C-Terminal HA Tags Compromise Function and Exacerbate Phenotypes of Saccharomyces cerevisiae Bloom’s Helicase Homolog Sgs1 SUMOylation-Associated Mutants

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ABSTRACT The Sgs1 helicase and Top3-Rmi1 decatenase form a complex that affects homologous recombination outcomes during the mitotic cell cycle and during meiosis. Previous studies have reported that Sgs1-Top3-Rmi1 function is regulated by SUMOylation that is catalyzed by the Smc5-Smc6-Mms21 complex. These studies used strains in which SGS1 was C-terminally tagged with three or six copies of a human influenza hemagglutinin-derived epitope tag (3HA and 6HA). They identified SGS1 mutants that affect its SUMOylation, which we will refer to as SGS1 SUMO-site mutants. In previous work, these mutants showed phenotypes consistent with substantial loss of Sgs1-Top3-Rmi1 function during the mitotic cell cycle. We find that the reported phenotypes are largely due to the presence of the HA epitope tags. Untagged SGS1 SUMO-site mutants show either wild-type or weak hypomorphic phenotypes, depending on the assay. These phenotypes are exacerbated by both 6HA and 3HA epitope tags in two different S. cerevisiae strain backgrounds. Importantly, a C-terminal 6HA tag confers strong hypomorphic or null phenotypes on an otherwise wild-type Sgs1 protein. Taken together, these results suggest that the HA epitope tags used in previous studies seriously compromise Sgs1 function. Furthermore, they raise the possibilities either that sufficient SUMOylation of the Sgs1-Top3-Rmi1 complex might still occur in the SUMO-site mutants isolated, or that Smc5-Smc6-Mms21-mediated SUMOylation plays a minor role in the regulation of Sgs1-Top3-Rmi1 during recombination.

DNA double strand breaks (DSBs) present a major threat to genome integrity and, if repaired incorrectly, can lead to a loss of genetic information. Cells have developed multiple mechanisms to repair these lesions, and the safest repair process is homologous recombination (HR). During HR, a broken DNA strand invades a homologous chromosome and uses it as a repair template. Repair can occur with or without exchange of chromosome arms, producing crossover (CO) or noncrossover (NCO) recombinants, respectively (Kowalczykowski 2015). During the mitotic cell cycle, cells repress CO formation and favor the NCO outcome (Larocque et al. 2011; Symington et al. 2014). During meiosis, cells use HR to promote homologous chromosome alignment and segregation during the first nuclear division. This requires the formation of regulated crossover products by only a subset of the initiating DSBs (Hunter 2015; Zickler and Kleckner 2015; Lam et al. 2017). A group of meiosis-specific and biochemically diverse factors, referred to collectively as the ZMM proteins, collaborate to stabilize strand invasion intermediates and to promote formation of double Holliday junctions (dHJs). ZMM-promoted dHJs are resolved predominantly as crossovers by the action of the Mlh1-Mlh3-Exo1 (MutLγ) complex (Fung et al. 2004; Snowden et al. 2004; Lynn et al. 2007; De Muyt et al. 2012; Zakharyevich et al. 2012; Hunter 2015).
The Sgs1-Top3-Rmi1 (STR) helicase-decatenase complex and its homologs are central regulators of recombination product formation during both the mitotic and meiotic cell cycles (Ira et al. 2003; Jessop et al. 2006; Oh et al. 2007; Jessop and Lichten 2008; Oh et al. 2008; Laroque et al. 2011; De Muyt et al. 2012; Zakharyevich et al. 2012; Hunter 2015; Kaur et al. 2015; Tang et al. 2015). STR and homologs are thought to promote NCO formation by unwinding strand invasion intermediates in a process known as synthesis dependent strand annealing (SDSA, Cejka and Kowalczykowski 2010; Fasching et al. 2015). STR and its homologs can also disassemble dHJs and form NCOs in a process known as dissolution (Wu et al. 2005; Cejka and Kowalczykowski 2010; Dayani et al. 2011; Kaur et al. 2019). In addition, the Top3-Rmi1 subcomplex has an Sgs1-independent role in the resolution of recombination intermediates (Kaur et al. 2015; Tang et al. 2015). During meiosis, the D-loop disassembly activity of the STR complex is hypothesized to lead to recycling of early strand invasion intermediates, which can promote NCO formation or promote recombination intermediate stabilization by the ZMM proteins and subsequent resolution as COs (Jessop et al. 2006; De Muyt et al. 2012; Zakharyevich et al. 2012; Hatkevich and Sekelsky 2017).

