Dephosphorylation and Subcellular Compartment Change of the Mitotic Bloom’s Syndrome DNA Helicase in Response to Ionizing Radiation*

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Bloom’s syndrome is a rare human autosomal recessive disorder that combines a marked genetic instability and an increased risk of developing all types of cancers and which results from mutations in both copies of the BLM gene encoding a RecQ 3′-5′ DNA helicase. We recently showed that BLM is phosphorylated and excluded from the nuclear matrix during mitosis. We now show that the phosphorylated mitotic BLM protein is associated with a 3′-5′ DNA helicase activity and interacts with topoisomerase IIIα. We demonstrate that in mitosis-arrested cells, ionizing radiation and roscovitine treatment both result in the reversion of BLM phosphorylation, suggesting that BLM could be dephosphorylated through the inhibition of cdc2 kinase. This was supported further by our data showing that cdc2 kinase activity is inhibited in γ-irradiated mitotic cells. Finally we show that after ionizing radiation, BLM is not involved in the establishment of the mitotic DNA damage checkpoint but is subjected to a subcellular compartment change. These findings lead us to propose that BLM may be phosphorylated during mitosis, probably through the cdc2 pathway, to form a pool of available active protein. Inhibition of cdc2 kinase after ionizing radiation would lead to BLM dephosphorylation and possibly to BLM recruitment to some specific sites for repair.

Mutations in both copies of the BLM gene give rise to Bloom’s syndrome (BS),1 a rare disorder characterized by marked genetic instability combined with a greatly increased predisposition to a wide range of cancers commonly affecting the general population. The BLM gene is located on chromosome 15 at 15q26.1 and encodes the BLM protein, which belongs to the DEH box-containing RecQ helicase subfamily (1) and displays ATP- and Mg2+-dependent 3′-5′-DNA helicase activity (2). The major cellular consequences of a BLM defect are an increase in homologous recombination and in the rate of widespread mutations. Indeed, BS cells display spontaneous hypermutability and several cytogenetic abnormalities including an increase in chromosome breaks, symmetric quadriradial chromatid interchanges between homologous chromosomes, and sister chromatid exchanges (for review, see Ref. 3). Until recently, very little information was available about the physiological function of BLM. Now, several lines of evidences strongly support the involvement of BLM during DNA replication and in the cellular response to DNA damage. Recently, BLM protein has been shown to accumulate during the S phase of the cell cycle (4), to interact selectively in vitro with Holliday junctions (5), and to communoprecipitate with hRAD51 from cells synchronized in early S phase (6). BLM has also been shown to participate in the BRCA1-associated genome surveillance complex (7), to be phosphorylated and to accumulate through an ATM-dependent pathway in response to ionizing radiation (8), to assemble with promyelocytic leukemia protein at sites of single-stranded DNA after γ-irradiation (9), and to be cleaved early during apoptosis (10).

We showed recently that BLM is phosphorylated during mitosis both in cells treated with microtubule-disrupting agents and in mitotic cells isolated from untreated asynchronous populations and that mitotic phosphorylated BLM is excluded from the nuclear matrix and is not degraded via the ubiquitin-proteasome pathway (4). These data prompted us to investigate further the possible role of BLM phosphorylation in mitosis. If BLM acts in mitosis-arrested cells in the same pathways as those described for exponentially growing cells, it could be involved in the cellular response to DNA damage. However, little is known about the cellular response to DNA damage in mitosis. Poon et al. (11) have shown that in mitosis-arrested cells, cdc2 kinase activity is inhibited by DNA damage. Very recently, Smits et al. (12) showed for the first time that responses to DNA damage in mammalian cells are not restricted to the interphase but also occur during mitosis through a mitotic DNA damage checkpoint that blocks the exit from mitosis.

