BLM Helicase Facilitates Mus81 Endonuclease Activity in Human Cells

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Abstract

Bloom syndrome is a rare, autosomal recessive inherited disorder in humans. The product of the Bloom syndrome mutated gene, designated BLM, is a member of the RecQ helicase family. BLM has been proposed to function at the interface of replication and recombination, and to facilitate the repair of DNA damage. Here, we report in vivo physical interaction and colocalization of BLM and a DNA structure–specific endonuclease, Mus81, at sites of stalled replication forks outside the promyelocytic leukemia nuclear bodies during the S-phase arrest of the cell cycle. Amino acids 125 to 244 of Mus81 interact with the C-terminal region (amino acids 1,007-1,417) of BLM. Whereas Mus81 does not have any effect on the helicase activity of BLM, BLM can stimulate Mus81 endonuclease activity on the nicked Holliday junctions and 3’ flap. This stimulation is due to enhanced binding of Mus81 to the DNA substrates. These data suggest a new function of BLM in cooperating with Mus81 during processing and restoration of stalled replication forks. (Cancer Res 2005; 65(7): 2526-31)

Introduction

Mus81, a highly conserved DNA structure–specific endonuclease, which shares homology with the XPF/Rad1 family of proteins involved in DNA nucleotide excision repair, is required for the survival of cells undergoing aberrant replication. Mus81, along with its heterodimeric partner, Mms4 or Emel, functions as an endonuclease by cleaving intact and nicked Holliday junctions (HJ), replication forks, and 3’ flap substrates under in vitro conditions (1, 2). mus81/mms4/eme1 yeast mutants show sensitivity to agents that stall replication forks, such as UV radiation, methyl methane sulfonate, hydroxyurea, and camptothecin (1, 3–5), indicating a role in fork restoration. These mutants do not exhibit sensitivity to ionizing radiation, which produces double-strand breaks. RNA interference experiments show that Mus81 is required for mitotic recombination in somatic human cells (2). Recently, it was reported that Mus81 homozygote and heterozygote knockout mice have a predisposition to develop cancer (6).

Members of the evolutionarily conserved RecQ helicase family can unwind various branched DNA structures (7). Defects in the human RecQ helicase genes BLM, WRN, and RecQ4 resulted in Bloom syndrome (BS), Werner syndrome, and Rothmund-Thomson syndrome, respectively. These genetic disorders are characterized by cancer predisposition and/or premature aging. BLM, found in both promyelocytic leukemia (PML) nuclear bodies and the nucleolus, responds to replication stress by accumulating at stalled DNA replication forks and modulates diverse functions like homologous recombination and mismatch repair by affecting the roles of process-specific proteins like p53, MSH2/6, RAD51, and RAD51D (7–11).

Yeast genetic studies indicate a functional overlap between Sgs1-Top3 and the Mms4-Mus81 endonuclease (12). Synthetic lethal mutants of Mus81 in combination with the deletion of RecQ helicases in fission and budding yeast implicate their cooperative role in the recovery from DNA synthesis arrest (13). Recently, we reported that BLM and WRN, which are required for recombination repair in human cells, colocalize with Mus81 in nucleoli (5). However, the nucleolar retention of Mus81 is not dependent on the presence of BLM or WRN (5). We also showed that human Mus81 is abundant in replicative and post-replicative cells (4). Like BLM, the level of Mus81 increased after exposure to agents that block DNA replication (4, 8). Here we report in detail the physical and functional interactions of BLM and Mus81 during replication stress.

Materials and Methods

Cell lines. hTERT-immortalized normal human fibroblasts (NHF), the human colon cancer cell line HCT116, and two pairs of isogenic BS cell lines, GM08505(GFP)/GM08505-GFP-BLM [termed BS(GFP)/BS(BLM)] and GM08505/GM08505-BLM [termed BS/BS(BLM)], have all been previously described (8, 14, 15).

