [72]. Telomeres are shortened after every division due to the end-replication problem of the lagging strand [73]. Critically short telomeres result in cellular senescence [74]. Therefore, telomeres have been suggested to function as a counting mechanism for cellular proliferation [74]. The majority of cancer cells overcome this limited proliferative potential by the transcriptional upregulation of telomerase, the reverse transcriptase that elongates telomeres [75]. However, some cancer cells are incapable of upregulating telomerase and rely on an alternative mechanism to elongate telomeres known as ALT (alternative lengthening of telomeres) [76]. ALT facilitates telomere elongation by promoting telomere recombination [77]. One hallmark of ALT cells that is speculated to be required for telomere elongation is the recruitment of telomeres into nuclear PML (promyelocytic leukemia) bodies [78]. The recruitment of telomeres to these specialized PML bodies, known as APBs (ALT-associated PML bodies), is thought to facilitate HR by bringing telomeres and HR proteins together. RAD51, RAD52, BRCA1, RAD9, MRE11, NBS1, BLM, and WRN have all been shown to localize to APBs in ALT cells [78–82]. Consistent with the role of the SMC5/6 complex in HR, SMC5, SMC6, and the SUMO ligase MMS21 also localize to APBs in ALT cells [38]. Knockdown of the SMC5/6 complex results in diminished telomere recombination, shortening of telomeres, and increased senescence in ALT cells [38]. One mechanism by which the SMC5/6 complex promotes telomere HR and elongation in ALT cells is by MMS21-induced sumoylation of the Shelterin/Telosome complex (Fig. 4) [38]. The Shelterin/Telosome complex consists of six core proteins, TRF1, TRF2, TIN2, TPP1, RAP1, and POT1, which bind to telomeric DNA and regulate its structure and accessibility [83]. Of these six components, sumoylation of TRF1, TRF2, TIN2, and RAP1 is enhanced by MMS21 [38]. Based on previous studies, sumoylation of the Shelterin/Telosome complex could promote the relocalization of telomeres to PML bodies due to the high affinity of proteins within PML bodies for SUMO (Fig. 4) [84]. In support of this model, inhibition of MMS21-induced sumoylation of the Shelterin/Telosome complex blocks recruitment of telomeres to APBs [38]. Whether the SMC5/6 complex also plays additional roles in telomere HR besides facilitating telomere recruitment to PML bodies is currently under investigation.

In addition to its role in telomere recombination in human ALT cells, the SMC5/6 complex has also been shown to localize to telomeres in budding and fission yeast [33,41,45]. Interestingly, MMS treatment of fission yeast enhances the localization of the SMC5/6 complex to telomeres in a MMS21 SUMO ligase dependent manner [33]. The relevant MMS21 SUMO substrates for enhanced localization of the SMC5/6 complex to telomeres are not precisely known. Intriguingly, treatment of fission yeast with MMS significantly enhances the sumoylation of both SMC6 and NSE4 in a manner dependent on the SUMO ligase activity of MMS21 [24,33]. These results suggest that MMS21-induced sumoylation of the SMC5/6 complex could stimulate recruitment of the complex to telomeres in telomerase-positive fission yeast. How these sumoylation events would target the SMC5/6 complex to telomeres is entirely unknown.

Although sumoylation is important for telomere length regulation in telomerase-positive fission yeast [85], the SUMO ligase MMS21 does not significantly affect telomere length in telomerase-positive budding or fission yeast or human cells [16,38,85]. In contrast, inhibition of MMS21 SUMO ligase activity in budding yeast results in impaired clustering of telomeres into telomeric bouquets during meiosis [16]. The clustering of telomeres during meiosis is an event that is widely conserved among fungi, plants, and animals [86]. When cells enter meiosis, telomeres move to and attach to the nuclear envelope [87]. Inhibition of telomere clustering delays the pairing of homologues, decreases recombination between homologues, and increases ectopic recombination in budding and fission yeast [88,89]. Thus, telomere bouquet formation has been proposed to facilitate pairing of homologous chromosomes to allow for meiotic recombination. The precise function of the MMS21 SUMO ligase in this process is unknown. Interestingly, the human meiosis-specific cohesin component SMC1β also plays a role in telomere attachment to the nuclear envelope in meiosis [90]. It will be interesting to determine whether MMS21 regulates the function of the cohesin complex in this process as it does in DSB repair. Therefore, the SMC5/6 complex has multiple roles at telomeres. It is required for telomere elongation by HR in human ALT cells, telomere clustering in budding yeast, and possibly maintaining the stability and/or restarting DNA replication forks at telomeres in fission yeast.