In the present study we show that in mitosis-arrested cells, the phosphorylated BLM protein is associated with 3′-5′ DNA helicase activity and interacts with topoisomerase IIIα. We present data demonstrating that mitotic BLM phosphorylation...
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is reversed in response to ionizing radiation and also after treatment with roscovitine, which is known to inhibitcdc2/cyclin B kinase activity in mitosis-arrested cells. We also show thatcdc2/cyclin B kinase activity is inhibited in mitosis-arrested cells subjected to ionizing radiation, suggesting that the BLM dephosphorylation that we observed in γ-irradiated mitotic cells could result in part from inhibition ofcdc2/cyclin B kinase activity. Finally, we show that in response to ionizing radiation, BLM is not involved in the establishment of the DNA damage mitotic checkpoint but is transferred from a soluble to an insoluble protein fraction, suggesting that during mitosis, phosphorylation modulates the localization of BLM to specific subcellular compartments.

Our observations led us to suggest that during mitosis, the phosphorylation of BLM protein, probably via thecdc2/cyclin B pathway, could disrupt its association with the nuclear matrix, allowing a pool of readily available active BLM protein to build up. In response to ionizing radiation, inhibition ofcdc2/cyclin B kinase activity and activation of an as yet unidentified phosphatase(s) could lead to BLM dephosphorylation and possibly to BLM recruitment to some specific sites for repair.

EXPERIMENTAL PROCEDURES

Chemicals

Demecolcine and nocodazole (Sigma) were resuspended in dimethyl sulfoxide to stock concentrations of 0.26 mM and 0.2 mg/ml, respectively, and used at dilutions of 1:1,000 and 1:4,000. Roscovitine (Sigma) was resuspended in dimethyl sulfoxide to a stock concentration of 150 mM and used at the dilutions indicated.

Antibodies

Rabbit anti-BLM antiserum 1340 and 1343 were generated and used as described (4). Anti-topoisomerase IIIα (D6) was a kind gift from Dr. Jean-François Riou (Aventis Pharma S.A., Vitry-sur-Seine) and used at a dilution of 1:1,000 as described (13). Goat anti-BLM antibody C18 and mouse monoclonal IgG2a anti-Cdc2 (used at a dilution of 1:500) were purchased from Santa-Cruz. Mouse monoclonal IgG1 anti-phospho-Ser-Thr-Pro MPM-2 (used at 10 μg/ml) was purchased from Upstate Biotechnology. Goat anti-rabbit IgG antiserum conjugated to peroxidase (Pierce) was used at a dilution of 1:10,000, goat anti-mouse IgG antiserum conjugated to peroxidase (Zymed Laboratories Inc.) was used at a dilution of 1:2,000, and goat anti-mouse IgG antiserum conjugated to fluorescein isothiocyanate (Jackson ImmunoResearch Labs, Inc.) was used at a dilution of 1:4,000.

Cell Lines

The Epstein-Barr virus-transformed lymphoblastoid B cell line GM03403D and D1 and HeLa cells were used as described previously (4, 8).

Flow Cytometry Analysis

Cell cycle analysis was performed as described previously (4).

Helicase Assay

Construction of the Helicase Substrate—Helicase substrate was prepared by mixing a 5-ng oligonucleotide corresponding to fragment 6218–6251 of the single-stranded M13mp18(+) DNA with 1 μg of single-stranded M13mp18(+) DNA in the presence of 25 mM NaCl and 2.5 mM MgCl2. The mixture was heated for 2 min at 100 °C and cooled slowly to room temperature for 30 min to allow annealing of the DNA heteroduplex. After EcoRI digestion, substrate labeling was performed using 5 units of Klenow fragment in the presence of 0.1 mM dTTP and 70 μCi of 3,000 Ci/mmol [α-32P]ATP (Amersham Biosciences, Inc.). After 20 min at 23 °C, 62.5 μCi dATP was added to the reaction mixture, which was then incubated for another 20 min at 23 °C. After phenol/ chloroform extraction, the labeled substrate was purified on a gel filtration column.

