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Bloom’s syndrome (BS) is an autosomal disorder characterized by predisposition to a wide variety of cancers. The gene product whose mutation leads to BS is the RecQ family helicase BLM, which forms a complex with DNA topoisomerase IIIα (Top3α). However, the physiological relevance of the interaction between BLM and Top3α within the cell remains unclear. We show here that Top3α depletion causes accumulation of cells in G2 phase, enlargement of nuclei, and chromosome gaps and breaks that occur at the same position in sister chromatids. The transition from metaphase to anaphase is also inhibited. All of these phenomena except cell lethality are suppressed by BLM gene disruption. Taken together with the biochemical properties of BLM and Top3α, these data indicate that BLM and Top3α execute the dissolution of sister chromatids.

Eukaryotic Top3 was first identified in Saccharomyces cerevisiae as a gene that is required to suppress recombination between repeated sequences (28). Deletion of TOP3 results in a slow-growth phenotype that is suppressed by the disruption of SGS1, the gene encoding the sole RecQ helicase in S. cerevisiae (5). Further analyses revealed that the function of Sgs1 is closely associated with that of DNA topoisomerase III (Top3) (2, 13, 19, 27). The close relationship between RecQ helicases and Top3 seems to be maintained in higher eukaryotes. Higher eukaryotic cells have two Top3s, Top3α and Top3β (8, 22, 23). Knocking out the Top3α gene in mice results in embryonic lethality (17), while knocking out Top3β does not affect development but reduces the life span (15). Various Top3 and RecQ helicase molecules have been reported to interact physically, including Top3α and BLM (35), one of the RecQ family helicases in higher eukaryotic cells (3). BLM is a causative gene for Bloom’s syndrome (3), which is an autosomal disorder characterized by predisposition to a wide variety of cancers (6). Biochemical analyses have suggested that BLM and Top3α together affect the in vitro resolution of a recombination intermediate containing a double Holliday junction (HJ) via a double-junction dissolution mechanism (34). However, the phenotypes of cells that lack Top3α have not been characterized precisely, since Top3α knockout is lethal. Furthermore, the phenotypes of Top3α-depleted cells before they die have not been examined. Moreover, the physiological relevance of the interaction between BLM and Top3α within the cell remains unclear. Therefore, elucidating higher eukaryotic Top3α function may enhance our understanding of the physiological roles of BLM.

In this study, to assess the function of Top3α and its interactions with BLM, we constructed cells whose expression of Top3α can be switched off by doxycycline hydrochloride (Dox) treatment. To our knowledge, we present the first evidence to support the hypothesis that vertebrate Top3α together with the BLM helicase executes the dissolution of sister chromatids during DNA replication.

MATERIALS AND METHODS

Plasmid construction. Fragments of chicken TOP3α and TOP3β cDNAs were obtained by PCR from a ZAPII chicken cDNA library using primers designed from the human TOP3α and TOP3β gene sequences. The terminal regions of the cDNAs were obtained from chicken testis RNA by 3′ or 5′ rapid amplification of cDNA ends. Genomic DNA fragments of the TOP3α and TOP3β genes were amplified by long-range PCR using genomic DNA from DT40 cells. Targeting constructs used to disrupt TOP3α were made by replacing the region encoding the active site of Top3α with a neomycin or blastidin selection marker cassette. Targeting constructs for TOP3β disruption were made in an analogous manner using a puromycin or blastidin selection marker cassette. Targeting constructs used to disrupt WRN and BLM have been described previously (12, 29). To construct an expression plasmid carrying mouse TOP3α cDNA (22) with the tet-off promoter, a cDNA encoding FLAG-tagged Top3α was inserted into the pUHG10-3 vector.

Gene disruption. DT40 cells (1 × 107) were electroporated with a Gene Pulser (Bio-Rad, Hercules, CA) at 550 V and 25 μF in the presence of 30 μg linearized targeting constructs. Drug-resistant colonies were selected in 96-well plates with medium containing 2 mg/ml neomycin, 1 mg/ml histidinol, 0.5 μg/ml puromycin, or 30 μg/ml blastidin. The disruption of the targeted gene(s) was checked by...
Southern blotting, genomic PCR, and reverse transcriptase PCR. The genotypes of all of the cell lines used in this study are listed in Table 1.

