

# The human Bloom syndrome gene suppresses the DNA replication and repair defects of yeast *dna2* mutants

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**Bloom syndrome is a disorder of profound and early cancer predisposition in which cells become hypermutable, exhibit high frequency of sister chromatid exchanges, and show increased micronuclei. *BLM*, the gene mutated in Bloom syndrome, has been cloned previously, and the BLM protein is a member of the RecQ family of DNA helicases. Many lines of evidence suggest that *BLM* is involved either directly in DNA replication or in surveillance during DNA replication, but its specific roles remain unknown. Here we show that *hBLM* can suppress both the temperature-sensitive growth defect and the DNA damage sensitivity of the yeast DNA replication mutant *dna2-1*. The *dna2-1* mutant is defective in a helicase-nuclease that is required either to coordinate with the crucial *Saccharomyces cerevisiae* (sc) FEN1 nuclease in Okazaki fragment maturation or to compensate for scFEN1 when its activity is impaired. We show that human BLM interacts with both scDna2 and scFEN1 by using coimmunoprecipitation from yeast extracts, suggesting that human BLM participates in the same steps of DNA replication or repair as scFEN1 and scDna2.**

Eukaryotic genomes encode hundreds of genes with homology to DNA helicases. Many of these show functional overlap, making it difficult to assign the precise DNA structures that are unwound by the respective gene products during DNA replication, recombination, and repair. One class of human helicase, the RecQ family, has received special attention in the past few years, because mutations in three different RecQ helicase family members give rise to three distinct human disorders leading to cancer predisposition and/or segmental premature aging, Bloom syndrome, Werner syndrome, and Rothmund–Thomson syndrome (1–3). The genes affected in the diseases, *BLM*, *WRN*, and *RECQ4*, have a single, sequence-specific homolog in yeast called *SGS1* (4, 5). Both *hBLM* and *hWRN* have been shown to be biologically active in yeast by demonstrating the effects of their expression on *sgs1Δ* mutants. *mWRN* suppresses the slow-growth phenotype and the inhibition of type II recombination at telomeres observed in *est2Δsgs1Δ* survivors (6). Both *hBLM* and *hWRN* suppress the excessive illegitimate and homologous recombination phenotypes of *sgs1Δ* mutants (7). However, *hBLM* and *hWRN* are not completely interchangeable in yeast. *hBLM*, but not *hWRN*, restores slow growth in the *sgs1Δtop3* mutants (7–10). Similarly, *hBLM*, but not *hWRN*, suppresses the premature aging phenotype of yeast *sgs1Δ* mutants (8). Differential function in yeast is consistent with structural and biochemical differences between *BLM* and *WRN*. *BLM* and *WRN* are homologous mainly in the helicase domain but are divergent in N-terminal and C-terminal domains. *WRN*, but not *BLM*, contains an intrinsic nuclease activity in addition to helicase activity (see ref. 2 for review). There are also clear differences in physiological function, because the symptoms of Werner and Bloom syndromes and types of cellular damage in humans differ. Taken together, the *sgs1* suppression studies clarify the function of *BLM* and *WRN* by suggesting that they suppress recombination. Nevertheless, the suppression studies leave many questions unanswered, because there are many mechanisms by which a gene product might suppress recombination.

*SGS1* interacts genetically with several other yeast helicase-encoding genes, including the *DNA2* helicase-nuclease identified in our laboratory (11–13). Although Dna2 is not a member of the RecQ helicase subfamily, and encodes both a nuclease and helicase activity, *sgs1Δdna2-2* mutants are synthetically lethal, suggesting that their defects are synergistic (12, 14). *DNA2* is essential and is required for the elongation stage of DNA replication *in vivo* and *in vitro* (11, 15–18). High-copy-number suppression of the temperature-sensitive growth defect of a yeast *rad27Δ* strain by *DNA2* (and vice versa), synthetic lethality of the *dna2-1* allele with a *rad27Δ* mutation and with a DNA ligase mutant, and biochemical evidence for an interaction between Dna2 and the *RAD27* gene product FEN1 suggest that Dna2 may be involved in Okazaki fragment maturation (19–22). Interaction of *DNA2* with lagging strand-replication functions has also been shown in *Schizosaccharomyces pombe* (23). A strand-displacement model for removal of RNA primers during Okazaki fragment processing was proposed that incorporated roles for both FEN1 and Dna2 (24). In this model, pol  $\delta$  displaces the RNA primer of the previously synthesized Okazaki fragment, providing a flap structure that is endonucleolytically processed by sequential action of Dna2 and FEN1 in preparation for ligation (24). *In vitro* reconstitution of the processing reaction showed that, if the flap was >30 nt, then Dna2 was required to stimulate the endonucleolytic activity of FEN1; however, Dna2 had no effect if the flap was <30 nt (24, 25). Thus, Dna2 is likely to be essential for Okazaki fragment maturation only in cases where FEN1 activity is somehow impaired (25, 26).