Assay Methods—The DNA helicase assay buffer contains 25 mM Tris-HCl (pH 7.4), 5 mM MgCl2, 1.25 mM dithiothreitol (DTT), 5 mM ATP, 250 μg/ml bovine serum albumin, and 1–3 ng of labeled DNA substrate. Immunoprecipitates or purified TFIIH was incubated in 25 μl of helicase assay buffer for 45 min at 37 °C, and the assay was stopped by the addition of 10 μl of a buffer containing 20 mM EDTA, 0.2% SDS, 10% glycerol, and 0.02% bromphenol blue. For immunoprecipitates, the supernatants were analyzed using a 14% non-denatured polyacrylamide gel. The gel was then dried and analyzed by autoradiography. The remaining pellets containing immunocomplexes were boiled and subjected to Western blot analysis.

The purified TFIH fraction was kindly provided by Frédéric Coin (IGBMC, Illkirch, France) and used as described (14).

Western Blot Analysis

Cells were cultured with or without drugs, scraped, and lysed in 1% SDS in water for 5 min at 95 °C and then sonicated. Samples equivalent to 5 × 105 cells were subjected to immunoblot analysis as described previously (4, 8).

Immunoprecipitations

Cells were lysed by incubation in 50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM MgCl2, 1 mM EDTA, 0.5% Igepal Ca-630 (Sigma), and a mixture of protease inhibitors (Roche) for 15 min at 4 °C. Membrane debris were eliminated by centrifugation at 14,000 rpm for 15 min. The protein concentration was measured by Coomassie protein assay according to the manufacturer’s instructions (Pierce).

For Helicase Assay—Immunoprecipitations were performed using 1.5 mg of lysisate for each point. Protein extracts were incubated with anti-BLM C18 or with irrelevant antibodies for 16 h at 4 °C. 50 μl of protein A/G-agarose beads (Santa Cruz) was then added, and the incubation continued for another hour. The beads were recovered by low speed centrifugation and washed five times in 0.7 ml of ice-cold wash buffer (50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 0.25% Igepal Ca-630, and a mixture of protease inhibitors), and then divided into two fractions. Each fraction was loaded onto a 5.5% SDS-polyacrylamide gel.

Kinase Assay

Cells were lysed in immunoprecipitation buffer (IP buffer: 50 mM Tris (pH 7.4), 150 mM NaCl, 0.5% Nonidet P-40, 0.5 mM sodium orthovanadate, 50 mM NaF, 80 μM β-glycerophosphate, 10 mM sodium pyrophosphate, 1 mM DTT, 5 mM EDTA, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 10 μg/ml aprotinin). Cell lysates were incubated by stirring gently with monoclonal anti-cdc2 antibody overnight at 4 °C. Immunocomplexes (bound to protein G-Sepharose) were collected by centrifugation and washed once in IP buffer and three times in kinase assay buffer (25 mM Hepes (pH 7.4), 25 mM MgCl2, 25 mM β-glycerophosphate, 2 mM DTT, 0.1 mM Na3PO4). The beads were then incubated with a kinase reaction mixture (5 μg of GST-Rb, 50 μM of cold ATP, and 5 μCi of 3,000 Ci/mmol [γ-32P]ATP (Amersham Biosciences, Inc.) in the kinase assay buffer) in a total volume of 30 μl for 30 min at 30 °C with shaking (purified GST-Rb was kindly provided by Serge Leibovitch, UMRS CNRS 1599, IG, Villejuif). The reaction was stopped with the addition of 2× sample buffer to the reaction, and the reaction mixture was then heated to 90 °C for 5 min. Proteins were resolved by 10% SDS-PAGE before being transferred. The membrane was analyzed by autoradiography, and the amount of cdc2 protein in immunoprecipitates was then determined by probing the membrane with anti-cdc2 antibody.

Radiation Treatment

Cells were irradiated at room temperature with 50 or 100 Gy using a 137Cs γ source at a dose rate of 1.95 Gy/min. After irradiation, cells were grown at 37 °C for the indicated times.