Antibodies. Antibodies used were rabbit anti-Mus81(4); rabbit polyclonal anti-BLM, ab476 (Novus Biologicals, Littleton, CO); goat polyclonal anti-BLM, C-18 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA); mouse monoclonal anti-RAD51, antibody 2 (Oncogene Research Products, Boston, MA); mouse monoclonal anti-PCNA (Oncogene Research Products); mouse monoclonal anti-hemagglutinin (Covance, Berkeley, CA); and normal rabbit immunoglobulin G (IgG; Santa Cruz Biotechnology).

Immunoblots and immunoprecipitation. Cells were lysed in lysis buffer (4). Immunoblots were incubated with the first antibody (1:1,000) and secondary antibody (1:5,000) conjugated to horseradish peroxidase (Santa Cruz Biotechnology). Chemiluminescence was used to develop immune antibodies. Quantitation of immunoprecipitated Mus81 was determined by scanning Western blot done using purified recombinant GST-Mus81 protein titration. Densitometric quantification was analyzed by Image Gauge V3.46 from Fuji Film.

Immunofluorescence. The immunofluorescence procedure and percentage of colocalization were done as previously described (8). Cells were visualized with a Zeiss Axioskop fluorescence microscope equipped with a high performance CCD imaging system and IP Lab Spectrum software. The percentage of colocalization in the quantitation indicates the percentage of cells in which the two proteins being analyzed colocalized, and is the average obtained from three independent experiments.

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Transfection, protein expression, and purification. GFP- and GFP-BLM–expressing cells were transiently transfected with wild-type pcDNA-3HA-Mus81 (4) using Lipofectamine 2000 (Invitrogen, San Diego, CA), according to the protocol of the manufacturer. Recombinant hexahistidine–tagged human BLM (rBLM) and full-length and truncated GST-Mus81 were expressed and purified from yeast and E. coli, respectively, as described (4, 16). Recombinant WRN (WRN) and recombinant replication protein A (RPA) were generously provided by Lawrence A. Loeb and Mark K. Kenny, respectively.

Endonuclease and helicase assays. Immunoprecipitated endogenous Mus81 was tested for its ability to cleave either a 32p-3' flap (17) or a 32p-nicked HJ (nX01; ref. 18) according to a previously described procedure (4). The reaction condition of the rBLM unwinding an X-junction, which mimics an HJ, was previously described (19). The DNA substrates and their cleavage products were resolved on 10% Tris-borate EDTA native polyacrylamide gel and detected by autoradiography. The percentages of DNA substrates either cleaved by Mus81 or unwound by rBLM were calculated according to densitometric quantification using Image Gauge V3.46 from Fuji Film.

Mus81-BLM 3' flap DNA binding. The 3' flap DNA was biotinylated at the 5' end of oligo 1 (BioServer Biotechnologies, Laurel, MD). In vitro translated HA-Mus81 (2 μL; Promega, Madison, WI) and biotinylated 3'-flap DNA (0.1 pmol) were incubated in the absence or presence of various amounts of rBLM for 30 minutes. The biotinylated 3' flap DNA substrates