Dna2 is conserved in primary sequence from yeast to human (23, 27, 28). In *Xenopus*, depletion of *Xenopus laevis* DNA2 (xDNA2) from egg extracts leads to inhibition of DNA replication, and *Caenorhabditis elegans dna2* mutants appear to be replication-deficient (29), so the function of Dna2 in DNA replication is also conserved (27). The human genome contains three genes related to *DNA2* in the helicase domain, although only one of them also shows conservation throughout the length of the protein, including the nuclease domain (27, 28). Yeast *dna2* mutants are sensitive to x-rays, methyl methanesulfonate (MMS), bleomycin, and hydroxyurea (HU) (15, 16). The mechanism by which Dna2 participates in base excision repair (MMS sensitivity) may be related to its role in DNA replication, because reconstitution of the repair reaction requires the same proteins as those involved in Okazaki fragment maturation (26). Some hypomorphic alleles of *dna2* induce high levels of recombination in the yeast rDNA, and yeast *dna2* mutants have short lifespans (12). Subnuclear localization of *Saccharomyces cerevisiae* (sc) Dna2 in yeast is notable. The bulk of scDna2 is localized to telomeres in the G<sub>1</sub> and G<sub>2</sub> phases of the cell cycle (16). The scDna2 protein is released in S phase and is then found

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Abbreviations: sc, *Saccharomyces cerevisiae*; xDNA2, *Xenopus laevis* DNA2; HU, hydroxyurea; MMS, methyl methanesulfonate; Ura, uracil; 5-FOA, 5-fluoroorotic acid.

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throughout the replicating chromatin. The scDna2 protein is also released from telomeres by treatment of cells with agents that cause DNA damage (16). The scDna2 relocation from telomeres in response to DNA damage requires the checkpoint gene *MEC1* (unpublished data). All of these phenomena suggest that scDna2 is a major suppressor of genome instability.

In this study, we show that both the human *DNA2* and *BLM* genes can suppress the temperature-sensitive growth and DNA repair defects of *dna2* mutants, suggesting that *hBLM* is involved in DNA replication.

## Materials and Methods

**Strains.** All of the strains used in this study were derived from W303 (*MATa ade2-1 can1-100 trp1-1 leu2-3,112 his3-11,15 ura3*). The *dna2-1* derivative has been described (12). The *rad27Δ* and BJ5459 strains have been described (15). Yeast cultures, transformations, and other techniques were as described (30). All media containing galactose also contained raffinose.

**Plasmids and Constructs.** *hBLM* was excised from pBluescript II KS(+)-human (h) BLM (gift of Y. Furuichi, AGENE Research Institute, Kanagawa, Japan) with *NotI/XhoI* and inserted into the yeast expression vector pRS316 behind the *GAL* promoter. The helicase defective derivative was made by subcloning the K695T mutation from pC4 BLM K695T (gift of N. Neff, New York Blood Center, New York) on the *EcoRI/SalI* fragment into the corresponding sites of the recipient plasmid. The *HindIII/XhoI* fragment of pcDNA3-FLAG-mWRN and the *NotI/XhoI* fragment of pcDNA3-FLAG-hRecQ4 were cloned into the corresponding sites of plasmid pRS316/*GAL*, respectively. (mWRN and hRECQ4 were obtained from Y. Furuichi.) A *hDNA2* (GenBank accession no. P51530) fragment containing the complete ORF was cloned by PCR by using pBluescript II SK(+)-hDNA2 (gift of N. Nobuo, Kazusa DNA Research Institute, Chiba, Japan) as a template, and constructed as follows. Based on the nucleotide sequence of the *hDNA2* cDNA, a sense primer, 5'-CGGGATCCCGTCCAGGATGGAGCAGCTGAC-3' (*Bam*HI site underlined) and an antisense primer, 5'-CCGCTCGAGCGGTTACTTGTTCATCGTCATCCTTGTA-GTCTTCTCTTTGAAAGTCACCCAA-3' (*Xho*I site underlined) were used to amplify the complete ORF. The antisense primer contains a stretch of 24 nt (boldface) that encodes a FLAG tag in-frame to Glu-1060 of hDna2. The PCR fragment was digested with *Bam*HI and *Xho*I and inserted into the yeast expression vector pRS316 behind the *GAL* promoter. pYEp24-3a, containing the *RAD27* gene on a YEp24-based vector, pJDG-Rad27-myc, encoding scFEN1-myc, and pJDG-DNA2-hemagglutinin (HA) have been described (15).

