Physical and Functional Interaction between the Bloom’s Syndrome Gene Product and the Largest Subunit of Chromatin Assembly Factor 1

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Bloom’s syndrome (BS) is a genomic instability disorder characterized by cancer susceptibility. The protein defective in BS, BLM, belongs to the RecQ family of DNA helicases. In this study, we found that BLM interacts with hP150, the largest subunit of chromatin assembly factor 1 (CAF-1), in vitro and in vivo. Colocalization of a proportion of the cellular complement of these two proteins is found at specific nuclear foci coinciding with sites of DNA synthesis in the S phase. This colocalization increases in the presence of agents that damage DNA or inhibit DNA replication. In support of a functional interaction between BLM and CAF-1, we show that BLM inhibits CAF-1-mediated chromatin assembly during DNA repair in vitro. Although CAF-1 activity is not altered in BLM-deficient cells, the absence of BLM does impair the ability of CAF-1 to be mobilized within the nucleus in response to hydroxyurea treatment. Our results provide the first link between BLM and chromatin assembly coupled to DNA repair and suggest that BLM and CAF-1 function in a coordinated way to promote survival in response to DNA damage and/or replication blockade.

Bloom’s syndrome (BS) is a rare recessive genetic disorder associated with cancer predisposition, immunodeficiency, proportional dwarfism, and a sunlight-induced facial erythema (16). Cells from BS patients display genomic instability, including an excessive number of locus-specific mutations as well as a high frequency of microscopically visible chromatid gaps, rearrangements, breaks, and sister chromatid exchanges (40). BS cells are not defective in the known DNA repair pathways (14, 26, 38) but show a retarded rate of nascent DNA chain elongation (18) and accumulate abnormal replication intermediates (29). The gene mutated in BS encodes a DNA helicase (BLM) belonging to the RecQ family (12). Recombinant BLM exhibits ATP-dependent 3'-5' DNA helicase activity (22). Immunolocalization studies have shown that BLM is found mainly in the nucleus at discrete foci (shown to coincide with promyelocytic leukemia protein (PML) bodies) (21, 35) and in nucleoli during S phase (55).

Recently, much attention has been paid to the characterization of proteins that associate with the RecQ family of DNA helicases in order to understand their exact roles in the maintenance of genome stability in human cells (19, 34). BLM has been reported to interact with several proteins, including replication protein A (RPA), a DNA binding protein that plays essential roles during DNA replication, repair, and recombination (8); topoisomerase IIIα, a protein that, together with BLM, can resolve intermediates that arise during homologous recombinational repair (53, 54); BRCA1, a protein required for efficient homologous recombination and which acts as a signal processor for DNA damage responses (50); RAD51 and RAD51L3, proteins required for DNA strand invasion and exchange during homologous recombination (6, 7, 52); p53, a tumor suppressor implicated in S-phase processes, DNA repair, and homologous recombination (11, 41, 49); TRF2, a telomere binding protein (36, 44); WRN, Werner syndrome protein, another member of the RecQ helicase family (48); ATM, one of the essential transducers of DNA damage checkpoint responses (5); MLH1 and MSH6, proteins involved in mismatch repair and recombination (26, 37, 38); and five Fanconi anemia proteins: FANCA, FANCG, FANCC, FANCE, and FANCF, a multiprotein complex suggested to function in a DNA damage response pathway (31). The phenotypes exhibited by BS cells, as well as the proteins that can associate with BLM protein, imply that BLM may be involved in DNA recombination and repair processes linked to DNA replication and in telomere maintenance. There are several lines of evidence to show the importance of BLM during DNA replication. First, in Xenopus egg extracts, depletion of the Xenopus BLM homolog (xBLM) leads to an inhibition of DNA replication that can be rescued by addition of recombinant xBLM (27). Second, BLM−/− cells possess replication defects that include the accumulation of abnormal replication fork intermediates, retarded replication fork progression, and the delayed maturation of Okazaki fragments (10). Third, BLM is localized to replication foci, particularly during late S phase (51). However, it remains unclear whether the involvement of BLM in DNA replication is direct or is a secondary effect of lacking efficient repair of stalled replication forks. It is therefore of considerable interest to study the mechanism of how BLM influences DNA synthesis.

