BLM Helicase Complements Disrupted Type II Telomere Lengthening in Telomerase-Negative *sgs1* Yeast

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Abstract

Recombination-mediated pathways for telomere lengthening may be utilized in the absence of telomerase activity. The RecQ-like helicases, BLM and Sgs1, are implicated in recombination-mediated telomere lengthening in human cells and budding yeast, respectively. Here, we show that *BLM* expression rescues disrupted telomere lengthening in telomerase-negative *sgs1* yeast. BLM helicase activity is required for this complementation, indicating BLM and Sgs1 resolve the same telomeric structures. These data support a conserved function for BLM and Sgs1 in recombination-mediated telomere lengthening. (Cancer Res 2005; 65(13): 5520-2)

Introduction

Bloom's syndrome is a rare autosomal recessive disorder characterized by a high predisposition to cancer of many tissues and types. Somatic cells display extraordinary chromosome instability with a high frequency of sister chromatid exchanges, chromosome configurations signifying recombination, and telomeric associations. *BLM*, the gene mutated in Bloom's syndrome, encodes a RecQ-like helicase that unwinds displacement loops, G-quadruplexes, and promotes the branch migration and resolution of Holliday junctions *in vitro* (1–3). The *Saccharomyces cerevisiae* genome encodes only one RecQ family member, *SGS1*, the deletion of which results in excessive sister chromatid exchanges and interchromosomal recombination reminiscent of the Bloom's syndrome phenotype (4). BLM can also suppress hyperrecombination and premature aging of *sgs1* mutants (5). Similar to BLM, Sgs1 unwinds G-quadruplexes and synthetic Holliday junctions *in vitro* (6, 7). Furthermore, BLM and Sgs1 share a conserved interaction with topoisomerase IIIα, which may facilitate non-crossover resolution of Holliday junctions (8, 9).

In the absence of telomerase, immortalized mammalian cells and yeast may utilize recombination-mediated pathways to maintain telomeres, termed alternative lengthening of telomeres (ALT), in mammalian cells. In human cells using ALT, BLM associates with telomeres and the telomere repeat factors TRF1 and TRF2 (10, 11). Overexpression of BLM in cells using ALT increases telomeric signals, suggesting that BLM promotes ALT (10). In addition to BLM, the related RecQ-like Werner's syndrome helicase, WRN, as well as Rad52, Rad51, RPA, and the Rad50/Mre11/Nbs1 complex, associate with telomeric foci in cells using ALT (12, 13). Telomerase-negative *S. cerevisiae* overcome telomere crisis by utilizing one of two Rad52-dependent recombination-mediated pathways, termed type I and type II (14). Type I telomere lengthening requires Rad51, Rad54, and Rad57, whereas type II telomere lengthening requires Rad50, Rad59, and Sgs1 (12, 15–17). $C_{1-3}A/TG_{1-3}$ terminal repeats in type II survivors are amplified and often heterogeneous in length, whereas type I survivors have amplified subtelomeric Y′-elements and very short tracts of $C_{1-3}A/TG_{1-3}$ terminal repeats (Fig. 1*A*). Like type II survivors, terminal telomeric repeats in human cells using ALT are long and heterogeneous, suggesting that these pathways involve similar mechanisms (18). The association of BLM and Sgs1 with the ALT and type II pathway, respectively, led us to hypothesize a conserved function of these RecQ helicases in recombination-mediated telomere lengthening.

Materials and Methods

Expression vectors. *pYES-BLM* expression construct (*pJK1*; ref. 19) was provided by I. Hickson (University of Oxford, Oxford, United Kingdom). The conserved lysine at amino acid 695 of the human BLM protein was mutated to glutamic acid by PCR-mediated site-directed mutagenesis of the *BLM* gene within the *pYES-BLM* vector to create *pYES-K695E BLM*. The presence of the mutation was confirmed by sequencing. Helicase assays with purified proteins expressed from *pYES* vector and purified from yeast confirmed unwinding activity of wild-type BLM (11) and lack of unwinding with K695E-BLM (data not shown).

