

A new deal for Holliday junctions

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BLM, the helicase implicated in Bloom's syndrome, and its yeast counterpart Sgs1 function in complexes with a type I topoisomerase to resolve double Holliday junctions to form non-crossover products. These data provide a direct explanation for the highly elevated sister chromatid exchange frequency in Bloom's syndrome cells.

Fifty years ago Bloom described a congenital disorder¹ that now bears his name. Since that time, Bloom's syndrome has become a classic example of genomic instability syndromes. Affected humans have significant predisposition for the development of many types of cancer, particularly non-Hodgkins lymphoma, leukemias, and carcinomas of the breast, gut and skin^{2,3}. The hallmark of Bloom's syndrome cells is an increase in spontaneous sister chromatid exchanges (SCE)—that is, crossover between sister chromatids (Fig. 1)⁴. Several studies have now made important contributions toward identifying one of the cellular roles of the Bloom's syndrome DNA helicase. In a recent issue of *Cell*, Haber and co-workers⁵ identified Sgs1, the budding yeast homolog of the human BLM DNA helicase, as critical for the resolution of recombination intermediates to non-crossover recombinants. In December of 2003, Hickson and colleagues⁶ reported in *Nature* direct biochemical evidence that human BLM helicase, in conjunction with TOPO III α , can process double Holliday junctions (dHJ) to non-crossover products. These studies provide a coherent molecular explanation for the SCE phenotype of Bloom's syndrome cells. In addition, the results are supported by Roeder and co-workers⁷, who reported that Sgs1 is localized to chromosomal sites of meiotic crossovers and that cells lacking Sgs1 have an increase in meiotic crossover formation. Together these data provide compelling evidence that dHJs can be processed to non-crossover products by the combined and specific function of BLM-TOPO III α and likely also by their homologs Sgs1-Top3.

An increase in sister chromatid exchange may seem innocuous, but loss of crossover

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suppression in recombination between homologs can have drastic genetic consequences. When levels of somatic crossovers are high between homologous chromosomes, allelic differences will be lost (loss of heterozygosity). In the case of repeated sequences, increased crossover frequencies will lead to chromosomal aberrations such as deletions, inversions and translocations. These genetic events represent the hallmark of the genomic instability seen in many cancer cells.

Biochemical functions of BLM helicase

BLM is a member of the RecQ family of 3'→5' DNA helicases that has four additional members in humans but only a single representative in bacteria (RecQ) or yeasts (budding yeast, Sgs1; fission yeast, Rqh1) (for recent reviews see refs. 3,8). Three of the human genes have been implicated in hereditary diseases and cancer predisposition: *BLM* in Bloom's syndrome, *WRN* in Werner's syndrome, and *RECQ4* in Rothmund-Thomson syndrome. The RecQ helicases in all organisms are essential for the maintenance of genomic stability and are thought to function during DNA replication, in particular in the reinitiation of DNA replication after replication fork stalling (Fig. 2a). Replication forks fail when DNA lesions like cyclobutane pyrimidine dimers (UV damage), interstrand crosslinks or other types of DNA damage stall the progress of the replicative DNA polymerase or helicase. Blockage on the lagging strand can be overcome by downstream initiation of another Okazaki fragment, leading to a single-stranded gap containing the blocking damage (Fig. 2). Repair of the resulting gap by homologous recombination can lead to a dHJ that requires resolution into the component duplexes. Resolution by an HJ resolvase, analogous to the bacterial RuvABC resolvase, leads to crossover and non-crossover products, depending on the orientation of cleavage. In mitotically growing cells, a crossover in replication-associated recombination is indicated by SCE, which can be directly visualized under a microscope (Fig. 1).

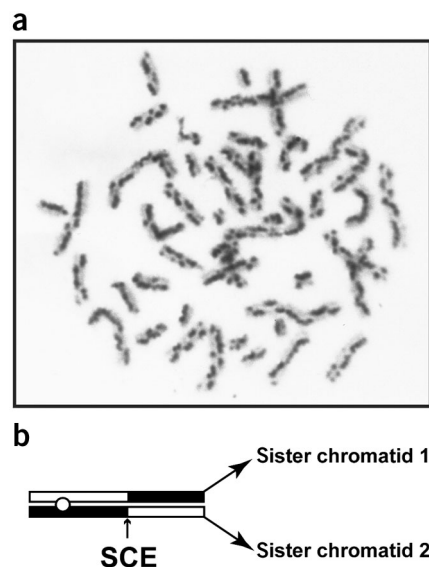


Figure 1 Elevated sister chromatid exchange in Bloom's cells. (a) Visualization (provided by J. German, Cornell University Medical School) and (b) explanatory panel of sister chromatid exchanges (SCE) in stimulated blood lymphocytes of a Bloom's syndrome patient. The completion of two cycles of 5-BrdU labeling results in color differences between the sister chromatids (dark and light). The alternating color pattern of each sister is created by SCEs.