In this paper, we report that BLM physically and function-
ally associates with hp150, the largest subunit of chromatin assembly factor I (CAF-1). CAF-1 is a three-subunit complex that promotes the incorporation of histones H3 and H4 into newly synthesized DNA during replication (15) or during nucleotide excision repair (15). Moreover, we show that BLM and hp150 colocalize to sites of DNA synthesis when cells are exposed to DNA replication inhibitors or DNA-damaging agents. In pursuit of a biological function of the BLM-hp150 interaction, we found that BLM specifically attenuates CAF-1-mediated chromatin assembly during DNA repair in vitro. Furthermore, we show that in BLM−/− cells, CAF-1 activity is not altered but that the absence of BLM affects the localization of CAF-1 following hydroxyurea (HU) treatment. Collectively, our data indicate that BLM and CAF-1 may function together in vivo during DNA metabolic processes and provide a novel insight into the role of BLM and CAF-1 in the maintenance of genome stability.

MATERIALS AND METHODS

Constructs. Construction of part of the BLM deletion mutant series (see Fig. 2) has been described previously (38). PCR products of BLM313–333 and BLM599–652, from plasmid pPK1 (22) were cloned into the pBTM116 vector. The presence of the correct clones was verified by sequencing and by checking expression in yeast cells by Western blotting, hp150 and its deletion mutants were cloned into pBTM116 and pACT2 vectors for yeast two-hybrid (YTH) testing and into the pCT4E vector (Novagen) for in vitro transcription-translation analyses. Precise details of sequences and construction schemes are available on request.

Yeast two-hybrid (YTH) assay. The screening of an activation domain (AD) cDNA library generated from human peripheral blood mRNA using the C-terminal 647 amino acids of BLM as a bait was performed as described previously (38). Standard yeast transformation and interaction tests were used to map the interaction domains for BLM and hp150. Yeast strain L40 (MATα his2-3 lys2 lexA-lacZ ura3 lacI-lacZ) was used.

In vitro binding assay. Different amounts (1.25, 2.5, and 5 pmol) of purified recombinant BLM, which was diluted in protein storage buffer (50 mM Tris-HCl, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 1 mM phenylmethylsulfonyl fluoride [PMSF]); 5 mM dithiothreitol [DTT], protease inhibitors) before the nuclei were resuspended in 3 volumes of buffer A+ (buffer A containing 10 mM HEPES [pH 7.9], 1.5 mM MgCl2, 10 mM KCl, 1 mM phenylmethylsulfonyl fluoride [PMSF], 5 mM dithiothreitol [DTT], protease inhibitors) before the pellet was resuspended in 3 volumes of buffer A− (buffer A containing an additional 0.1% NP-40). After incubation for 5 min, the cells were centrifuged and the pellet was resuspended in 3 volumes of buffer C (20 mM HEPES [pH 7.9], 450 mM NaCl, 1.5 mM MgCl2, 25% [vol/vol] glycerol, 80 mM PMSF, 5 mM DTT, other protease inhibitors) and incubated on ice for 15 min. Finally, after the mixture was pelleted, the supernatant was taken as the soluble fraction of nuclear proteins (the concentration of NaCl was adjusted to 120 mM with buffer D, which contains 20 mM HEPES [pH 7.9], 1 mM PMSF, 5 mM DTT, and protease inhibitors) and the pellet was either subjected to ultrasonication to dissolve the insoluble fraction of nuclear proteins in SDS sample buffer or digested with DNase I in DIG buffer (50 mM NaCl, 10 mM Tris [pH 6.8], 3 mM MgCl2, 1 mM EGTA, 0.5% Triton, 100 μg of DNase I per ml, 50 μg of RNase A per ml, 1,2 mM PMSF) for 30 min at room temperature before the chromatin fraction was separated from the nuclear matrix fraction.