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Mus81 and GFP-BLM physically interact and colocalize at stalled replication forks. **A,** Mus81 and GFP-BLM colocalize at replication foci in BLM (WT) cells. The cells were incubated with hydroxyurea (HU) for 3 hours. Immunofluorescence was visualized as (1) GFP-BLM/PCNA, (2) GFP-BLM/RAD51, (3) PCNA/Mus81, (4) RAD51/Mus81, (5) PML/Mus81, and (6) GFP-BLM/Mus81. B, Mus81 colocalized with PCNA and RAD51 in BS cells. BLM-deficient cells were treated as mentioned in A, and immunofluorescence was visualized as (7) PCNA/Mus81 and (8) RAD51/Mus81. C, quantitation of PCNA/Mus81 and RAD51/Mus81 colocalizations in BLM (WT) and BLM (null) cells. D, Mus81 and GFP-BLM physically interact in vivo. Top, a and b, BS(GFP) and BS(GFP-BLM) cells were left either untreated (lanes 1 and 3) or treated (lanes 2, 4, and 5) with hydroxyurea (2 mM/L) for 16 hours. Cell lysates (500 μg) were immunoprecipitated with either anti-Mus81 antibody (lanes 1-4) or corresponding IgG (lane 5). Coimmunoprecipitated BLM was detected with anti-BLM antibody (ab476). Input indicates 10% of the lysates used for immunoprecipitation. Approximately 20% of the BLM, from the cell lysate inputs of BS(GFP-BLM) cells, but not BS(GFP) cells, was detected in Mus81 immunoprecipitates (b, lanes 1-4). Bottom, c and d, BS(GFP) and BS(GFP-BLM) cells were transfected with HA-Mus81 and were either left untreated (lanes 1 and 3) or treated (lanes 2, 4, and 5) with hydroxyurea for 16 hours. The immunoprecipitation was done with either anti-BLM antibody (ab476; lanes 1-4) or corresponding IgG (lane 5). Coimmunoprecipitated HA-Mus81 was detected by immunoblotting using mouse anti-hemagglutinin antibody. Approximately 12% of HA-Mus81, from cell lysate inputs of BS(GFP-BLM) cells, but not from BS(GFP) cells, was coimmunoprecipitated with the anti-BLM antibody (d, lanes 1-4).
were retrieved with streptavidin beads, and the unbound proteins were washed away with the binding buffer. Buffers and binding procedures were described previously (20). HA-Mus81 bound to the biotinylated 3' flap DNA was detected by immunoblotting.

Results and Discussion

Colocalization and physical interaction of Mus81 with BLM.

Two pairs of isogenic cell lines, BS(GFP)/BS(GFP-BLM) and BS/BS(BLM), were used to investigate the localization of Mus81 and BLM. BS and BS(GFP) are null for BLM, whereas BS(BLM) and BS(GFP-BLM) are restored with BLM wild-type (WT). In these cell lines, in the absence of replication stress, BLM was mostly present in the nucleolus and PML nuclear bodies. Mus81 staining was heterogeneous, both nucleolar and diffuse nucleoplasmic staining were observed, indicating a low level of BLM-Mus81 colocalization even in asynchronous cultures (Supplementary Fig. 1, \( \text{HU} \)). However, the localization pattern of both BLM and Mus81 changed within 3 hours of replication arrest. After hydroxyurea treatment, both BLM and Mus81 accumulated as foci at stalled replication forks, as confirmed by their colocalization with PCNA and RAD51. Consequently, both Mus81 and BLM also colocalized. These replication foci, containing both BLM and Mus81, were present outside the PML nuclear bodies (Fig. 1A and Supplementary Fig. 1, +HU). Mus81-Rad51 and Mus81-PCNA colocalizations were also observed in BLM-null cells treated with hydroxyurea (Fig. 1B and Supplementary Fig. 2). However, the extent of the colocalization was always significantly enhanced in cells containing wild-type BLM (Fig. 1C). These results indicate that whereas Mus81 can independently accumulate at sites of stalled DNA replication forks following replication stress, its accumulation is facilitated by BLM.