Nonidet P-40 Extraction

Demecolcine-treated HeLa cells (0.26 μM for 16 h) were γ-irradiated or not, washed with phosphate-buffered saline (PBS), and then resuspended in Nonidet P-40 buffer (15 mM NaCl, 60 mM KCl, 5 mM MgCl2, 0.1 mM EDTA, 15 mM Tris (pH 7.4), 0.5 mM DTT, 300 mM sucrose, and a mixture of protease inhibitors (Roche) containing 0.3% Nonidet P-40). After incubating for 3 min at room temperature, the samples were centrifuged, and the supernatant was kept as the Nonidet P-40-soluble fraction.

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The nondenaturing gel. Cleotide (tide) migrates more slowly than the 21-nucleotide oligonucleotide (lane TFIIH). Immuno-
inated on the autoradiography. The directions of the helicase translocation are indicated. The helicase activity was used to
clearly immunoprecipitated by C18 goat antibodies. However, we cannot formally exclude the possibility that a protein interact-
ing specifically with BLM could also display this helicase activity.

BLM Phosphorylation in Mitosis-arrested Cells Does Not Prevent Its Interaction with Topoisomerase IIIα—It has been shown that BLM protein associates with topoisomerase III α in somatic and meiotic cells, and it has been proposed that these proteins may cooperate to antagonize recombination (13, 15). To investigate the possibility that BLM mitotic phosphorylation could prevent its interaction with topoisomerase IIIα, immunoprecipitates were prepared from either unsynchronized or demecolcine-arrested HeLa cells using either 1343 (anti-BLM) or D6 (anti-hTOPOIIIα) antibodies. As controls, immunoprecipitations were carried out under the same conditions from protein extracts prepared from either unsynchronized or demecolcine-arrested HeLa cells using irrelevant antibodies. These immunoprecipitates were fractionated in 5.5% SDS-PAGE and then subjected to Western blot analysis to check for the presence of BLM and topoisomerase IIIα. As shown in Fig. 2, A and B, BLM and hTOPOIIIα can be coimmunoprecipitated both from unsynchronized and from demecolcine-arrested HeLa cells. These results show that the BLM-topoisomerase IIIα complex is not disrupted by mitotic BLM phosphorylation.

Mitotic BLM Protein Is Dephosphorylated in Response to Ionizing Radiation—Our results showing that the mitotic BLM protein is associated with a helicase activity and interacts with topoisomerase IIIα raised the question of the biological significance of BLM phosphorylation during mitosis. We showed recently that in unsynchronized cells BLM participates in the cellular response to ionizing radiation (8). To find out whether BLM could be involved in a similar pathway during mitosis, we analyzed BLM expression in mitotic cells exposed to ionizing radiation. Thus, nocodazole-arrested HeLa cells (data not shown) and demecolcine-arrested HeLa cells were subjected or not to 100-Gy ionizing radiation. 2 h or 8 h after exposure, BLM protein expression was analyzed by Western blotting using the BLM-specific antibody 1343. In parallel, we confirmed that the γ-irradiated demecolcine-arrested cells were still arrested in mitosis 8 h after exposure by determining the DNA content by flow cytometry (Fig. 3, center panels) and by immunostaining with BLM antibodies that recognize mitosis-specific epitopes (Fig. 3, bottom panels) (16). Untreated or γ-irradiated unsynchronized HeLa cells were used as controls. As shown in Fig. 3 (top panel), 8 h after subjecting demecolcine-arrested cells to ionizing radiation, the migration of BLM protein (lane 3) was

