PERSPECTIVE

RecQ helicases queuing with Srs2 to disrupt Rad51 filaments and suppress recombination

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Homologous recombination (HR) is an important mechanism for the maintenance of genome integrity. HR functions to repair double-strand breaks (DSBs) and single-strand gaps formed during replication or created by DNA damaging agents or from processing DNA lesions. In addition, HR is implicated in the restart of damaged replication forks and functions in telomere length maintenance in cells lacking telomerase. Increasing evidence suggests that HR plays an important role in cancer prevention (Thompson and Schild 2002; Sung and Klein 2006). However, recombination can also be harmful and have oncogenic and mutagenic consequences. It is known that inappropriate or untimely recombination can generate damaging genome rearrangements, such as somatic loss of heterozygosity (LOH), chromosome deletions, inversions, or translocations. Therefore, cells have evolved specific mechanisms to control recombination and to coordinate HR with other responses to DNA damage as well as with replication and progression through the cell cycle. Indeed, several cancer-prone genetic diseases, including the ones caused by mutations in human RecQ helicases, are associated with HR dysfunction. In this issue of *Genes & Development*, two studies by Bugreev et al. (2007b) and Hu et al. (2007) demonstrate a novel function for two human RecQ helicases, BLM and RECQL5, in regulating an early step of HR, which is related to their role in protecting genome stability.

An important early step of all HR reactions is to use a Rad51 presynaptic filament on single-stranded DNA (ssDNA) (Fig. 1A) to invade a homologous duplex, giving rise to a three-stranded structure called a D-loop (Fig. 1B). HR can then bifurcate into two main subpathways called DSB repair (DSBR) and synthesis-dependent strand annealing (SDSA) (Fig. 2; Allers and Lichten 2001; Hunter and Kleckner 2001). In DSBR, the second DSB end can be captured to form an intermediate with two Holliday junctions (HJs), usually called a double HJ (dHJ),

that can be resolved at the HJs by endonucleases to crossover and noncrossover products (Paques and Haber 1999; Sung and Klein 2006), or dissolved to noncrossover products by the concerted actions of a helicase and topoisomerase (Fig. 2; see below; Sung and Klein 2006 and references therein). In SDSA, however, the extended D-loop is dissolved, likely by a DNA helicase, and the extended single-stranded end anneals to the ssDNA of the other break end, followed by gap-filling DNA synthesis and ligation, to restore a contiguous chromosome always in noncrossover configuration (Fig. 2; Paques and Haber 1999; Sung and Klein 2006). Crossover is essential for proper chromosome disjunction during meiosis, but it is likely suppressed in mitotic cells as it can lead to LOH and chromosome rearrangements. Conceivably, cells are endowed with systems that regulate HR so as to generate exclusively or predominantly noncrossover products. Results of several studies have suggested that an important enzymatic function in both promoting SDSA and regulating dHJ resolution to noncrossover products is achieved through the BLM helicase (Fig. 2; see below; Adams et al. 2003; Wu and Hickson 2003; McVey et al. 2004; Johnson-Schlitz and Engels 2006).

BLM is a member of the highly conserved RecQ family of helicases with crucial roles in the maintenance of genome stability. Consequently, no eukaryotic organism has been found that lacks a *RECQ* gene. While *Saccharomyces cerevisiae* has one RecQ gene (*Sgs1*), humans have five (WRN, BLM, RECQL4, RECQL1, and RECQL5). Deficiencies in three of them—WRN, BLM, and RECQL4—cause WS (Werner syndrome), BS (Bloom syndrome), and RTS (Rothmund-Thomson syndrome), respectively. These genetic disorders are characterized by cancer predisposition, premature aging, and/or developmental abnormalities (Sharma et al. 2006; Hanada and Hickson 2007).