The elegant biochemical work of Wu and Hickson⁶ makes use of oligonucleotide substrates that model dHJs. The key result is that BLM-TOPO III α processes dHJ oligonucleotide substrates to non-crossover products. The helicase activity of BLM likely collapses the two junctions of the dHJ into a hemicatenane, where the two single-strands interlock (Fig. 2a, step 5). BLM helicase also provides the necessary strand separation for TOPO III α to resolve the hemicatenanes, as this type I topoisomerase is only able to pass single strands through each other. The combined BLM-TOPO III α activity is highly specific, as neither *Escherichia coli* uvrD, another 3'→5' helicase such as BLM, nor eukaryotic TOPO I, another type I topoisomerase similar to TOPO III α , could substitute for BLM or TOPO III α , respectively, in this *in vitro*

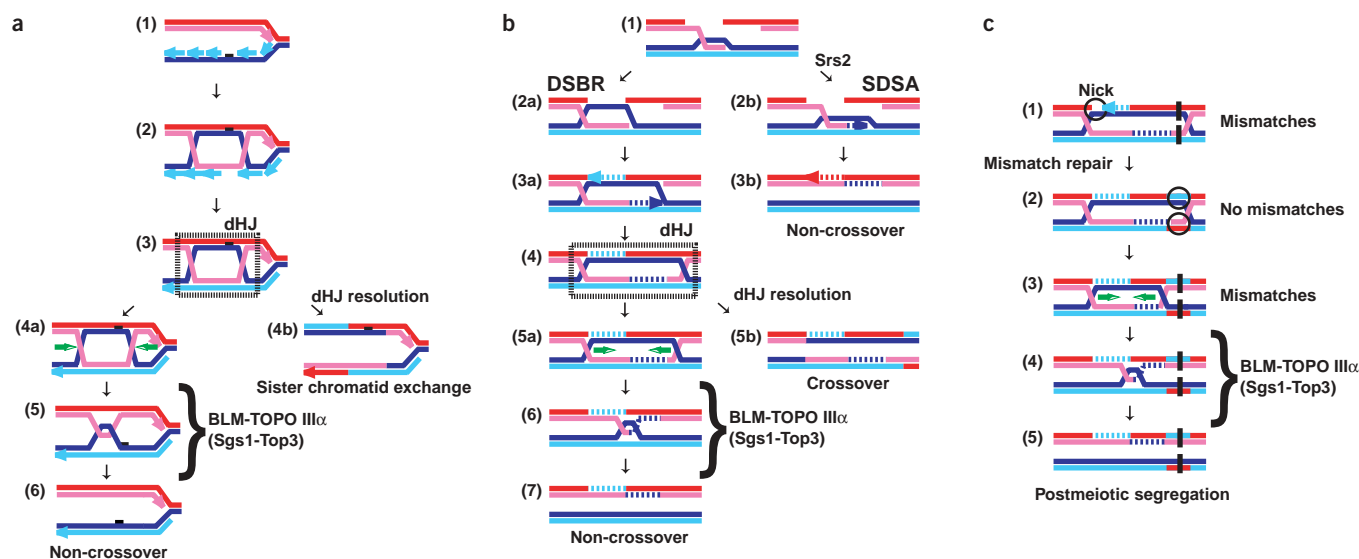


Figure 2 Function of BLM-TOPO III α /Sgs1-Top3 in homologous recombination. (a) Role of BLM-TOPO III α (and probably their *Saccharomyces cerevisiae* homologs Sgs1-Top3) in the recombinational repair of replication-induced gaps. Lagging strand blockage (black box) and resumption of lagging strand replication generates a gap (step 1). Post-replicative repair (step 2) can lead to the formation of a double Holliday junction (dHJ) (step 3). Resolution by an HJ resolvase leads to crossover (step 4b, sister chromatid exchange), whereas BLM-TOPO III α (step 4a) will collapse the dHJ (reverse branch migration indicated by green arrows) to a single hemicatenane (step 5) leading to the exclusive formation of non-crossover products (step 6). (b) Recombinational repair of double-strand breaks and meiotic recombination can proceed by double-strand break repair (DSBR; steps 2a–7) involving the formation of dHJs leading to crossover (step 5b) and non-crossover (step 7) recombinants or by synthesis-dependent strand annealing (SDSA; step 2b) leading to non-crossovers (step 3b). (Some models of SDSA include the possibility of crossover formation, although the effect of *srs2* on crossover suggests that such a contribution is minor in vegetative yeast cells.) BLM-TOPO III α (and probably their *S. cerevisiae* homologs Sgs1-Top3) process dHJs (step 4) by collapsing them (reverse branch migration indicated by green arrows) to a single hemicatenane (step 6) leading to the exclusive formation of non-crossover recombinants (step 7)⁵. (c) Role of mismatch repair in dHJ resolution by BLM-TOPO III α /Sgs1-Top3. Mismatches formed in heteroduplex DNA during recombination are subject to mismatch repair, presumably in a nick-directed fashion (step 1). Any asymmetry during mismatch repair will recreate mismatches during reverse branch migration (indicated by green arrows; step 3), enhancing the probability for postmeiotic segregation (PMS; step 5).