To further evaluate the physical interaction between Mus81 and BLM, we did immunoprecipitations of endogenous Mus81 and GFP-BLM in the BS(GFP)/BS(GFP-BLM) isogenic cell lines. Approximately 20% of BLM, from the cell lysate inputs of BS(GFP-BLM) cells, but not BS(GFP) cells, was detected in Mus81 immunoprecipitates (Fig. 1D, \( \text{b} \), lanes 1-4). Immunoprecipitation with the corresponding IgG did not reveal the presence of BLM, indicating the specificity of the Mus81-BLM interaction (Fig. 1D, \( \text{b} \), lane 5). This interaction was specific for BLM as WRN, another member of the RecQ helicase family, did not coimmunoprecipitate with Mus81 (Supplementary Fig. 3A). To confirm this interaction, HA-Mus81 was transfected into the BS(GFP)/BS(GFP-BLM) isogenic pair. Approximately 12% of HA-Mus81, from cell lysate inputs of BS(GFP-BLM) cells, but not BS(GFP) cells, was coimmunoprecipitated with the anti-BLM antibody (Fig. 1D, \( \text{d} \), lanes 1-4). Lack of immunoprecipitation with the corresponding IgG (Fig. 1D, \( \text{d} \), lane 5) again revealed the specificity of the interaction. Under the same experimental conditions, immunoprecipitation with anti-hemagglutinin antibody revealed the presence of BLM. This interaction was no longer detectable when the immunoprecipitation was carried out in the presence of the peptide, against which the HA antibody was raised (Supplementary Fig. 3B). Similar coimmunoprecipitation results

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**Figure 2.** Mapping of the binding sites of Mus81 and BLM interaction. A, schematic diagram of the full-length and truncated GST-Mus81 deletion constructs. B, interaction of full-length and truncated GST-Mus81 with in vitro translated full-length BLM (\( ^{35} \text{S-BLM} \)). Recombinant GST fusion Mus81 full-length and truncated derivatives from (A) were expressed in bacteria and purified. Equal amount of the GST fusion protein (lanes 2-7) or GST alone (lane 8) was immobilized and incubated with 10 \( \mu \)L of in vitro translated \( ^{35} \text{S-BLM} \). Fifteen percent of input (lane 1) and resin-bound proteins were resolved by 10% SDS-PAGE and \( ^{35} \text{S-BLM} \) was detected by autoradiography. C, schematic diagram of BLM fragment constructs. D, BLM C-terminal domain (amino acids 1,007-1,417) interacts with GST-Mus81. In vitro translated \( ^{35} \text{S-labeled full-length (FL) and three fragments (I, II, and III) of BLM were incubated with either GST-Mus81 (lanes 5, 7, 8, and 9) or GST alone (lane 6). Fifteen percent of input (lanes 1-4) and bound proteins were analyzed by 8% SDS-PAGE and detected by autoradiography.**
were also obtained when cell lysates were treated with DNase I (data not shown). Collectively, these results indicate that BLM and Mus81 physically interact in vivo.

Mapping of the binding sites of Mus81 and BLM interaction. To identify the BLM binding region in Mus81, we did pull-down assays using an in vitro translated 35S-labeled full-length BLM (35S-BLM) and recombinant GST fusion proteins containing either full-length Mus81 or its truncated derivatives (Fig. 2A). BLM bound to full-length Mus81 and to its truncated derivatives only when they contained the amino acids 125 to 244 (Fig. 2B). To determine the region in BLM that interacted with Mus81, pull-down assays using full-length GST-Mus81 and 35S-labeled full-length or fragments (I, II, and III) of BLM (Fig. 2C) were done. Mus81 strongly bound to BLM only at its C-terminal domain encompassing the amino acids 1,007 to 1,417 (Fig. 2D). The site of Mus81 binding to BLM partially overlapped with its interaction site to the checkpoint kinase, Cds1 (21). Similarly, the C-terminal region of BLM that binds to Mus81 also binds to proteins involved in DNA repair and recombination like RAD51, TopoIIIa, MLH1, and FEN-1 (7, 22). These results indicate that BLM-Mus81 interaction may not only have a direct consequence on the processing of different types of DNA junctions, but also indirectly may serve as a platform for diverse functions like damage recognition, signal transduction, DNA repair, and recombination.