Cell synchronization and immunofluorescence microscopy. HeLa cells, BS cells, and PSNF cells (BS cells containing the BLM cDNA [BLM+/−/+pBLM]) were grown on chamber slides (Lab-Tek) to up to 50% confluence before being subjected to synchronization or treatment with variant DNA-damaging agents. For a G2/S block, 5 mM thymidine and 2 μg of aphidicolin per ml were added to the cells for 28 and 24 h, respectively; for S-phase synchronization, 2 mM HU was used for 24 h; for a G2/M block, 1 μg of nocodazole per ml was incubated with the cells for 24 h. Synchronized cells or cells taken 20 h after treatment with Methyl-N′-nitro-N-nitrosoguanidine (MNNG) or 4 to 18 h after UV treatment were permeablized with 0.5% (vol/vol) Triton in PBS (PBS supplemented with 5 mM MgCl2) plus 5 mM PMSF for 5 min at room temperature before being fixed in paraformaldehyde for 20 min. After 20 min of further treatment with 0.5% (vol/vol) Triton, and following washing with PBSS (PBS with 0.1% [vol/vol] Tween 20), the slides were blocked for 20 min in PBST (PBBS plus 5% [vol/vol] BSA) for 2 h at room temperature. To visualize the primary antibodies, Cy3-conjugated goat anti-rabbit IgG (1:200; Jackson ImmunoResearch) and 5-(4,6-dichlorotiazin-2-yl)aminofluorescein (DTAF)-coupled goat anti-mouse IgG (1:100; ANAWA) or fluorescein isothiocyanate-coupled goat anti-mouse IgG (1:100; Jackson ImmunoResearch) were used, and 1 μg of 4,6-diamidino-2-phenylindole (DAPI) per ml was added to stain the DNA. The stained cells were mounted in Vectashield (Vector laboratories) and observed under a Leica TCS 4D confocal microscope. Imaris software was used to process the images.

Chromatin assembly assay. An in vitro chromatin assembly assay with a human cell-free system was utilized to analyze the effect of recombinant BLM on DNA repair-coupled chromatin assembly (15). Briefly, UV (500 J/m2)-irradiated circular pBluescript plasmid DNA was incubated for 3 h at 37°C with cytosolic extract derived from HeLa cells (38) supplemented with purified recombinant CAF-1 complex or with nuclear extract from either HeLa or BS cells (47) in the presence or absence of recombinant native BLM or BLM preincubated with anti-MLH antibody (32). The extent of supercoiling was examined by agarose gel electrophoresis followed by dried of the gel and exposure to X-ray film. Incorporation of [α-32P]dCTP indicates newly synthesized DNA after nucleotide excision repair.

RESULTS

BLM and hp150 interact in vitro and in vivo. To identify proteins that interact with BLM, we screened an AD cDNA library generated from human peripheral blood mRNA, using the C-terminal 647 amino acids of BLM as a bait (38). Two interacting clones contained amino acids 39 to 938 and 238 to 938 of hp150 fused in frame to the AD (Fig. 1A). To ascertain the specificity of this interaction, we analyzed whether either of the AD-hp150 fusions could interact with a nonspecific control protein, yeast Dna2p. The resulting phenotype of the trans-
formed yeast was His3<sup>−</sup> LacZ<sup>−</sup>, indicating that hp150 of human CAF-1 does not interact with Dna2p. We conclude that hp150 specifically binds to the C terminus of BLM in the YTH system (Fig. 1A).

To substantiate this interaction further, we tested whether BLM and hp150 could interact in vitro. To this end, full-length recombinant BLM protein was immobilized on a nitrocellulose filter, which was then incubated with in vitro-translated hp150 or its derivatives (Fig. 1C). As shown in Fig. 1B, the two truncated forms of hp150 found in the YTH screen, as well as the full-length hp150, interacted with BLM. Since in vitro-translated hp150 and the two N-terminally truncated forms did not interact with either of two control proteins, BSA and the bacterial helicase UvrD (Fig. 1B), we conclude that the interaction between BLM and hp150 is specific and direct, not requiring additional accessory proteins. This direct interaction was also confirmed by an in vitro co-IP assay by incubating the purified recombinant BLM with in vitro-translated hp150 and then with the MAb against hp150 (data not shown, available on request).

