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Sgs1 Truncations Induce Genome Rearrangements but Suppress Detrimental Effects of BLM Overexpression in *Saccharomyces cerevisiae*

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RecQ-like DNA helicases are conserved from bacteria to humans. They perform functions in the maintenance of genome stability, and their mutation is associated with cancer predisposition and premature aging syndromes in humans. Here, a series of C-terminal deletions and point mutations of Sgs1, the only RecQ-like helicase in yeast, show that the Helicase/RNase D C-terminal domain and the Rad51 interaction domain are dispensable for Sgs1's role in suppressing genome instability, whereas the zinc-binding domain and the helicase domain are required. BLM expression from the native *SGS1* promoter had no adverse effects on cell growth and was unable to complement any *sgs1*Δ defects. BLM overexpression, however, significantly increased the rate of accumulating gross-chromosomal rearrangements in a dosage-dependent manner and greatly exacerbated sensitivity to DNA-damaging agents. Co-expressing *sgs1* truncations of up to 900 residues, lacking all known functional domains of Sgs1, suppressed the hydroxyurea sensitivity of BLM-overexpressing cells, suggesting a functional relationship between Sgs1 and BLM. Protein disorder prediction analysis of Sgs1 and BLM was used to produce a functional Sgs1–BLM chimera by replacing the N-terminus of BLM with the disordered N-terminus of Sgs1. The functionality of this chimera suggests that it is the disordered N-terminus, a site of protein binding and posttranslational modification, that confers species specificity to these two RecQ-like proteins.

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Introduction

RecQ-like DNA helicases, named after the DNA repair protein RecQ of *Escherichia coli*,[1–3] are evolutionarily highly conserved. These 3′- to 5′-helicases function at the interface between DNA replication and recombination to maintain genome integrity. Sgs1 is the only known member of this helicase family in *Saccharomyces cerevisiae*.[4] Sgs1-deficient cells show increased sensitivity to the DNA-damaging agents hydroxyurea (HU) and methyl methanesulfonate (MMS), missegregate chromosomes, accumulate gross-chromosomal rearrangements (GCRs),

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Abbreviations used: HRDC, Helicase/RNase D C-terminal; GCR, gross-chromosomal rearrangement; HU, hydroxyurea; MMS, methyl methanesulfonate; RQC, RecQ helicase conserved; BS, Bloom's syndrome; HR, homologous recombination; TCA, trichloroacetic acid; ssDNA, single-stranded DNA; YPD, yeast–peptone–dextrose; YP, yeast–peptone.

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and have a shortened life span.[5–8] In contrast, five RecQ-like helicases (RecQL1, BLM, WRN, RecQL4, and RecQL5) are known in humans, and mutations in the *BLM*, *WRN*, and *RECQL4* genes are associated with the rare, cancer-prone Bloom's syndrome (BS), Werner syndrome, and Rothmund–Thompson syndrome, respectively.[9–13] All RecQ-like helicases share a seven-motif helicase domain with Walker A and DEAH motifs. The RecQ-helicase-conserved (RQC) domain, located C-terminal to the helicase domain, is thought to be involved in DNA binding and conferring specificity of binding to DNA structures, such as G4 tetrads.[14–17] The Helicase/RNase D C-terminal (HRDC) domain is the most C-terminal of the conserved domains and resembles domains in other proteins that are involved in nucleic acid metabolism, such as RNase D and UvrD; however, similar to the RQC domain, it is not found in all RecQ-like helicases.[18,19] The HRDC domain has been implicated in binding and resolving DNA structures, such as Holliday junctions, and in mediating protein–protein interactions.[18,20–23] Two acidic regions have also been identified N-terminal of the helicase domain and may be involved in mediating protein–protein interactions.[10,24,25] Sgs1 is found in a complex with Top3 and Rmi1, and there is also evidence of physical interactions of the N-terminal half of Sgs1 with Top2, Srs2, and Rad16 and interactions of the C-terminus with Mlh1 and Rad51.[26–31]

Defects in BLM, the human RecQ helicase considered to be most closely related to Sgs1, cause BS, an autosomal recessive disorder characterized by chromosome gaps and breaks, elevated sister chromatid exchange, mitotic hyper-recombination, and aberrant DNA replication events.[32–34] Affected individuals suffer from a high incidence and wide variety of cancers, infertility, and dwarfism (reviewed in Ref. 33). BLM catalyzes ATP-dependent 3′- to 5′-DNA unwinding, with a preference for DNA structures that may arise spontaneously during DNA replication or as a result of homologous recombination (HR).[35] For example, by unwinding unusual secondary DNA structures, BLM may aid replication fork progression, prevent illegitimate recombination during replication, and assist in restarting stalled forks.[36–39] Evidence supporting a role of BLM in maintaining genome integrity has been accumulating. For example, BLM-defective cells exhibit a retarded rate of strand elongation during DNA replication,[40] accumulate abnormal replication intermediates,[41] and are hypersensitive to agents that impair DNA replication.[42] BLM physically interacts with several proteins that play important roles during DNA replication and repair, such as replication protein A, flap endonuclease 1, chromatin assembly factor 1, the mismatch repair protein Mlh1, HR factor Rad51, and topoisomerase III α.[43–49] BLM peaks in S phase and it localizes to replication foci, most likely through its physical interaction with a subunit of DNA polymerase δ.[50–54]

Here, we have determined the role of C-terminal domains and protein interaction sites of Sgs1 in suppressing GCR accumulation by expressing point mutants and truncations of Sgs1, lacking as few as 20 residues and as many as 1428 residues.'s Human *BLM* cDNA was expressed under control of the native *SGS1* promoter and overexpressed from a galactose-inducible promoter to investigate BLM's ability to complement *sgs1*Δ defects (such as increased genome instability and sensitivity to HU and MMS), revealing that BLM could suppress *sgs1*Δ defects neither in haploid nor in diploid cells. However, using computational protein disorder prediction tools, we have designed a yeast–human chimera that consists of two nonfunctional segments of BLM and Sgs1. The ability of this chimera to suppress all *sgs1*Δ defects that we tested suggests a functional relationship between BLM and Sgs1, which is also supported by our finding that short N-terminal fragments of Sgs1, which are devoid of all known functional domains for helicase activity and DNA binding, suppress severely detrimental effects of BLM overexpression in yeast.

Results

Requirement of the RQC domain of Sgs1, but not the HRDC domain, for GCR suppression

Sgs1 contains a conserved DEAH helicase domain, a conserved HRDC domain, two acidic regions (AR1 and AR2), and an RQC domain composed of zinc-binding and winged-helix domains. Several protein interaction sites have also been located in the 1447-amino-acid-long protein (Fig. 1a). To determine the role of these domains in the maintenance of genome stability, we generated systematic deletions to the 3′-end of the chromosomal *SGS1* gene, such that truncations of the C-terminus of Sgs1, ranging from 20 to 1428 amino acids, were expressed as fusions to a myc epitope. Truncations of up to 80 amino acids were constructed to not affect any known functional domain of Sgs1, while Δ*C100* and Δ*C200* deletions partially and completely removed the HRDC domain and Δ*C300* and Δ*C400* deletions partially and completely removed the RQC domain, respectively. The largest deletions (Δ*C700*, Δ*C800*, Δ*C900*, Δ*C1000*, and Δ*C1100*) eliminate the entire helicase domain, including the Walker A motif (residues 803-812), with the Δ*C800*–Δ*C1100* deletions also affecting the part of the N-terminal half of Sgs1 that contains protein interaction sites (e.g., Rad16, residues 421–792; Top2, residues 432–724; and Srs2, residues 422–722) and two acidic regions (AR1, residues 321–447; AR2, residues 502–648), whereas