Yeast strains and crosses. *sgs1::HIS3; URA3* yeast (RDKY3813; ref. 20) was provided by R. Kolodner (University of California-San Diego, La Jolla, CA). The *URA3* marker present in this strain was eliminated by selection on 5-FOA, confirmed by PCR and lack of growth on media lacking uracil, to create *sgs1::HIS3*, which was used for all subsequent experiments. *est2::KANMX4/EST2* (BY4743-YLR318W) was obtained from American Type Culture Collection Yeast Genetic Stock Center, Manassas, VA. The latter was streaked onto standard sporulation media to obtain *MATα; est2::KANMX4* spores, which were mated to *MATa; sgs1::HIS3* to produce a double-heterozygous diploid strain that was subsequently transformed with *pYES-BLM, pYES-K695E*, or *pYES* vector (Invitrogen, Carlsbad, CA). The diploid strain was sporulated and tetrads dissected. Individual spores with genotypes *SGS1 EST2, sgs1::HIS3 EST2, SGS1 est2::KANMX4*, and *sgs1::HIS3 est2::KANMX4* were selected on the appropriate media and passaged on uracil dropout plates with minimal base containing 2% galactose at 30°C. After restreaking 10 times (~250 cell generations), single colonies were selected and grown in standard liquid uracil dropout medium with minimal base containing 2% galactose. Yeast were diluted 1:1,000 every 24 to 72 hours (when cultures reached $\sim 1 \times 10^8$ cells/mL) into fresh media for an additional 10 passages (~100 cell generations) and were subsequently collected for telomere analysis.

Telomere Southern blot analysis. Yeast were pelleted by centrifugation, resuspended in 200 μL lysis buffer [1% (w/v) SDS, 50 mmol/L EDTA, 0.1 mol/L Tris (pH 8.0)] with acid-washed glass beads, and lysed by vortexing for 2 minutes. Genomic DNA was extracted with phenol/chloroform, ethanol-precipitated, and resuspended in Tris-EDTA buffer. Genomic DNA (7.5 μg) was digested with *Xho*I (New England Biolabs, Beverly, MA) at 37°C overnight and resolved by electrophoresis on a 1% Tris-borate EDTA agarose gel. Digested DNAs were transferred onto a positively charged Hybond-N+ nylon membrane (Amersham Biosciences, Piscataway, NJ) and

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Figure 1. *BLM* rescues the ability of *sgs1 est2* mutants to use the type II pathway to maintain telomere ends. *A,* expected telomere composition in telomerase-negative survivors utilizing the type I and type II pathways. Telomeres in type I survivors are typified by amplification of Y′-elements and very short $C_{1-3}A/TG_{1-3}$ terminal repeat fragments (Y′-TRF). Telomeres in type II survivors are typified by a normal number of Y′-elements and heterogeneous, often very long, $C_{1-3}A/TG_{1-3}$ terminal repeat fragments (type II-TRF). Gray arrowheads represent placement of $C_{1-3}A/TG_{1-3}$ repeats. *B,* structure and analysis of telomere length in yeast. Y′-elements (5.2 and 6.7 kb, 0-4 copies) are present internal to the $C_{1-3}A/TG_{1-3}$ terminal repeats (~250-400 bp). Y′-elements are frequently separated by short stretches of $C_{1-3}A/TG_{1-3}$ repeats. *Xho*I restriction sites are present within Y′-elements as indicated. Digestion with *Xho*I releases individual Y′-elements and the TRF. Other subtelomeric elements may be present but are not shown. Gray arrowheads represent placement of $C_{1-3}A/TG_{1-3}$ repeats. *C, BLM* rescues the type II pathway in *sgs1 est2* yeast mutants. Telomeres from *SGS1 est2* and *sgs1 est2* cultures expressing *BLM, K695E BLM*, or empty vector were analyzed by digestion with *Xho*I and Southern blot analysis with a ^{32}P-labeled probe that hybridizes to TG_{1-3} repeats. Type II survivors are indicated by an asterisk below the autoradiograph. *Lane 1,* DNA from *SGS1 EST2* (*wt*); *lanes 2* to *9, sgs1 est2* survivors expressing *BLM*; *lanes 10* to *17, sgs1 est2* survivors transformed with empty vector; *lanes 18* to *25, sgs1 est2* survivors expressing *K695E BLM*; and *lanes 26* to *33, SGS1 est2* survivors transformed with empty vector.

probed by standard Southern blotting procedures. A probe for the TG_{1-3} repeats was created by random priming with [α-^{32}P]dCTP using poly(dA-dC)·poly(dG-dT) template (Amersham Biosciences) as described by others (12). Southern blots were developed and analyzed by autoradiography.