**Measurement of MMS and HU Sensitivity.** Overnight cultures were diluted to an OD of 0.2 and grown for an additional 4–5 h. Tenfold serial dilutions from each strain were spotted onto synthetic complete plates containing galactose with or without MMS (0.005%) or HU (150 mM). The plates were then incubated for the time indicated. For each genotype tested, at least two independent strains were assayed.

**Protein Blotting.** The *dna2-1* cells transformed with pRS316, pGAL-hBLM, or pGAL-hDNA2-FLAG were grown in SC raffinose minus uracil (Ura) to an OD of 0.5, and the expression was induced by adding 2% galactose. The cells were then cultured for the time indicated, and extracts were prepared. The cells, 0.5 ml of culture, were pelleted, washed, and resuspended in 100  $\mu$ l of 50 mM NaOH (pH 10.5) containing 2 mM EDTA, 1 mM PMSF, 2% SDS, 10% glycerol, 5% mercaptoethanol, and a mixture of protease inhibitors (Roche Molecular Biochemicals), and boiled for 5 min. Debris was removed by centrifugation and the supernatant was adjusted to pH 7.0 with 1 M HCl and

supplemented with bromophenol blue. Equal amounts of crude lysates were electrophoresed in SDS/7.5% polyacrylamide gels, blotted to nitrocellulose membranes, and analyzed with monoclonal anti-FLAG M2 (Sigma) or polyclonal anti-BLM antibodies (gift of N. Neff). scFen1-myc was detected by anti-myc (9E10, Babco, Richmond, CA) antibodies. scDNA2-HA was detected by anti-HA (12CA5, Roche Molecular Biochemicals) antibodies. Secondary antibodies were purchased from Bio-Rad and detected by using an ECL kit (Amersham Pharmacia) following the manufacturer's instructions.

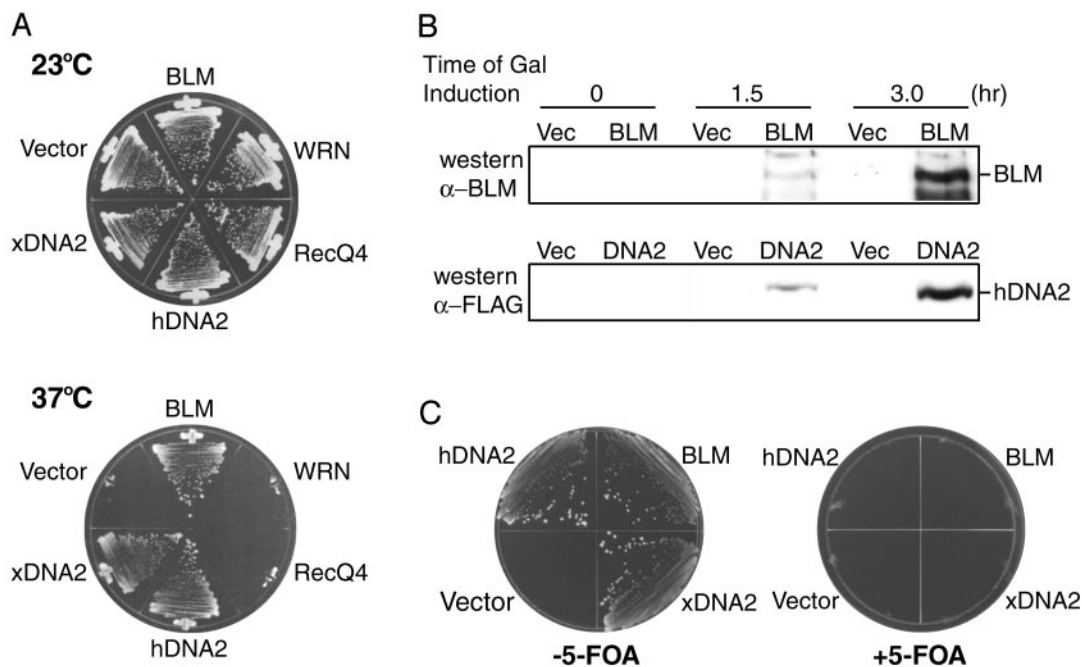
**Immunoprecipitation.** Yeast cells were grown in yeast extract/peptone/raffinose (YPR) at 30°C until they reached an OD of 0.5, at which point 2% galactose was added. The cells were then cultured for a further 4 h and pelleted by centrifugation. The pellets were washed with sterile water twice, then resuspended in 200  $\mu$ l of lysis buffer (50 mM Tris-HCl, pH 7.6/150 mM NaCl/0.2% Nonidet P-40/10% glycerol/15 mM DTT/2 mM EDTA/1 mM PMSF, and a mixture of protease inhibitors). Glass beads (500  $\mu$ l) were added, and the cells were lysed by vortexing for 5 min. The extracts were clarified by 15 min of centrifugation at 4°C. The supernatant was diluted with lysis buffer and incubated at 4°C for 2 h with protein G-Sepharose beads bound to anti-BLM or anti-myc antibodies or with protein A-Sepharose beads bound to anti-HA antibodies. The beads were washed four times with lysis buffer and boiled in sample buffer, and the immunoprecipitates were resolved by SDS/7.5% PAGE.