To gain insight into the BLM-hp150 interaction in vivo, we performed co-IP experiments with nuclear extracts of human BJAB cells, using a MAb directed against hp150 (Fig. 1D). Endogenous BLM was effectively coprecipitated by the hp150
tracts. As shown in Fig. 1D, hp150 could be specifically immunoprecipitated with anti-BLM (middle panel, lanes 6 and 7) but not with the control IgG antibody (lane 5). The results of this co-IP experiment have indicated that in soluble HeLa nuclear cell extracts, less than 10% of total hp150 is associated with BLM (data not shown, available upon request). Moreover, in addition to hp150, anti-BLM antibody efficiently co-immunoprecipitated topoisomerase IIIα (Fig. 1D, bottom panel, lane 6), a protein known to interact with BLM (53). Importantly, the anti-BLM antibody (C-18) could not immunoprecipitate hp150 from BS (BLM<sup>−/−</sup>) cells (Fig. 1E, lane 3) whereas it efficiently immunoprecipitated hp150 from BS cells containing the BLM cDNA (BLM<sup>−/−</sup> + pBLM) (lane 4). We conclude from these different protein interaction experiments that BLM associates with hp150 of CAF-1 in yeast, in vitro, and in human cells.

Mapping of interaction regions on BLM and hp150. To identify the region of BLM that mediates the interaction with hp150, we generated a series of BLM deletion mutants. These deletion mutants were tested for interaction with the full-length hp150 in the YTH assay (Fig. 2A). Two independent regions in BLM were found to interact with hp150. The first interacting region resides at the N terminus of BLM and comprises amino acids 131 to 333, and the second region is located in the middle of the helicase domain and comprises amino acids 782 to 952 of BLM (Fig. 2A). The relatively weaker interaction of BLM amino acids 131 to 333 and BLM amino acids 782 to 952 with hp150 compared to that of full-length BLM could be due to ineffectively folding these two short forms. A similar YTH approach combined with an in vitro binding assay was used to map the BLM interaction region on hp150 (Fig. 2B). One region of hp150, comprising amino acids 551 to 714 containing the ED domain (amino acids 564 to 641) (24) and the dimerization domain (amino acids 642 to 782) (39), was found to be necessary and sufficient for interaction with BLM. Interestingly, this region is different from the region in hp150 that interacts with proliferating-cell nuclear antigen in vitro (33) and from the region critical for the interaction with p60, another subunit of CAF-1 (24).

BLM and hp150 colocalize on DNA replication block or DNA-damaging signals. Next, we analyzed whether BLM and hp150 colocalize within the nucleus of intact human cells by indirect immunofluorescence of exponentially growing HeLa cells. Previous studies using HeLa cells have shown that CAF-1 is present at replication foci, since it localizes at sites of 5-bromodeoxyuridine incorporation (25, 45). In these cells, BLM forms nuclear foci corresponding to PML nuclear bodies (21, 52). The observation of nuclear foci containing BLM with PML, RPA, and 5-bromodeoxyuridine led to the suggestion that these sites represent sites of DNA synthesis and repair (6). In untreated HeLa cells, we detected some nuclear foci that were enriched in both BLM and hp150. Under these conditions, only 7% of the cells contained a mean of 1.8 colocalizing BLM and hp150 foci per nucleus (Fig. 3A and Table 1). This observation suggested that the two proteins might associate at sites of endogenous damage during the replication process. Therefore asked whether the number of colocalizing BLM-hp150 foci increased in response to treatment of the cells with HU. Indeed, following a 20-h HU treatment, the proportion of cells containing BLM-hp150 colocalizing nuclear foci increased significantly, such that 40% of the population contained a mean of 7.6 colocalizing foci per nucleus (Fig. 3A; Table 1). We then tested whether other DNA-damaging agents resulted in increased BLM-hp150 colocalization. At 4 h after treatment of HeLa cells with UV light (50 J/m<sup>2</sup>), 25% of the cells contained a mean of 4.2 BLM-hp150 colocalizing foci per...
nucleus. In addition, after exposure of HeLa cells to MNNG for 20 h, 36% of the HeLa cells contained a mean of 6.9 BLM-hp150 colocalizing foci (Fig. 3A; Table 1), demonstrating that this significant increase in BLM-hp150 colocalization is irrespective of the type of DNA damage present.

