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BLM is an early responder to DNA double-strand breaks

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Abstract

Bloom syndrome (BS) is an autosomal recessive disorder characterized by a marked predisposition to cancer and elevated genomic instability. The defective protein in BS, BLM, is a member of the RecQ helicase family and is believed to function in various DNA transactions, including in replication, repair, and recombination. Here, we show that both endogenous and overexpressed human BLM accumulates at sites of laser light-induced DNA double-strand breaks within 10 s and colocalizes with γH2AX and ATM. Like its RecQ helicase family member, WRN, the defective protein in Werner syndrome, dissection of the BLM protein revealed that its HRDC domain is sufficient for its recruitment to the damaged sites. In addition, we confirmed that the C-terminal region spanning amino acids 1250–1292 within the HRDC domain is necessary for BLM recruitment. To identify additional proteins required for the recruitment of BLM, we examined the recruitment of BLM in various mutants generated from chicken DT40 cells and found that the early accumulation of BLM was not dependent on the presence of ATM, RAD17, DNA-PKcs, NBS1, XRCC3, RAD52, RAD54, or WRN. Thus, HRDC domain in DNA helicases is a common early responder to DNA double-strand breaks, enabling BLM and WRN to be involved in DNA repair.

Keywords: DNA double-strand break repair; BLM protein; RecQ helicase; Laser micro-irradiation; HRDC domain; DT40 cells

Bloom syndrome (BS) is a rare autosomal recessive disorder characterized by sunlight sensitivity, short stature, and a very high incidence of various types of neoplasia [1,2]. Cells from BS patients exhibit chromosomal instability typified by elevated rates of sister chromatid exchanges, insertions, deletions, telomere associations, and quadriradials. The gene mutated in BS, *BLM*, encodes a member of the RecQ helicase family [3]. To date, five human RecQ helicases have been identified, three of which are associated with genetic disorders, namely, BS, Werner's syndrome, and Rothmund–Thomson syndrome; the latter two syndromes are both characterized by premature aging and a predisposition to cancer. Inactivation of any of three helicases resulted in specific cellular defects, which suggests that these RecQ helicases have specialized functions in the cell [4]. The mechanism that results in the hyper-recombination phenotype of BS has not yet been clarified at the cellular level. However,*in vitro* studies suggest that BLM is a DNA structure-specific helicase that functions to resolve specific DNA structures that resemble recombination intermediates and that may arise during the processing of DNA double-strand breaks (DSBs) or daughter-strand gaps [5].

DSBs are a major threat to the cell and the broken ends should be joined properly to avoid subsequent genomic

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instability. The repair of DSBs is usually executed by two major pathways, namely, non-homologous end joining (NHEJ) and homologous recombination (HR). Several sub-pathways of DSB repair may also exist, since many proteins have been reported to be involved in DSB repair and the coordinated functions of these proteins are necessary for successful DSB repair [6,7]. Studies on BLM and WRN, which is encoded by the gene that causes Werner's syndrome, revealed that these proteins are somehow involved in DSB repair; in accordance with this, the loss of function of these RecQ helicases augments the cell's sensitivity to the DNA-damaging agents that directly or indirectly generate DSBs [8–10]. Moreover, both WRN and BLM proteins have been shown to associate with proteins that are involved in HR and NHEJ and it is believed they may function together at sites of DNA damage [9,11–13]. However, although it is known that human BLM (hBLM) interacts with several repair/recombination proteins, including RPA, Rad51, Rad51D, and MLH1 [14–17], as well as with DNA-damage signaling proteins such as ATM [18], the precise role of BLM remains largely unknown.

The recent development of the laser light micro-irradiation system has led to the identification of proteins that accumulate at DNA sites that were damaged by the laser irradiation. For example, TRF2 was shown to be recruited to the damaged sites early after laser micro-irradiation [19]. In addition, WRN was found to associate immediately with the DSB sites after laser irradiation; this association was shown to be mediated by its HRDC (Helicase-and-RNaseD-C-terminal) domain, which is conserved in some of RecQ family helicases including *Escherichia coli* recQ, budding yeast Sgs1, hWRN, and hBLM [20]. The latter study also carefully defined the damage that is produced by different laser light irradiation conditions. Since BLM is part of the human telomeric complex [21] and interacts with WRN [22], we were interested in determining the cellular dynamics of BLM after DNA damage is induced by the laser irradiation system. Here, we report that, in response to laser-induced DNA damage, both endogenous BLM and exogenously expressed EGFP (enhanced green fluorescence protein)-linked BLM are immediately recruited to the sites of irradiation and persist there for more than 6 h. We further show that the C-terminal region spanning amino acid residues 1250–1292 within the HRDC domain of hBLM is essential for its recruitment. The significance of the rapid recruitment of BLM to sites of DNA damage will be discussed.

Experimental procedures

Cell lines and culture. HeLa cells, U2OS cells, and the *BLM*-mutated fibroblasts derived from a BS patient (GM 03498) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere with 5% CO_2. The various DT40 cell lines used have been described previously [23–30] and were cultured in suspension with RPMI1640 supplemented with 10% FBS and 1% chicken serum.