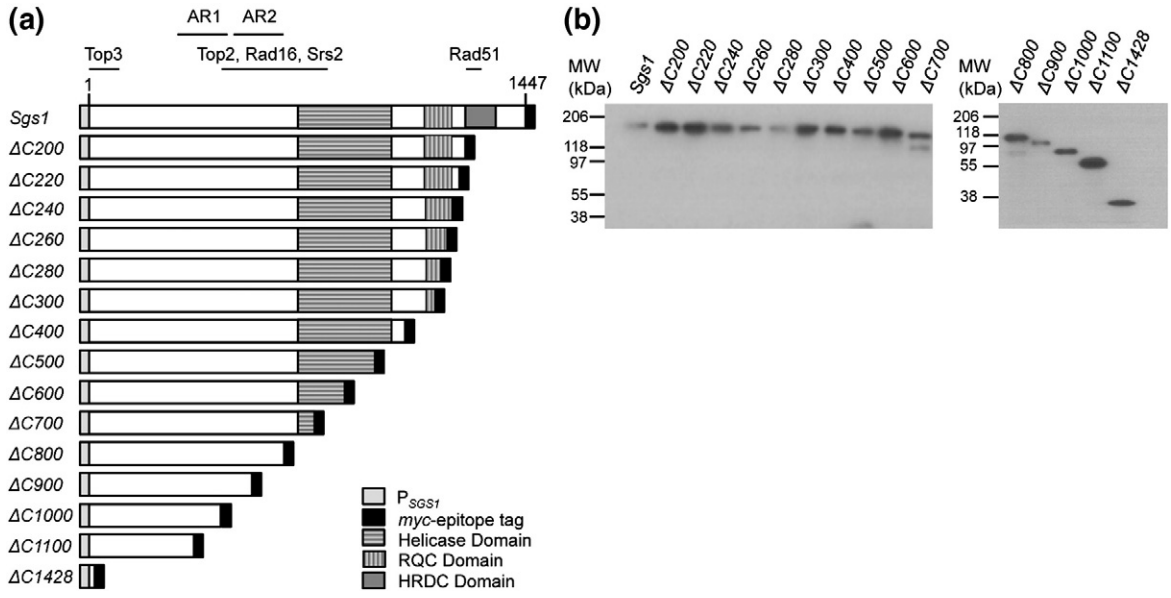


Fig. 1. C-terminal truncations of Sgs1 used in this study. (a) Full-length Sgs1 contains a DEAH helicase domain, an RQC domain, and an HRDC domain in its C-terminal half and acidic regions AR1 and AR2 in its N-terminal half; interaction sites with Top3, Top2, Srs2, Rad51, and Rad16 are indicated. C-terminal truncations ranging in size from 200 residues to 1428 residues were constructed by fusion to a myc epitope tag. All truncations were introduced at the endogenous *SGS1* locus on chromosome VIII. (b) Expression of wild-type Sgs1 and truncation alleles from the endogenous *SGS1* promoter (P_{SGS1}) was confirmed by Western blotting, using a myc antibody. Molecular weights (MW) are indicated on the left.

Δ*C500* and Δ*C600* deletions partially remove the helicase domain while leaving the Walker A motif intact (Fig. 1a). All truncation alleles were stably expressed from the chromosomal *SGS1* locus under control of the native *SGS1* promoter (Fig. 1b). C-terminal fusion to the myc epitope did not adversely affect Sgs1 function, as indicated by equal sensitivity to HU and MMS of strains expressing tagged and untagged Sgs1 (wild type) (Fig. 2a). The largest deletion, leaving intact only the 19 N-terminal amino acids of Sgs1 (*sgs1*Δ*C1428*), was as sensitive to HU and MMS as a complete *SGS1* deletion (*sgs1*Δ), thus behaving similar to a null allele (Fig. 2a). Loss of up to 200 C-terminal amino acids did not increase sensitivity to HU or MMS, whereas loss of 300 or more amino acids led to sensitivity similar to that of the *sgs1*Δ*C1428* and *sgs1*Δ mutants (Fig. 2a). The construction of additional 20-amino-acid truncations extended the C-terminal region that is dispensable for HU/MMS resistance to 240 amino acids (Fig. 2b).

It was previously shown that cells lacking the DNA helicase Srs2 (*srs2*Δ) depend on functional Sgs1 for their viability.[55] To assess the ability of *sgs1* truncation alleles to support growth of the *srs2*Δ mutant, we constructed diploid strains heterozygous for the *srs2*Δ deletion and heterozygous for the *sgs1*Δ*C200*, *sgs1*Δ*C260*, or *sgs1*Δ*C300* allele. The meiotic products of the sporulated diploids were spread on nonselective, rich media [yeast–peptone–dextrose (YPD)], allowing all spores to grow (Fig. 2c). Diploids heterozygous for the *srs2*Δ deletion and for the *sgs1*Δ*C200* truncation yielded spores that grew into colonies of the same size, suggesting that the 200 C-terminal amino acid residues of Sgs1, which harbor the HRDC domain and an interaction site with the HR factor Rad51, are not required for the viability of the *srs2*Δ mutant. In contrast, sporulation of diploids heterozygous for the *srs2*Δ deletion and *sgs1*Δ*C260* or *sgs1*Δ*C300* allele yielded mixtures of normal-sized and small colonies. Genotyping revealed that the small colonies were *srs2*Δ *sgs1*Δ*C260* or *srs2*Δ *sgs1*Δ*C300* mutants, whereas the normal-sized colonies corresponded to wild-type spores or single mutants. Thus, Sgs1 that lacks 260 or more C-terminal residues and therefore does not contain a complete RQC domain cannot support normal growth of cells lacking Srs2.

When we tested the effect of the C-terminal deletions on the accumulation of GCRs, we found that 240 C-terminal amino acids were dispensable for maintaining genome integrity, whereas deleting as little as an additional 20 amino acids (*sgs1*Δ*C260*) caused the GCR rate to increase to that exhibited by the null mutant without a discernable intermediate phenotype (Table 1). Combining the *sgs1*Δ*C300* truncation allele with a deletion of the DNA-damage checkpoint sensor *MEC3* led to a synergistic GCR rate increase, while, as expected, combining the *sgs1*Δ*C200* allele with a *mec3*Δ mutation did not.