To examine the BLM-hp150 interaction on inhibition of DNA replication, we treated HeLa cells with HU, an agent that inhibits ribonucleotide reductase and thereby halts DNA replication fork progression, and coimmunoprecipitated proteins from soluble nuclear extracts with an anti-hp150 MAb. This treatment did not appear to dramatically change the amount of the BLM-hp150 complex (data not shown). Similar results were observed following treatment of HeLa cells with the alkylating agent MNNG (data not shown). Next, we examined if the amount of BLM and/or hp150 increases in the fraction of insoluble nuclear proteins after HU treatment. To that end, HeLa cell nuclear proteins were resolved into soluble (Fig. 3B, lanes 1 and 2) and insoluble (lanes 3 and 4) fractions, which were subjected to SDS-PAGE followed by Western blotting for BLM (upper panel), hp150 (middle panel), or SP1 (lower panel), the last of which was used as a loading control (28). Interestingly, the amount of both BLM and hp150 increased, although to different extents, in the insoluble (lane 4), but not the soluble (lane 2) fraction of nuclear proteins after HU treatment of HeLa cells. To determine the fraction of insoluble nuclear proteins in which BLM and CAF1-hp150 were associated, we separated chromatin from the nuclear matrix fraction. As shown in Fig. 3C, most of the functional BLM and hp150 were found to be associated with the nuclear matrix fraction of the insoluble nuclear structure, as judged by the presence of the nuclear matrix marker protein lamin B. To define further the sites in the nucleus where BLM and hp150 colocalize, additional immunofluorescence experiments were performed with antibodies to proteins involved in replication, repair, and recombination. RPA is a nuclear protein that interacts with BLM (8) and localizes at sites of DNA replication in nuclei of different cell types (3, 9). BRCA1, another BLM-interacting protein (50), is required for efficient homologous recombination and serves as a signal processor for DNA damage responses (46). We found that following treatment with HU, UV, or MNNG, both BLM and hp150 colocalized with BRCA1 in discrete nuclear foci (Fig. 3D and data not shown). Similarly, both BLM and hp150 co-localized with RPA in HU-

![FIG. 3. BLM and hp150 colocalize within distinct nuclear foci in response to DNA-damaging signals. (A) Indirect immunofluorescence of BLM (red) and hp150 (green) in untreated and HU-, MNNG-, and UV-treated HeLa cells. Representative pictures are shown for a portion of cells analyzed. For details, see Table 1. In the merged image, colocalization is seen as yellow. The panel labeled DNA indicates the position of the nucleus, as judged by DAPI staining. Bars, ~10 μm. (B) The amount of BLM and hp150 increases after HU treatment in the insoluble fraction of nuclear proteins. Untreated (lanes 1 and 3) and HU-treated (lanes 2 and 4) HeLa nuclear proteins were resolved into soluble (lanes 1 and 2) and insoluble (lanes 3 and 4) fractions, and these were subjected to SDS-PAGE followed by Western blotting for BLM (top panel), hp150 (middle panel), and SP1 (bottom panel [loading control]). (C) The increased amount of BLM and hp150 is mainly associated with the nuclear matrix. After DNase 1 digestion, the insoluble nuclear fraction was separated into two fractions: chromatin (lanes 1 and 2) and nuclear matrix (lanes 3 and 4). Lanes 1 and 3 contain untreated HeLa cells; lanes 2 and 4 contain HU-treated HeLa cells. Lamin B is shown as a fractionation and loading control. (D) BLM and hp150 colocalize with BRCA1 after HU treatment of HeLa cells. Red foci represent either BLM or hp150, and green foci reveal BRCA1 staining. DNA is revealed by DAPI staining. Bars, ~10 μm. (E) BLM and hp150 colocalize with RPA after HU treatment of HeLa cells. Red represents either BLM or hp150, as indicated, and green indicates RPA protein. DNA indicates the position of nucleus. Bars, ~10 μm. See Results and Discussion for details.]
UV-, or MNNG-treated HeLa cells (Fig. 3E and data not shown). Taken together, these immunofluorescence data suggest that after treatment of cells with DNA-damaging agents or in response to endogenous damage, a proportion of BLM and hp150 colocalize at sites of ongoing DNA synthesis and repair.