BLM's role in controlling HR and suppressing SCE formation

One of the most characteristic symptoms of BS patients is a dramatic cancer predisposition. BS cells have an abnormally high rate of HR, and about a 10-fold increased

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Figure 1. Schematic representation of 3'-overhang formation and strand invasion. (A) DSBs or DNA gaps are processed nucleolytically or by the action of DNA helicases to expose 3'-overhangs of ssDNA. (B) The ssDNA of 3'-overhangs or generated at stalled forks is immediately coated by RPA, but RPA can be removed and replaced by Rad51 with the help of mediator proteins such as Rad52, Rad55, and Rad57 to form what is called the Rad51 presynaptic filament. This process is counteracted by helicases such as Srs2, BLM, and RECQL5 that disrupt the Rad51 filaments. Once formed, the Rad51 filament can engage in a homology search and invade the homologous region in the duplex to form a DNA joint called a D-loop. The D-loop can be then extended through DNA synthesis.

rate of sister chromatid exchanges (SCEs), a feature that is used as a molecular diagnosis of the disorder (Ray and German 1984; Hanada and Hickson 2007). When HR uses the homologous chromosome instead of the sister chromatid as a repair template, LOH may occur. Indeed, mutations in both human *BLM* and mouse *Blm* also result in elevated rates of LOH (German 1993; Luo et al. 2000; Goss et al. 2002). Furthermore, SCEs using non-identical repeat sequences or occurring unequally between identical sequences can lead to chromosome duplications and deletions, an important source of genomic instability. Although the mechanism of SCE formation is not fully understood, previous studies suggest that a large part of SCEs require HR for their formation (Sonoda et al. 1999; Wang et al. 2000). Hence, it follows that one important function of BLM is to suppress HR events leading to SCEs and LOH. Indeed, the relevance of BLM suppressing SCEs in cancer avoidance is well established (German 1993; Luo et al. 2000; Goss et al. 2002). Since SCEs represent crossover events, conceivably BLM may act to suppress their formation through D-loop unwinding of unwanted strand invasion events (Bachrati et al. 2006), by promoting SDSA (Adams et al. 2003), or in DSBR by favoring the formation of noncrossover products (Fig. 2; Wu and Hickson 2003). Studies in *Drosophila* have suggested that BLM is required for SDSA (Adams et al. 2003), perhaps by acting downstream from strand invasion to unwind the D-loop intermediate and free the newly synthesized strand (McVey et al. 2004).

This model explains that in the absence of DmBlm, alternative pathways of D-loop disassembly result in short repair synthesis tracts or flanking deletions.

In this issue, Bugreev et al. (2007b) show biochemical evidence using in vitro reconstitution of HR reactions that BLM might indeed promote late steps of SDSA through D-loop disruption and by stimulating DNA repair synthesis of DNA polymerase η (Fig. 2). However, there is also recent evidence that argues against the role of BLM in SDSA based on observations that BLM deficiency leads to structural alterations in both the template and the donor sequences, which would not be predicted by the SDSA model (Johnson-Schlitz and Engels 2006). Rather, analysis of the template disruptions that occur in the absence of BLM points to a failure to dissolve dHJs (Johnson-Schlitz and Engels 2006), consistent with a model previously proposed to explain the role of BLM in promoting formation of noncrossover recombinants (Wu and Hickson 2003). In this dHJ dissolution model (Fig. 2), it is proposed that BLM drives the convergence of the dHJs via branch migration into a hemicatenane structure, which is resolved using the ssDNA passage activity of Top3 α (Wu and Hickson 2003). Also, consistent with this biochemical activity of Blm, genetic studies in yeast have brought support to this model (Ira et al. 2003; Robert et al. 2006) and shown that mutations in Sgs1 and Top3 lead to accumulation of X-shaped DNA structures at damaged replication forks that might represent hemicatenane-like molecules (Liberi et al. 2005).