Two recent studies have proposed a mechanism for STR complex activity regulation by the Smc5-Smc6-Mms21 complex (Bermúdez-López et al. 2016; Bonner et al. 2016). The Smc5-Smc6-Mms21 complex is a member of the SMC (Structural Maintenance of Chromosomes) family with structural similarities to cohesin and condensin, and is important in chromosome transactions such as DNA replication and repair. The Smc5-Smc6-Mms21 complex is unique among SMC complexes because it contains an essential subunit, Nse2/Mms21 (referred to as Mms21 here), with an SP-RING domain in its C-terminus that contains E3 SUMO ligase activity (Andrews et al. 2005; Potts and Yu 2005; Zhao and Blobel 2005; Aragón 2018). In budding yeast, mutants lacking this E3 SUMO ligase activity are viable but are highly sensitive to DNA damage (Zhao and Blobel 2005). The two studies of SUMO-mediated STR regulation referred to above (Bermúdez-López et al. 2016; Bonner et al. 2016) suggested that DNA lesions promote Mms21-mediated SUMOylation of Smc5-Smc6-Mms21 components, which then act as a platform to recruit STR through Sgs1's SUMO Interaction Motifs (SIMs). This Sgs1-Smc5 interaction is then suggested to result in Mms21-mediated modification of STR components, which in turn promotes STR activity during homologous recombination (Bermúdez-López et al. 2016; Bonner et al. 2016). In these studies, which used either a 6HA (Bermúdez-López et al. 2016) or a 3HA (Bonner et al. 2016) epitope tag at the Sgs1 C-terminus, Sgs1 was found to be SUMOylated at 6 lysines, with lysine 621 (K621) being the major site. Lysine to arginine mutations at either K621 or at all 6 lysines substantially reduced MMS-induced SUMOylation without affecting Sgs1-Smc5 interaction (Bermúdez-López et al. 2016; Bonner et al. 2016). The two groups mutated different sets of residues in Sgs1 SIMs, but both sets of mutants blocked Sgs1-Smc5 interaction while leaving Sgs1-Top3 interaction intact (Bermúdez-López et al. 2016; Bonner et al. 2016).

Work in human cells has also revealed a role for SUMOylation of the Sgs1 homolog, BLM, in rescuing of stalled replication forks (Ouyang et al. 2009). This study proposed that SUMOylation of BLM relieves the inhibition of RAD51 at a stalled replication fork, and allows for homologous recombination to proceed. Furthermore, this activity was found to be dependent on the NSMCE2 protein, the human homolog of Mms21 (Pond et al. 2019). These studies suggest that SUMOylation of BLM/Sgs1 may be evolutionarily conserved. However, the nature of the contribution of this modification to BLM/Sgs1 activity and the different contexts in which this modification is required are not well understood.

The SUMO ligase activity of Mms21 is required for the Smc5-Smc6-Mms21 complex's role in destabilizing aberrant intermediates formed during the early stages of meiotic recombination (Xaver et al. 2013), and many of the meiotic phenotypes of Smc5-Smc6-Mms21 mutants closely resemble those of mutants lacking STR activities (Jessop et al. 2006; Oh et al. 2007; Jessop and Lichten 2008; Oh et al. 2008; De Muyt et al. 2012; Copsay et al. 2013; Lilienhal et al. 2013; Xaver et al. 2013; Kaur et al. 2015; Tang et al. 2015). However, it is not known if the requirement for Mms21 SUMO E3 ligase activity reflects SUMOylation of the STR complex or of other proteins involved in recombination.