Plasmid construction. The plasmids harboring *EGFP* conjugated to full-length *BLM*, truncated *BLM*, or *XRCC1* have been described earlier [31,32]. The DNA fragment encoding the HRDC and NLS domains of BLM was amplified by PCR from full-length *BLM* and inserted into the *Xho*I/*Kpn*I sites of the pEGFP-C1 vector to produce the HRDC construct. To make the HRDCΔC construct, we first introduced a *Hin*dIII site at amino acid residue 1293 by using the Quick Change Site Directed Mutagenesis Kit (STRATAGENE). Since a *Hin*dIII site also exists at amino acid residue 1248, when the mutagenized plasmid was digested with *Hin*dIII and self-ligated, it encoded GFP-BLM that bears a deletion spanning amino acids 1250–1292. To make the HRDC construct in which the lysine at amino acid 1270 has been substituted by glutamine (HRDC-K1270Q), the plasmid carrying the full-length *BLM* gene was mutated by using the Quick Change Site Directed Mutagenesis Kit. All mutations were confirmed by DNA sequencing.

Transfection. To transfect cells with DNA, 1–2 μg of DNA was used. Cells were grown in poly-L-lysine-coated glass dishes overnight and transfected with lipofectamine (Life Technology) according to the manufacturer's instructions. After transfection, the cells were incubated for 24–48 h before being subjected to laser light irradiation.

Laser light irradiation and microscopy. Fluorescence images were obtained and processed by using a FV-500 confocal scanning laser microscopy system (Olympus). The microscope is coupled with micro-irradiation facilities to emit 365 or 405 nm laser light as described in detail previously [20,32]. Both 365 and 405 nm laser lights are focused through a 40× objective lens. We mainly used the 405 nm scan laser system for irradiating the cells in the epifluorescence path of the microscope system. The power of the scan laser can be controlled by altering the scanning times and/or the laser power. The scan laser light of 405 nm wavelength delivers within 5 ms around 1600 nW of energy at full power (total energy delivered on the path is 40 μJ for 500 scans), while one pulse of 365 nm laser through F25 filter used in this experiment delivers 2.5 μJ. We used the 405 nm laser at full power only and regulated the dose by changing the scanning times. Thus, cells were incubated on a 37 °C hot plate in glass-bottomed dishes placed in chambers to prevent evaporation and then irradiated. After irradiation, images were captured at 10–20 s intervals for up to 5–10 min. The energy of the fluorescent light at the irradiated site was measured with a laser power/energy monitor (ORION, Ophir Optronics, Israel). The mean intensity of the track in the irradiated cell was obtained after subtracting the background intensity.

Immunolabeling. Cells were incubated for different time periods after irradiation and then fixed with freshly prepared 4% paraformaldehyde for 15 min on ice. After washing, the cells were permeabilized with 0.25% Triton X-100 for 10 min on ice, then treated with blocking buffer for 30 min at room temperature, followed by incubation with a primary antibody for 16 h at 4 °C. Rabbit polyclonal anti-phosphorylated H2AX (1:400, Upstate Biotechnology), goat polyclonal anti-BLM (1:100, Santa Cruz Biotech), rabbit polyclonal anti-ATM (1:200, Rockland, USA), and rabbit polyclonal anti-RecQL1 (1:200) [33] were used. Bound antibodies were visualized by incubating the cells with secondary antibodies, namely, Alexa 488-labeled donkey anti-goat and Alexa 594-labeled chicken anti-rabbit antibodies. The cells were observed with a laser scan confocal microscope.

TUNEL assay. After laser irradiation cells were washed and fixed with 4% paraformadehyde. DNA double-stranded break was detected by terminal deoxynucleotidyl transferase mediated dUTP-biotin end labeling (Trevigen) followed by incubation with avidin-FITC (1:400).

Detection of incorporated BrdU. After laser irradiation, cells were incubated for 60 min and then for 10 min in the presence of 100 μM BrdU. The cells were fixed and treated with 0.2 M HCl for 1 h to denature DNA. Incorporated BrdU was detected with mouse anti-BrdU monoclonal antibody which was visualized with Alexa 594 labeled donkey anti-mouse antibody.

Photosensitization assay using BrdU. For incorporation of BrdU as photosensitizer during laser irradiation, BrdU at a final concentration of 10 μM was added to the medium 16 h before laser irradiation. Cells were irradiated without washing.

Results

It has been shown that UVA laser irradiation through a microscope lens can be used to introduce various types of DNA damage including strand breaks and base damage at limited area within the nucleus of a cell under microscope, thus leaving the ability of the cell to execute repair processes [20,32]. This system has opened up the possibility of visualizing the accumulation and the dissociation of proteins involved in the damage responses at sites of irradiation immediately after the DNA has been damaged. Making use of this system, we wanted to investigate the *in situ* response of hBLM after introducing DNA damage by UVA laser. We first overexpressed a full-length EGFP-tagged hBLM protein in HeLa cells and irradiated the cell nucleus with either linear 500 scans of 405 nm laser light or two pulses of 365 nm laser light. As shown in Fig. 1a for 405 nm scan laser and Fig. 1b for 365 nm pulse laser, EGFP-BLM accumulated at the irradiated sites as linear tracks or as a dot immediately after irradiation, respectively. The average fluorescence intensity of the irradiated sites over the background reached a plateau within 1 min for the laser pulses (Fig. 1c). We next analyzed the effect of different doses of laser light on the accumulation of BLM by changing the scan times of the 405 nm laser light. As shown in Fig. 1d, more than 90% of the cells showed EGFP-BLM-specific lines in their nuclei after irradiation with 500 or 1000 scans of 405 nm laser light, while quite a weak accumulation of EGFP-BLM was detected in only 10–20% of cells irradiated with 100–200 scans, indicating dose of laser is important for efficient accumulation of the protein. Therefore, in the remaining studies described here, we mainly used 405 nm scan laser with 500 scans, which were delivered within a few seconds and we will refer to as 'line laser.'