**Western blotting analysis.** Cells (1 x 10^6) were cultured in the presence or absence of Dox, a derivative of tetracycline, harvested, washed with phosphate-buffered saline (PBS), precipitated, and suspended in sodium dodecyl sulfate sample buffer containing 20 mM N-ethylmaleimide. Samples were fractionated in a linear 4 to 20% gradient sodium dodecyl sulfate-polyacrylamide gel. Proteins were transferred onto a polyvinylidene difluoride membrane (Millipore) and immunoblotted with anti-RecQ-like helicase 1 (BLM) mouse monoclonal antibody (American Type Culture Collection; 1:5,000), rabbit anti-Top3alpha (TOP3α) mouse monoclonal antibody (1:500), mouse monoclonal anti-BrdU, and rabbit anti-Ser10-phosphorylated histone H3. Binding of primary antibodies was then detected using fluorescein isothiocyanate-conjugated goat or rabbit anti-mouse IgG or Cy3-conjugated goat anti-rabbit IgG. Nuclei were counterstained with 0.2 μg/ml DAPI (4′,6-diamidino-2-phenylindole).

**Detection of chromosome aberrations.** Cells were cultured in the presence of 0.1 μg/ml colcemid for 2 h. The cells were harvested and treated with 75 mM KCl for 11 min at room temperature and fixed with methanol/acetic acid (3:1) for 30 min. The cell suspension was dropped onto ice-cold wet glass slides and air dried. The cells were stained with 2% Giemsa solution at pH 6.8 for 25 min and examined by light microscopy.

**FISH analysis.** Chromosomal aberrations were examined using fluorescent in situ hybridization (FISH) analysis. The microchromosomes, 1, 2, 3, 4, 5, and 7, and other smaller chromosomes were distinguished by FISH analysis after three-colorized painting of chromosomes, as described previously (7). Chromosomes were counterstained with 0.2 μg/ml DAPI.

**Detection of early apoptotic cells by flow cytometry.** Apoptotic cells were detected using Vybrant apoptosis assay kit no. 3 (Molecular Probes). Cells (1 x 10^6) were washed with ice-cold PBS and suspended in 100 μl annexin-binding buffer, and 5 μl of FITC-conjugated annexin V and 1 μl of 100 μg/ml PI were added to the cell suspension. After a 15-min incubation at room temperature, 400 μl annexin-binding buffer was added to the cell suspension, and cells were filtered through nylon mesh and analyzed by FACScan. The obtained data were processed with Cell Quest software (Becton Dickinson).

**RESULTS**

**TOP3α is an essential component of vertebrate cells.** To understand the physiological roles of vertebrate Top3α, we generated TOP3α and -β knockout cells using chicken DT40 cells (Fig. 1a and c) (33). The TOP3α^−/− DT40 cells were constructed by transfecting two TOP3α targeting vectors sequentially into wild-type DT40 cells. The TOP3α^−/− cells were generated by first disrupting one TOP3α genomic locus, transf ecting the cells with a plasmid expressing the FLAG-tagged mouse TOP3α gene from the tet-off promoter, and then disrupting the second TOP3α genomic locus. Treatment of these cells with Dox suppresses the expression of the mTOP3α protein and results in cells with the TOP3α^−/− genotype. This circumvents the lethality problem engendered by disruption of both alleles of the TOP3α gene. Gene disruption was confirmed by Southern blotting (Fig. 1b and d).

**TABLE 1. DT40 strains used in this study**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Disrupted gene(s) (selective marker)*</th>
<th>Expression plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>BLM (His/Hsr)</td>
<td>FLAG-mTOP3α:Hyg</td>
</tr>
<tr>
<td>WRN^−/−</td>
<td>WRN (His/Hsr)</td>
<td></td>
</tr>
<tr>
<td>TOP3α^−/−</td>
<td>TOP3α (Neo/His)</td>
<td>FLAG-mTOP3α:Hyg</td>
</tr>
<tr>
<td>TOP3β^−/−</td>
<td>TOP3β (Puro/Brsr)</td>
<td></td>
</tr>
<tr>
<td>TOP3α^−/−/TOP3β^−/−</td>
<td>TOP3β (Neo/His)</td>
<td></td>
</tr>
<tr>
<td>TOP3α^−/−/BLM^−/−</td>
<td>TOP3α (Neo/His)</td>
<td></td>
</tr>
<tr>
<td>TOP3β^−/−/BLM^−/−</td>
<td>TOP3β (Puro/Brsr)</td>
<td></td>
</tr>
<tr>
<td>TOP3α^−/−/WRN^−/−</td>
<td>TOP3α (Neo/His)</td>
<td></td>
</tr>
<tr>
<td>RAD51^−/−</td>
<td>RAD51 (Neo/Hsr)</td>
<td></td>
</tr>
<tr>
<td>MRE11^−/−</td>
<td>MRE11 (His/Hsr)</td>
<td></td>
</tr>
</tbody>
</table>

* Selective markers: Neo, neomycin; His, histidinol; Brsr, blasticidin; Hyg, hygromycin; Puro, puromycin; Eco, mycophenolic acid.