**BLM-hp150 colocalization is cell cycle dependent.** Since the subcellular localization patterns for BLM and CAF-1 are not identical during a normal, unperturbed cell cycle (25, 55), we set out to determine the stage of the cell cycle at which BLM and hp150 colocalize to the greatest extent. For this purpose, we synchronized HeLa cells in the G1/S, early S, and G2/M phases (Fig. 4A) and immunostained the cells with anti-BLM and anti-hp150 antibodies. Figure 4B and C show the pattern of BLM-hp150 colocalization in G1/S- and S-phase cells, respectively. As before, only a portion of cells exhibited BLM-hp150 colocalizing foci: only 3% of the G1/S phase cells contained BLM-hp150 colocalizing foci, while 38% of the S-phase cells displayed BLM-hp150 colocalization. During the G2/M stage, no cells showed any BLM-hp150 colocalization (data not shown). This reveals that colocalization between BLM and hp150 occurs most frequently during the S phase, where the majority of DNA replication and repair events are occurring.

**BLM inhibits CAF-1-mediated chromatin assembly coupled to DNA repair.** Next, we investigated the functional consequences of the interaction between BLM and hp150 by analyzing whether BLM affects the activity of hp150 in terms of its critical role in chromatin assembly. To address this, we used the in vitro chromatin assembly assay coupled to repair of UV-damaged DNA. This assay takes advantage of simultaneous analysis of DNA repair and chromatin assembly processes on the same damaged circular DNA template (15). HeLa cytosolic extracts complemented by recombinant CAF-1 efficiently mediated DNA repair-linked chromatin assembly, as indicated by the appearance of radioactively labeled form I DNA molecules corresponding to supercoiled, repaired DNA (Fig. 5A, lane 2). In this reaction, chromatin assembly that occurs independently of DNA synthesis or repair is very inefficient and appears to be CAF-1 independent (data not shown). Addition of purified BLM alone, in the absence of recombinant CAF-1, did not result in the formation of supercoiled molecules (Fig. 5B, lane 1). However, preincubation of CAF-1 complex with recombinant BLM led to a significant decrease in the amount of supercoiled product (Fig. 5A, lanes 3 and 4), indicating that BLM acted as an inhibitor of CAF-1-mediated chromatin assembly coupled to DNA repair. When recombinant BLM was preincubated with anti-BLM antibody before addition of the mixture to the chromatin assembly reaction mix, the BLM no longer inhibited CAF-1-dependent DNA repair-coupled chromatin assembly (Fig. 5A, lanes 5 and 6). This is most probably due to an inability of the antibody-complexed BLM to interact productively with hp150. No effect on CAF-1-mediated chromatin assembly was observed when anti-BLM antibody alone was added to the reaction mix (lane 7). Importantly, chloroquine gel analysis demonstrated that there was no difference in the topology of the DNA repaired by the cytosolic extracts in the presence or absence of BLM (data not shown), thus excluding the possibility that BLM-mediated inhibition of chromatin assembly is due to the effect of BLM on topoisomerases in our system.

Next, we asked whether the inhibitory effect of BLM on CAF-1-mediated chromatin assembly could be relieved by the addition of an excess of purified CAF-1-holoenzyme complex. Figure 5B (lane 4) shows that a significant and CAF-1 concentration-dependent relief of BLM-mediated inhibition of chromatin assembly was detected on the addition of the CAF-1 holoenzyme complex. Most importantly, this inhibition of CAF-1-mediated chromatin assembly is specific for BLM, since the bacterial helicase UvrD, which does not interact with hp150 (Fig. 1B), showed no inhibitory effect on CAF-1-mediated chromatin assembly (Fig. 5C, lane 3). Collectively, these results argue for a role of BLM in inhibiting CAF-1-dependent chromatin assembly coupled to DNA repair through a direct protein-protein interaction.

We also tested how p150 affects BLM helicase activity. Although hp150 inhibited BLM-catalyzed DNA unwinding, this appeared to be a nonspecific effect, probably mediated via the binding of hp150 to the DNA substrate, because hp150 also inhibited UvrD (data not shown, available on request).