To understand the response of endogenous BLM to line laser and to exclude the possibility that overexpression of EGFP-BLM forces the protein to accumulate at damage sites, we assessed the accumulation of endogenous BLM at irradiation-induced DNA damage sites by indirect immunostaining. Since histone H2AX is rapidly phosphorylated in the chromatin micro-environment surrounding DSBs and is essential for the accumulation of numerous proteins at DSB sites [34], we also immunostained γH2AX. As shown in Fig. 2a, both BLM and γH2AX accumulated very quickly at the damage sites after irradiation. The accumulation of BLM and γH2AX at the damage sites was detected by immunostaining for at least 4 h after the irradiation event (data not shown), indicating that BLM stayed at the irradiated site for quite a long period. We also

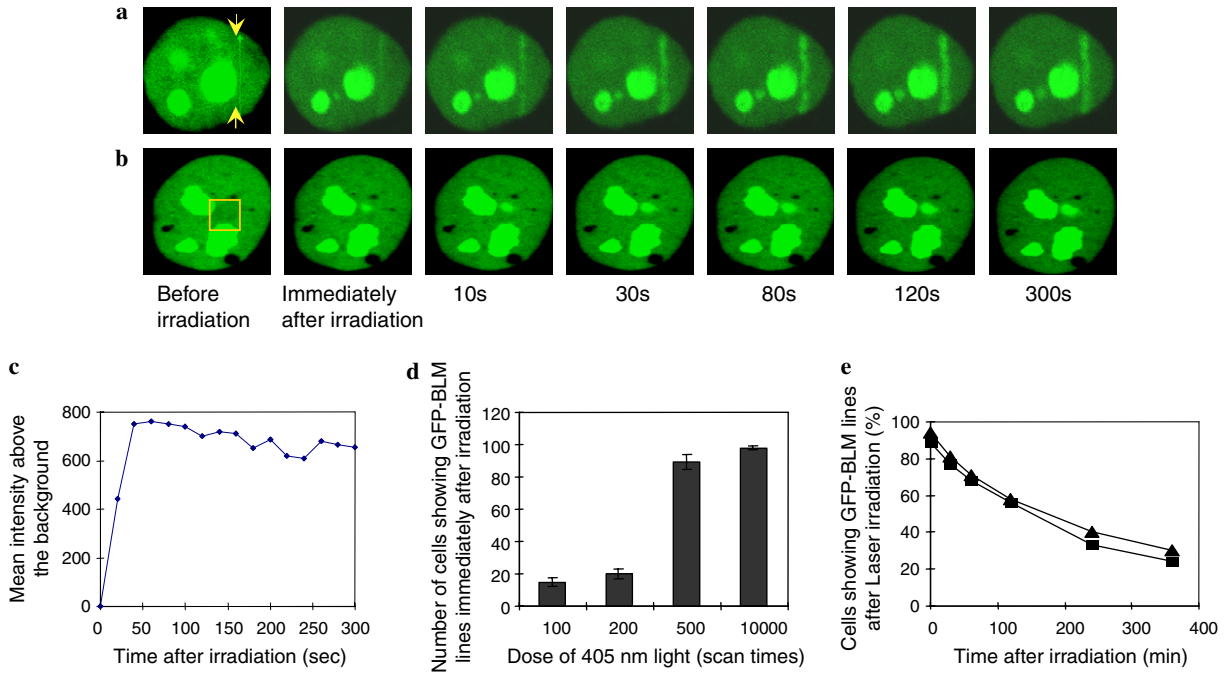


Fig. 1. Association of GFP-hBLM with laser-induced sites of DNA damage. (a,b) Typical images of irradiated nuclei. HeLa cells were irradiated with 405 nm laser light by 500 scans (a) or with two successive pulses of 365 nm laser light through F25 filter producing 0.49 μJ per pulse (b). The first column represents the cells before irradiation, while the subsequent columns show the cells at various times after irradiation, as indicated. (c) Time course with which GFP-hBLM accumulates in irradiated nuclei. The mean intensity of fluorescence in irradiated sites was plotted against time after two successive pulses with 365 nm laser light irradiation. The intensity of the non-irradiated portion of the same area of the nucleus was subtracted from the intensity of the spot. The graph represents the mean intensity of one typical nucleus. (d) Dose-dependency of the nuclear accumulation of GFP-BLM. The average number of nuclei showing GFP-BLM accumulation immediately after irradiation was calculated and plotted against the dose of 405 nm laser light, which was varied by changing the scan times. At least 50 nuclei were examined for each dose. (e) Decrease in nuclear levels of GFP-BLM over time. Cells were irradiated with 405 nm laser light by 500 scans and the GFP-BLM-positive nuclei were scored at various time points. (▲): HeLa cells; (■): U2OS cells. At least 100 irradiated nuclei were examined for each cell line. The arrowheads and box indicate the sites of damage that will be introduced by laser irradiation.