We wished to test the hypothesis that the defects of mms21 mutants can be attributed to an absence of STR SUMO modification. We reasoned that mutations in Sgs1 that block its SUMOylation should not alter STR recruitment by Smc5-Smc6-Mms21 or modification of Top3 and Rmi1, whereas mutations in Sgs1 SIMs would prevent STR recruitment and thus abolish modification of all of the STR components. Therefore, we recreated lysine to arginine mutants at either the major SUMOylation site (sgs1-K621R) or all 6 lysines previously identified as being SUMOylated (hereafter called sgs1-6KR, referred to by Bermúdez-López et al. 2016 as sgs1-3KR), and also mutated the residues within the SIMs previously mutated by the Zhao (Bonner et al. 2016) and Aragon (Bermúdez-López et al. 2016) groups (referred to as sgs1-ZSIM and sgs1-ASIM, respectively; see Figure 1A for details of all mutants). We integrated these mutants, which we will refer to collectively as SGS1 SUMO-site mutants, at the endogenous SGS1 locus, either with or without a C-terminal epitope tag, and examined the resulting strains for both mitotic and meiotic STR function.

Our findings suggest that the previously reported phenotypes of the SGS1 SUMO-site mutants are largely due to the presence of C-terminal HA epitope tags. In particular, a 6HA tag rendered an otherwise wild-type SGS1 gene nonfunctional, and SUMO-site mutant strains lacking an epitope tag showed only mildly hypomorphic phenotypes with regards to MMS sensitivity and synthetic interactions with slx4 mutation. We also find that untagged SUMO-site mutant strains do not show the meiotic viability defects and the ZMM mutant bypass phenotypes displayed by sgs1 loss-of-function mutants, nor do they display the synthetic nuclear division defects that sgs1 loss-of-function mutants do when combined with mms4 mutants. These results point to a role for the Sgs1 C-terminus in STR function. They also raise the possibilities that SUMOylation may not play an essential role in regulation of the STR complex, and that regulation of recombination by the E3 SUMO ligase function of Mms21 may operate through targets other than the STR complex.

MATERIALS AND METHODS

Yeast strains

Strains used in this study are S. cerevisiae of SK1 (Kane and Roth 1974) or W303 (Bonner et al. 2016) backgrounds (Table S1). Strains were constructed by transformation or genetic crosses. SGS1 alleles were made by transforming an sgs1A strain (deleted for all SGS1 coding sequences) with an allele replacement fragment that contains, in the following order: 500 nt immediately upstream of the SGS1 start codon; the entire SGS1 coding sequence; 175 nt immediately downstream of the SGS1 stop codon; a hygMX cassette (Jankc et al. 2004); and the next 361 bp downstream of SGS1 (Figure 1B). In some cases, a C-terminal tag was included. Mutant alleles were inserted into a plasmid with this fragment by Gibson assembly, and
were confirmed by sequencing. The 3HA tag in the W303 strains was removed by transformation with a PCR product containing the last 890 bp of SGS1 through TEF terminator sequences in the hygMX cassette from the wild-type allelic replacement plasmid (Figure 1C). Yeast transformants were confirmed by Southern blotting to ensure proper integration, and PCR fragments from transformants were sequenced to confirm 3’ end and terminator structures. 

**Sporulation and spore viability**

Diploid strains were grown in pre-sporulation media and sporulated as previously described (Goyon and Lichten 1993; Börner and Cha 2015). For spore viability analysis, tetrads were collected after 24 h of sporulation, and at least 70 tetrads per genotype were dissected. For experiments examining synthetic interactions, haploids were mated to diploids, and at least 70 tetrads per genotype were dissected. For spore viability analysis, tetrads were collected after 24 h of sporulation, and at least 70 tetrads per genotype were dissected.

**Cytology**

Nuclear divisions were monitored by DAPI staining of cells from liquid sporulations as previously described (Kaur et al. 2018). At least 200 cells were scored per time point.

**MMS and HU sensitivity assays**

Cells were grown overnight in YPD liquid media (Kaur et al. 2018). After adjusting cell concentrations to the same OD$_{600}$, cells were diluted in a 10-fold series, spotted on YPD plates containing the indicated drug concentrations, incubated at 30°C for 2-3 days, and imaged.