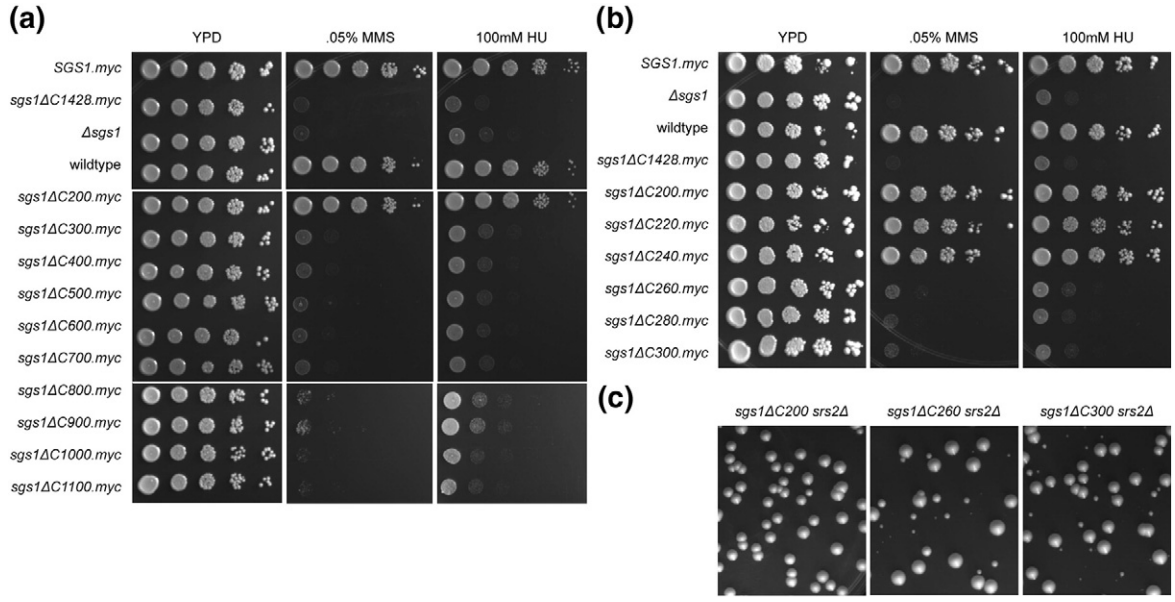


Fig. 2. Sensitivity of cells expressing Sgs1 truncation alleles to the DNA-damaging agents HU and MMS. Tenfold dilutions of exponentially growing cultures ($OD_{600}=0.5$) were spotted on YPD for viable cell count and on YPD containing 100 mM HU or 0.05% MMS, followed by incubation at 30 °C. (a) Haploid cells expressing *sgs1* alleles lacking 300 or more residues from the C-terminus are as sensitive to HU and MMS as the null allele. (b) Additional incremental 20-amino-acid deletions reveal that cells expressing *sgs1* alleles lacking up to 240 residues are as resistant to HU and MMS as wild-type cells, whereas those lacking 260 or more residues are as sensitive as the *sgs1*Δ mutant. (c) Spores from diploids heterozygous for an *srs2*Δ deletion and heterozygous for the *sgs1*Δ*C200*, *sgs1*Δ*C260*, or *sgs1*Δ*C300* were spread on YPD to allow for growth of spores of all possible genotypes. Similar-sized colonies obtained from the spores of the diploid heterozygous for *sgs1*Δ*C200* and *srs2*Δ mutations (left) indicate that the *sgs1*Δ*C200 srs2*Δ mutant and the single mutants grow, suggesting that deletion of 200 C-terminal amino acid residues does not negatively affect the growth of the *srs2*Δ mutant. In contrast, spores from diploids heterozygous for the *srs2*Δ mutation and the *sgs1*Δ*C260* allele (middle) or the *sgs1*Δ*C300* allele (right) grew into a mixture of normal-sized colonies (corresponding to single mutants and wild type) and small-sized colonies (corresponding to *srs2*Δ *sgs1*Δ*C260* or *srs2*Δ *sgs1*Δ*C300* mutant as determined by genotyping), demonstrating that an intact RQC domain in Sgs1 is required for the viability of the *srs2*Δ mutant.

Thus, these findings show that the HRDC domain and the previously reported C-terminal interaction with Rad51 are not required for Sgs1's role in preventing the accumulation of GCRs and supporting normal growth of the *srs2*Δ mutant, whereas the integrity of the RQC domain, which has been suggested to span residues 1075–1207 based on the alignment of three-dimensional structures,[57] is essential.

BS-associated RQC domain mutations cause loss of Sgs1 function *in vivo*

Of the 32 exonic base substitutions that are causative of BS, 13 are missense mutations,[9,13,58,59] with 6 of these mutations affecting conserved residues that have been shown *in vitro* to participate in zinc binding and G-tetrad DNA binding activity (Fig. 3a). Studies, however, have been limited to biochemical and biophysical analyses of mutant proteins and were hampered by the inability to purify some mutant BLM proteins.[16,17,61] Since the cysteine residues are highly conserved between RecQ-like helicases, including Sgs1, we replaced the corresponding cysteine residue in Sgs1 with the BS-associated mutation (*sgs1*-C1047F). Unlike BLM with mutations in any of the three conserved cysteine residues C1036, C1063, or C1066, which degraded upon purification and could therefore not be characterized,[61] the *sgs1-C1047F* mutant allele was stably expressed *in vivo* from the native *SGS1* locus (Fig. 3b). The *sgs1-C1047F* mutant showed an increased HU and MMS sensitivity, which, however, did not reach the level of the *sgs1*Δ allele, and exhibited levels of GCR accumulation comparable to the *sgs1*Δ mutant, demonstrating that the C1047F mutation severely impairs Sgs1 function (Fig. 3c; Table 1). In addition to conserved cysteine residues and immediately adjoining arginine (R1037) and aspartic acid (D1064) residues, ClustalW2 alignments showed F1056 to be the only other fully conserved amino acid residue in the zinc-binding domain of Sgs1 (Fig. 3a). Although the corresponding residue in BLM (F1045) is not associated with a BS mutation, the BLM-F1045A mutation has been shown to cause a severe helicase defect and a single-stranded DNA (ssDNA) binding deficiency *in vitro*.[61] When we introduced the corresponding mutation into Sgs1

Table 1. Accumulation of GCRs in cells expressing mutant alleles of *SGS1*

Relevant Genotype[a]	GCR rate (Can^r 5-$FOA^r \times 10^{-10}$)	95% CI[b] (Can^r 5-$FOA^r \times 10^{-10}$)
Wild type	1.1	<1–6.2
sgs1Δ	251	80–310
sgs1ΔC200	7	<6–23
sgs1ΔC220	31	5–41
sgs1ΔC240	10	<6–27
sgs1ΔC260	159	85–362
sgs1ΔC280	244	166–387
sgs1ΔC300	145	76–204
sgs1ΔC400	106	60–180
sgs1ΔC500	102	53–252
sgs1ΔC600	152	26–283
sgs1ΔC700	189	49–271
sgs1ΔC800	133	71–225
sgs1ΔC1428	206	97–273
sgs1-C1047F	64	35–131
sgs1-F1056A	<16	<10–26
mec3Δsgs1ΔC200	11	<7–22
mec3Δsgs1ΔC300	1003	691–1500
mec3Δsgs1ΔC800	758	645–895
mec3Δsgs1ΔC800-blmΔN647[c]	361	330–419

[a] All *sgs1* truncations (*sgs1ΔC*) are C-terminally fused to a myc epitope tag.
[b] We calculated 95% confidence intervals (CIs) according to Nair.[56]
[c] The *sgs1ΔC800-blmΔN647* allele expresses a chimeric protein that consists of the 647 N-terminal residues of Sgs1 and the 770 C-terminal residues of human BLM.

(F1056A), however, the mutant was no more sensitive to HU and MMS than wild-type cells (Fig. 3c) but instead appeared fully functional with a wild-type GCR rate (Table 1).

Expression of human BLM cDNA from the endogenous *SGS1* promoter does not complement *sgs1Δ* defects

RecQ-like DNA helicases are evolutionarily conserved from bacteria to humans. Since cells from BS patients share defects seen in *sgs1Δ* cells, including increased sensitivity to DNA-damaging agents, increased levels of aberrant genetic exchange, and reduced life span, it has been suggested that RecQ-like DNA helicases from different phyla or even kingdoms might complement each other, thus allowing the development of simple model organisms for the functional and mutational characterization of disease-associated human RecQ-like helicases, such as BLM and WRN.[62] Thus, to assess the ability of BLM to suppress genome instability in the *sgs1Δ* mutant, BLM cDNA was inserted in-frame with the start codon of *SGS1* at its chromosomal locus ($P_{SGS1}BLM$). We reasoned that insertion at the wild-type *SGS1* locus would promote cell-cycle-dependent regulation of BLM expression and expression levels similar to those previously shown for Sgs1.[63] Stable expression of BLM was confirmed by Western blot analysis, using a yeast strain expressing myc-tagged BLM (Fig. 4a); however, all subsequent experiments were carried out with untagged BLM. Expression of a single copy of BLM ($P_{SGS1}BLM$) neither led to a statistically significant difference in the GCR rate compared to the *sgs1Δ* mutant (Tables 1 and 2) nor alleviated HU sensitivity (Fig. 4b), demonstrating that *BLM* can be successfully expressed in yeast under control of the native *SGS1* promoter without detrimental effects on cell growth, but is unable to complement the tested *sgs1Δ* defects to any extent.