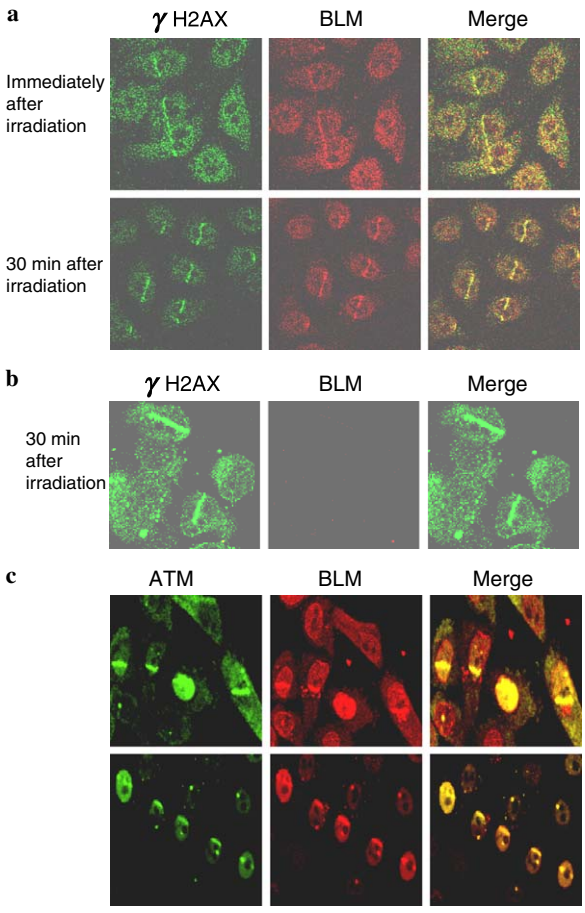


Fig. 2. Analysis of the behavior of endogenous BLM in irradiated HeLa and BLM-negative cells as determined by indirect immunostaining. Colocalization of BLM with γH2AX (a,b) or ATM (c) in HeLa cells (a,c) or BLM-negative fibroblast cells (b). These cells were irradiated with 405 nm laser at 500 scans, fixed at the indicated time after irradiation, and immunostained for the simultaneous detection of BLM (red) and γH2AX (green) or ATM (green).

immunostained the BLM-negative cells derived from a BS patient; this allowed us to assess the specificity of the anti-BLM antibody as well as to examine the dependency of the accumulation of γH2AX on the presence of BLM. We found that while the BLM signal was absent, γH2AX nevertheless accumulated at the damage sites. This indicates that the antibody used in this experiment is indeed BLM-specific and that the nuclear accumulation of γH2AX is independent of BLM (Fig. 2b). ATM is a member of the phosphoinositide-3-kinase-related protein kinase family that is recruited to sites bearing DSBs and is essential for DNA-damage signaling [35]. When we immunostained ATM along with BLM in laser-irradiated HeLa cells, we found that, like BLM, ATM accumulated at the sites of DNA damage just after irradiation (Fig. 2c). This suggests that BLM accumulates at least at sites that bear DSBs.

That the laser light generates DNA-strand breaks at which hBLM accumulates was then substantiated by TUNEL analysis of irradiated HeLa cells. For this purpose, after laser irradiation the cells were fixed and subjected to the terminal deoxytransferase reaction for 30 min using the biotin-conjugated nucleotide. The incorporated nucleotide and endogenous BLM were then detected with avidin-FITC and anti-BLM antibody, respectively. BLM was found to colocalize with the incorporated nucleotide, as shown in Fig. 3a. To confirm the damage substrate of BLM accumulation, we incubated cells overnight in medium containing bromodeoxyuridine (BrdU), irradiated with 100 scans of 405 nm laser, and compared the accumulation of EGFP-BLM with that of cells without BrdU. BrdU incorporated in DNA produces strand breaks by absorption of UV light [36,20]. Fig. 3b shows that addition of BrdU significantly increased the BLM accumulation, while the same scans without BrdU produce almost no accumulation of BLM. The same scans without BrdU resulted in a significant XRCC1 accumulation at SSBs [32]. Thus, BLM appears to be recruited to the sites where DSBs were produced.

To characterize the relationship between the sites of BLM localization and the sites where repair DNA synthesis occurs, HeLa cells were pulse-labeled with BrdU 1 h after laser irradiation. Although visualization of incorporated BrdU requires the denaturation of DNA with hydrochloric acid, which may weaken the BLM signal, we could clearly detect the colocalization of BLM with the incorporated BrdU along the track of photodamage (Fig. 3c). Thus, BLM having accumulated at DSBs immediately after irradiation is involved also in the later stages of DNA repair during DNA synthesis occurred, which may partially explain the prolonged accumulation of BLM at damaged sites (Fig. 1e).

