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Functions of RecQ Family Helicases: Possible Involvement of Bloom's and Werner's Syndrome Gene Products in Guarding Genome Integrity during DNA Replication

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***Escherichia coli* RecQ helicase is a component of the RecF pathway of recombination whose components are required to reassemble a replisome complex at the site of the replication fork after the removal of a lesion. There are at least five RecQ homologues in human cells, including BLM and WRN. The genes encoding BLM and WRN are mutated in the cancer-prone disorder Bloom's syndrome (BS) and the plogeroid disorder Werner's syndrome (WS), respectively. These syndromes are characterized by a high degree of genomic instability, including chromosomal breaks, multiple large deletions, and translocations, and cells derived from BS and WS patients show defects in DNA replication. Recently, it has become clear that a Holliday junction-like structure is formed at stalled replication forks to result in the formation of double-stranded breaks, and recombination plays an important role in the repair of stalled or broken replication forks, leading to the reinitiation of replication. Defects in the processing of stalled replication forks could lead to aberrant recombination events resulting in genetic instability. Recent studies on BLM, WRN, and the RecQ homologue of *Saccharomyces crevisiae*, Sgs1, indicate that these RecQ homologues interact with proteins involved in DNA replication, and function in a pathway from the DNA replication check point to homologous recombination.**

Key words: Bloom's syndrome, DNA topoisomerase III, RecQ helicase, Sgs1, Werner' syndrome.

Bloom's syndrome (BS) is a rare genetic disorder characterized by retarded growth, sunlight sensitivity, immunodeficiency, male infertility, and a predisposition to a wide variety of malignant tumors (*1*). The most characteristic feature of BS cells is genomic instability which is manifested as an elevated frequency of chromosome breaks, interchanges between homologous chromosomes, and sister chromatid exchanges (SCEs). The gene responsible for BS, *BLM*, encodes a protein belonging to the RecQ helicase family (*2*). The gene responsible for Werner's syndrome (WS) also encodes a protein belonging to the RecQ helicase family (*3*). WS is characterized by accelerated aging and an early onset of age-related diseases such as arteriosclerosis, melituria, and cataract (*4*). WS is also associated with a predisposition to malignant tumors although of a limited range compared with BS. The cells derived from WS patients show genomic instability, and a shorter life span in *in vitro* culture (*5*).

Early studies using BS cells showed a slow replication fork progression and the accumulation of abnormal replication intermediates (*6, 7*). The S phase in WS cells was prolonged with a reduced frequency of replicon initiation (*8, 9*). Thus the functions of these RecQ family helicases seem to be somehow related to DNA replication.

Five genes encoding a RecQ homologue have been identified in human cells. In contrast, a lower eukaryote, *Saccharomyces cerevisiae*, has a sole gene that encodes a RecQ homologue, *SGS1*. Deletion mutants of *SGS1* showed a reduction in the fidelity of chromosome segregation during mitosis and meiosis (*10, 11*), a mitotic hyper-recombination phenotype in interchromosomal homologous recombination, intrachromosomal excision recombination, ectopic recombination (*11*), unequal sister-chromatid recombination (*12*), and illegitimate recombination (*13*). They also showed poor sporulation (14) and premature aging phenotypes (*15*) and were sensitive to methyl methanesulfonate (MMS), hydroxyurea (HU), and UV light (*12–14, 16*). We found that *sgs1* genes containing equivalent missense mutations to those found in BS patients, were not able to suppress the higher sensitivity to MMS and HU and the increased frequencies of interchromosomal recombination and SCE of *sgs1* disruptants (*12*). *BLM* and *WRN* partially suppressed the increased homologous and illegitimate recombination in the *sgs1* mutants (*13*), suggesting that structures and functions are partially conserved among these proteins. In this review, I will focus on three RecQ family helicases, BLM, WRN, and Sgs1 with reference to their roles in DNA replication and recombination.