Fig. 1. 3′-5′ DNA helicase activity of the mitotic BLM protein. A, the helicase bidirectional DNA substrate used in this study is de-
picted. The helicase substrate contains a duplex region at each end, which is either 20 or 21 nucleotides in length. The 21-nucleotide strand can only be displaced by a helicase translocating from 3′ to 5′ and the 20-nucleotide strand by a helicase translocating in the opposite direction. B, left panel, helicase assay. Proteins extracted from demecolcine-
treated HeLa cells (0.26 μg for 16 h) were immunoprecipitated (IP) using C18 anti-BLM antibody (lane IP BLM) or normal goat serum as an irrelevant antibody (irr) control (lane IP irr). BLM immunoprecipitates were then incubated with labeled DNA substrate for 45 min at 37 °C. A purified TFIIH fraction with a 5′-3′ helicase activity was used as control. All reactions were loaded on a 14% nondenatured polyacryl-
amide gel. Nonreacted labeled DNA substrate, either native or heat-
denatured, was run in parallel. The gel was then dried and analyzed by autoradiography. The directions of the helicase translocation are indicated on the right. We should note that the 20-nucleotide oligonucleotide (lane TFIIH) migrates more slowly than the 21-nucleotide oligonucleotide (lane IP BLM). This is probably because of the structural conformation adopted by the oligonucleotides during the migration in the nondenaturing gel. Right panel, Western blot (WB). The immuno-
precipitates used for the helicase assay were boiled in Laemmli buffer, separated on 5.5% polyacrylamide gel, and transferred onto a polyvi-
nylidenefluoride membrane. The membrane was probed with 1340 anti-BLM antibody.

Immunofluorescence Labeling

Cells were transferred onto poly-l-lysine-coated glass slides and fixed in 3.7% formaldehyde in PBS at 15 min, room temperature. All subsequent procedures were performed at room temperature. Cells were rinsed in PBS and permeabilized with 0.5% Triton X-100 for 5 min. After a wash in PBS, cells were blocked with a solution containing PBS, 0.1% Tween, 0.1% bovine serum albumin (1 h). After three washes in PBS, the slides were incubated with primary antibody (MPM-2) for 1 h. After being washed three times with PBS, the slides were incubated with the secondary antibodies (goat anti-mouse IgG antiserum conjugated with fluorescein isothiocyanate) for 1 h at room temperature. After three washes in PBS, nuclear DNA staining was carried out by incubating with 1 μg/ml 4,6-diamidino-2-phenylindole solution for 5 min (Sigma). Confocal fluorescent images were collected using a Leica TCS confocal system (Wetzler, Germany).

RESULTS

In Mitosis-arrested Cells, the Phosphorylated BLM Protein Is Associated with a 3′-5′ DNA Helicase Activity—We had already shown that BLM protein was hyperphosphorylated during mitosis (4). To find out whether BLM protein is inactivated during mitosis by phosphorylation, we assayed mitotic BLM protein for helicase activity. To do this, BLM protein was immunoprecipitated from demecolcine-arrested HeLa cell protein extracts using goat C18 anti-BLM antibody and subjected to a bidirec-
tional helicase assay using the substrate shown in Fig. 1A (see “Experimental procedures”). The immunoprecipitate used for the helicase assay was then checked by Western blot analysis, using rabbit 1340 anti-BLM antibody (Fig. 1B, right panel). A parallel control immunoprecipitation was carried out under the same conditions using an irrelevant antibody. Furthermore, for the helicase assay, we used a purified TFIIH fraction with a bidirectional helicase activity, but which has been shown to exhibit preferential 5′-3′ helicase activity in a similar assay (14). This purified TFIIH fraction displaced the 20-nucleotide fragment in our assay, as expected (Fig. 1B). As shown in Fig. 1B (left panel), immunoprecipitate from demecolcine-arrested HeLa cells catalyzed the displacement of the 21-nucleotide fragment (but not the 20-nucleotide fragment) from the linearized substrate, whereas no significant helicase activity was detected in the immunoprecipitate used as control. These results indicate that BLM immunoprecipitate from mitotic ar-
rested cells displays a 3′-5′ DNA helicase activity. The same results were obtained using rabbit 1343 anti-BLM antibody (data not shown), confirming that the helicase activity we de-
tected (Fig. 1B) is specifically associated with the BLM protein and not the result of an unrelated protein that is nonspecifically immunoprecipitated by C18 goat antibodies. However, we cannot formally exclude the possibility that a protein interacting specifically with BLM could also display this helicase activity.