BLM stimulates Mus81 endonuclease activity in vitro and in vivo. The physical interaction between Mus81 and BLM suggested the two proteins might modulate mutual activities during replication stress. To test this hypothesis, we first determined whether BLM regulated Mus81 endonuclease activity. Mus81 has been shown to act on and process different types of DNA junctions with varying efficiencies. Recent studies have indicated that the preferred structures on which Mus81 can act are the four-way DNA junctions that contain an exposed 5' DNA strand end or 3' flap structures (23, 24). Therefore, the effect of BLM on Mus81 endonuclease activity was assayed using either nicked HJs (Fig. 3A) or 3' flap structures (Fig. 3C). Equivalent amounts of immunoprecipitated Mus81 from NHF, determined by quantitative Western blot done using purified recombinant GST-Mus81 protein titration (data not shown), were used for these assays (Fig. 3A and C, bottom). Immunoprecipitated Mus81 (3.8 fmol for Fig. 3A, and 7.6 fmol for Fig. 3C) from NHF alone could cleave around 30% of the total substrates. However, the addition of recombinant BLM to the endonuclease assays enhanced the cleavage of both substrates to 60% to 70% (Fig. 3A and C). Another human RecQ helicase, WRN, as well as an unrelated protein like RPA, did not stimulate Mus81-mediated cleavage of nicked HJs, indicating the specificity of the BLM-mediated stimulation (Fig. 3B). The endonuclease activity by the

![Figure 3](https://via.placeholder.com/150)

**Figure 3.** BLM stimulates Mus81 endonuclease activity in vitro. A, rBLM stimulates Mus81 endonuclease activity on nicked HJ in vitro. The endonuclease activity assay was carried out by incubating the 32P-nicked HJ substrate (1 nmol/L) with immunoprecipitates of either anti-Mus81 (lanes 2–4) or IgG (lane 5) from NHF cell lysates (250 μg) in the presence [50 fmol (lane 3) and 250 fmol (lane 4)] or absence (lanes 2 and 5) of rBLM at 30°C for 30 minutes. Equivalent amounts of immunoprecipitated Mus81 (3.8 fmol), determined by quantitative Western blot done using purified recombinant GST-Mus81 protein titration (data not shown), were used. rBLM itself did not cleave the nicked HJ substrate (lane 6). Lanes 2 to 5, the immunoprecipitated proteins were probed with Mus81 antibody (bottom). B, WRN and RPA did not stimulate Mus81 endonuclease activity on nicked HJ in vitro. The endonuclease activity assay was carried out as described in A with immunoprecipitates of anti-Mus81 in the absence (lane 2) or presence of the indicated amounts of WRN (lanes 3 and 4), RPA (lanes 6 and 7), or rBLM (lane 9). Neither WRN nor RPA alone cleaved the nicked HJ (lanes 5 and 8). Lanes 2 to 4, 6, 7, 9, and 10, immunoprecipitated proteins were probed with Mus81 antibody (bottom). C, rBLM stimulates Mus81 endonuclease activity on the 3' flap structure in vitro. The endonuclease activity assay was carried out by incubating the 32P-3' flap DNA substrate (0.25 nmol/L) with immunoprecipitates of either anti-Mus81 (lanes 4 and 5) or IgG (lane 3) from NHF cell lysates (1 mg) in the presence (100 fmol, lane 5) or absence (lanes 3 and 4) of rBLM at 30°C for 30 minutes. Immunoprecipitated Mus81 (7.8 fmol), determined as in A, was used. Lane 1, denatured (Δ) at 95°C for 3 minutes; lane 2, nondenatured 32P-3' flap substrate. Lanes 3 to 5, immunoprecipitated proteins were probed with Mus81 antibody (bottom). D, rBLM dose dependently stimulates Mus81 endonuclease activity from HCT116 cells on the 3' flap structure in vitro. Mus81 (4 fmol) was immunoprecipitated from the cell lysates (1 mg) of HCT116 cells (lanes 2–6). The endonuclease activity of Mus81 was analyzed as mentioned in C in the presence of indicated amounts of rBLM (lanes 2–6). IgG served as a negative control for immunoprecipitation (lane 5). rBLM itself did not cleave the 3' flap DNA substrate (lane 6). Lanes 2 to 5, immunoprecipitated proteins were probed with Mus81 antibody (bottom).
immunoprecipitated Mus81 is specific as the control immunoprecipitates with the IgG showed no detectable endonuclease activity. The BLM-dependent stimulation of equivalent amounts of immunoprecipitated Mus81 was also evident in the colon cancer cell line HCT116. The cleavage activity of immunoprecipitated Mus81 (4 fmol) from HCT116 cells on 3' flap structure was also enhanced by rBLM in a dose-dependent manner (Fig. 3D). These results revealed that BLM stimulated Mus81 endonuclease-mediated cleavage of DNA substrates. BLM-mediated enhancement of Mus81-dependent cleavage of intact HJs has also been observed. However, the effects were modest as Mus81 cleaves intact HJs much less efficiently than 3' flaps and nicked HJs (data not shown).