**Statistical analysis**

GraphPad Prism was used for comparisons of spore viability using Fisher’s exact test, applying the Bonferroni correction for multiple comparisons.

**Data availability**

All strains and plasmids are available upon request without restrictions. Supplementary files are available on Figshare: Table S1 contains genotypes of strains; File S1 contains underlying data for spore viability and DAPI progression in Figure 3; Figure S1 shows MMS sensitivity of SGS1 SUMO-site mutant alleles from Bonner et al. (2016); Figure S2 shows MMS sensitivity of SGS1 SUMO-site mutant alleles from Bonner et al. (2016). Supplemental material available at figshare: https://doi.org/10.25387/g3.12181998.

**RESULTS**

**Validation of the allelic replacement strategy**

As part of a project investigating whether the meiotic defects of Smc5-Smc6-Mms21 complex mutants can be attributed to a loss of STR complex recruitment or modification, we analyzed several SGS1 mutants that were characterized in two recent studies of Sgs1 SUMOylation (Figure 1A). These two studies used SGS1 alleles with C-terminal epitope tags and non-native terminators (Bermúdez-López et al. 2016; Bonner et al. 2016). To avoid potential complications of these non-native configurations, all mutant alleles used in our study, as well as a wild-type control, were generated as allelic replacements that retain the native SGS1 promoter and terminator (Figure 1B and Materials and Methods). sgs1A mutants are sensitive to DNA damaging agents, such as MMS and HU, and exhibit synthetic lethality with sds4Δ (Figure 2A), nor was it sensitive to MMS or hydroxyurea (HU) (Figure 2C), indicating that this allelic replacement strategy did not provide a selective advantage.
not abrogate SGS1 function (Mullen et al. 2001; Ui et al. 2001). Using this approach, we recreated the SGS1 SUMO-site mutants and set out to analyze whether these mutants recapitulate previously reported STR or Smc5-Smc6-Mms21 mutant phenotypes.

Phenotypes of sgs1 mutants are exacerbated by a C-terminal HA tag

We first sought to reproduce some of the SGS1 SUMO-site mutant phenotypes previously reported (Bermúdez-López et al. 2016; Bonner et al. 2016). We found that the C-terminal 6HA tag used by Bermúdez-López et al. (Bermúdez-López et al. 2016) seriously compromised mitotic function of an otherwise wild-type SGS1 gene in strains of the SK1 background. sgs1-6HA strains displayed synthetic lethality with sde4Δ (Figure 2A), and also displayed MMS and HU sensitivity, although not to the same degree as an sgs1Δ mutant (Figure 2C). Conversely, when present in an untagged context, sgs1-ASIM (which mutates SIMs in Sgs1) and sgs1-6KR (which lacks six lysines that are substrates for SUMOylation) did not show synthetic interaction with sde4Δ and were not sensitive to MMS or HU (Figure 2A and 2C). However, the addition of a 6HA tag to these two mutants increased MMS and HU sensitivity relative to the tagged wild-type gene (Figure 2C, Fig. S1). Thus, at least in the SK1 strain background, a C-terminal 6HA tag interferes with Sgs1 protein function and exacerbates SUMO-site mutant phenotypes.

We also found that the C-terminal 3HA tag used by Bonner et al. (Bonner et al. 2016) compromises Sgs1 function. Bonner et al. used W303 strains to examine mutants in the major Sgs1 SIMs (sgs1-ZSIM, Figure 1A) and a mutant lacking the major Sgs1 SUMOylation target lysine (sgs1-K621R, Figure 1A), all in the context of an SGS1 gene containing a C-terminal 3HA tag. They reported that sgs1-K621R-3HA exhibits synthetic sickness with sde4Δ, and that sgs1-ZSIM-3HA is synthetically lethal with sde4Δ. We examined the same mutants in the absence of an epitope tag in the SK1 background and observed no synthetic interaction between sgs1-K621R and sde4Δ, and reduced growth, but not lethality, when sgs1-ZSIM is combined with sde4Δ (Figure 2A). Furthermore, unlike the 3HA-tagged W303 mutants, the untagged mutants in SK1 displayed wild-type sensitivity to MMS and HU (Figure 2D, Fig. S2A). These results further suggest that SGS1 function is, at most, only partially affected by the SGS1 SUMO-site mutants.