9. Summary and perspective

In summary, the SMC5/6 complex has been intimately tied to HR. Mutation of the SMC5/6 complex results in the hypersensitization of cells to a broad spectrum of DNA damaging agents. The SMC5/6

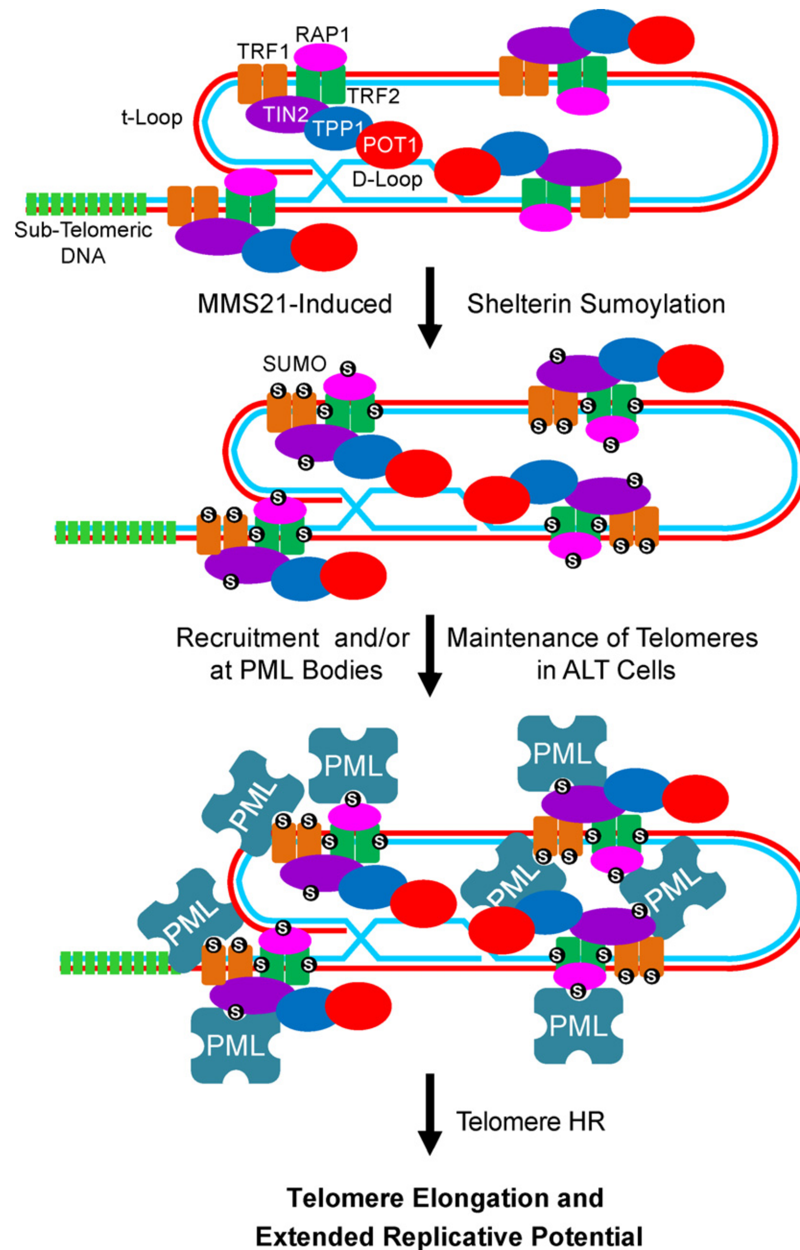

Fig. 4. Model for the role of MMS21 in recruitment/maintenance of telomeres at PML bodies in ALT cells. The Shelterin/Telosome complex, consisting of TRF1, TRF2, TIN2, TPP1, POT1, and RAP1, binds telomeric DNA to protect chromosome ends from being recognized as DSBs. The SUMO ligase MMS21 stimulates the sumoylation of TRF1, TRF2, TIN2, and RAP1. Sumoylation of the Shelterin/Telosome complex promotes recruitment and/or maintenance of telomeres at PML bodies (labeled PML) in ALT cells, possibly due to the high affinity of PML and/or other components of PML bodies for SUMO. The localization of telomeres to PML bodies in ALT cells promotes telomere elongation by HR.

complex localizes to endonuclease-induced DSBs and promotes sister chromatid cohesion. In addition, it localizes to collapsed replication forks to promote fork restart through HR. Consistently, the SMC5/6 complex is enriched at multiple repetitive DNA regions of the genome which are prone to replication fork collapse. In its absence, cells accumulate branched DNA structures generated by aberrant HR. Therefore, the SMC5/6 complex is important for maintaining genomic integrity and preventing potential oncogenic mutations or translocations. On the other hand, the SMC5/6 complex has recently been implicated in the elongation of telomeres by HR in a subset of cancer cells. As a result, the SMC5/6 complex extends the replication potential of ALT cancer cells. Therefore, the SMC5/6 complex has both Yin and Yang characteristics (Fig. 5).