Results and Discussion

The Sgs1 helicase is required for the type II pathway such that telomerase-negative *sgs1* mutants give rise to type I survivors exclusively (12, 16, 17). To test whether BLM and Sgs1 have conserved functions in recombination-mediated telomere lengthening, rescue of the type II pathway by *BLM* expression was tested in yeast lacking expression of Sgs1 and Est2, the catalytic subunit of telomerase. *sgs1::HIS3/SGS1*; *est2::KAN2/EST2* diploid yeast were transformed with empty expression vector or expression vector encoding BLM or K695E-BLM. K695E-BLM, created by site-directed mutagenesis, encodes a BLM protein that is mutated within the conserved ATP-binding site resulting in loss of helicase activity (21). The diploid strain was sporulated and tetrads were dissected to identify individual *sgs1 est2, SGS1 est2, sgs1 EST2*, and *SGS1 EST2* (wild-type) spores from which individual survivor colonies were established and subsequently cultured in liquid media. DNAs were isolated and digested with *Xho*I to release the $C_{1-3}A/TG_{1-3}$ terminal repeats, the terminal restriction fragment (TRF), from the subtelomeric 6.7 and 5.2 kb Y′-elements (Fig. 1*B*). Digested DNAs were analyzed by Southern blot hybridization with a probe that identifies TG_{1-3} repeats. The separation of Y′-elements by short tracts of $C_{1-3}A/TG_{1-3}$ allows identification of Y′-elements and TRFs simultaneously.

Southern blot analysis of telomeres is shown in Fig. 1*C*. Seven of eight *SGS1 est2* survivors transformed with vector only (*est2* + vector) utilized the type II pathway, identified by the presence of TRFs of heterogeneous distribution (type II-TRFs). A single type I *SGS1 est2* survivor was identified by the presence of intense Y′-element bands and a short TRF fragment (Y′-TRF). *SGS1 est2* survivors expressing *BLM* utilized type I and type II pathways as well, although a smaller number of type II survivors were identified compared with *SGS1 est2* yeast transformed with empty vector (data not shown). *sgs1 est2* yeast transformed with empty vector

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(*sgs1 est2* + vector) gave rise to type I survivors only, consistent with the requirement of Sgs1 for type II telomere lengthening. In contrast, *BLM* expression induced in *sgs1 est2* yeast (*sgs1 est2* + BLM) resulted in the appearance of three type II survivors out of eight cultures, demonstrating that BLM can rescue the type II telomere lengthening defect associated with the absence of Sgs1 in some cultures. Finally, all *sgs1 est2* survivors expressing a helicase-dead BLM mutant (*sgs1 est2* + K695E BLM) utilized the type I pathway, demonstrating that BLM helicase activity is required for rescue of the type II pathway.

Our experiments show that BLM can complement disrupted type II telomere lengthening in *sgs1 est2* mutants. Complementation requires BLM helicase activity, indicating that BLM and Sgs1 resolve common homologous structure(s) to promote type II telomere lengthening. Given that BLM promotes telomere signal amplification in human cells using ALT (10), these data are consistent with a conserved function of BLM and Sgs1 in recombination-mediated telomere lengthening in mammalian and yeast cells, respectively. Furthermore, these data support the hypothesis that ALT and the type II pathway extend telomeres by a common mechanism.

Three of eight *sgs1 est2* survivors expressing *BLM* utilized the type II pathway compared with seven of eight *SGS1 est2* survivors transformed with vector alone. Rescue of the type II pathway by BLM may be partial due to differences in *pYES-BLM* copy number, and thus variable *BLM* expression in individual cultures. Notably, when *BLM* expression was induced in *SGS1 est2* yeast, only two of eight survivors utilized the type II pathway (data not shown). Therefore, it is likely that very high levels of BLM expression in some cultures can interfere with normal functioning of the type II machinery by a dominant-negative effect of overexpression that results in a lower than expected number of type II survivors.

The precise mechanisms by which recombination-mediated telomere lengthening occur and the functions of BLM and Sgs1 in these pathways remain speculative. The preferential unwinding of branched DNA substrates by these helicases *in vitro* (6, 22) suggests the possibility that they process recombination intermediate(s), such as Holliday junctions and displacement loops, at telomeres undergoing recombination. Additionally, these RecQ-like helicases may be required to unwind secondary structures, such as G-quadruplex DNA, that occur during recombination within the GC-rich $C_{1-3}A/TG_{1-3}$ terminal repeats in yeast and the $TTAGGG_n$ telomeric repeats in mammalian cells. G-quadruplex DNA is readily unwound by Sgs1, BLM, and WRN helicases *in vitro* (1, 7, 22). WRN can also partially rescue the type II pathway in *sgs1 est2* mutants and colocalizes with telomeric proteins in human cells using ALT (12, 16), although its contribution to ALT remains unknown. An inability to unwind G-quadruplexes may prohibit replication of the lengthy $C_{1-3}A/TG_{1-3}$ terminal repeats in the absence of Sgs1 such that the type I pathway is required for prolonged survival without telomerase. Notably, no comparable type I mechanism has been uncovered in telomerase-negative mammalian tumors or cell lines. By extension, cells lacking BLM, or possibly WRN, may be unable to utilize ALT in the absence of telomerase, predicting that all tumors and stem cell populations in persons with Bloom's or Werner's syndrome use telomerase for telomere maintenance.