reaction. Moreover, the catalytic activities of BLM and of TOPO III α were essential for product formation. These results argue strongly for the biological relevance of these biochemical observations. These results also shed a different light on the previously identified interactions of BLM with Rad51⁹ and the Rad51-D paralog¹⁰, which are involved in forming the pivotal presynaptic filament that functions in homology search and DNA strand exchange during recombination. This interaction may not only be relevant at the onset of recombination in the assembly of the filament, but also after DNA strand exchange, where stable binding of Rad51 (and perhaps Rad51-D) to the product heteroduplex DNA could provide the appropriate intermediate to direct BLM-TOPO III α to its dHJ substrate.

A dramatic 10-fold increase in SCEs typifies the genome instability in Bloom's syndrome⁴ and various models have been proposed to link this phenotype to the defects in DNA replication observed in Bloom's syndrome cells^{3,8}. The finding by Wu and Hickson⁶ that BLM-TOPO III α specifically processes dHJs to non-crossover products provides a very satisfy-

ing explanation for the SCE increase in Bloom's syndrome cells. In the absence of the BLM-TOPO III α pathway, dHJs are resolved to crossover products, which manifest as SCEs (Fig. 2a). This model suggests that most recombination between sister chromatids in wild-type cells cannot be observed under the microscope, as it does not lead to SCE.

Role of Sgs1 in crossover suppression

Outside of S-phase, dHJs are also prominent intermediates in double-strand break (DSB) repair and meiotic recombination (Fig. 2b). Two studies in budding yeast address crossover control and implicate Sgs1, the budding yeast BLM homolog, in the processing of recombination intermediates (presumably dHJs) to non-crossover products.

In another fruitful exploitation of the mating-type system, Haber and colleagues⁵ provide evidence that Sgs1-Top3, the budding yeast homologs of BLM-TOPO III α , suppresses crossover formation. Mutations in *SGS1* or *TOP3* increased crossovers in vegetatively growing cells by 2- to -3-fold but did not have much effect on the efficiency of DSB repair.

In an independent study, Roeder and colleagues⁷ reported a 40% increase in meiotic crossovers in *sgs1* mutants. The recombination-dependent meiotic defects of *top3* mutants suggest that Sgs1 and Top3 act together in the processing of meiotic dHJs in yeast¹¹. Similar to the activity of BLM on dHJs⁶, crossover suppression in vegetative and meiotic cells requires the Sgs1 helicase activity, which is dispensable for many of Sgs1's functions during vegetative growth^{5,7}. Consistent with the genetic data, cytological analysis of meiotic *sgs1* cells identified an increase in the connections (so called axial associations) between homologous chromosomes, which likely represent sites of future meiotic crossovers⁷. The localization of Sgs1 (ref. 7) and BLM¹² on meiotic chromosomes suggests a role in crossover control. An increase in crossover could reflect a general hyper-recombination phenotype, rather than a dHJ processing defect. However, Roeder and colleagues⁷ demonstrated that this increase in crossovers was not accompanied by an increase in meiotic gene conversions, showing that *sgs1* specifically affected crossover control.