To determine whether the position in the cell cycle influences the recruitment of BLM to the damage sites, both cells which were synchronized by double thymidine block and were subsequently released into S phase, were irradiated and the accumulation of BLM at the damage sites was monitored. Endogenous BLM accumulated at the damage sites in the cells regardless of whether they were in S or G1 phase, as 75% and 52% of the irradiated cells in the S and G1 phases showed the accumulation of BLM, respectively (not shown). The difference between the two cycle phases may be due to the difference in the amount of endogenous BLM that is present in the different phases of the cell cycle [37].

Since BLM has several domains that are conserved among the RecQ family helicases, we sought to identify the domain that is responsible for the recruitment of BLM to the damage sites. To do this, we constructed a series of EGFP-tagged BLM mutant clones as shown in Fig. 4a and examined the time course with which the EGFP accumulated in the nucleus after laser irradiation. The first column in Fig. 4b shows cells before irradiation with yellow arrowheads indicating the sites of damage that will be introduced by laser irradiation. The results shown in Fig. 4b show clearly that neither the acidic regions at the N-terminus nor the helicase domain is necessary for the accumulation of BLM at damaged sites, but that the

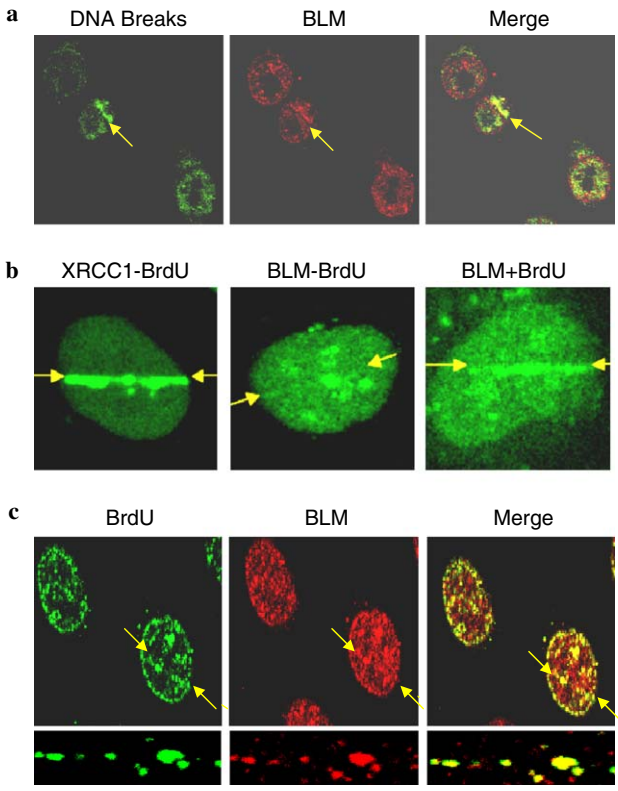


Fig. 3. (a) Localization of endogenous BLM in sites bearing DNA breaks. After irradiation with 405 nm laser light 500 scans, the HeLa cells were fixed and subjected to the TUNEL assay, which detects DSBs, and indirect immunostaining to monitor BLM accumulation. The incorporated biotin-labeled nucleotide in the TUNEL assay was detected by avidin- FITC. (b) Photosensitization assay using BrdU:EGFP-XRCC1 (left) or EGFP-hBLM (middle) transfected HeLa cells were irradiated with 405 nm laser 100 scans without BrdU photosensitization. (right) EGFP-hBLM transfected HeLa cells that had been pre-labeled with BrdU were irradiated with 405 nm laser light 100 scans. (c) Colocalization of BLM at sites bearing DNA breaks and those undergoing repair synthesis. After irradiation with 405 nm laser light 500 scans, HeLa cells were incubated for 60 min before being pulsed with BrdU for 10 min. The incorporated BrdU (green) and BLM (red) were simultaneously detected by indirect immunostaining to determine the colocalization of BLM at the sites incorporating BrdU in the photo-induced track. Lower panels indicate enlarged images of the immunostained tracks. The arrows indicate the position of the irradiation.

C-terminal region containing the HRDC domain is sufficient for the recruitment of the protein. Further dissection of the HRDC domain showed that the amino acid residue strip 1250–1292 is necessary for the recruitment. It was recently reported that the lysine residue at amino acid residue 1270 is important for the ability of BLM to resolve double Holliday junctions with Top3α, which appear as a recombination intermediate [38,39]. Therefore, we introduced a point mutation that changes this lysine residue into glutamine; however, we found that the mutation did not affect the accumulation of BLM (Fig. 4b). Thus, the recruitment of BLM to sites of DNA damage is independent of its helicase and Holliday junction resolvase activities.