Overexpression of BLM leads to increased sensitivity to DNA-damaging agents and rapid accumulation of GCRs

Since a single copy of *BLM* ($P_{SGS1}BLM$) did not complement *sgs1Δ* defects, we examined the effect of increasing BLM expression levels on *sgs1Δ* mutant phenotypes. For this purpose, the native *SGS1* promoter was replaced with a *GAL1* promoter and galactose-dependent expression of BLM was verified by fusing *BLM* to a myc epitope tag (Fig. 4a). Overexpression of BLM did not compensate for the lack of Sgs1 when cells were exposed to HU but instead led to a further increase in sensitivity to HU compared to the *sgs1ΔC1428* cells or cells expressing BLM under the *SGS1* promoter (Fig. 4b). We found that maximum induction of *BLM* expression led to a 1665-fold increase in the GCR rate compared to wild type and a 34-fold increase compared to the *sgs1Δ* mutant assayed under the same conditions (Table 2). In contrast, overexpression of Sgs1 from the *GAL1* promoter did not lead to GCR accumulation (Table 2). The GCR rate increase upon BLM overexpression was dependent on induction levels, with the GCR rate gradually decreasing to that of the *sgs1Δ* mutant as the galactose concentration in the media decreased (Table 2). Thus, *sgs1Δ* defects cannot be complemented by any level of BLM expression; in fact, increasing BLM expression levels induce higher sensitivity to DNA-damaging agents and significantly higher genome instability compared to that of the *sgs1Δ* mutant.

N-terminus of Sgs1 suppresses detrimental effects of BLM overexpression

Since Sgs1 is important for the suppression of illegitimate recombination between identical sequences, such as those found in related genes, on homologous chromosomes, and on sister chromatids, we tested the HU sensitivity of diploid strains expressing truncated *sgs1* alleles in the presence or in the absence of the *SGS1* wild-type allele (Fig. 5). HU sensitivity was fully suppressed for all alleles if a single copy of wild-type *SGS1* was expressed from the other allele (Fig. 5a), demonstrating that the *sgs1*

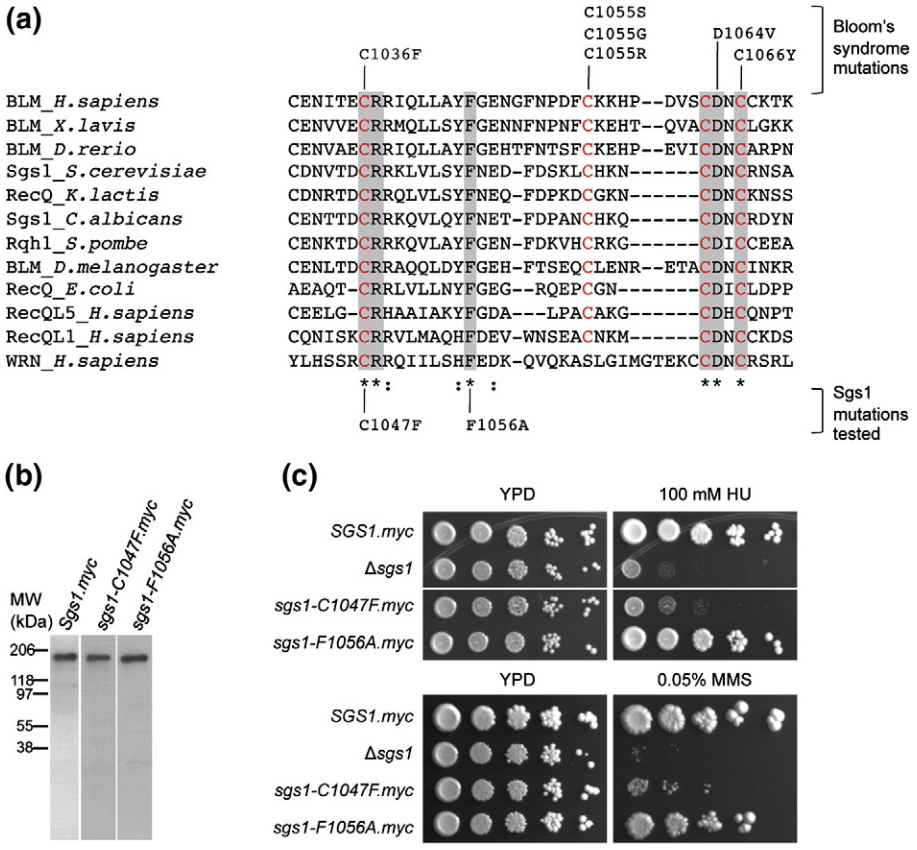


Fig. 3. Effect of zinc-binding domain mutations on Sgs1 function *in vivo*. (a) Zinc-binding domain is conserved from bacterial to human RecQ-like DNA helicases. Protein sequences were aligned with ClustalW2.[60] The alignment of RecQL1 was manually adjusted. Amino acid residues identical in all sequences are highlighted in gray and indicated by "∗" below the alignment, conserved substitutions are indicated by ":" below the alignment, and cysteine residues thought to be involved in zinc binding are shown in red. At least six different missense mutations in the zinc-binding domain are associated with BS. (b) C1047F and F1056A mutations were introduced into Sgs1, and expression was confirmed by Western blot using antibody against the C-terminal myc epitope. Molecular weights (MWs) are indicated on the left. (c) Mutation of the highly conserved F1056 does not impair Sgs1 function, whereas the C1047F mutation leads to an increase in sensitivity to HU and MMS but not to the level seen in the *sgs1*Δ mutant.

truncation alleles did not have a dominant effect. As in haploid cells, only the *sgs1*Δ*C200* allele complemented HU sensitivity of the *sgs1*Δ diploid completely (Fig. 5b); however, cells expressing the *sgs1*Δ*C300* to *sgs1*Δ*C900* alleles were less sensitive than diploids that expressed larger truncations or the *sgs1*Δ*C1428* null allele (Fig. 5b). This ability of *sgs1*Δ*300–sgs1*Δ*C900* truncation alleles to at least partially suppress HU sensitivity indicates that there may be N-terminal segments in Sgs1 that contribute to HU resistance.

Diploids expressing *BLM* from native *SGS1* promoters on both alleles were as sensitive to HU as diploids not expressing Sgs1, whereas diploids overexpressing BLM from one allele or from both alleles were severely HU sensitive, with the highest expression level lacking any growth on 100 mM HU (Fig. 5c), reflecting the severe HU sensitivity of haploid cells expressing the P_{GAL}*BLM* allele (Fig. 4b). Diploids overexpressing BLM also appeared to grow more slowly than any other diploid tested here (Fig. 5c). Remarkably, expression of a single copy of *SGS1* from its endogenous promoter (*SGS1*/P_{GAL}*BLM*) completely eliminated the severe HU sensitivity conferred by overexpression of BLM. To determine if full-length Sgs1 was required for this suppression, we crossed the haploid strain overexpressing BLM with haploids expressing various Sgs1 truncations. We found that a single copy of the *sgs1*Δ*C200* allele was as sufficient as wild-type Sgs1 in suppressing HU sensitivity and slow growth of the BLM-overexpressing strain, and as few as 547 N-terminal amino acids remaining in the *sgs1*Δ*C900* allele were sufficient for significant suppression of HU sensitivity and slow growth caused by BLM overexpression (Fig. 5c). These findings suggest that none of the known enzymatic activities or functional and conserved domains are required for suppressing the HU sensitivity of the BLM-overexpressing diploids but that 547 N-terminal amino acids are sufficient for