**BLM is required for an optimal CAF-1 response to HU.** To further study the in vivo functional relationship between BLM and CAF-1, we examined CAF-1 function in BLM-deficient cells. To determine whether CAF-1-mediated chromatin assembly is affected in BS cells, we prepared nuclear extracts from BS cells and compared them with the nuclear extracts from wild-type (HeLa) cells for their ability to mediate chromatin assembly. The presence and absence of BLM in these cell lines was controlled by Western blotting (Fig. 6C). As shown in Fig. 6A, the endogenous CAF-1 activity was not obviously altered in BS cells (lane 2) compared with the CAF-1 activity from wild-type cells (lane 1).

Next, we investigated whether CAF-1 forms normal nuclear foci in untreated or HU-treated BS cells. Immunofluorescence analysis of the subcellular localization of hp150 indicated that CAF-1 formed normal nuclear foci in both untreated and HU-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of cells containing:</th>
<th>Mean no. of foci/cell (range)</th>
<th>% of cells containing BLM and CAF-1 colocalizing foci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BLM foci</td>
<td>CAF-1 foci</td>
<td>BLM foci</td>
</tr>
<tr>
<td>None</td>
<td>36 9</td>
<td>4.2 (0-27)</td>
<td>3.1 (0-29)</td>
</tr>
<tr>
<td>HU (5 mM)</td>
<td>85 68</td>
<td>22.2 (0-51)</td>
<td>29.6 (0-54)</td>
</tr>
<tr>
<td>MNNG (5 M)</td>
<td>94 57</td>
<td>26.8 (0-66)</td>
<td>23.3 (0-49)</td>
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<tr>
<td>UV</td>
<td>45 37</td>
<td>6.8 (0-30)</td>
<td>6.3 (0-32)</td>
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*In each case, 150 cells were scored.*
treated BS cells (Fig. 6B). In untreated BS cells, the percentage of cells containing CAF-1 foci (6.7%) did not significantly differ compared with that in untreated PSNF5 cells [BS cells containing the BLM cDNA (BLM−/− + pBLM)] (7.1%), suggesting that BLM is not required for recruitment of CAF-1 to nuclear foci. In HU-treated BS cells, the percentage of cells containing CAF-1 foci (18%) increased compared with that in untreated BS cells (6.7%). In PSNF5 cells which contain the BLM cDNA, the percentage of cells containing CAF-1 foci (43%) also increased in the presence of HU compared to that.
in untreated cells (7.1%). However, this increase in the percentage of CAF-1-foci containing cells observed in HU treated PSNF5 cells was greater than that seen in BS cells alone. This result suggests that BLM is required for an optimal CAF-1 response to HU and is consistent with the cellular fractionation experiment showing an increase in the amount of hp150 associated with nuclear matrix in PSNF5 cells containing the BLM cDNA compared to that in BS cells alone (Fig. 6D).

**DISCUSSION**

To maintain genome stability, both genetic and epigenetic information has to be correctly inherited from mother cells after cell division. BLM and other members of the RecQ helicase family play a crucial role in maintaining genome stability through their ability to suppress inappropriate recombination (4, 19). Consistent with this notion, BS cells show increased rates of homologous recombination and the aberrant resolution of this event (14, 41). However, the action of BLM per se does not suffice to preserve genome integrity. The newly-synthesized DNA, either newly-replicated or repaired, has to be assembled into chromatin to make sure that the genome is also epigenetically stable, and CAF-1 plays a critical role in this process (17). Recent results clearly showed not only that CAF-1 is directly involved in DNA replication (20) but also that it ensures the maintenance of epigenetic information by acting at the sites of DNA repair (17). The results presented in this paper suggest a cross talk between the genetic and epigenetic pathways in the maintenance of genome stability. In particular, we have shown that BLM physically and functionally associates with hp150, the largest subunit of CAF-1. The results of our interaction region mapping experiments indicate that BLM interacts with hp150 via two separate sites on BLM, the first located near the N terminus of BLM (amino acids 131 to 333) and the second located in the middle of the helicase domain (amino acids 782 to 952) of BLM. Furthermore, we have shown that one region of hp150 (amino acids 551 to 714) containing the ED domain and the dimerization domain is necessary and sufficient for interaction with BLM.