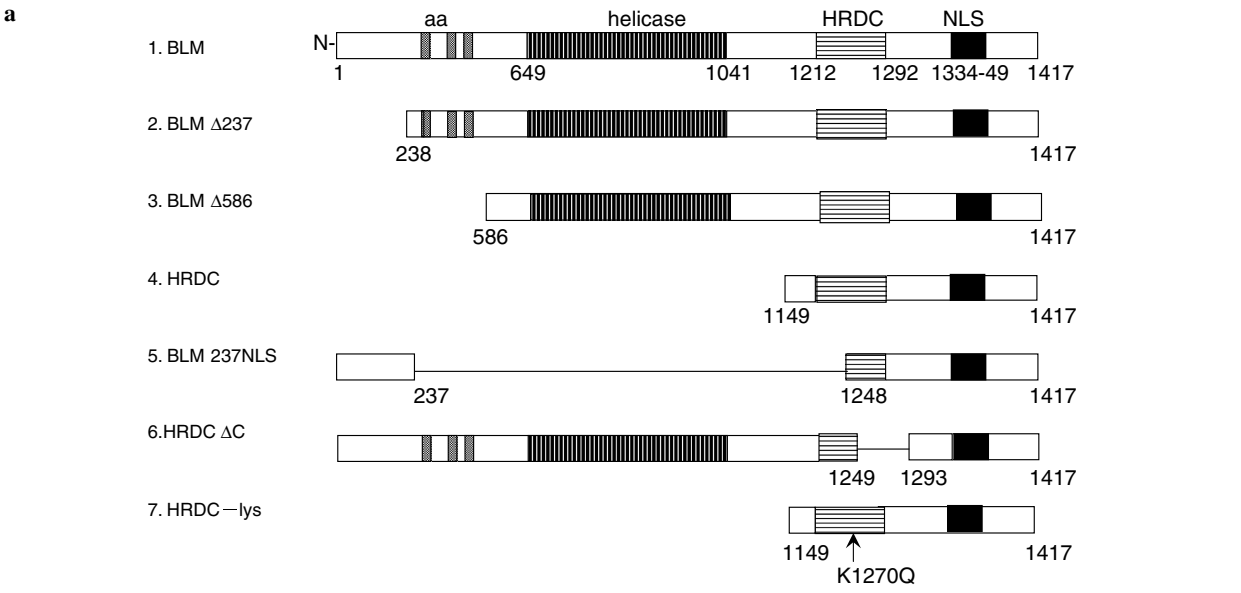
Interestingly, the HRDC domain of WRN is also responsible for its recruitment to DSB sites [20]. Thus, to test whether the HRDC domain is generally responsible for the swift accumulation of RECQ at damaged sites, we examined the accumulation of RECQL1, a RECQ helicase that lacks a HRDC domain. In HeLa cells transfected with the plasmid carrying *GFP-human RECQL1*, hRECQL1 failed to accumulate at the damaged sites immediately after irradiation (Fig. 4c). However, given that hRECQL1 may be involved in the repair of DNA damage [40], we analyzed the behavior of endogenous hRECQL1, along with that of endogenous BLM, by indirect immunolabeling. This showed that although the BLM-specific line was evident immediately after irradiation, only a very faint hRECQL1-specific line appeared considerably later after irradiation (data not shown). These results suggest that the swift accumulation of BLM in damaged sites is not a general feature of RecQ family helicases; rather, it is due to the existence of the HRDC domain.

We next examined whether other proteins are required for the recruitment of BLM. To address this issue, we used nine DT40 cell lines bearing knockout genes encoding various proteins involved in DNA repair or repair-related processes, namely, the $ATM^{-/-}$,$RAD17^{-/-}$, $DNA\text{-}PKcs^{-/-/-}$, $NBS1^{-/-/-}$,$XRCC3^{-/-}$,$RAD52^{-/-}$, $RAD54^{-/-}$,$WRN^{-/-}$, and $BLM^{-/-}$ cells. These cells were transfected with GFP-hBLM and then irradiated with laser light. As shown in Fig. 5a, hGFP-BLM accumulated at the damage sites in all mutant cell lines as efficiently as in the wild type cells. This was confirmed when we estimated the number of cells with a GFP-hBLM-specific line after laser irradiation, since no significant differences between the mutant and wild type cells were observed (Fig. 5b). Thus, the accumulation of BLM at the damaged sites seems to be independent of the presence of the proteins that we have examined.

Discussion

BS is considered as one of the chromosome instability syndromes since the fibroblasts or lymphocytes of BS