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similar to that from unsynchronized cells (lane 4) but not to that from γ-irradiated unsynchronized HeLa cells (lane 5). We had shown previously that adding λ phosphatase to BLM immunoprecipitates from unsynchronized cells did not affect BLM migration, whereas λ phosphatase treatment of BLM immunoprecipitated from mitotic cells resulted in the recovery of a band migrating in a way similar to BLM from unsynchronized cells (4). These results (Fig. 3) clearly show that virtually all of the mitotic BLM proteins had been dephosphorylated within 8 h after ionizing radiation of demecolcine-arrested HeLa cells.

Mitotic BLM Protein Is Dephosphorylated after Roscovitine Treatment—Dephosphorylation of the mitotic BLM protein in response to ionizing radiation probably results from the combined effects of the inactivation of kinase(s) directly involved in BLM phosphorylation during mitosis and the activation of phosphatase(s). Cdc2 kinase triggers the entry of cells into mitosis by direct phosphorylation of numerous proteins (17). The BLM protein presents two potential phosphorylation sites that fit the consensus sequence X-S/T-P-X/R/K for protein kinase p34cdc2 (18), at positions 711–717 and 763–769, respectively. To determine whether inhibition of the cdc2 kinase pathway could be involved in vivo in the reversion of mitotic BLM phosphorylation, demecolcine-arrested HeLa cells were treated or not with roscovitine, a highly selective inhibitor of cdc2/cyclin-B kinase specifically in mitosis (19, 20). Moreover, roscovitine has been shown to inhibit cdc2/cyclin B kinase specifically in mitosis-arrested HeLa cells (21). Using demecolcine-arrested HeLa cells, we carried out a dose response and then a time course investigation of the effect of roscovitine on the BLM migration shift. As shown in Fig. 4A, at 75 and 150 μM roscovitine (lanes 6 and 7, respectively), BLM phosphorylation was completely reversed, recovering an electrophoretic migration similar to BLM in unsynchronized cells (lane 1). Furthermore, as shown in Fig. 4B, when mitotic cells were treated for 30 min with 150 μM roscovitine, we observed a partial reversion of BLM phosphorylation, whereas 60 and 120 min after roscovitine treatment, BLM protein phosphorylation had been completely reversed. To ensure that extracellular signals-regulated kinase, which has been shown to be inhibited by much higher doses of roscovitine than cyclin-dependent kinases (22), was not involved in mitotic BLM phosphorylation, the same experiments were conducted using specific inhibitors (UO126, Calbiochem), and we did not observe any reversion of BLM phosphorylation (data not shown).

These results show that the inhibition of cdc2 kinase by roscovitine treatment is associated with reversion of mitotic BLM phosphorylation, which suggests that BLM could be dephosphorylated in mitosis through cdc2 kinase pathway. We should note that the mitotic BLM protein seems to be dephosphorylated within two steps, as illustrated by the intermediate BLM species observed 30 min after roscovitine treatment (Fig. 4B, lane 3). Once cdc2 kinase is inactivated, dephosphorylation of...
the mitotic BLM protein could result from the sequential action of two independent phosphatases. Future experiments will help to address this question.