**Nucleotide sequence accession numbers.** The chicken TOP3α and -β cDNA sequences have been deposited in GenBank with accession numbers AB215104 and AB215105, respectively.
Figure 2 shows the growth curves of the different DT40 cell lines. The TOP3β−/− cells grew at the same rate as DT40 wild-type cells (Fig. 2A, panel a). In the absence of Dox, the TOP3α−/− and TOP3α−/−/TOP3β−/− cells grew at the same rate, which was slightly lower than that of wild-type cells. However, both TOP3α−/− and TOP3α−/−/TOP3β−/− cells ceased growing within 3 days after Dox addition (Fig. 2A, panel b). The amount of mTop3 in the cells was markedly decreased after Dox treatment (Fig. 2B). Top3α-depleted cells arrest primarily in G2 phase. Flow cytometric analysis indicated that the cell cycle distribution of TOP3α−/− cells was largely unchanged over 2 days following the addition of Dox (Fig. 3A). However, on the third day, the peak corresponding to G2/M phase became prominent and cells containing less DNA than G1 cells appeared. These changes increased markedly by the fourth day. To investigate the point of cell cycle arrest more precisely, the proportion of cells in different phases was calculated relative to the total number of interphase cells and plotted. The fraction of interphase cells that could not be classified into the above three categories was plotted as “all negative.” (C) Enlargement of nuclei in Top3α-depleted cells. TOP3α−/− cells were cultured in the absence or presence of Dox for 1, 2, 3, or 4 days and observed by microscopy after being stained with DAPI. Representative morphologies of enlarged nuclei are indicated by arrows (giant nuclei) and arrowheads (disrupted nuclei).
cell cycle stages was quantified using cell cycle marker-specific antibodies. Thus, cells in G$_1$ to S phase, S phase, and late G$_2$ phase were identified by the presence of nuclear staining of MCM4, BrdU, and Ser-10-phosphorylated histone H3, respectively. There was a remarkable increase in the proportion of cells that did not stain with any of these antibodies 3 days after the addition of Dox (Fig. 3B). These data suggest that depletion of Top3$\alpha$ caused a large population of the cells to arrest in G$_2$ phase before histone H3 was phosphorylated. Moreover, the nuclei of a significant number of the cells were enlarged after the 3-day incubation with Dox and disrupted nuclei were also observed. On the fourth day, there was a marked increase in the number of cells with disrupted nuclei (Fig. 3C).

### Appearance of metaphase cells with highly aberrant chromosomes following depletion of Top3$\alpha$

Although the number of M-phase cells was decreased by the depletion of Top3$\alpha$, a small but appreciable number of metaphase cells continued to be detected on the third and fourth days after Dox addition. When we examined the chromosomes of these cells, we found a significant increase in chromosome-type gaps and breaks (Table 2 and Fig. 4A, panels b and c). An increase in chromatid-type gaps and breaks was also observed in the Top3$\alpha$-depleted cells but was much smaller than the increase in chromosome-type aberrations. Top3$\alpha$ deficiency also led to the emergence of metaphase cells with highly aberrant karyotypes which could not be scored (Fig. 4A, panel d, and Table 2). In these cells, the 12 macrochromosomes found in typical metaphase DT40 cells (Fig. 4A, panel a) could not be identified. Chromosome painting revealed the fragmentation of the macrochromosomes in these Top3$\alpha$-depleted metaphase cells (Fig. 4B). Similar chromosome-type gaps and breaks have been observed in Rad51- or Mre11-depleted cells (25, 36), but almost no “unscorable” metaphase cells were detected in these cells (Table 3). The extent of chromosomal aberrations found in Top3$\alpha$-depleted cells was much higher than that found in Rad51- or Mre11-depleted cells.