## Results

**Suppression of Strain *dna2-1*.** Because of the genetic interaction between *dna2* and *sgs1*, we tested whether any of the human RecQ helicase disease genes could suppress any of the phenotypes of *dna2* mutants. The putative human *DNA2* ORF, *hDNA2* (28), the human *BLM* gene, *hBLM* (31), the murine *FLAG-WRN* gene, *mWRN* (32), and the human *FLAG-RECQ4* gene, *hRECQ4* (33), were each subcloned into the yeast single-copy, centromeric vector pRS316 under the control of the *GAL1,10* yeast promoter. As we had previously shown for the yeast *DNA2* gene and the *Xenopus DNA2* gene (*xDNA2*) (11, 27, 34), human *DNA2* (*hDNA2*) suppressed *dna2-1* (Fig. 1A), suggesting that this is the functional human Dna2 homolog. Suppression by *xDNA2* is shown as a control. As shown in Fig. 1A, *hBLM* also suppressed the temperature-sensitive growth defect of a *dna2-1* mutant. Surprisingly, the complementation of yeast *dna2-1* by *hBLM* is as efficient as complementation by *hDNA2* (Fig. 1A). Suppression by *hBLM* required galactose induction. Western blotting revealed that the hBLM protein was not expressed at detectable levels on glucose medium (data not shown), but was present at significant levels after 3 h of galactose induction (Fig. 1B). We conclude that high levels of hBLM can function in yeast to compensate for loss of *dna2* function in DNA replication.

To ensure that the complementation was caused by the gene introduced on the plasmid and not by chromosomal reversion or suppression, the transformants were also tested on 5-fluoroorotic acid (5-FOA) plates, on which only cells that have lost the *URA3* vector plasmid and have become Ura<sup>-</sup> can grow. There was no growth at 37°C on 5-FOA, indicating that suppression requires the presence of the *hBLM*, *hDNA2*, and *xDNA2* plasmid-encoded genes (Fig. 1C).

Neither *mWRN* nor *hRECQ4* suppressed the temperature-sensitive growth defect of a *dna2-1* mutant (Figs. 1A and 2C). *mWRN* and *hRECQ4* were expressed under these conditions of induction, but at much lower levels than *hBLM* (data not shown). Because *mWRN* and *hRECQ4* were not expressed at high levels, and because several attempts to improve expression were unsuccessful, we can draw no conclusions at this time about their



**Fig. 1.** (A) *hBLM* and *hDNA2* complement the *dna2-1* temperature-sensitive phenotype. Strain *dna2-1* was transformed with the following plasmids: empty vector (pRS316), pGAL-*hBLM*, pGAL-FLAG-*mWRN*, pGAL-FLAG-*hRecQ4*, pGAL-*hDNA2*-FLAG, and pGAL-*xDNA2*. Transformants were streaked onto SC galactose minus Ura and incubated at 23°C (Upper) and 37°C (Lower) for 10 days. (B) Analysis of expression levels for the *hBLM* and *hDNA2* proteins in *dna2-1* cells. Strains *dna2-1* transformed with empty vector (pRS316), pGAL-*hBLM*, and pGAL-*hDNA2*-FLAG were grown in SC galactose minus Ura and harvested at the indicated times to prepare protein extracts. Extracts were prepared by the alkaline lysis method. Equal amounts of protein were loaded onto SDS/7.5% PAGE and immunoblots were probed with either anti-*BLM* (Upper) or anti-FLAG (Lower) antibodies. The positions of the *hBLM* and *hDNA2* bands are indicated on the right. (C) Strain *dna2-1* carrying empty vector (pRS316), pGAL-*hBLM*, pGAL-*hDNA2*-FLAG, or pGAL-*xDNA2* was streaked onto SC galactose minus Ura plates with (Right) or without (Left) 5-FOA, and grown at 37°C for 7 days.