To test whether these phenotypic differences could be attributed to strain background differences, we removed the 3HA tag from the original W303 strains (Bonner et al. 2016) and restored the native SGS1 terminator (Figure 1C and Materials and Methods). When tested for synthetic interactions with sde4Δ, these untagged W303 strains behaved identically to the untagged SK1 strains, while 3HA-tagged strains recapitulated previously published phenotypes (Figure 2B). In addition, the 3HA-tagged SGS1 SUMO-site mutants, but not SGS1-3HA, displayed a modest increase in MMS sensitivity, while the untagged mutants were no more MMS-sensitive than wild type (Figure 2E). These results indicate that both 3HA and 6HA C-terminal tags interfere with normal Sgs1 function, and that many of the phenotypes reported for SGS1 SUMO-site mutants are due, in large part, to the presence of the epitope tag. We therefore performed all further analyses in SK1 strains lacking C-terminal tags.

SGS1 SUMO-site mutants do not recapitulate the meiotic defects of mms21 SUMO ligase-defective or sgs1 loss-of-function mutants

Similar meiotic phenotypes are observed in sgs1 loss-of-function mutants and SUMO ligase-defective mms21-11 mutants, including reduced spore viability, partial suppression of some zmm mutant phenotypes, and recombination and nuclear division defects when combined with mutants lacking the Mus81-Mms4 nuclease (Rockmill et al. 2003; Jessop and Lichten 2008; Oh et al. 2008; De Muyt et al. 2012; Zakharyevich et al. 2012; Xaver et al. 2013; Kaur et al. 2015; Tang et al. 2015). We first examined spore viability as a test of whether or not SGS1 SUMO-site mutants show similar defects. As a control, we used sgs1-md (pCLB2- SGSI), in which Sgs1 is expressed during vegetative growth but is progressively depleted from the cells...
during meiosis (Lee and Amon 2003; Jessop and Lichten 2008; Oh et al. 2008). sgs1-md strains display a modest reduction in spore viability (90% compared to 98% in the wild-type and 97% in SGS1-WT allelic replacement, P < 0.0001; ≥ 70 tetrads per genotype analyzed), similar to the defect observed in mms21-11 mutants (89%; (Xaver et al. 2013). None of the untagged SGS1 SUMO-site mutants showed reduced spore viability, while a strain with a 6HA-tagged wild-type SGS1 (sgs1-6HA) showed reduced spore viability, to a greater extent than did sgs1-md (76% compared to 90% for sgs1-md, P < 0.0001) (Figure 3A). We do not understand the basis of the more severe phenotype of sgs1-6HA. It could be due to pre-meiotic defects that arise during vegetative growth; alternatively, it is possible that the 6HA tag creates an unproductive STR complex that sequesters Top3Rm1 and prevents it from performing its Sgs1-independent functions in joint molecule resolution (Kaur et al. 2015; Tang et al. 2015).

sgs1 and mms21 loss-of-function mutants partially suppress several meiotic phenotypes of zmm mutants, which have defects in the stabilization of regulated DHJ intermediates and their resolution as crossovers (Rockmill et al. 2003; Börner et al. 2004; Jessop et al. 2006; Lynn et al. 2007; Oh et al. 2007; De Muyt et al. 2012; Zakharyevich et al. 2012; Xaver et al. 2013). We asked if SGS1 SUMO-site mutants suppress the spore inviability phenotype of strains lacking the ZMM protein Msh4 (Figure 3B, Supplementary File 1). sgs1-6HA restored spore viability to msh4Δ to the same extent as sgs1-md (77% for msh4Δ sgs1-md; 80% for msh4Δ sgs1-6HA, compared to 30% for msh4Δ SGS1-WT). In contrast, most untagged SGS1 SUMO-site mutants did not significantly increase msh4Δ spore viability. The only exception was sgs1-ZSIM, which showed partial suppression of the msh4Δ spore viability defect, but not to the same extent as sgs1-md (52% for msh4Δ sgs1-ZSIM, 77% for msh4Δ sgs1-md). The partial suppression seen with untagged sgs1-ZSIM is consistent with the synthetic slow growth phenotype of sgs1-ZSIM sxs4Δ double mutants, and suggests that this mutation confers a greater loss-of-function than the other SGS1 SUMO-site mutants.