The major challenge for the future will be to determine the precise mechanisms by which the SMC5/6 complex facilitates HR. To do so, we will need a better understanding of the components of the SMC5/6 complex and how they are regulated. Specifically, we will need to determine the relevant targets of the MMS21 SUMO ligase and how the sumoylation of these proteins contributes to proper HR. We will also need to determine the function of the other NSE proteins in the complex, specifically NSE1, NSE3, NSE5, and NSE6. Finally, structural studies on the architecture of the complex will yield valuable information regarding its potential role at DSBs. Through these approaches we will gain fruitful insight into the mechanisms of homologous recombination and genomic stability.

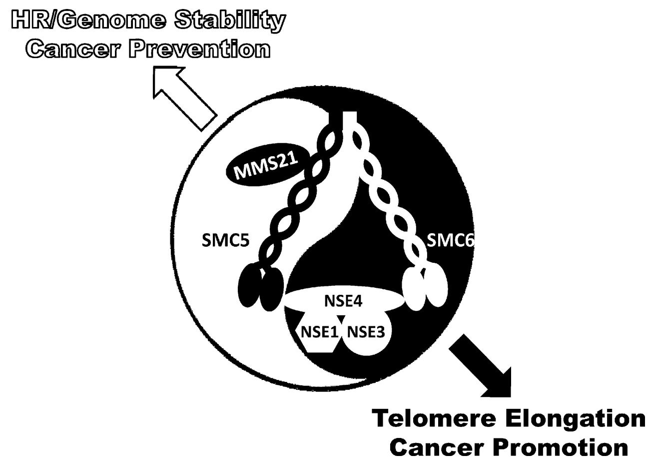

Fig. 5. The SMC5/6 complex has both Yin and Yang characteristics. The SMC5/6 complex, depicted as a Yin–Yang symbol, promotes genome stability through HR and therefore protects against cancer. However, the SMC5/6 complex also promotes telomere elongation by HR in ALT cancer cells and therefore extends the replicative potential of ALT cancer cells. The NSE5–NSE6 components of the complex are omitted for aesthetic purposes.

Conflict of interest statement

The author declares that there are no conflicts of interest.

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