Fig. 4. Requirement of the HRDC domain for the recruitment of BLM to DSB sites. (a) GFP-BLM mutant constructs used in this study. The domains in BLM are shown as boxes. The relative amino acid positions are indicated. (b) Images of irradiated cells transfected with each of the mutant BLM constructs. HeLa cells transfected with plasmids carrying mutant *GFP-BLM* were irradiated with 405 nm laser light at 500 scans and images were taken at regular intervals. Only some of the time points are shown in the figure, as indicated. The left-hand column shows the images of the cells before irradiation and the arrowheads and lines indicate the position of the irradiation. Each experiment was repeated at least four times and 10–15 nuclei were examined in each experiment. (c) The HRDC domain-lacking RECQL1 protein does not immediately accumulate in damaged sites. HeLa cells transfected with the plasmid carrying *GFP-RECQL1* were irradiated with 405 nm laser light at 500 scans and images were taken at the indicated times.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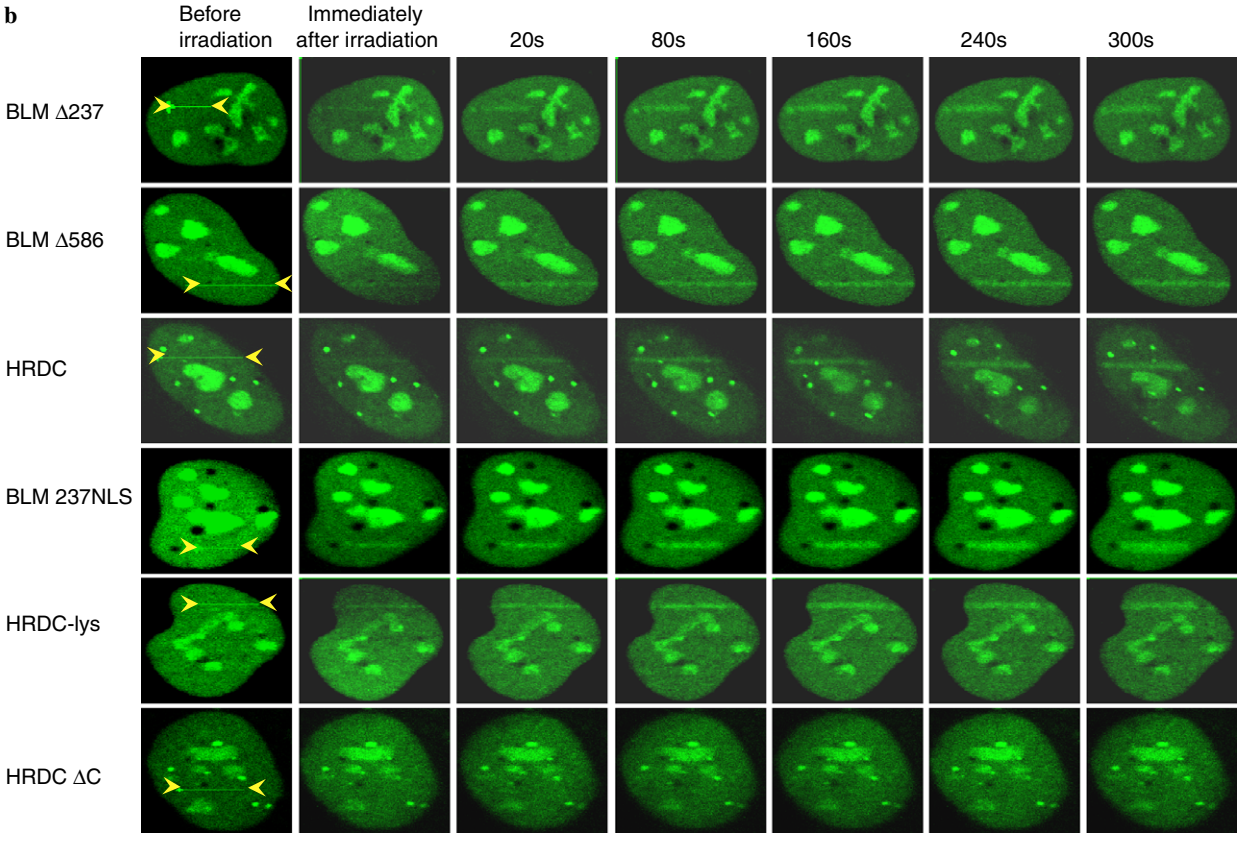
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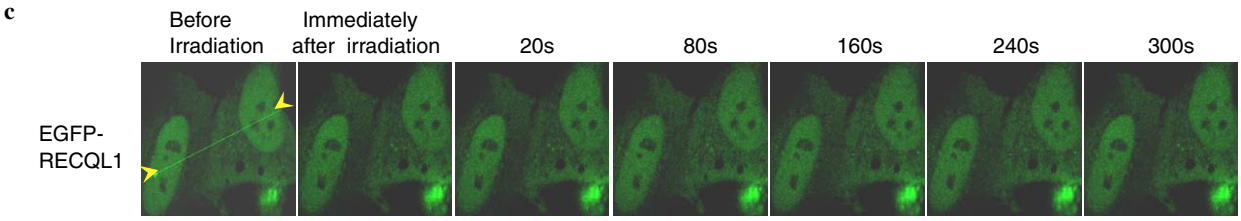
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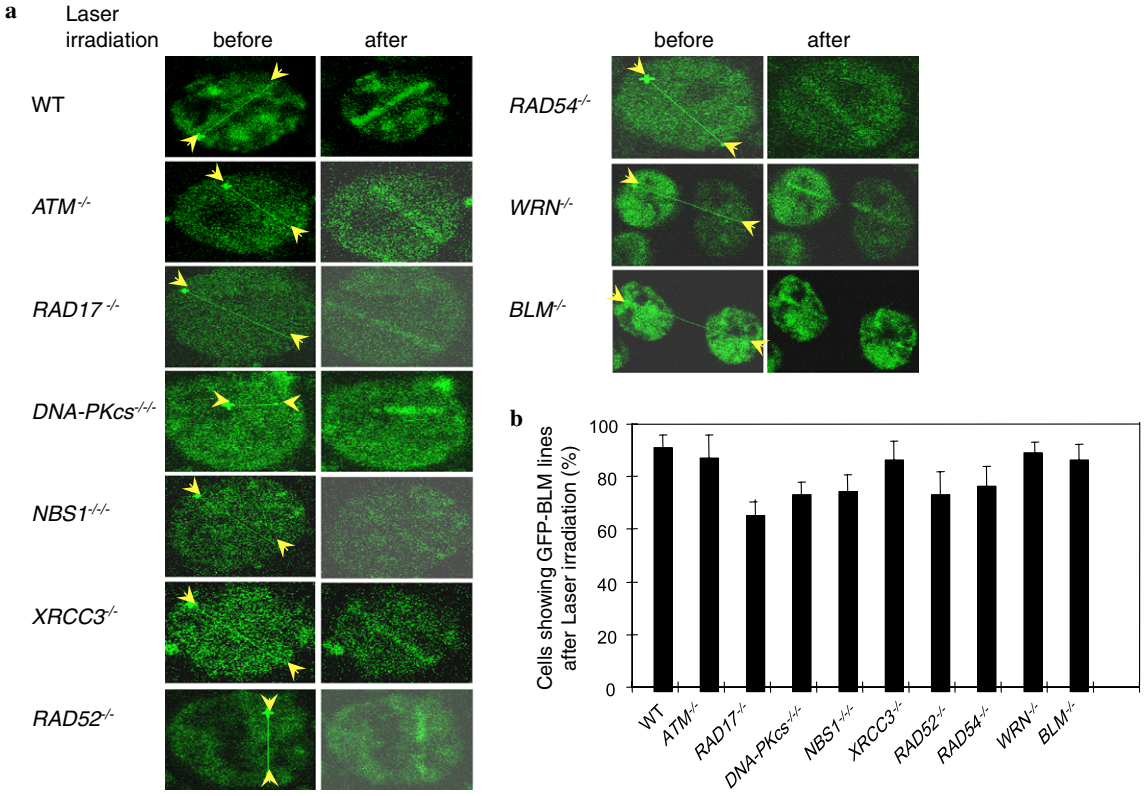