To more stringently test meiotic Sgs1 function, we examined genetic interactions between SGS1 SUMO-site mutants and mms4-md (pCLB2-MMS4), which does not express Mms4 during meiosis (De Muyt et al. 2012). When STR activity is absent, efficient meiotic recombination intermediate resolution requires Mus81-Mms4. Importantly, sgs1 mus81 and sgs1 mms4-md double mutants display nuclear division failure caused by unresolved recombination intermediates (Jessop and Lichten 2008; Oh et al. 2008; De Muyt et al. 2012; Zakharyevich et al. 2012). The same phenotype is observed in mms4-md mms21-11 double mutants (Xaver et al. 2013), so we asked if any of the SGS1 SUMO-site mutants recapitulate this synthetic interaction (Figure 3C). As expected, sgs1-md mms4-md double mutants displayed a complete block to nuclear divisions, as did sgs1-6HA mms4-md double mutants, consistent with the C-terminal HA tag disrupting SGS1 meiotic function. mms4-md single mutants displayed a slight delay in nuclear division relative to wild-type, and strains where mms4-md was combined with any of the untagged SGS1 SUMO-site mutants displayed a further 1-hour delay (Figure 3C). The delayed nuclear divisions in mms4-md SGS1 SUMO-site double mutants suggests that an increased fraction of recombination intermediates utilize the non-ZMM resolution pathway in these strains, consistent with the SUMO-site mutants conferring a partial loss of SGS1 meiotic function.

**DISCUSSION**

This report shows that the presence of a C-terminal HA tag is largely responsible for the previously reported phenotypes of SGS1 mutants proposed to influence Mms21-mediated modification of the STR complex (Bermúdez-López et al. 2016; Bonner et al. 2016). We found that both 3HA and 6HA C-terminal tags on Sgs1 sensitize cells to MMS and HU and exacerbate SUMO-site mutant phenotypes; this effect was observed in two different *S. cerevisiae* genetic backgrounds. It is likely that these effects were missed in previous studies because tagged and untagged mutant alleles were not compared. For example, in W303 strains, an SGS1-3HA construct did not display a synthetic
interaction with slx4Δ or increased sensitivity to either MMS or HU (Bonner et al. 2016). However, we found that the presence of a 3HA tag causes, for both sgs1Δ-K621R-3HA and sgs1Δ-ZSIM-3HA, a more severe synthetic interaction with slx4Δ and increased MMS sensitivity than when the tag is absent (Figure 2). We also find that, in SK1 strains, a C-terminal 6HA tag on 1 otherwise wild-type SGS1 causes complete synthetic lethality with slx4Δ and sensitivity to both MMS and HU (Figure 2); this allele also causes reduced spore viability, suppresses the reduced spore viability of msh4Δ, and blocks meiotic nuclear division when combined with mms4-md (Figure 3). These phenotypes are suggestive of substantial loss of Sgs1 function. It therefore appears that a C-terminal HA tag can compromise Sgs1 function, but this compromised function may not be detected until the HA tag is combined with other mutants or until the strain is sufficiently challenged by other means.