In Top3$\alpha$-depleted cells, inhibition of the transition from G$_2$ to M phase and from metaphase to anaphase is released by the protein kinase inhibitor 2-aminopurine. We next examined the transition from metaphase to anaphase in Top3$\alpha$-depleted cells by scoring anaphase cells that had condensed chromosomes and dephosphorylated histone H3. The Top3$\alpha$ depletion markedly decreased the ratio of cells in anaphase to the total number of mitotic cells, suggesting that the metaphase-to-anaphase transition was inhibited (Fig. 5A). This result is consistent with the dramatic increase in chromosome-type gaps and breaks, which indicates that double-strand breaks are occurring at the same position in sister chromatids. These data suggest a possibility that the decatenation of sister chromatids is defective in Top3$\alpha$-depleted cells, reflecting an impediment at a very late stage of DNA replication. If the decatenation of sister chromatids is impaired, the decatenation checkpoint should be activated in Top3$\alpha$-depleted cells. Treatment with 2-aminopurine (2-AP), an inhibitor of protein kinases, has been reported to override G$_2$ arrest induced by the failure of DNA topoisomerase II (Topo II)-dependent decatenation (1). To assess whether Top3$\alpha^{-/-}$ cells are arrested in G$_2$ by a 2-AP-sensitive checkpoint mechanism, Top3$\alpha^{-/-}$ cells were treated with 2-AP after a 3-day incubation with Dox. The proportion of cells in M phase increased remarkably within 1 h after the addition of 2-AP and then declined after 3 h (Fig. 5B). In addition, the number of cells containing irregular-shaped, but not enlarged, nuclei increased 3 h after the addition of 2-AP (data not shown). This result further supports the hypothesis that Top3$\alpha$ depletion may result in an ineffective decatenation of sister chromatids. The reduced metaphase-to-anaphase transition of 2-AP-treated Top3$\alpha^{-/-}$ cells may also be explained by a defect in the decatenation of sister chromatids. The 2-AP treatment decreased the frequency of enlarged nuclei induced by Dox treatment (Fig. 5C), presumably due to entry into M phase, as described above.

### Generation of Top3$\alpha^{-/-}$/BLM$^{-/-}$, Top3$\beta^{-/-}$/BLM$^{-/-}$, and Top3$\alpha^{-/-}$/WRN$^{-/-}$ double mutant cells.

To elucidate the functional relevance of Top3$\alpha$s and RecQ helicases, we generated Top3$\alpha^{-/-}$/BLM$^{-/-}$, Top3$\beta^{-/-}$/BLM$^{-/-}$, and Top3$\alpha^{-/-}$/WRN$^{-/-}$ double mutant cells. WRN, which encodes a RecQ family helicase, is the causative gene of Werner syndrome, which is characterized by premature aging (37). Disruption of BLM and WRN was confirmed by PCR (Fig. 1e and f). While Top3$\alpha^{-/-}$/BLM$^{-/-}$ and Top3$\alpha^{-/-}$/WRN$^{-/-}$ cells proliferated at a slightly lower rate than Top3$\alpha^{-/-}$ cells in the absence of Dox, the viable cell numbers of all three Top3$\alpha$-deficient cell lines declined after

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**Table 2. BLM gene disruption suppresses the emergence of unscorable metaphase karyotypes in Top3$\alpha$-depleted cells**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of days after Dox addition</th>
<th>Total no. of cells observed$^a$</th>
<th>No. of unscorable cells</th>
<th>No. of scored cells</th>
<th>No. of aberrant cells$^b$</th>
<th>No. of cells with the following chromatic type:</th>
<th>No. of cells with the following chromatic type:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>200</td>
<td>200</td>
<td>16</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>BLM$^{-/-}$</td>
<td>200</td>
<td>200</td>
<td>19</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Top3$\alpha^{-/-}$/BLM$^{-/-}$</td>
<td>200</td>
<td>200</td>
<td>18</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Top3$\alpha^{-/-}$</td>
<td>3</td>
<td>241</td>
<td>41</td>
<td>200</td>
<td>79</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Top3$\alpha^{-/-}$/BLM$^{-/-}$</td>
<td>4</td>
<td>329</td>
<td>129</td>
<td>200</td>
<td>104</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>Top3$\alpha^{-/-}$/BLM$^{-/-}$</td>
<td>3</td>
<td>201</td>
<td>1</td>
<td>200</td>
<td>54</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Top3$\alpha^{-/-}$/BLM$^{-/-}$</td>
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<td>203</td>
<td>3</td>
<td>200</td>
<td>64</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

*Since unscorable metaphase cells were markedly increased in Top3$\alpha$-depleted cells, we examined from 200 to 329 metaphase cells of various mutants in order to score a total of 200 metaphases per cell line.