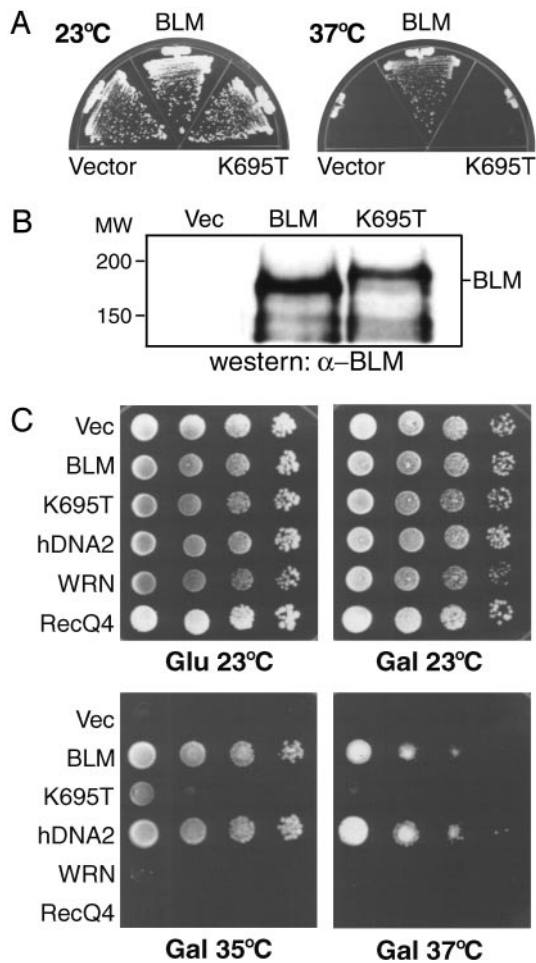
ability or inability to suppress *dna2-1* replication defects. Thus, the remainder of our studies address primarily *hBLM*.

**Failure of *hBLM*(K695T) to Suppress Strain *dna2-1*.** To explore the mechanism of suppression by *hBLM*, a mutation that inactivates the *hBLM* helicase, K695T (9), was expressed in the same way in the *dna2-1* strain. No complementation occurred at 37°C (Fig. 2A). Because the mutant *hBLM* and wild-type proteins were expressed at similar levels (Fig. 2B), the helicase activity of *hBLM* is essential to suppress the *dna2-1* defect. We next examined the complementation by a more quantitative dilution assay. The suppression of yeast *dna2-1* by *hBLM* is as efficient as suppression by *hDNA2*, and the *hBLM*(K695T) mutant was unable to suppress the *dna2-1* defect at 37°C (Fig. 2C). A very low level of viability ( $10^{-3}$ ) was restored to *dna2-1* by *hBLM*(K695T) at 35°C, which might be caused by stabilization of partially active Dna2 protein by the helicase-dead *hBLM* protein at the lower temperature (see below).

**Suppression of HU and MMS Sensitivity of *dna2-1* Mutants by *hBLM*.** *dna2-1* mutants, like other *dna2* mutants (15, 16), are sensitive to MMS, a DNA alkylating agent, and HU, an inhibitor of ribonucleotide reductase, even at the temperature permissive for growth in the absence of drugs (Fig. 3). *hDNA2* suppresses both the MMS and HU sensitivity of *dna2-1* at the permissive temperature (Fig. 3). Wild-type *hBLM* also suppresses sensitivity to 0.005% MMS (Fig. 3A) and 150 mM HU (Fig. 3B), but the *hBLM* helicase mutant (K695T) does not. This finding demonstrates that the ability of *hBLM* to act as a suppressor of *dna2-1* is not uniquely specific for DNA replication, but also includes the ability to suppress sensitivity to DNA-damaging agents. The low level of MMS used here is thought to cause lethal damage during S phase, because it induces the S-phase checkpoint (35). At

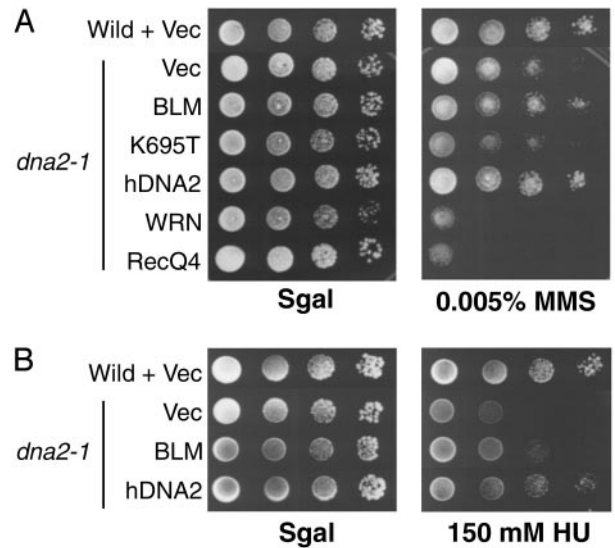
higher levels of MMS, even *hDNA2* fails to complement the damage sensitivity of *dna2-1* mutants (data not shown). Both *mWRN* and *hRECQ4* expression caused increased sensitivity of *dna2-1* to MMS.