Our results show that the proportion of detergent-resistant BLM and CAF-1 foci that colocalize in the nucleus increases after replication fork inhibition and treatment of cells with DNA-damaging agents. Together with the nuclear fractionation experiments, this suggests that the function of the BLM-hp150 interaction is associated with the matrix fraction of nuclear proteins. The increase in the number of foci containing
both BLM and hp150 after HU treatment suggests that the BLM-hp150 complex concentrates in the vicinity of stalled replication forks. These replication forks probably contain nonduplex DNA structures, which might provoke a DNA damage response. In addition, BLM and hp150 are recruited together to discrete foci after DNA damage induction with MNNG or UV light. One interpretation of these phenomena is that in each case, the foci represent DNA repair processes that are initiated at a replication fork. In the case of HU treatment, as well as of UV- and MNNG-induced damage, stalled DNA replication may also be accompanied by a recombinational DNA damage response. The colocalization of BLM and hp150 at sites containing RPA and BRCA1 further supports the contention that the BLM-hp150 complex plays a repair role during DNA synthesis. Although the results of our indirect immunofluorescence experiments show that BLM is not required for recruitment of CAF-1 to nuclear foci, BLM seems to be required for an optimal CAF-1 response to HU. That the physical interaction between BLM and hp150 is of functional significance is indicated by the inhibitory effect of BLM on CAF-1-mediated chromatin assembly coupled to repair. This inhibitory effect appeared specific because a control helicase, UvrD, was unable to inhibit chromatin assembly.

Given all these observations, we propose that the BLM-hp150 interaction is important during times when DNA synthesis is perturbed in human cells. On the basis of our results, we propose a model suggesting a functional link between BLM and hp150 in restoring functional replication forks following fork stalling. Progression of a replication fork might be impeded by DNA secondary structures such as G-quadruplexes and DNA hairpins, thus causing the regression of the fork and formation of a four-way junction, a structural analog of a Holliday junction. It has been suggested previously that BLM and other RecQ helicases process such lesions that arise at replication forks (or are generated by the translocating fork itself) and, in doing so, reset the fork by “reverse” branch-migrating the four-way junction. This has the effect of preventing cleavage of the four-way junction and hence suppressing subsequent “promiscuous” recombination (10, 19, 25, 51). A second potential role for BLM at replication forks has been suggested by our recent observation that BLM and topoisomerase IIIα together catalyze the dissolution of a recombination intermediate containing a double Holliday junction (54). Such double Holliday junctions are predicted to arise during post-replication gap filling when lesions on the lagging-strand template are bypassed. It is known that CAF-1-dependent chromatin assembly occurs on newly replicated DNA (43) and that CAF-1 is required in vitro for chromatin assembly following DNA repair (15). CAF-1 binds directly to PCNA, a DNA polymerase processivity factor that forms a sliding clamp around DNA, and the CAF-1–PCNA interaction promotes chromatin assembly (33). Although it remains unclear exactly how BLM and CAF-1 act after damage at a replication fork, our data suggest that the recruitment of BLM, probably not by CAF-1, may inhibit CAF-1, and hence chromatin assembly, in the vicinity of a stalled replication fork, perhaps to allow more efficient lesion processing. We envisage that such inhibition would continue until the point where normal replication structures are restored. Once the DNA lesion is bypassed and the replication fork is restored, the CAF-1 complex can be reactivated (it again assembles the newly synthesized DNA into chromatin) and DNA replication fork progression can resume.

This sequential scheme requires a level of regulation that may be achieved by mechanisms that involve physical modification. Interestingly, BLM is phosphorylated following DNA damage (1, 2, 13), and UV damage induces changes in the amount of chromatin-bound phosphorylated CAF-1 complex (30). It is possible that either of these phosphorylation events or some other postranslational modification(s) could be responsible for modulating both physical and functional interactions between BLM and CAF-1. The inhibitory effect of BLM on CAF-1 might also be modulated by associations with other interacting proteins. A candidate would be PCNA, which can bind to both CAF-1 (33) and BLM (S. L. Davies, J.-L. Li, and I. D. Hickson, unpublished data).

While the precise biochemical relationship between BLM and CAF-1 remains to be fully elucidated, the physical and functional interactions documented here provide a basis to further understand how BLM and hp150 function together to facilitate DNA repair and/or replication.

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REFERENCES