Cdc2 Kinase Activity Is Inhibited by Ionizing Radiation—
These findings suggest that reversion of mitotic BLM phosphorylation in response to ionizing radiation could result in part from inhibition of cdc2 kinase activity, suggesting that cdc2 kinase activity could be inhibited by ionizing radiation. It has been shown that cdc2 kinase is inactivated by DNA damage during mitosis and that its inactivation outweighs the stabilization of cdc2 activity by nocodazole or taxol (11). However, data presented by Smits et al. (12) show that cdc2/cyclin B kinase activity is not inhibited in response to DNA damage in mitotic cells. To resolve this apparent controversy and to find out whether cdc2 kinase activity is inhibited in response to ionizing radiation in mitotic cells, we immunoprecipitated cdc2 kinase from protein extracts prepared from demecolcine-arrested HeLa cells, and the cdc2 kinase activity was measured through GST-Rb phosphorylation (23). The amount of cdc2 immunoprecipitated in the kinase assay was checked by Western blot analysis, using anti-cdc2 antibody (Fig. 5, lower panel). Comparable amounts of GST-Rb were present in the different lanes as verified by staining the membrane with Ponceau solution (data not shown). The reversion of mitotic BLM phosphorylation was also checked using 1343 anti-BLM antibody (data not shown). As shown in Fig. 5, upper panel, cdc2 kinase activity is stabilized in demecolcine-arrested cells, as expected, but exposing demecolcine-arrested cells to ionizing radiation resulted in inhibition of cdc2 kinase activity. We confirmed that cyclin B coimmunoprecipitates with cdc2 in both demecolcine-arrested cells and in γ-irradiated demecolcine-arrested HeLa cells, whereas cyclin A is undetectable and thus probably degraded, as expected (24) (data not shown).

These results clearly show that cdc2/cyclin B kinase activity is inhibited in response to ionizing radiation in mitosis-arrested cells.

BLM-deficient Cells Display a Mitotic DNA Damage Checkpoint Similar to That of Control Cells—Recently, Smits et al. (12) showed that DNA damage blocks mitotic exit. To investigate the possible consequences of BLM dephosphorylation in response to ionizing radiation during mitosis, we analyzed the block to mitotic exit (as described in Ref. 12) in BLM-deficient cells. Thus, nocodazole-blocked wild-type (D1) and BS cells (GM03403D (4)) were left untreated or γ-irradiated (50 Gy) (BLM phosphorylation is identical in nocodazole- and demecolcine-arrested cells; data not shown). The cells were then released from the block by removing nocodazole and were harvested and fixed 4 h later. As shown in Fig. 6, 4 h after the removal of nocodazole, untreated wild type and BS cells had exited from mitosis and entered the next G1 phase. However, exposure to ionizing radiation prevented the exit from mitosis of both wild type cells and BS cells, and a large proportion of cells remained arrested within a 4 N DNA content 4 h after the release (Fig. 6). These results confirm the data published by Smits et al. (12) showing that a DNA damage checkpoint is activated in mitotic cells. Moreover, these results clearly show that, like wild type cells, BLM-deficient cells exhibit an intact block to mitotic exit in response to ionizing radiation, showing that BLM does not play a critical role in establishing such a mitotic DNA damage checkpoint.

The Reversion of Mitotic BLM Phosphorylation in γ-Irradiated Mitotic Cells Is Associated with a Subcellular Compartment Change—We showed previously that mitotic BLM was extracted totally within a soluble cellular fraction and is not found associated with the nuclear matrix, unlike BLM from unsynchronized cells (4). To find out whether the dephosphorylation of mitotic BLM could be associated with a change of subcellular compartment after ionizing radiation, we permeabilized the cells with the nonionic detergent Nonidet P40, which leaves proteins bound to chromatin and to the nuclear scaffold in place while other proteins are solubilized (25–27). As shown in Fig. 7, phosphorylated BLM from nonirradiated demecolcine-arrested HeLa cells was extracted totally in the Non-
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The striking feature of Bloom’s syndrome is that it predisposes patients to all kinds of cancer that commonly affect the general population, which suggests that identification of the molecular basis of tumor development in BS patients could also help in deciphering of new pathways of carcinogenesis in the general population.

We had shown previously that BLM protein is hyperphosphorylated during mitosis (4), and the aim of the present study was to characterize further the possible role of this phosphorylation of mitotic BLM.