*Data are presented as the number of aberrations per 200 metaphases.
the addition of Dox. However, the cessation of growth of the TOP3α−/−/BLM−/− cells was slightly delayed compared with that of the TOP3α−/− or TOP3α−/−/WRN−/− cells (Fig. 2A, panel c). This suggests that unlike WRN, BLM functionally interacts with Top3α. TOP3β−/−/BLM−/− cells grew at the same rate as BLM−/− cells (Fig. 2A, panel a). These data indicate that BLM but not WRN functionally interacts with Top3α, while BLM and Top3β do not functionally interact.

**Effect of disruption of the BLM gene on the phenotypes of TOP3α−/−-depleted cells.** Since our data showed that the disruption of BLM did not suppress the lethality of Top3α-depleted cells but did delay the decline of viable cell numbers, we examined in detail the effect of disruption of BLM on the phenotypes of Top3α-depleted cells. The mechanism of cell death in TOP3α−/−, TOP3α−/−/BLM−/−, or TOP3α−/−/WRN−/− cells was examined by flow cytometry. In the case of TOP3α−/−/BLM−/− cells, the number of cells that could be stained with an anti-annexin V antibody was markedly increased 4 days after the addition of Dox. In contrast, Dox treatment of TOP3α−/− and TOP3α−/−/WRN−/− cells did not lead to a significant increase of annexin V-positive cells (Fig. 6A). These observations indicate that Top3α-depleted BLM−/−
cells died from apoptosis, while Top3α-depleted cells and Top3α-depleted WRN−/− cells seem to die by a nonapoptotic pathway.

The occurrence of enlarged nuclei in Dox-treated Top3α-depleted cells was also suppressed by the additional disruption of BLM but was not affected by the disruption of WRN (Fig. 6B). Both the number of S-phase cells and the number of cells with enlarged nuclei after the addition of Dox were quantified. While Top3α−/− cells showed a decrease in the number of DNA-synthesizing cells and the emergence of enlarged nuclei after Dox treatment for 48 h, Top3α−/−/BLM−/− cells showed a decrease in S-phase cells only after 78 h and no enlarged nuclei even after 96 h (Fig. 6C). The disruption of BLM also suppressed the G2 arrest (data not shown) and metaphase arrest observed in Top3α-depleted cells (Fig. 5A). Moreover, the disruption of BLM in Top3α-depleted cells remarkably reduced the frequency of chromosome-type aberrations and, more importantly, nearly completely suppressed the emergence of “unscorable” metaphase cells which contained chromosomal aberrations too severe to allow us to identify individual chromosomes (Table 2).

DISCUSSION

We show here that Top3α depletion causes accumulation of cells in G2 phase, enlargement of nuclei, chromosome gaps and breaks that occur at the same position in sister chromatids, and the emergence of “unscorable” metaphase cells. Additionally, the transition from metaphase to anaphase is inhibited. All of these phenotypes were suppressed by BLM gene disruption in Top3α-depleted cells.

DNA damage or the failure of sister chromatid dissolution (described below) may cause cells to arrest in G2 phase, and prolonged arrest in G2 may subsequently trigger the enlargement of nuclei, similar to that seen with Mre11-depleted cells (36). However, the size of the nuclei is extremely enlarged in Top3α-depleted cells after Dox addition for 48 h, DNA-synthesizing cells and the emergence of enlarged nuclei (described below) may cause cells to arrest in G2 phase, and prolonged arrest in G2 may subsequently trigger the enlargement of nuclei, similar to that seen with Mre11-depleted cells. Furthermore, the disruption of BLM in Top3α-depleted cells remarkably reduced the frequency of chromosome-type aberrations and, more importantly, nearly completely suppressed the emergence of “unscorable” metaphase cells which contained chromosomal aberrations too severe to allow us to identify individual chromosomes (Table 2).

TABLE 3. No appearance of unscorable metaphase karyotypes in Rad51- and Mre11-depleted cells

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of cells with aberrant chromatid type:</th>
<th>No. of cells with the following chromosome type:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gap</td>
<td>Break</td>
</tr>
<tr>
<td>Rad51−/−</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rad51+/−</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rad51+/+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mre11−/−</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mre11+/−</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The occurrence of enlarged nuclei in Dox-treated Top3α-depleted cells remarkably was also suppressed by the additional disruption of BLM, but was not affected by the disruption of WRN (Table 3). Based on the results obtained in this study, we present two possible mechanisms of BLM and Top3α function, as follows.