**Lack of Suppression of Yeast *rad27Δ* by *hBLM*.** *hBLM* might function at either of two phases in the replication process to suppress the growth defect of *dna2-1* mutants. *hBLM* might reduce the need for Dna2 during its otherwise essential role in fork propagation, or *hBLM* might efficiently mediate repair of toxic structures arising because of *dna2* insufficiency. For instance, *BLM* has been proposed to function in the resolution of regressed DNA replication forks (see ref. 2 for review). If *hBLM* were involved in repair of toxic intermediates, then *hBLM* might suppress defects in mutants affecting proteins that participate in Okazaki fragment processing along with scDNA2. As described in the introduction, the essential function of *DNA2* can be provided by overproduction of scFEN1, and mutants lacking both FEN1 and Dna2 are inviable, suggesting that they interact or perform redundant functions in the same step of DNA replication. scFEN1 is encoded by the *RAD27* gene. *rad27Δ* mutants are viable at 30°C, presumably because another protein can perform a redundant function at this temperature, but *rad27Δ* mutants are not viable at 37°C. We next asked if *hBLM* might also suppress the temperature-sensitive growth of a *rad27Δ* strain. As shown in Fig. 4A, *hBLM* does not suppress temperature-sensitive growth of the *rad27Δ* mutant. *rad27Δ* mutants are also sensitive to MMS. *hBLM* fails to suppress the MMS sensitivity of the *rad27Δ* mutant at 30°C (Fig. 4B). We conclude that either the damage in *rad27Δ* mutants is repaired by a different mechanism than damage in *dna2-1* mutants, or *hBLM* is specifically compensating for Dna2 in propagating the yeast-replication fork.



**Fig. 2.** The helicase activity of hBLM is required for complementation of *dna2-1*. (A) hBLM helicase activity is required for complementation. The *dna2-1* cells carrying empty vector (pRS316), pGAL-hBLM, or pGAL-hBLM(K695T) were streaked onto SC galactose minus Ura plates and grown at 23°C or 37°C for 7 days. The K695T mutation changes lysine to arginine at amino acid position 695 within the helicase domain of hBLM. (B) Expression of hBLM(K695T). Strain *dna2-1* transformed with various vectors were analyzed by Western blotting with anti-hBLM antibody as described in Fig. 1B. Lane Vec, *dna2-1* transformed with empty vector (pRS316); lane BLM, pGAL-hBLM; lane K695T, pGAL-hBLM(K695T). The position of hBLM is indicated. The helicase mutant protein always migrates slightly more slowly than the wild-type protein, as noted (9). (C) Complementation assay at different temperatures. Exponentially growing yeast cultures of *dna2-1* cells carrying empty vector (pRS316), pGAL-hBLM, pGAL-hBLM(K695T), pGAL-FLAG-mWRN, pGAL-FLAG-hRecQ4, and pGAL-hDNA2-FLAG were spotted in 10-fold serial dilutions onto SC glucose minus Ura or SC galactose minus Ura plates and grown at 23°C, 35°C, and 37°C for 5–7 days.

**Physical Interaction Between hBLM and scFEN1 and Between hBLM and scDna2.** To obtain further evidence that hBLM is associated with the replication fork, we wanted to show coimmunoprecipitation of hBLM with a yeast-replication fork protein. We chose scFEN1, because the human WRN helicase/nuclease has been shown to interact with FEN1 (36, 37), and the same might also be true of hBLM, although it has not been reported. As shown in Fig. 5A, we did find convincing coimmunoprecipitation of hBLM and scFEN1-myc, which suggests that both scFEN1 and hBLM reside in the same complex, although we cannot determine whether the interaction is direct or indirect. We also examined the interaction of scFEN1-myc with the hBLM K695T mutant protein, which did not complement the *dna2-1* phenotypes (Figs. 2A and 3A). Similar to the wild-type hBLM, the



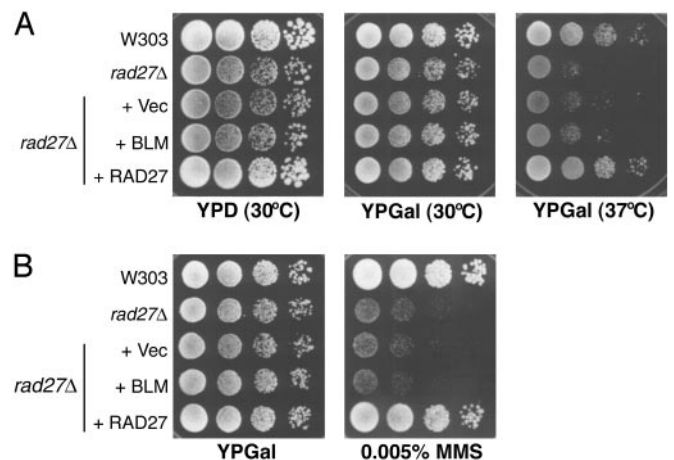
**Fig. 3.** Complementation of sensitivity of *dna2-1* to MMS and HU. Exponentially growing yeast cultures of wild-type cells carrying empty vector (pRS316) and *dna2-1* cells carrying empty vector, pGAL-hBLM, pGAL-hBLM(K695T), pGAL-FLAG-mWRN, pGAL-FLAG-RecQ4, and pGAL-hDNA2-FLAG were spotted in 10-fold serial dilutions onto SC galactose minus Ura plates with or without MMS or HU at the concentrations indicated and grown at the permissive temperature for 6 days. This experiment was performed in duplicate with identical results.