Figure 2. DSBR by SDSA and DSBR pathways. Resected DSBs expose 3' ssDNA overhangs that initiate strand invasion followed by DNA synthesis. Strand displacement of this intermediate by a DNA helicase (BLM, Sgs1, and possibly Srs2) channels the reaction toward SDSA. However, if the second DSB end is captured, an intermediate with two HJs, called a dHJ, forms. A dHJ can be dissolved by means of a helicase (BLM) and topoisomerase (Top3 α) to give a noncrossover product, or resolved at HJs by endonucleases to give both crossover and noncrossover recombinants.

RecQ helicases and Srs2 in suppressing recombination

Mutations in human *RECQL1* and *RECQL5* are not yet linked to a disease, but studies using DT40 cells or knockout mice suggested that deficiencies in these genes might also predispose individuals to cancer or chromosome instability disorders (Wang et al. 2003; Sharma et al. 2006; Hanada and Hickson 2007). In this issue, Hu et al. (2007) provide evidence that deletion of *Recql5* in mice results in increased susceptibility to cancer and gross chromosomal rearrangement (GCR) accumulation, likely due to a failure to regulate HR, and show through biochemical experiments that RecQL5 functions to disrupt Rad51 filaments thereby attenuating HR initiation. RecQL5 is not the only RecQ helicase capable of such a feat. In fact, Bugreev et al. (2007b) demonstrate that in addition to its previously discovered anti-recombination activities (see Sharma et al. 2006; Hanada and Hickson 2007), BLM can also function to disrupt Rad51 filaments, thereby suppressing HR at an early stage.

A potent and well-studied mechanism to control recombination in yeast involves the Srs2 protein (Sung and Klein 2006). Srs2 is a helicase with similarities to the bacterial UvrD/Rep helicases (Rong and Klein 1993). Mutations in the *SRS2* gene lead to increased recombination and suppression of the damage sensitivity of post-replication repair mutants in a manner dependent on HR genes (Aboussekhra et al. 1989; Schiestl et al.

1990). These genetic observations suggested that Srs2 functions to restrict recombination, and, indeed, later biochemical evidence provided the mechanistic basis of the Srs2 function, by revealing its ability to bind Rad51 and disrupt Rad51 presynaptic filaments (Krejci et al. 2003; Veaute et al. 2003). So far, an Srs2 ortholog has not yet been found in other eukaryotes, although the newly identified Fbh1 helicase shows some structure similarities to Srs2 in the helicase domain (Morishita et al. 2005; Osman et al. 2005; Chiolo et al. 2007). Genetic studies conducted in *Schizosaccharomyces pombe* and chicken DT40 cells suggest that Fbh1 plays a role in processing HR intermediates and in suppressing SCE formation (Morishita et al. 2005; Osman et al. 2005; Kohzaki et al. 2007). However, it remained unclear whether disruption of the Rad51 presynaptic filament represents a significant mechanism of HR attenuation and control in higher eukaryotes.

The findings reported in this issue by Hu et al. (2007) and Bugreev et al. (2007b) are particularly important as they show that two human RecQ helicases, BLM and RecQL5, can also act as translocases to dislodge Rad51 from presynaptic filaments, thus functioning similarly to Srs2 to suppress the initiation of HR. In fact, previous genetic data suggested a possible functional overlap between Srs2 and the yeast RecQ helicase Sgs1 in counteracting recombination. For instance, in *sgs1 srs2* double mutants, Rad51-mediated recombination causes cell