BLM promotes HJ branch migration, which is necessary for restoring stalled replication forks (25). We next investigated whether Mus81 affected BLM helicase activity. We measured rBLM helicase activity on the unwinding X-junction with or without adding an equivalent amount of immunoprecipitated Mus81 (Supplementary Fig. 3C, bottom). We found that there is no significant difference in BLM-mediated helicase activity due to the presence of Mus81 (Supplementary Fig. 3C).

To determine whether BLM affected the Mus81 endonuclease activity in vivo, equivalent amounts of Mus81 (4 fmol), immunoprecipitated from BS(GFP) or BS(GFP-BLM) cells, were assayed for their ability to cleave the 3' flap substrate. The presence of BLM stimulated Mus81-mediated cleavage (Fig. 4A). These results suggest that Mus81 and BLM cooperated in processing the stalled replication forks during replication stress. BLM facilitates Mus81 binding to the 3'-flap DNA substrate.

To determine the mechanism of the BLM-mediated stimulation of the endonuclease activity of Mus81, we tested whether rBLM stimulated the binding of HA-Mus81 to the 3' flap DNA structure. Immunoblot analysis with anti-hemagglutinin antibody showed that in the absence of rBLM, a low level of HA-Mus81 was associated with biotinylated-3' flap DNA, as retrieved by streptavidin beads (Fig. 4B, lane 3). The addition of rBLM increased the binding of HA-Mus81 to the DNA in a dose-dependent manner (Fig. 4B, compare lane 3 with lanes 4 and 5). The densitometry quantification analysis indicates that the ratios of the enhanced binding of HA-Mus81 to the 3' flap by rBLM were 1.9- and 3.1-fold, with 50 and 250 fmol of rBLM, respectively (lanes 3-5). Bovine serum albumin did not affect BLM-facilitated Mus81 binding to the 3' flap substrate (lanes 6 and 7). HA-Mus81 was detected by immunoblotting with the anti-hemagglutinin antibody. Input, 25% of the total HA-Mus81 used in each reaction (lane 1).

Due to the functions of the RecQ helicases at the junction of DNA repair, recombination, and replication, it can be postulated that the helicases can functionally interact with a variety of specific proteins, including endonucleases, involved in the above processes. For example, WRN and BLM physically interact with FEN-1, a 5' flap