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References

1. Sun H, Karow JK, Hickson ID, Maizels N. The Bloom's syndrome helicase unwinds G4 DNA. J Biol Chem 1998; 273:27587–92.

2. van Brabant AJ, Ye T, Sanz M, German JL III, Ellis NA, Holloman WK. Binding and melting of D-loops by the Bloom syndrome helicase. Biochemistry 2000;39: 14617–25.

3. Karow JK, Constantinou A, Li JL, West SC, Hickson ID. The Bloom's syndrome gene product promotes branch migration of Holliday junctions. Proc Natl Acad Sci U S A 2000;97:6504–8.

4. Onoda F, Seki M, Miyajima A, Enomoto T. Elevation of sister chromatid exchange in *Saccharomyces cerevisiae sgs1* disruptants and the relevance of the disruptants as a system to evaluate mutations in Bloom's syndrome gene. Mutat Res 2000;459:203–9.

5. Heo SJ, Tatebayashi K, Ohsugi I, Shimamoto A, Furuichi Y, Ikeda H. Bloom's syndrome gene suppresses premature ageing caused by Sgs1 deficiency in yeast. Genes Cells 1999;4:619–28.

6. Bennett RJ, Keck, JL, Wang JC. Binding specificity determines polarity of DNA unwinding by the Sgs1 protein of *S. cerevisiae*. J Mol Biol 1999;289:235–48.

7. Sun H, Bennett RJ, Maizels N. The *Saccharomyces cerevisiae* Sgs1 helicase efficiently unwinds G-G paired DNAs. Nucleic Acids Res 1999;27:1978–84.

8. Ira G, Malkova A, Liberi G, Foiani M, Haber JE. Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. Cell 2003;115:401–11.

9. Wu L, Hickson ID. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. Nature 2003;426:870–4.

10. Stavropoulos DJ, Bradshaw PS, Li X, et al. The Bloom syndrome helicase BLM interacts with TRF2 in ALT cells and promotes telomeric DNA synthesis. Hum Mol Genet 2002;11:3135–44.

11. Lillard-Wetherell K, Machwe A, Langland GT, et al. Association and regulation of the BLM helicase by the telomere proteins TRF1 and TRF2. Hum Mol Genet 2004;13:1919–32.

12. Johnson FB, Marciniak RA, McVey M, Stewart SA, Hahn WC, Guarente L. The *Saccharomyces cerevisiae* WRN homolog Sgs1p participates in telomere maintenance in cells lacking telomerase. EMBO J 2001;20: 905–13.

13. Henson JD, Neumann AA, Yeager TR, Reddel RR. Alternative lengthening of telomeres in mammalian cells. Oncogene 2002;21:598–610.

14. Teng SC, Zakian VA. Telomere-telomere recombination is an efficient bypass pathway for telomere maintenance in *Saccharomyces cerevisiae*. Mol Cell Biol 1999;19:8083–93.

15. Chen Q, Ijpma A, Greider CW. Two survivor pathways that allow growth in the absence of telomerase are generated by distinct telomere recombination events. Mol Cell Biol 2001;21:1819–27.

16. Cohen H, Sinclair DA. Recombination-mediated lengthening of terminal telomeric repeats requires the Sgs1 DNA helicase. Proc Natl Acad Sci U S A 2001;98: 3174–9.

17. Huang P, Pryde FE, Lester D, et al. SGS1 is required for telomere elongation in the absence of telomerase. Curr Biol 2001;11:125–9.

18. Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR. Telomere elongation in immortal human cells without detectable telomerase activity. EMBO J 1995; 14:4240–8.

19. Karow JK, Chakraverty RK, Hickson ID. The Bloom's syndrome gene product is a 3′-5′ DNA helicase. J Biol Chem 1997;272:30611–4.

20. Myung K, Kolodner RD. Suppression of genome instability by redundant S-phase checkpoint pathways in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 2002;99:4500–7.

21. Neff NF, Ellis NA, Ye TZ, et al. The DNA helicase activity of BLM is necessary for the correction of the genomic instability of Bloom syndrome cells. Mol Biol Cell 1999;10:665–76.

22. Mohaghegh P, Karow JK, Brosh RM Jr, Bohr VA, Hickson ID. The Bloom's and Werner's syndrome proteins are DNA structure-specific helicases. Nucleic Acids Res 2001;29:2843–9.

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