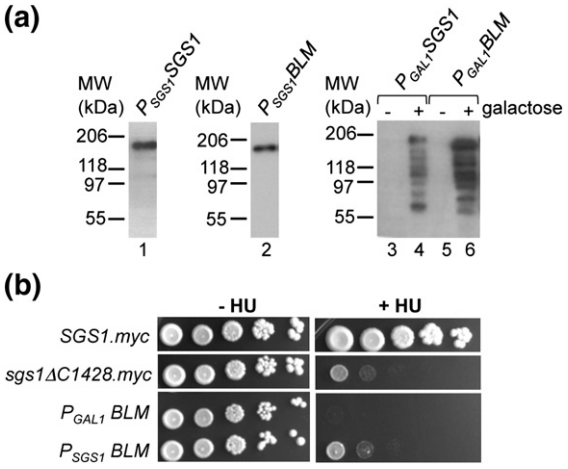


Fig. 4. BLM expression does not suppress *sgs1*Δ defects and BLM overexpression is detrimental to yeast cells. (a) Expression of myc-epitope-tagged Sgs1 (lane 1) and BLM (lane 2) from the native chromosomal *SGS1* locus or galactose-inducible overexpression of myc-epitope-tagged Sgs1 (lane 4) and BLM (lane 6) in yeast cells grown in YP supplemented with 1% sucrose and 1% galactose (to induce expression, lanes 4 and 6) or without galactose (lanes 3 and 5). Both BLM and Sgs1 show signs of degradation upon overexpression (lanes 4 and 6), whereas expression from the native *SGS1* promoter is stable (lanes 1 and 2). Molecular weights (MWs) are indicated on the left. (b) Cells expressing BLM from the *SGS1* promoter on chromosome VIII are as sensitive to HU as cells lacking Sgs1 (*sgs1*Δ). Replacement of the natural *SGS1* promoter with a galactose-inducible *GAL1* promoter induces BLM overexpression and leads to increased HU sensitivity. Tenfold dilutions of cells were spotted on media containing 1% sucrose and 1% galactose (to induce BLM overexpression) with and without 100 mM HU.

suppressing the detrimental effects of BLM overexpression in a diploid. That the *sgs1*Δ*C1000* and *sgs1*Δ*C1100* alleles were clearly less effective at suppressing HU sensitivity shows that 447 N-terminal amino acids, which contain the Top3 interaction site, are necessary but not sufficient for complementation.

Design of a functional Sgs1–BLM chimera

Sgs1 and BLM share about 21% of their amino acid residues in a pairwise alignment of the full-length proteins (ClustalW2), with most of the identical residues in the helicase domain. In fact, the N-terminal segment of Sgs1 expressed by the *sgs1*Δ*C800* allele, which is able to suppress the HU sensitivity of BLM-overexpressing diploids, shares only 11% with the corresponding N-terminal segment of BLM. Devoid of conserved domains and known enzymatic activities, the N-terminus of Sgs1 has been shown to be required for physical interactions with Top3, Top2, Srs2, and Rad16.[6,26–30] Using IUPred, an algorithm for the prediction of intrinsically disordered proteins, we found that the 650 N-terminal residues contain a similar distribution of ordered and intrinsically disordered segments (Fig. 6a and b). In disorder prediction algorithms, such as IUPred,[64,65] a score of >0.5 predicts a disordered amino acid residue and a score of <0.5 predicts an ordered residue, with 30 consecutive disordered amino acids commonly being used as a lower limit for detecting disorder in whole proteome searches.[64–67] The helicase domains of Sgs1 and of BLM coincide with the predicted ordered regions in both proteins, starting at around residue 648, and are surrounded by a long N-terminal and a short C-terminal segment, which contain mostly disordered residues. In fact, based on the IUPred output scores, 83% of the 648 N-terminal residues of Sgs1 (538/648) are disordered, with 70% of all 648 residues being located in segments of more than 30 consecutive disordered residues, whereas only 16% of the 800 C-terminal residues of Sgs1 are predicted to be disordered, with only a single disordered segment that is longer than 30 residues (residues 1396–1447). Based on the IUPred prediction, BLM can also be divided into a disordered N-terminus and an ordered C-terminus (Fig. 6a and b). For BLM, 52% of 648 N-terminal residues are predicted to be disordered, but only 15% of these residues are found in stretches of more than 30 disordered residues. The difference in the pattern of disorder predicted for the N-terminal segments of Sgs1 and BLM led us to hypothesize that this region may be involved in conferring species specificity to BLM and Sgs1 function and, thus, prevent BLM from

Table 2. Effect of BLM expression on GCR accumulation in the *sgs1*Δ mutant

Relevant genotype[a]	Galactose concentration in media (%)	GCR rate (Can^r 5-FOA^r $\times 10^{-10}$)	95% CI[b] (Can^r 5-FOA^r $\times 10^{-10}$)
Wild type	0	1.1	<1–6.2
P_{SGS1}*BLM*	0	70	56–151
P_{GAL}*BLM*	0	61	30–153
P_{GAL}*BLM*	0.1	335	233–576
P_{GAL}*BLM*	0.5	382	170–777
P_{GAL}*BLM*	2	1832	1090–2910
P_{GAL}*SGS1*	2	<11	<9–12
*sgs1*Δ	2	54	23–104

[a] Human *BLM* cDNA was inserted at the endogenous *SGS1* locus and fused to the native *SGS1* promoter (P_{SGS1}) or fused to a galactose-inducible promoter (P_{GAL}). In P_{GAL}*SGS1*, the native *SGS1* promoter region was disrupted by fusing the *SGS1* open reading frame to a galactose-inducible promoter. If strains expressing *BLM* or *SGS1* genes from the galactose-inducible GAL1 promoter were grown in less than 2% galactose (to lower protein expression levels), media were supplemented with sucrose to reach a total sugar concentration of 2%.

[b] We calculated 95% confidence intervals (CIs) according to Nair.[56]