Figure 4. rBLM enhances the binding of Mus81 to the 3' flap DNA substrate. A, BLM elevates the Mus81 endonuclease activity in vivo. BS(GFP)/BS(GFP-BLM) cells were treated with lanes 3, 5, and 6 or without lanes 2 and 4 hydroxyurea for 16 hours. Mus81 was immunoprecipitated from 500 μg of cell lysates and endonuclease activity of Mus81 (4 fmol) was measured. Immunoprecipitation with pre-immune serum did not show any cleavage (lane 6). Lanes 2 to 6, the immunoprecipitated proteins were probed with Mus81 antibody (bottom). B, rBLM facilitates Mus81 binding to the 3' flap DNA substrate. The biotin-3' flap DNA was incubated with in vitro translated HA-Mus81 in the absence (lane 3) or presence of various amounts of rBLM (50 and 250 fmol, lanes 4 and 5). The biotinylated 3' flap DNA and its bound proteins on the beads were analyzed by SDS-PAGE gel. The densitometry quantification analysis indicates that the ratios of the enhanced binding of HA-Mus81 to the 3' flap by rBLM were 1.9- and 3.1-fold, with 50 and 250 fmol of rBLM, respectively (lanes 3-5). Bovine serum albumin did not affect BLM-facilitated Mus81 binding to the 3' flap substrate (lanes 6 and 7). HA-Mus81 was detected by immunoblotting with the anti-hemagglutinin antibody. Input, 25% of the total HA-Mus81 used in each reaction (lane 1).
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endonuclease/5’-3’ exonuclease involved in the base excision repair pathway and DNA replication (22, 26). Interestingly, the C-terminal regions of both BRM and BLM interact with FEN-1. WRN and BLM stimulate both the endonucleolytic and exonucleolytic cleavage activities of FEN-1. WRN recruits FEN-1 to HJs and unwinds the chicken-foot structure, thereby enabling FEN-1 to cleave the unwound structure (26). Likewise, the data presented here suggest that BLM may both recruit Mus81 to sites of DNA damage and stimulate its endonuclease activity on at least the 3’ flap structure, and possibly on other DNA structures too. It will be interesting to know whether BLM, like WRN, can also affect the Mus81-mediated cleavage efficiency.

The requirement for Sgs1-Top3 in yeast can be bypassed by the use of the structure-specific endonuclease activity of Mus81-Mms4 (12, 27). According to this model, it has been hypothesized that DNA structures formed during replication can be acted on either by Sgs1 or Mus81-Mms4, leading to the processing of these intermediates. Hence, absence of both pathways leads to a severe growth defect that can be suppressed by recombination defects. According to this yeast-based model, Sgs1 and Mus81 do not necessarily need to physically interact. However, these results do not definitely indicate that physical interaction between Mus81 and Sgs1 did not occur. More importantly, it is not known which of the mammalian helicases is functionally equivalent to yeast Sgs1 or Rqh1. Hence, the extrapolation of the yeast model to human cells may not be necessarily accurate.

A variety of models predict the formation and resolution of different DNA structures during homologous recombination that probably occur following replication stress. These structures include 3’ flaps, forks, D-loops, nicked, gapped, and intact HJs (24). Indeed, recent studies have indicated that these structures are possible in vivo substrates of Mus81 (18, 23, 24, 28). Our results show that human Mus81 and BLM interact in vivo and the interaction results in the modulation of Mus81 activity. We envisage that BLM can act on DNA intermediates that are formed during replication stress, leading to the formation of DNA structures of which processing may require an endonuclease like Mus81. In this latter step, BLM recruits Mus81 more efficiently to these intermediates. Moreover, BLM also stimulates Mus81 to more efficiently bind to the 3’ flap structure, possibly by utilizing its helicase activity to unwind the 5’ end of the junction.

We had previously hypothesized that a stalled replication fork is restored by either reverse branch migration, mediated by the BLM helicase in a non-recombinogenic pathway, or by a recombinogenic pathway that involves RAD51/RAD54-mediated homologous recombination (8). Here, we provide further evidence of BLM being a multifunctional protein: not only can it affect the homologous recombination process by transporting p53 to sites of stalled replication (8), but it can also affect the localization and function of the Mus81 endonuclease (this report). Hence, apart from its own helicase function, BLM can also modulate the functions of several key proteins, thereby living up to its characterization as a “caretaker tumor suppressor” (7, 11).

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