death (Gangloff et al. 2000), and Sgs1 overexpression can suppress recombination and repair defects of *srs2* (Mankouri et al. 2002; Ira et al. 2003). These data suggest that in *srs2* cells, potentially toxic recombination intermediates may form, which are substrates for Sgs1. Srs2 becomes essential also in other mutant contexts such as the one of *rad54*, encoding a double-stranded DNA (dsDNA) translocase that both promotes D-loop formation and enhances DNA branch migration (Sung and Klein 2006). In addition, as in the case of *sgs1 srs2*, the synthetic lethality of *srs2 rad54* is suppressed by *rad51* mutation (Rong and Klein 1993; Sung and Roberson 1995). These observations led to the current view that holds that Srs2 disrupts Rad51 nucleofilaments essentially when subsequent recombination steps are impaired. Sgs1 and Srs2 do not seem to be redundant, and while they could sometimes substitute for each other, it is conceivable that they may act preferentially at different steps in HR or, for instance, at different stages in the cell cycle or with respect to the timing of DNA replication. While BLM likely represents the Sgs1 ortholog, Bugreev et al. (2007b) hypothesized that it may also bear similarities to Srs2, while Hu et al. (2007) started with the presumption that the other RecQ helicases may represent potential candidates as the functional equivalent for Srs2 in humans. It turned out that both hypotheses were right, and both BLM (Bugreev et al. 2007b) and RecQL5 (Hu et al. 2007) resemble Srs2 in respect to their ability to disrupt Rad51 filaments, although with some differences that are discussed below.

Similarities and dissimilarities between BLM, RecQL5, and Srs2

Although the human RecQ helicases share many biochemical activities (Sharma et al. 2006), it appears that the ability to disrupt Rad51 filaments is restricted to BLM (Bugreev et al. 2007b) and RecQL5 (Hu et al. 2007), as WRN (Hu et al. 2007) and RecQL1 (Bugreev et al. 2007b; Hu et al. 2007) were not able to inhibit the D-loop reaction even at concentrations much higher than those used for RecQL5. Although RecQL4 ability to act as a translocase is not yet ruled out, it is important to note that this is the only RecQ inactive as a helicase (Sharma et al. 2006).

Two additional questions come to mind: How similar to the Srs2 activity are the ones now reported for BLM and RecQL5? And are the translocase activities of these two RecQ helicases also implicated in other cellular pathways involving protein–DNA complexes? Like Srs2 (Krejci et al. 2003; Veaute et al. 2003), both BLM's and RecQL5's ATPase activities are strongly stimulated by ssDNA (Bugreev et al. 2007b; Hu et al. 2007). However, while Srs2 can also remove RecA, the *Escherichia coli* relative of Rad51 (Krejci et al. 2003; Veaute et al. 2003) and hRad51 (Krejci et al. 2003) from DNA, BLM can only remove hRad51, and it is unable to inhibit strand exchange activity of a meiotic hRad51 homolog, hDmc1, or of yeast Rad51 protein (Bugreev et al. 2007b). These results indicate that while Srs2 can recognize a general,

common feature of the presynaptic filaments formed by the RecA/Rad51 class of recombinases, the effect of BLM in this reaction is quite specific. Since BLM interacts physically with hRad51 (Wu et al. 2001), the displacement might also involve protein interactions. BLM forms a stable complex with Top3 α (Wu et al. 2000), which is required for dHJ dissolution (Wu and Hickson 2003), and thus it would be of interest to address whether Top3 affects BLM activity with regard to Rad51 filaments' disruption. Intriguingly, Bugreev et al. (2007b) show that BLM is only able to disrupt inactive hRad51 filaments present in an ADP-bound form. It was previously shown that ATP hydrolysis leads to spontaneous hRad51 filament inactivation (Bugreev and Mazin 2004) and that some auxiliary proteins may help to maintain the filament in an active form (Shim et al. 2004), thus corroborating the idea that hRad51 displacement by BLM occurs if the cells are not fully prepared for the later steps of HR. In this situation, the hRad51 filaments may remain inactive and susceptible to BLM dissociation. In contrast to the limited ability of BLM to disrupt only inactive, ADP-bound Rad51 filaments, Hu et al. (2007) show that RecQL5 is capable of displacing both Rad51 and Rad51 K133R protein from ssDNA. Rad51 K133R binds ATP and is greatly attenuated for ATP hydrolysis, thus forming a very stable presynaptic filament (Chi et al. 2006). The D-loop disruption activity of RecQL5 is greatly stimulated by the single-stranded binding protein RPA, in a manner reminiscent of Srs2 (Krejci et al. 2003), and Hu et al. (2007) suggest that this enhancement is perhaps due to the ability of RPA to sequester the ssDNA after RecQL5-mediated Rad51 removal, to prevent Rad51 renucleation on DNA (Fig. 1B).