We first showed that the mitotic BLM protein is associated with a 3′-5′ DNA helicase activity and interacts with topoisomerase IIIα, and this led us to wonder whether BLM could play a functional role during mitosis. Our previous work showing that in interphase cells, BLM participates in the cellular response to ionizing radiation (8) led us to analyze the expression of mitotic BLM in response to ionizing radiation. We found that the mitotic BLM protein was dephosphorylated after ionizing radiation, suggesting that BLM could also be involved in the cellular response to DNA damage during mitosis. This was supported further by recent data showing that responses to DNA damage in mammalian cells are not restricted to the interphase but also occur during mitosis (12). We therefore suggested that dephosphorylation of mitotic BLM protein could result in part from the inactivation of a kinase playing a major role in BLM phosphorylation during mitosis after exposure of the mitotic cells to ionizing radiation. The best candidate was the kinase p34cdc2 because it is known to phosphorylate a large number of proteins in mitosis and because BLM carries two potential phosphorylation sites that fit the consensus sequence X-S/T-P-X-R/K for protein kinase p34cdc2 (18), at positions 711–717 and 763–769, respectively. Interestingly, we found that the mitotic BLM protein is dephosphorylated by exposure to roscovitine, which further supports our hypothesis. Indeed, roscovitine displays high efficiency and selectivity toward cdc2/cyclin B kinase (19, 20) and has been shown to be useful for studying molecular events conducted through the cdc2/cyclin B pathway in mitosis-arrested cells (11, 21). Altogether, these results suggest that cdc2 kinase may play a key role in BLM phosphorylation during mitosis and indicate that cdc2 kinase activity could be inhibited after ionizing radiation.

Inhibition of cdc2 kinase activity in response to DNA damage in mitosis is not well documented. It has been shown that exposing nocodazole-arrested cells to UV irradiation or adriamycin resulted in the loss of cdc2/cyclin B kinase activity (11). In contrast, the data recently published by Smits et al. (12) showed that exposing nocodazole-arrested cells to adriamycin does not inhibit cdc2/cyclin B kinase activity and suggested similar effects after camptothecin or ionizing radiation treatment of mitotic cells. In the present study, we showed clearly that the cdc2 kinase activity was inhibited when mitosis-arrested cells were treated with ionizing radiation. These results resolve the controversy reported in the literature for at least one type of genotoxic stress and support our hypothesis that dephosphorylation of the mitotic BLM protein after ionizing radiation results, at least in part, from inactivation of cdc2 kinase. However, we cannot exclude the possibility that inhibition of cdc2 kinase activity may depend on the type of DNA damage.

Smits et al. (12) show that exposure to γ-irradiation prevents exit from mitosis, possibly by inactivating the polo-like kinase-1. In confirming those results, we showed that exposing mitosis-arrested cells to ionizing radiation prevents the exit from mitosis in wild-type cells and also in BLM-deficient cells, demonstrating that BLM is not involved in the establishment of a mitotic DNA damage checkpoint of this type.

Finally, we showed that all of the mitotic phosphorylated BLM is extracted in the Nonidet P-40-soluble fraction, whereas reversion of mitotic BLM phosphorylation in response to ionizing radiation is concurrent with the relocalization of BLM in the Nonidet P-40-insoluble fraction. These findings confirm our previous results, showing that phosphorylation modifies the extractability of BLM during mitosis (4) but also demonstrate that the phosphorylation of BLM during mitosis modulates its subcellular localization.

Our findings led us to propose a model for the phosphorylation of BLM during mitosis. BLM is phosphorylated during mitosis, probably via the cdc2 pathway. Exclusion of the mitotic phosphorylated BLM from the Nonidet P-40-insoluble cellular fraction containing proteins bound to chromatin and to the nuclear scaffold would avoid possible interference of BLM (and probably other proteins) with the mitotic process such as the condensation of chromosomes. However, the mitotic phospho-
mitotic BLM phosphorylation and localization

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Dephosphorylation and Subcellular Compartment Change of the Mitotic Bloom's Syndrome DNA Helicase in Response to Ionizing Radiation
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