While we do not know the exact nature of the defects caused by C-terminal HA tags, our findings suggest that the Sgs1 C-terminus may have an important biological function. The Sgs1 C-terminus contains a region predicted to be highly unstructured. This region follows immediately after the conserved HRDC (Helicase-and-RNaseD-like-C-terminal) domain, which is important for substrate binding and regulation of helicase activity (Liu et al. 1999; Yankiwski et al. 2001; Hickson 2003; Wu et al. 2005; Chu and Hickson 2009; Vindigni and Hickson 2009; Harami et al. 2017). The HA epitope has a net negative charge, and could conceivably interfere with Sgs1-DNA interaction, especially in the HRDC domain. Thus, it is possible that the tag interferes with the biochemical function of this HRDC domain and/or the disordered C-terminus, thereby compromising Sgs1 function during D-loop disruption or D/HJ dissolution. This would result in the persistence of DNA structures whose resolution requires endonucleases such as Mus81-Mms4 and Slx1-Slx4, which in turn could account for the synthetic lethality observed in sgs1Δ-HA slx4Δ strains and the meiotic nuclear division failure observed in sgs1Δ-HA mms4-md diploids.

The absence of strong mitotic or meiotic defects in untagged SGS1 SUMO-site mutants prompted us to examine genetic interactions of these alleles with mutants in genes known to confer synthetic phenotypes when combined with sgs1 mutants. While sgs1 loss of function suppresses the spore inviability of msh4Δ (Jessop et al. 2006), of the four SUMO-site mutants examined, only sgs1Δ-ZSIM significantly suppressed msh4Δ, and this suppression was partial (Figure 3B). In a similar vein, while meiotic Sgs1 depletion (in sgs1-md) led to a complete block to nuclear division in mms4-md mutants, the SGS1 SUMO-site mutants delayed division by 1h (Figure 3C), suggesting that these mutants confer, at most, a partial defect in STR complex activity.

The suggestion that SUMO-site mutants only slightly impinge upon STR complex function could be explained in a number of ways. For example, if the SUMO-site mutations studied do substantially reduce STR complex SUMOylation, as was suggested in previous studies (Bermúdez-López et al. 2016; Bonner et al. 2016), it is possible STR activity is regulated by factors or post-translational modifications other than Mms21-mediated SUMOylation. In this regard, it is worth noting a recent study reporting that phosphorylation regulates Sgs1 helicase activity during both meiosis and the mitotic cell cycle (Grigaitis et al. 2020).

Alternatively, it is possible that, in the absence of epitope tags, the SUMO-site mutants we studied only partially disrupt Mms21-mediated SUMOylation of the STR complex, either because alternative SUMOylation sites (Klug et al. 2013; Pichler et al. 2017) or because other SUMO ligases (Klug et al. 2013; Hendriks and Vertegaal 2016; Pichler et al. 2017) come into use. Thorough testing of these possibilities will require isolation of antibodies to the native Sgs1 protein, identification of epitope tags that do not disrupt Sgs1 function, or proteomic analyses of STR complex SUMO modification in mutant strains. Finally, since SGS1 SUMO-site mutants did not recapitulate, in any of the assays used, the phenotypes of SUMO E3 ligase-null mms21Δ mutants, it is possible that Mms21 modification of proteins other than the STR complex is required for proper recombination and repair during mitosis and meiosis. In this regard, it will be of interest to determine the full set of factors that Mms21 acts through to exert its functions. We note that a recent analysis of the yeast meiotic SUMO proteome found SUMO conjugated to many recombination-associated proteins, including several subunits of the Smc5-Smc6-Mms21 complex (Bhgawat et al. 2019).

A recent report (Zapata et al. 2019) has proposed that Mms21-mediated SUMOylation of Smc5 is involved in error-free bypass of damaged replication forks. It is worth noting that, in this study, mutations in SMC5 thought to interfere with its SUMOylation did not have strong phenotypes, but exhibited synthetic chromosome segregation defects when combined with mms4Δ. This finding is reminiscent of our finding that meiotic nuclear divisions are delayed in mms4-md SGS1 SUMO-site double mutants. (Figure 3C).

In summary, our data show that the phenotypes of mutants thought to affect the STR interaction with and modification by the Smc5-Smc6-Mms21 complex are exacerbated by a C-terminal tag on SGS1 and reveal a possible role for the C-terminus of Sgs1 in recombination. These finding reinforce the importance of testing genetic interactions of mutants without a protein tag to ensure that the effects seen are not due to an exacerbating effect caused by the tag, but rather represent true biological function.

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