Both Srs2 and RecQ helicases in different organisms have been suggested to play an important role in processing intermediates that arise at damaged or stalled replication forks (Sharma et al. 2006; Sung and Klein 2006). Previous studies have suggested that regulation of Srs2 is achieved via its recruitment to stalled replication forks through its interaction with sumoylated PCNA (proliferating cell nuclear antigen) (Papouli et al. 2005; Pfander et al. 2005), an important replication and repair factor. Based on genetic evidence, it has been hypothesized that this recruitment favors translesion DNA synthesis or error-free post-replication repair mechanisms that are thought to involve template-switching pathways, and to disfavor HR (Stelter and Ulrich 2003; Papouli et al. 2005; Pfander et al. 2005). BS cells have a prolonged S phase and sensitivity to DNA damaging agents and replication inhibitors, and BLM interacts with several proteins involved in DNA replication, including DNA polymerase δ , RPA, and PCNA (for review, see Sharma et al. 2006). In agreement with a role for BLM in DNA replication, the absence of BLM in *Xenopus* leads to chromosomal breaks during S phase (Liao et al. 2000; Li et al. 2004), and an accumulation of Rad51-dependent cruciform structures at damaged replication forks in budding yeast *sgs1* mutants (Liberi et al. 2005). On the other hand, *RECQL5* knockout DT40 cells do not show slow-growth phenotypes and sensitivity to DNA damaging agents (Wang et

al. 2003), and *Recql5* knockout mouse ES cells show only a mild growth defect and a slightly higher level of sensitivity to γ -rays (Hu et al. 2005). However, recent studies show that RecQL5 also interacts with PCNA and colocalizes with the replication machinery in S-phase nuclei (Kanagaraj et al. 2006). The interactions of BLM and RecQL5 with PCNA thus resemble the one reported for Srs2 (Papouli et al. 2005; Pfander et al. 2005), but at present it is not known whether sumoylation affects this interaction. Nevertheless, both BLM and Sgs1 are sumoylated (Eladad et al. 2005; Branzei et al. 2006). Sumoylation was shown to affect the ability of BLM to localize to PML bodies and suppress SCE accumulation (Eladad et al. 2005), and the ability of Sgs1 to resolve the cruciform structures that arise in a Rad51-dependent manner during replication of damaged templates (Branzei et al. 2006). Interestingly, Hu et al. (2007) report that in response to camptothecin (CPT)-induced replication stress, *Recql5* knockout cells, but not wild-type or *Blm* knockout ES cells, exhibit a dramatic increase in the frequency of GCRs, and they provide evidence that the GCR phenotype of *Recql5* is likely due to failure to regulate HR. Taken together, these findings support the idea that RecQL5 might function preferentially in response to certain types of replication problems (as in CPT-induced intra-S lesions) to prevent HR via Rad51 presynaptic filament disruption (Hu et al. 2007), while BLM might function at least at two different stages in the HR process (Bugreev et al. 2007b) and maybe at replication forks that need to restart, as it has also been suggested for its yeast ortholog, Sgs1 (Liberi et al. 2005).