Dissolution of sister chromatids. It has been reported that catenanes are formed after DNA replication and are decat- enated by Topo II (10, 26). In addition, silencing of human Topo II by small interfering RNA leads to a defect in the dissolution of sister chromatids in human cells (21). Thus, it seems likely that Topo II plays a major role in the dissolution of sister chromatids in eukaryotic cells.

A decade ago, an alternative process for the dissolution of sister chromatids was proposed. An in vitro study using Escherichia coli proteins indicated that Topo III efficiently decat- enates precatenanes and that when this decatena- tion does not occur, the daughter molecules (catenanes) remain catenated after DNA replication is completed (9). In addition, it has been proposed that S. cerevisiae Sgs1 creates a deleterious topological substrate that Top3 preferentially resolves and that such substrates should be considered precatenanes (5). There is no direct evidence to prove the involvement of Top3 in the dissol- ution of sister chromatids in the cell. However, our data obtained in this study seem to strongly support the hypothesis that both Top3α and BLM are involved in the dissolution of sister chromatids in vertebrate cells.

If this is the case, the termination intermediates that normally arise when replication forks converge are effectively processed by Top3α and BLM, resulting in the dissolution of sister chromatids. Thus, it is speculated that in the absence of Top3α, the termination intermediates created by BLM are not reso- lved properly to result in the formation of double-strand breaks in both sister chromatids, which are observed as chromosome- type aberrations and “unscorable” metaphase cells (Fig. 7A). In the absence of BLM, however, catenanes are formed after DNA replication and can be decatened by Topo II.

Dissolution of aberrant recombination structures during DNA replication. In Schizosaccharomyces pombe, Top3, in con- junction with Rqh1, the sole RecQ helicase in fission yeast, is required for processing or disrupting aberrant recombination structures that arise during S phase (31). Failure of this function leads to the accumulation of aberrant DNA structures, particularly at the ribosomal DNA locus (32), and finally results in unfaithful chromosome segregation (31, 32). Recently, S. cerevisiae sgs1 mutant cells, which lack the sole RecQ heli- case, were found to accumulate damage-induced pseudo dou- ble HJs. This suggests that reestablishment of the normal re-
lication fork could be mediated by Sgs1 and Top3, which could collapse the pseudo double HJs back into the four-way sister chromatid junctions that resemble hemicatenanes (Fig. 7B) (18). Interestingly, the pseudo double HJs resemble the termi-
nutation intermediates that arise when replication forks converge (18). Thus, the mechanism that Top3α and BLM resolve sister chromatids is compatible with the above model that Sgs1 and Top3 collapse the pseudo double Holliday junctions created by recombination proteins to restore normal replication forks. This model is a modified version of Sgs1/Top3β in budding yeast (18).

Why the lethality of Top3α-depleted cells cannot be suppressed by disruption of BLM. Previous studies demonstrated that the lethality of the top3 mutation in fission yeast was suppressed by deletion of the rgh1 gene (31). However, disruption of the BLM gene did not rescue the lethality of the Top3α depletion in chicken DT40 cells, although several phenotypes observed in the Top3α-depleted cells were suppressed by disruption of BLM. This discrepancy may be due to the fact that vertebrate cells carry multiple RecQ helicases (4) which presumably together conduct the functions of the sole RecQ helicases found in unicellular organisms. We have previously suggested that both RECQL1 and RECQL5 may partially replace the function of BLM when it is absent in DT40 cells (30). In addition, human RECQL1 or mouse RECQL5 is required for efficient suppression of sister chromatid exchange even in the presence of their BLM counterparts (11, 16). Given that human RECQL1 interacts with Top3α (14) and human RECQL5 interacts with both Top3α and Top3β (24), it seems likely that vertebrate RECQL1, RECQL5, and BLM perform some overlapping functions. Thus, it is speculated that the partial replacement of BLM function by RECQL1 and/or RECQL5 is responsible for the incomplete suppression by BLM deletion of the lethality induced by the loss of TOP3α. In spite of the suppression of several phenotypes observed in the Top3α-depleted cells by disruption of BLM, TOP3α−/−/BLM−/− cells are still inviable, while BLM−/− cells are viable. Thus, it seems likely that Top3α has additional roles separate from those of BLM. Indeed, we previously showed that budding yeast Top3 has roles independent of those of Sgs1 (20).

In summary, this is the first detailed report of the phenotype of Top3α-depleted higher eukaryotic cells and has revealed possible roles played by BLM and Top3α in replication. The results obtained in this study help us to understand the function of Top3α and BLM at the molecular level and account for the chromosome instability of Bloom’s syndrome cells that arises from defects in BLM function.

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