Interestingly, *sgs1* and *top3* were not the only mutations affecting crossover formation in the yeast DSB repair assay. The physical analysis also identified that mutations in *SRS2*, which encodes an unrelated 3'→5' DNA helicase, enhance crossover formation by 2–3-fold⁵. Genetic and kinetic analysis showed that *Sgs1* and *Srs2* act in different pathways of crossover control⁵. While *Sgs1*-*Top3* processes dHJs in the DSB repair pathway (DSBR; Fig. 2b, left), *Srs2* is proposed to function in the synthesis-dependent strand annealing (SDSA) pathway (Fig. 2b, right), which leads to non-crossover products. By eliminating SDSA in *srs2* mutants, the balance shifts to DSB repair pathways, leading to the observed increase in crossovers.

Some implications

The proposed mechanism of how BLM-TOPO III α process dHJs has an interesting implication for mismatch repair (Fig. 2c). Mismatches formed during DNA strand exchange and branch migration are subject to mismatch repair, and the repair direction and efficiency is probably directed by the nick present during repair DNA synthesis. If the ensuing dHJ intermediate undergoes reverse branch migration (the collapsing of the dHJ to a hemicatenane catalyzed by BLM-TOPO III α /*Sgs1*-*Top3*), a second round of mismatches can arise (Fig. 2c). The dHJ intermediate has been demonstrated to contain contiguous strands¹³, suggesting that the absence of nicks may lead to less efficient repair of mismatches resulting from reverse branch migration. This would lead to postmeiotic segregation (PMS). Thus, in mutations that affect resolution to crossovers, dHJs would be shunted to the BLM-TOPO III α /*Sgs1*-*Top3* pathway and one might expect a curious increase in PMS. Such an increase in PMS was documented for *Drosophila mei-9* mutants¹⁴. *MEI-9* is

the *Drosophila* XPF homolog, the catalytic subunit of a DNA structure-specific endonuclease, that is critical for meiotic crossover formation and may act as an HJ resolvase in flies (reviewed in ref. 15).

Sgs1 in yeast and BLM in humans likely have other substrates in addition to dHJs and perform additional functions besides dHJ processing. The phenotypes of *sgs1* and *top3* cells overlap but are not identical, and it is evident that *Sgs1* (and perhaps BLM) functions also without its *Top3* partner^{3,8}. Mutations in *SGS1* display the puzzling phenotype characterized by higher than wild-type frequencies of spontaneous recombination but lower than wild-type frequencies of induced recombination¹⁶. *Sgs1* possibly restricts unwanted recombination or suppresses the formation of recombinogenic lesions (nicks, DSBs) under low or absent genotoxic stress but is required for recombination under high genotoxic stress. This paradoxical situation is reminiscent of *Srs2*, which is required for SDSA⁵, a Rad51-mediated process, but is also known to inhibit Rad51-mediated recombination^{17,18}. There is much evidence for a function of BLM, *Sgs1* and bacterial RecQ early in recombination, leading to the suppression of unwanted recombination^{5,7,19,20}. Interestingly, BLM in the absence of TOPO III α was found to bind and reverse D-loops²¹, which represent the first pairing intermediate for DSB repair and SDSA (Fig. 2b, step 1). Possibly, *Sgs1* performs some kind of quality check on this initial pairing intermediate. Moreover, BLM promotes branch migration of single HJs²², which can occur at stalled replication forks. Thus, the large increase in SCEs in Bloom's cells could be the compound effect of high frequency of recombination initiation and a dHJ processing defect. In addition, *Sgs1* functions in the S-phase checkpoint and in telomere maintenance.

It is not yet clear how these roles relate to its functions in recombination^{3,8}.

While a defined molecular function for human BLM is identified, the precise molecular functions of the other four human RecQ-like helicases remain to be understood. Genetic evidence suggests a role of the WRN helicase in the late phases of recombination²³, similar to the role proposed for BLM⁶. Why does WRN not substitute for BLM? Obviously many questions regarding the functions of this exciting group of proteins remain, and we are looking forward to their resolution.

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