K695T mutant protein coprecipitated with scFEN1 (Fig. 5B). Thus, we speculate that the K695T protein is present at the replication fork but does not suppress *dna2-1* phenotypes because it is catalytically inactive.

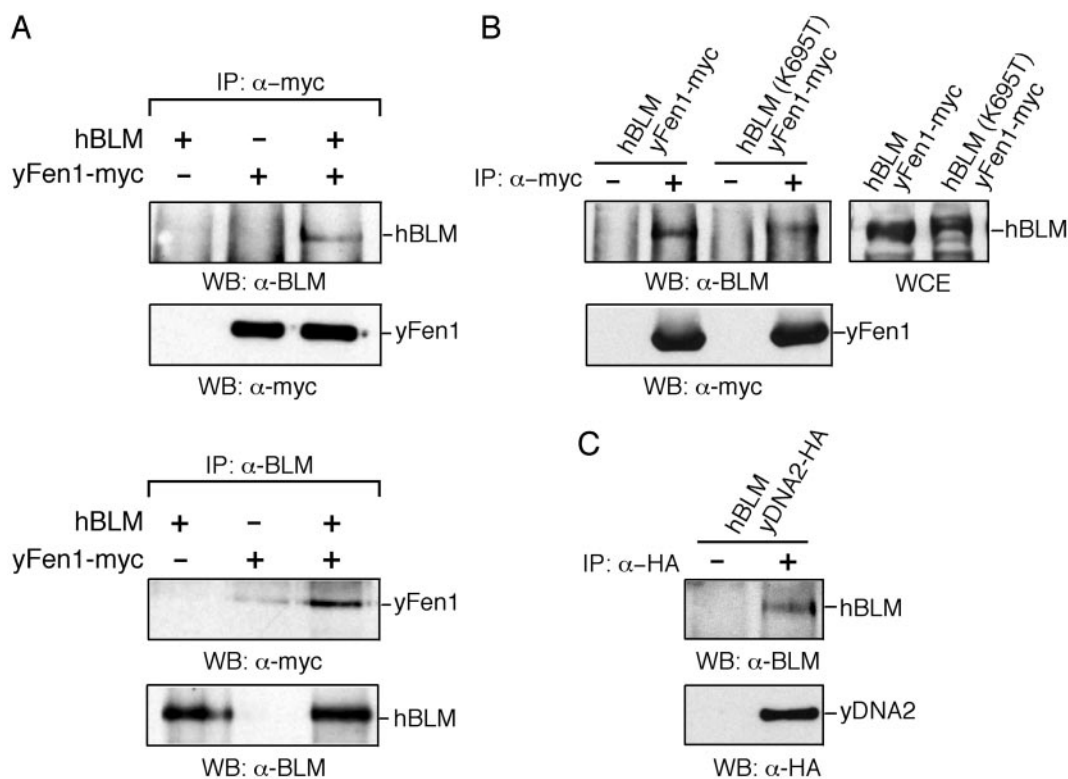
scFEN1 associates with the scDna2 helicase/nuclease when they are overexpressed (19). We therefore examined whether hBLM interacts with scDna2. As shown in Fig. 5C, hBLM also coimmunoprecipitates with scDna2-HA in extracts. These results suggest that hBLM participates in the same steps of DNA replication or repair as scFEN1 and scDna2.

## Discussion

In summary, expression of hBLM in *dna2* mutants suppresses both their replication and DNA repair defects, and with a similar



**Fig. 4.** Lack of complementation of temperature-sensitive growth and MMS sensitivity of strain *rad27Δ*. Exponentially growing yeast cultures of W303 wild type, *rad27Δ*, *rad27Δ*/vector, *rad27Δ*/pGAL-hBLM, and *rad27Δ*/Yep24-Rad27 were spotted in 10-fold serial dilution onto plates. (A) Growth at 30°C or 37°C. (B) Growth in the presence and absence of 0.005% MMS. Cells were grown for 3 days.