RecQL5 role in SCE

The ability of RecQL5 to disrupt presynaptic filaments is likely relevant also for the process leading to SCE formation (Hu et al. 2007). Indeed, previous genetic studies conducted in chicken DT40 cells and mouse ES cells indicated that RecQL5 might function in control of SCEs in a manner that, at least in chicken cells, may be partly redundant to that of BLM (Wang et al. 2003; Hu et al. 2005). *Recql5*^{-/-} mouse ES cells display a significantly higher number of SCEs (Hu et al. 2005), while *RECQL5*^{-/-} DT40 cells are comparable to wild-type cells for all phenotypes, including SCE levels (Wang et al. 2003). However both chicken and mouse double knockout *blm recql5* showed a significant higher number in SCEs (Wang et al. 2003; Hu et al. 2005), pointing to a role of RecQL5 in suppressing crossovers. *Recql5* knockout mice are very similar to their wild-type siblings (Hu et al. 2005), except for the striking cancer susceptibility phenotype that Hu et al. (2007) report in this issue. Interestingly, while mutations in both human *BLM* and mouse *Blm* result in elevated levels of LOH (see also above), Hu et al. (2007) find that *Recql5* knockout mouse ES cells have similar frequencies of LOH to wild-type ES cells as measured at two different loci. This finding indicates that *Blm* and *Recql5* contribute through different mechanisms to suppressing crossovers, in agreement with a previous model (Hu et al. 2005). However, the findings in

chicken DT40 cells (Wang et al. 2003) indicate there could be differences between organisms with respect to this.

The biochemical functions of RecQL5 also resemble those of BLM in several aspects. The *RECQL5* gene encodes three isoforms, but only RecQL5 β localizes to the nucleus (Shimamoto et al. 2000). Like BLM, RecQL5 β has 3'-5' DNA helicase activity, ssDNA annealing activity, can catalyze branch migration of HJs (Garcia et al. 2004; Kanagaraj et al. 2006), and interacts with Top3 α (Shimamoto et al. 2000). BLM together with Top3 α was shown to catalyze dHJ dissolution on model DNA substrates to lead exclusively to noncrossover products (Wu and Hickson 2003). In this reaction, BLM cannot be substituted by *E. coli* UvrD helicase, and Top3 α cannot be substituted by human topoisomerase I (Wu and Hickson 2003). Since genetic and biochemical evidence suggested that RecQL5 might serve as a backup system for BLM (Wang et al. 2003; Garcia et al. 2004; Kanagaraj et al. 2006), one possibility was that also RecQL5 could act in combination with Top3 in dissolving dHJs. However, as Hu et al. (2007) discuss in their study, they could find no evidence for such an activity of RecQL5 even when BLAP75, a component of the BLM/Top3 α complex that enhances BLM activity in dHJ dissolution (Wu and Hickson 2003; Raynard et al. 2006; Wu et al. 2006), was included in the reaction. The ability of RecQL5 to influence BLM/Top3 α activity in the dHJ dissolution process remains to be analyzed, but nevertheless these data collectively suggest that RecQL5 acts to regulate SCE formation through a mechanism primarily distinct from dHJ dissolution.

Dissociation of D-loops by Sgs1/BLM: relevance for promoting SDSA and preventing SCE

Dissociation of the extended D-loop formed in an HR reaction is expected to shunt this intermediate to the SDSA pathway and prevent crossover recombinants. A mechanism through which BLM/Sgs1 acts to prevent crossover is likely through its ability to dissolve dHJs (Fig. 2; Wu and Hickson 2003). Genetic data in yeast and *Drosophila* are supportive of this model (Adams et al. 2003; Ira et al. 2003), but could also be interpreted with the SDSA model, in which BLM/Sgs1 would prevent crossovers by promoting dissolution of the D-loop intermediate (Fig. 2). Genetic data suggest that also Srs2 is required for efficient DSB repair through the SDSA pathway (Aylon et al. 2003; Ira et al. 2003; Robert et al. 2006). However, while biochemical analysis of Srs2 could not yet recapitulate such activity (Veaute et al. 2003), possibly because other cofactors are required, human BLM was shown to dissociate D-loops by itself (van Brabant et al. 2000). Bugreev et al. (2007b) confirm this observation, although they find that again, this activity depends on the conformation of the hRad51 filament; that is, that BLM can efficiently dissociate D-loops following hRad51 inactivation, but is unable to do so when hRad51 is maintained in an active ATP-bound form in the presence of Ca²⁺. This activity parallels that of hRad54 (Bugreev et