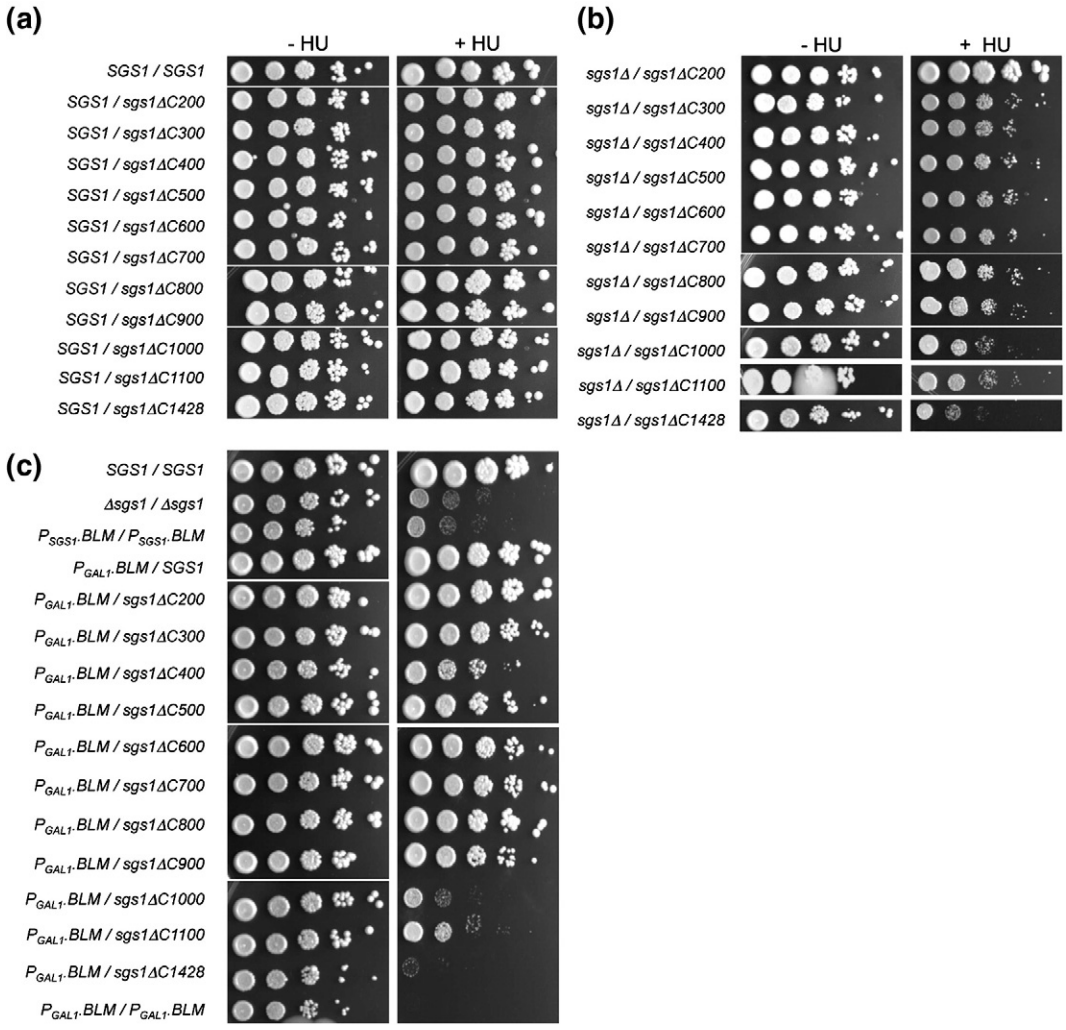
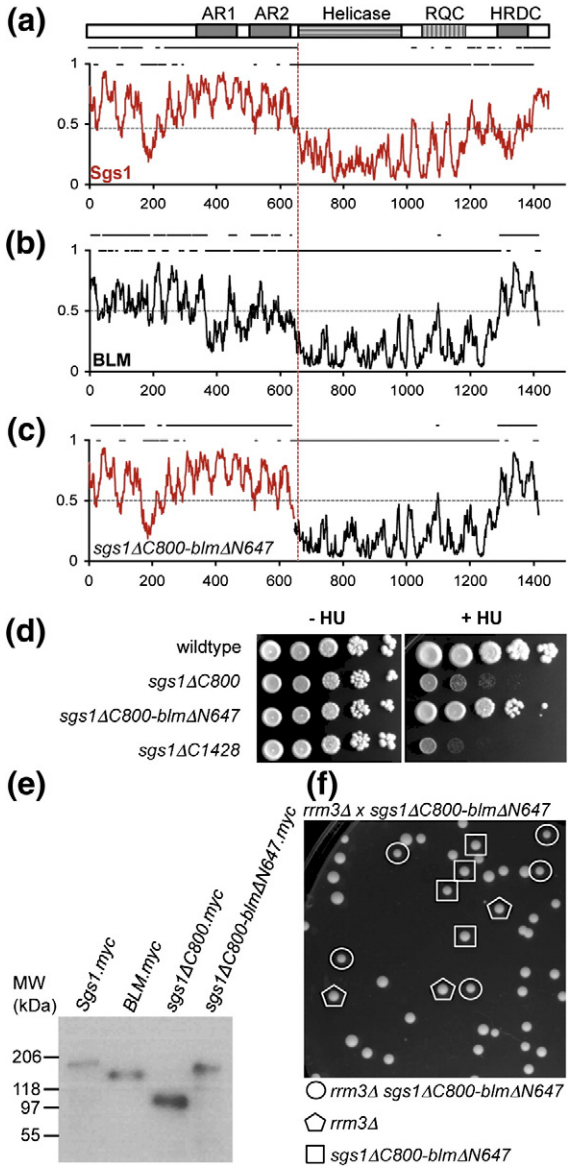


Fig. 5. HU sensitivity of diploid cells expressing *BLM* and mutant alleles of *SGS1*. (a) Tenfold dilutions of exponentially growing diploids expressing truncation alleles of *SGS1* in the presence of a wild-type allele were spotted on YPD media with and without 100 mM HU. (b) Tenfold dilutions of exponentially growing diploids expressing truncation alleles of *SGS1* in the absence of a wild-type allele were spotted on YPD media with and without 100 mM HU. (c) Tenfold dilutions of exponentially growing diploids overexpressing BLM from a *GAL1* promoter inserted at the native *SGS1* locus and expressing truncation alleles of *SGS1* under control of the native *SGS1* promoter on the other allele were spotted on media containing 1% galactose (to induce gene expression) and 1% sucrose with or without 100 mM HU.

functioning in yeast. This hypothesis is supported by the fact that the N-terminus of Sgs1 is sufficient for the complementation of the HU sensitivity induced by overexpression of BLM. To test this hypothesis, we constructed a yeast–human chimera in which the 647 N-terminal residues of BLM were replaced by the 647 N-terminal residues of Sgs1 (*sgs1ΔC800-blmΔN647*) (Fig. 6c). To express this chimera from the native *SGS1* promoter, we replaced nucleotides 1941 to 4344 of the endogenous *SGS1* gene with nucleotides 1941 to 4254 of *BLM* cDNA (Fig. 6e). Remarkably, the chimera was nearly as effective as wild-type *SGS1* in conferring resistance to HU, whereas the N-terminal segment of Sgs1 by itself was ineffective (Fig. 6d). Moreover, when we combined the chimeric allele with a *mec3*Δ mutation, GCRs accumulated at a significantly lower rate than in the *mec3*Δ mutant carrying the GCR-deficient *sgs1ΔC300* or *sgs1ΔC800* allele, albeit not at the low rate of the *mec3*Δ mutant carrying the GCR-proficient *sgs1ΔC200* allele, signifying partial functionality of the chimerical protein in the suppression of chromosomal rearrangements (Table 1). Finally, besides Srs2, the *sgs1*Δ mutant also requires the DNA helicase Rrm3 for viability. Synthetic lethality between *sgs1*Δ and *rrm3*Δ mutations is suppressed by disrupting HR factors such as Rad51 and Rad55, suggesting that the lethality is due to accumulation of aberrant HR intermediates.[68–70] To assess if the Sgs1–BLM chimera was capable of preventing the

accumulation of lethal levels of aberrant recombination intermediates, we constructed a diploid heterozygous for the *rrm3*Δ mutation and heterozygous for the *sgs1*Δ*C800-blm*Δ*N647* allele, expressing the Sgs1–BLM chimera. Spreading of spores from this diploid on YPD, which allows all spores to grow, showed that the *rrm3*Δ mutant expressing the chimera grows normally with the diameter of double-mutant colonies measuring approximately 90% of that of the single mutants (Fig. 6f). These findings indicate that the Sgs1–BLM chimera is functional and, while not capable of fully suppressing chromosomal rearrangements, prevents the accumulation of lethal levels of aberrant recombination intermediates when Rrm3 helicase is absent.