**Fig. 5.** Interaction between hBLM and replication fork proteins. (A) Interaction between hBLM and scFEN1. Extracts from BJ5459 transformed with pGAL-hBLM, pJDG-Rad27-myc encoding scFEN1-myc, or both were prepared, and immunoprecipitations were performed with either anti-myc (Upper) or anti-BLM (Lower) antibodies. Washed immunoprecipitates were loaded onto SDS/7.5% PAGE and immunoblotted with either anti-BLM or anti-myc antibodies, as indicated. (B) Interaction of the hBLM(K695T) mutant protein with scFEN1. Extracts were prepared from BJ5459 cells carrying pJDG-Rad27-myc and pGAL-hBLM or pGAL-BLM(K695T) and subjected to immunoprecipitation with anti-myc antibodies. The immunoprecipitates were immunoblotted with either anti-BLM or anti-myc antibodies, as indicated. BLM(K695T) always migrates slightly more slowly than wild-type BLM protein (see also Fig. 2B). (C) Interaction of hBLM with scDNA2. Extracts were prepared from BJ5459 cells carrying pGAL-hBLM and pJDG-DNA2-HA and subjected to immunoprecipitation with anti-HA antibodies. The immunoprecipitates were immunoblotted with either anti-BLM or anti-HA antibodies, as indicated. WCE, whole-cell extract before immunoprecipitation; IP, immunoprecipitation/immunoprecipitate; WB, Western blot of immunoprecipitate; -, control with no antibody in IP mix; +, presence of indicated antibody in IP mix.

efficiency to *hDNA2*. *hBLM* does not suppress *rad27Δ* mutants, however, suggesting that hBLM is specifically suppressing *dna2* defects. This finding suggests that hBLM may be at the replication fork, and, supporting this, hBLM coimmunoprecipitates with scFEN1 and scDna2. This result forges a direct link between hBLM and the replication apparatus. Similarly, depletion of xBLM from *Xenopus in vitro* replication extracts suggests that xBLM is involved in DNA replication (38).

Suppression of the *dna2-1* temperature-sensitive growth and repair defect by *hBLM* helicase is intriguing. The question arises whether hBLM can functionally substitute for scDna2 protein or if the role of the hBLM protein is indirect, involving additional molecular transactions leading to genomic stability of the *dna2* strain. The Dna2 and BLM helicases have opposite directionalities on simple duplex substrate DNAs (39, 40). This finding alone may suggest that the suppression is not caused by direct replacement of Dna2 activities. Because scDna2 and hBLM interact, one form of indirect suppression might be stimulation or stabilization of scDna2 activities. Several lines of evidence involving BLM, WRN, and FEN1 proteins may provide additional clues as to the function of BLM in suppression of the *dna2* defect. (i) We have demonstrated that overproduction of scFEN1 nuclease suppresses the *dna2-1* defect (19). (ii) We have shown that hBLM and scFEN1 interact physically. (iii) WRN and FEN1 interact and the interaction enhances FEN1 cleavage activity (36). (iv) hBLM is thought to recruit several repair/checkpoint proteins to arrested replication forks (41, 42). There-

fore, it is conceivable that the hBLM/scFEN1 interaction at the replication fork leads to either enhanced cleavage by scFEN1 or enhanced recruitment of scFEN1 to the flap, effectively raising the local concentration of the protein and reducing the need for scDna2. Suggesting that some component of the maturation system, such as a helicase, may be missing *in vitro*, pol  $\delta$  cannot strand displace rapidly enough in a reconstituted model system containing purified pol  $\delta$ , proliferating cell nuclear antigen, RFC, FEN1, Dna2, and ligase to account for rates of Okazaki fragment maturation *in vivo* (25, 26). Thus, hBLM might stimulate flap formation. By stimulating scFEN1 in these or in other ways, hBLM might offset the need for full scDna2 activity. Yeast *rad27Δsgs1* double mutants are inviable, suggesting a similar possible role for the yeast hBLM homolog Sgs1 helicase (43). Fibroblasts from patients with Bloom syndrome show a retarded rate of DNA chain growth that might be explained by a model in which BLM is essential for efficient Dna2/FEN1-mediated maturation of replication intermediates (44, 45).

It has recently been suggested that an important function of hBLM and its yeast ortholog, Sgs1, may be to aid the replisome in copying DNA sequences that form alternative structures (46, 47). Both hBLM and Sgs1 proteins were shown to have significantly greater affinity for G quartet DNA and more rapid kinetics of unwinding of G DNA than of Holliday junctions (47). Dna2 helicase might perform a similar function, in addition to or instead of its proposed role in Okazaki fragment processing, which might account for the suppression

of *dna2-1* by *hBLM* and the synthetic lethality of *sgs1* and *dna2-2*. *dna2-2* mutants show increased pausing in the ribosomal DNA (48), and Dna2 is localized to telomeres and participates in their stable maintenance (13). Both of these DNA regions have G-rich repeats. *hBLM* has also recently been linked to telomere function in several studies (49, 50). Taken together, we conclude that *BLM* plays an important role in DNA replication and repair, possibly directly, or, alterna-

tively, as a molecular matchmaker at a crossroad between DNA replication and repair.

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