al. 2007a). Thus, both BLM and hRAD54 could act to promote HR through the SDSA mechanism leading to noncrossover products. This role for BLM in SDSA was also previously proposed based on genetic studies in *Drosophila* that suggested a model of multiple invasion/dissolution cycles during SDSA, which in the absence of BLM would result in short repair synthesis tracts or flanking deletions (McVey et al. 2004). The findings of Bugreev et al. (2007b) in this issue support this model, and in addition they show that BLM can stimulate polymerase η -mediated DNA synthesis on a model replication fork, possibly to promote SDSA or in general HR at late stages.

Protein interactions and RecQ-like helicases in controlling recombination

Although certain biochemical activities and functions of RecQ helicases are partially redundant, the distinct clinical characteristics and cellular phenotypes of RecQ helicase disorders or knockout cells indicate unique roles of these proteins. Increasing evidence suggests that many, if not all, of the RecQ proteins can function both alone or in combination with other proteins, and we suggest that their distinct functions may be achieved through specialized protein interactions or post-translational modifications. This trend is also reflected by biochemical studies. For instance, BLM can dissociate D-loops by itself (van Brabant et al. 2000; Bugreev et al. 2007b), while dissolution of dHJs requires both BLM and Top3 α and is stimulated by BLAP75/Rmi1 (Wu and Hickson 2003; Raynard et al. 2006; Wu et al. 2006). In support of this view, Bugreev et al. (2007b) show that BLM activities in HR depend on hRad51 protein conformation, which could also be modulated via protein interactions. In addition, BLM opposing roles in stimulating or counteracting HR could also be controlled by the availability of other interacting proteins. Likewise, RecQL5 function in suppressing initial steps of HR during replication (Hu et al. 2007) may be regulated by PCNA and RPA.

In addition to the potentially overlapping roles of RecQ helicases, there are additional helicases that, like Srs2, function in the same or parallel pathways and sometimes also show physical interactions or colocalization with one or a subset of RecQ helicases. In respect to the roles of these "RecQ-like" DNA helicases in controlling or promoting HR and DSBR, we envisage that at least two proteins might deserve special attention for future studies, FBH1 and BACH1. FBH1 shows structural similarity to Srs2, the mutation in *S. pombe* leads to phenotypes and genetic interactions that resemble those of *srs2* (Morishita et al. 2005; Osman et al. 2005), and budding yeast *srs2* phenotypes can be partly complemented by hFBH1 (Chiolo et al. 2007). In addition, FBH1 mutation in DT40 cells leads to a slight increase in SCE, and in a BLM knockout context, FBH1 disruption in DT40 cells results in a further increase in SCE (Kohzaki et al. 2007), resembling in this aspect RECQL5 (Wang et al. 2003). BACH1, also known as FANCI and BRIP1, is a

DNA helicase that displays sequence identity with BLM in the DNA helicase domain, and is implicated in maintenance of genome stability, DSBR, and Fanconi anemia (FA) (Kumaraswamy and Shiekhattar 2007; Niedernhofer 2007 and references therein).

Again, the physical and functional links between BLM, HR factors, and FA proteins (Niedernhofer 2007) suggest that these cooperations could be achieved via protein interactions. Also in line with this view, recent findings show that BACH1 is required for timely progress through S phase and its activation is achieved through a dephosphorylation event that occurs as cells enter S phase (Kumaraswamy and Shiekhattar 2007). Based on the available information, we think it likely that the dazzling complexity of the pro- and anti-recombination activities of the RecQ helicases is related to the presence in the cell of other RecQ-like DNA helicases and their engagement in different dynamic protein complexes that could also be influenced or regulated by the cell cycle phase and additional post-translational modifications. The understanding of how these factors affect HR regulation and RecQ biology will be a key challenge for the future.

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