Discussion

Yeast cells that lack Sgs1 exhibit upregulated and aberrant recombination in mitosis, increased sensitivity to DNA-damaging agents, accumulation of GCRs, synthetic lethality with mutations in other DNA metabolic genes, such as the *SRS2* and *RRM3* helicase genes, and meiotic defects that lead to poor spore viability.[8,30,55,63,69,71–75] Sgs1 contains several conserved domains (DEAD helicase, RQC, HRDC, AR1, and AR2), and protein interaction sites (Top2, Top3, Srs2, Rad16, Rad51, and Mlh1) have been identified by two-hybrid screens.[27,29,30,46,76] How the integrity of these conserved motifs and protein–protein interaction sites affects the role of Sgs1 in suppression of aberrant genome rearrangements has not been determined. The requirement of some domains and/or protein interaction sites, but not others, may shed light on the poorly understood mechanism(s) by which Sgs1 contributes to the maintenance of genome integrity in yeast. Here, we find that the segment made up of 240 C-terminal amino acids, which contains Rad51 and Mlh1 interaction sites and the conserved HRDC domain thought to be involved in DNA binding and in recognition and processing of double Holliday junctions,[20,23] is dispensable for Sgs1's role in

Fig. 6. Construction of a functional chimerical protein composed of the N-terminus of Sgs1 and the C-terminus of BLM. (a and b) Protein disorder prediction of Sgs1 (red) and BLM (black), using the IUPred algorithm. Values above 0.5 indicate a disordered residue, whereas values below 0.5 indicate ordered residues; amino acid residue numbers (1–1447) are indicated on the abscissa. Black lines above the graph show a simplified order and disorder distribution along the length of the protein with values above 0.5 being assigned a "1" and values below 0.5 being assigned a "0". The vertical red line indicates the site in Sgs1, BLM, and the chimera where the disordered N-terminal segment transitions into the ordered helicase domain at residues 647–648. This site was chosen as the fusion site for the chimera. The approximate location of Sgs1 domains is indicated above (a). (c) Disorder prediction for the Sgs1–BLM chimera in which the 647 N-terminal residues of BLM (black) were replaced with the 647 N-terminal residues of Sgs1 (red). (d) Tenfold dilutions of exponentially growing haploids were spotted on YPD with or without 100 mM HU. (e) The C-terminus of the Sgs1–BLM chimera was fused to a myc epitope tag, and expression was confirmed by Western blotting. Molecular weight marker bands are indicated on the left. (f) A diploid heterozygous for the *rrm3*Δ mutation and the *sgs1*Δ*C800-blm*Δ*N647* allele expressing the chimera was sporulated, and random spores were plated on YPD to allow all spores to grow. An open circle indicates the haploid double mutant, and the open square and the open pentagon indicate haploid *sgs1*Δ*C800-blm*Δ*N647* and *rrm3*Δ single mutants, respectively.

suppressing GCRs. The integrity of the RQC domain, however, is essential for GCR suppression. That zinc binding is crucial for Sgs1 activity, as well as the fact that loss of function of the C-terminal truncation allele was not due to disruption of protein structure/function because of such a large deletion, was further confirmed by the finding that the point mutation of a conserved zinc-coordinating cysteine, which has also been observed in BS patients,[59] led to the loss of Sgs1's ability to suppress HU sensitivity and GCR accumulation. This loss of function was not due to degradation of the mutant protein as had been previously observed for some cysteine mutants of BLM during attempts at overexpression and purification from *E. coli*. However, we cannot exclude the possibility that the loss of function resulted from intracellular mislocalization of the mutant protein. Previously, modeling of the zinc-binding domain of BLM and instability of purified mutant proteins had indicated that hydrogen bonds between three conserved residues, Y1029 (Y1040 in Sgs1), R1037 (R1048 in Sgs1), and D1064 (D1070 in Sgs1), are required for the folding of the zinc-binding domain and overall protein stability.[17] Although F1056 of Sgs1 does not appear to be involved in this zinc-domain stabilization and the Sgs1-F1056A mutant protein appears stable in this study, F1056 is the only other fully conserved residue in the zinc-binding domain of RecQ-like helicases, suggesting functional significance. However, introduction of the F1056A mutation had no effect on Sgs1 function *in vivo* when we assessed HU sensitivity, consistent with a previous study,[77] or GCR accumulation. That, in a previous *in vitro* study,[61] the corresponding BLM mutation (F1045A) had severely impaired helicase and ssDNA binding activities could be either due to differences in the importance of this residue for enzymatic activity of BLM and Sgs1 or, more likely, due to the fact that only the helicase-core segment of BLM, lacking 769 residues of N- and C-termini, was purified. The *in vitro* function of this isolated domain could be more strongly affected by a mutation than the *in vivo* function of the full-length Sgs1 mutant protein assessed here. Although nearly half of all BLM alleles that are associated with single-amino-acid changes (7 of 17 alleles) in BS patients are located in the RQC domain,[9,13,58,59] none affect F1045, consistent with our finding that mutation of this conserved residue may not be associated with significant loss of function *in vivo*.

We find that Sgs1 retains partial functionality even when it lacks the HRDC, RQC, and DEAH helicase domains, as demonstrated by the greater HU resistance of diploids that only express 547 N-terminal amino acid residues compared to those alleles expressing fewer than 447 residues of Sgs1. One possible explanation for this finding is that protein–protein interactions conferred by the N-terminus could contribute to the structural stability of multi-protein complexes, such as the Sgs1–Top3–Rmi1[31] complex or, even more relevant to HU resistance, DNA-damage-specific complexes with Srs2 and Mre11.[29] In these multi-protein complexes, enzymatic activity of Sgs1 may be dispensable. Indeed, *sgs1* alleles with point mutations in the helicase domain have been shown to be capable of performing some functions of the wild-type allele, including those carried out during meiosis and checkpoint activation.[75,78]

In contrast to two previous reports,[62,79] which both used the same yeast strain that constitutively expressed *BLM* from a *GAPDH* promoter and showed partial suppression of some *sgs1*Δ defects, including HU sensitivity, we found that neither *BLM* expression under control of the natural *SGS1* promoter nor varying levels of *BLM* expression under control of a galactose-inducible promoter had any positive effect on the *sgs1*Δ mutant. That a single copy of BLM, when expressed under control of the native *SGS1* promoter, cannot alleviate *sgs1*Δ defects initially suggested to us that BLM had no functionality in yeast. In fact, the strong increase in genome instability, accompanied by severe HU sensitivity and some growth retardation upon overexpression of BLM, indicated that BLM expression is detrimental to yeast cells. The absence of any GCR accumulation upon Sgs1 overexpression suggests that increased accumulation of GCRs in BLM-overexpressing cells is not simply due to increased unwinding. Rather, we propose that BLM may possess helicase activity in yeast, leading to increased unwinding upon overexpression, but fails to elicit proper downstream responses, for example, due to lack of proper N-terminal protein–protein interactions, which ultimately leads to an overabundance of aberrantly repaired lesions. That endogenous levels of N-terminal segments of Sgs1 as short as 547 residues suppressed the slow growth phenotype and the severe HU sensitivity of BLM-overexpressing cells argues in favor of a functional relationship between Sgs1 and BLM. For example, co-expression of Sgs1 and BLM could alleviate HU sensitivity in BLM-overexpressing cells by acting as a bridge between BLM and Top3 (and/or other protein complexes interacting with the Sgs1 N-terminus), thereby linking enzymatic activity to appropriate upstream and downstream events. Remarkably, even relatively short N-terminal fragments of Sgs1 are sufficient for the suppression of the increased HU sensitivity of BLM-overexpressing cells, further supporting the importance of the Sgs1 N-terminus with its role in mediating interaction with other DNA metabolic factors. HU

resistance comparable to wild-type cells and significantly reduced GCR accumulation of cells expressing a chimeric fusion of the Sgs1 N-terminus, which is devoid of enzymatic function and dispensable for helicase activity and ssDNA binding *in vitro*, and the BLM C-terminus, which contains helicase/RQC and HRDC domains, are consistent with helicase activity of BLM in yeast and a biologically significant, functional interaction between BLM and Sgs1. That fusion of the Sgs1 and BLM segments provides HU resistance as well as co-expression of BLM and Sgs1 polypeptides from separate alleles in the same cell may indicate that the N-terminus of Sgs1 can physically interact with BLM. Our findings also suggest that it is the inability of the N-terminus of BLM to interact with or be modified by yeast proteins that leads to the inability of BLM to function in yeast. A previous report that BLM expression in yeast alleviates several *sgs1*Δ phenotypes, including partial suppression of HU sensitivity,[62,79] could be explained by the fact that, in the earlier study, BLM was expressed from a *GAPDH* promoter, whereas here it was expressed either from the native *SGS1* promoter or from a galactose-inducible promoter. However, in light of the findings presented here, there could also be an alternative explanation. Since the *GAPDH* promoter–*BLM* construct appears to have been inserted into the middle of the wild-type *SGS1* gene, an N-terminal segment of Sgs1 could have been expressed from the native *SGS1* promoter in addition to BLM being expressed from the *GAPDH* promoter. As shown here for haploids expressing the chimera and for diploids co-expressing the N-termini of Sgs1 and full-length BLM, such co-expression of an N-terminal Sgs1 segment from the native *SGS1* promoter and *BLM* from the *GAPDH* promoter could be an explanation for the reported increase in HU resistance of BLM-expressing cells compared to *sgs1*Δ cells.