Fig. 5. Independence of the recruitment of BLM on various proteins involved in DNA repair. (a) The recruitment of BLM in various mutants that lack various genes involved in DNA repair was tested by transfecting various DT40 mutant cells with full-length GFP-BLM and irradiating them with 405 nm laser light at 500 scans. Images were taken before (left) and after (right) irradiation. The location of the irradiation track is shown in the left-hand images. (b) Percent of cells showing GFP-BLM-specific lines. After irradiation with 405 nm laser light at 500 scans, cells showing a GFP-BLM-specific line were scored for each mutant cell line. At least 100 nuclei were examined for each cell line. The arrowheads and lines indicate the sites of damage that will be introduced by laser irradiation.

patients show excessive spontaneous chromosome instability. The causative gene of BS (*BLM*) was identified as a RecQ helicase. BLM interacts physically and functionally with several proteins involved in the maintenance of genome integrity and cellular response involving DNA damage. In this report we showed that BLM accumulates at the site of DNA double-strand breaks locally produced by laser micro-irradiation in living human cells as well as fixed cells. The accumulation of BLM is rapid and is strongly colocalized with γH2AX, the latter is known to accumulate instantly after DNA damage [34]. Our results suggest that BLM is recruited to sites of DNA damage that contain DSBs. It has been shown previously [32] that after laser irradiation under specified conditions that produce single stranded breaks (SSBs), polyADP-ribosylation occurs by Poly (ADP-ribose) polymerases (PARP) at the SSB-bearing sites; moreover, activation of PARP is necessary for the recruitment of XRCC1 to the damage sites [32,41]. To test the involvement of PARP in the recruitment of BLM, we treated cells with 5-dihydroxyisoquinoline (DIQ), an inhibitor of PARP, 1 h before laser irradiation and then immunostained the irradiated cells for endogenous BLM. The accumulation of both BLM and γH2AX was not affected by DIQ (data not shown), which suggests that the recruitment of BLM to the damage sites is not dependent on the activation of PARP.

This recruitment event appears to depend on its HRDC domain and does not require its helicase or Holliday junction resolvase activities. In addition, even though BLM is known to interact with several proteins, including WRN and ATM [18,22], none of these proteins appear to be necessary for the accumulation of BLM at the damaged sites since the disruption of the genes encoding these proteins did not influence the recruitment of BLM. One possible explanation for the swift recruitment of BLM is as follows. The laser light irradiation produces a large number of DSBs in a small region of the nucleus, which results in open-ended DNA that could become the target of illegitimate recombination, nuclease attack, or telomerase attack. To maintain the integrity of the genome, it is thus imperative to protect and repair such open-ended DNAs immediately. This protection could be provided by specialized proteins that are pooled in the nucleus and rapidly accumulate at the damage sites, thereby covering the massive DSBs and preventing attacking molecules from acting. Interestingly, in relation to this hypothesis, WRN and TRF2 have been reported to be recruited to sites of DNA damage as an early response [19,20]. Here, we provide evidence that

BLM is also recruited to DNA damage sites as early as TRF2 and WRN. Taken together, it appears that RecQ helicases, in particular WRN and BLM, have an additional function, namely, to guard the genome through their HRDC domain. The catalytic activity of BLM appears to be required at a later time point, after various proteins involved in DNA repair or the restart of DNA replication have been recruited.

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