Of the five human RecQ-like DNA helicases, BLM is considered to be most closely related to Sgs1. Even though we show here that BLM cannot suppress any defects of the *sgs1*Δ mutant, the functional chimera does provide evidence for a functional relationship between the two RecQ-like helicases and provides a model system for the further characterization of BLM functional domains in yeast. In fact, all BS-associated missense mutations and numerous polymorphisms are located within the 770-residue C-terminal fragment of BLM that is part of the chimera, so that they are now accessible to further functional and mutational characterization in yeast. The *in vivo* functionality of the Sgs1–BLM chimera also demonstrates the remarkable utility of protein disorder prediction as a tool for the construction of functional mutants. It will be interesting to see whether domains of any of the other human RecQ-like helicases, are capable of forming functional chimeras with the Sgs1 N-terminus.

Materials and Methods

Yeast strains and media

All strains are derived from KHSY802, a derivative of S288C. Yeast strains expressing truncations of Sgs1 helicase were constructed by HR-mediated integration of PCR products, replacing the desired 3′-segment of *SGS1* on chromosome VIII with a myc epitope coding sequence (from pFA6a-13Myc.His3MX6,[80] a gift from Mark Longtine, University of Washington) in-frame with the *SGS1* coding sequence. The expression of all truncation alleles and the myc-epitope-tagged wild-type allele of *SGS1* was confirmed by Western blot analysis. All gene replacements, insertions, and truncations were performed via the standard LiAc protocol,[81] using PCR products with at least 50 nt on each end, which matched the chromosomal target locus. A PCR fragment containing *BLM* cDNA (Open Biosystems) and a *HIS3* cassette was amplified by PCR from plasmid pKHS293 using primers that include 50-nt homology to the chromosomal *SGS1* locus to express BLM from the native *SGS1* promoter (P_{SGS1}). This PCR product was fused to the native chromosomal *SGS1* promoter by HR-mediated integration.[81] A PCR fragment coding for a 13Myc epitope tag was amplified from pFA6a-13Myc-kanMX6[80] and integrated in-frame at the 3′-end of cDNAs or *sgs1* alleles for detection of protein expression by Western blot analysis. In strains KHSY3350 and KHSY3218, galactose-inducible promoters amplified from plasmids pFA6a-kanMX6-PGAL1 and pFA6a-TRP1-PGAL1,[80] respectively, were used to replace the native *SGS1* promoter. To construct KHSY3355, we amplified the 3′-terminal 2313 bp of BLM cDNA linked to a *HIS3* cassette by PCR from plasmid pKHS293 and used them to replace the 3′-terminal 2400 bp of *SGS1* in KHSY802. The accuracy of PCR-derived *SGS1* or *BLM* integrations was confirmed by sequencing. Amino acid changes C1047F and F1056A in Sgs1 were made by site-directed mutagenesis (QuikChange, Stratagene) of pKHS360 and integrated at the *sgs1*::*HIS3* locus in KHSY1338. All yeast strains used in this study are listed in Table S1. Cells were grown in YPD consisting of 10 g/l yeast extract (Fisher Scientific), 20 g/l Bacto-peptone (BD Diagnostic Systems), and 2% glucose (Fisher Scientific), unless indicated otherwise. For plates, agar (BD Diagnostic Systems) was added at a concentration of 20 g/l.

Western blot analysis

Cells were grown to an $OD_{600} = 0.5$ in YPD, and whole-cell extracts were prepared from 5 ml of culture ($\sim 3.5 \times 10^7$ cells) by standard trichloroacetic acid (TCA, Fisher Scientific) extraction to confirm expression of myc-epitope-tagged *BLM* and *SGS1* alleles.[82] Five microliters of TCA extract was separated on 10% polyacrylamide gels, transferred to a PVDF membrane (Bio-Rad), probed with anti-c-myc monoclonal antibody (9E10, Covance Research Products), and visualized by chemiluminescence (ECL

Plus, GE Healthcare). To confirm expression of *SGS1* and *BLM* from the *GAL1* promoter, we used the same Western blot procedure, but cells were grown overnight in yeast–peptone (YP) [(10 g/l yeast extract (Fisher Scientific), 20 g/l Bacto-peptone (BD Diagnostic Systems)] supplemented with 2% sucrose (Fisher Scientific), diluted to an $OD_{600}=0.2$ in YP supplemented with either 2% sucrose (uninduced sample) or 2% galactose (induced sample), and harvested for TCA extraction when cultures reached $OD_{600}=0.5$. Molecular weight marker (Broad Range) was from Bio-Rad.

Sensitivity to DNA-damaging agents HU and MMS

Cell cultures were grown in YPD to an $OD_{600}=0.5$, and 10-fold serial dilutions were spotted on YPD supplemented with 0.05% MMS (Sigma Aldrich) or with 50 mM or 100 mM HU (US Biological), as indicated. For experiments that included strains expressing BLM or Sgs1 from the *GAL1* promoter (Fig. 5), cultures were grown in YP-2% sucrose instead of YPD and spotted on YP-1% sucrose + 1% galactose (to induce gene expression) supplemented with 100 mM HU or without HU as the growth control.

GCR rate measurements

Rates of accumulating GCRs in YPD were determined as previously described.[83] For GCR rate measurements of yeast strains expressing BLM or Sgs1 from the *GAL1* promoter, the same procedure was followed, except that media were supplemented with 2% galactose to induce gene expression. Briefly, 10 ml of YP-2% galactose was inoculated with a single colony, which had been grown on YPD agar for 3 days. After 3 days of growth in liquid media at 30 °C with vigorous shaking, cells were plated on GCR plates[83] supplemented with 2% galactose instead of 2% glucose, and 10^{-6} dilutions were plated on YPD to obtain the viable cell count. Colonies on GCR plates were counted after 5 days of incubation at 30 °C. For GCR rate measurements in the presence of varying BLM expression levels (Table 2), 0.1% or 0.5% galactose instead of 2% galactose was added to liquid YP media and to GCR plates, and sucrose was supplemented to reach a total of 2% sugar in the media. We calculated 95% confidence intervals according to Nair.[56]

Random spore analysis

Diploids heterozygous for the desired mutant alleles were grown overnight at 30 °C in YPD, washed, transferred to 0.1% potassium acetate (Fisher Scientific), and incubated for 5 days at 30 °C with vigorous shaking. Asci were incubated in the presence of 500 μg/ml zymolase (MP Biomedicals) in 1 M sorbitol (Fisher Scientific) for 20 min at 30 °C and enriched for haploid spores as previously described.[84] Spores were plated on YPD, incubated at 30 °C, and genotyped by spotting on synthetic drop-out media (US Biological) to detect the presence of *TRP1* and *HIS3* marker cassettes linked to the mutant alleles. The presence of mutant alleles linked to the kanMX6 cassette was detected by the ability of haploids to grow on YPD supplemented with 200 μg/ml G418 (Axxora LLC, San Diego, CA).

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