RecQ family helicases

The product of the *Escherichia coli recQ* gene is a compo-

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nent of the RecF pathway of recombination. RecQ is required both for the initiation of the RecF pathway recombination and for the suppression of illegitimate recombination. This gene was identified during a search for mutants resistant to thymine starvation in 1984 (*17*). Ten years later, the first RecQ homologue in higher eukaryotic cells was identified by us (*18*), and Puranam and Blackshear (*19*). Before cloning the cDNA encoding the RecQ homologue, we purified this protein and designated it as DNA-dependent ATPase Q1 and later as DNA helicase Q1 because of its DNA helicase activity (*20, 21*). Puranam and Blackshear named the gene encoding this protein as *RECQL*. Because of these circumstances and the existence of multiple RecQ homologues in higher eukaryotic cells, we propose to designate this gene *RECQL1*. The second *RECQ* homologue, *BLM*, and third *RECQ* homologue, WRN, were identified by positional cloning as the gene responsible for BS in 1995 (*2*), and for WS in 1996 (*3*), respectively. The fourth and fifth *RECQ* homologue, originally called *RecQ4* and *RecQ5* and recently called *RECQL4* and *RECQL5*, were cloned in 1998 after a search for sequences similar to the RecQ helicase motifs in the EST (expressed sequence tag) data base (*22*). Mutations in the *RECQL4* gene have been found in patients with Rothmund-Thomson syndrome, a rare genetic disorder characterized by premature aging and a predisposition to cancers like WS (*23*). At present, no genetic disorder has been found, which is caused by mutations in the *RECQL1* or *RECQL5* gene.

To date, a number of RecQ homologues have been reported from prokaryotes such as *Bacillus subtilis* (L47648 in GenBank) and *Haemophilus influenzae* (HI32756 in GenBank), to eukaryotes such as *S. pombe* (*24*) as well as *S. cerevisiae* and higher eukaryotes including *Homo sapiens*. Prokaryotes and unicellular eukaryotes possess only a single RecQ helicase. These helicases are characterized by their central domain which contains seven conserved helicase motifs, including a DExH-box in motif II. Thus, the RecQ family is a subfamily of the DExH helicase family. Although significant homology is present within the consensus helicase domains, these RecQ homologues are classified into two groups according to size (Fig. 1). One group includes prokaryotic RecQ homologues as well as *E. coli* RecQ, RecQL1, and RecQL5 which consist of about 400–650 amino acids, and the other group includes BLM, WRN, RecQL4, Sgs1, and Rqh1 of S. pombe, consisting of about 1,200–1,450 amino acids. Recently, it has been reported that a splicing variant of RecQL5, RecQL5β, consists of 991 amino acids (*25*). The latter RecQ homologues have one or two highly acidic regions in the N- or C-terminal domain, which might be involved in protein interactions and except RecQL4 share sequence homology in the C-terminal domain outside the helicase domain. Motifs homologous to the nuclease domains in certain proofreading DNA polymerases exist in the N-terminal domain of WRN.

A number of studies have shown functional connections between DNA replication and the RecF pathway in which RecQ is involved. It has been reported that RecF pathway proteins are required for the resumption of replication at DNA replication forks (*26*). It is considered that DNA double-strand breaks at the replication fork could be repaired by a mechanism involving homologous recombination using intact sister chromatids. In this context, it is interesting that RecQ can initiate homologous DNA pairing in the presence of RecA and SSB.

A single allele of *rqh1* (*hus2-22/rqh1-h2*) was identified in the screening for HU-sensitive mutants. *rqh1*$^-$ cells arrest DNA replication and cell division normally in response to HU but then display significant defects in chromosome segregation in the subsequent mitosis, indicating the requirement of *rqh1*$^+$ for recovery from S phase arrest (*24*).

Saccharomyces cerevisiae Sgs1

SGS1 (*slow growth suppressor 1*) was identified as a suppressor of the slow growth phenotype of DNA topoisomerase III mutants when it was mutated (*27*). Sgs1 was shown to actually have DNA helicase activity that translocates in the 3′-5′ direction (*28*). Sgs1 also unwinds G-quartet DNA (G4 DNA) (*29*) and Holliday junctions (*30*), indicating its role in suppressing DNA recombination.

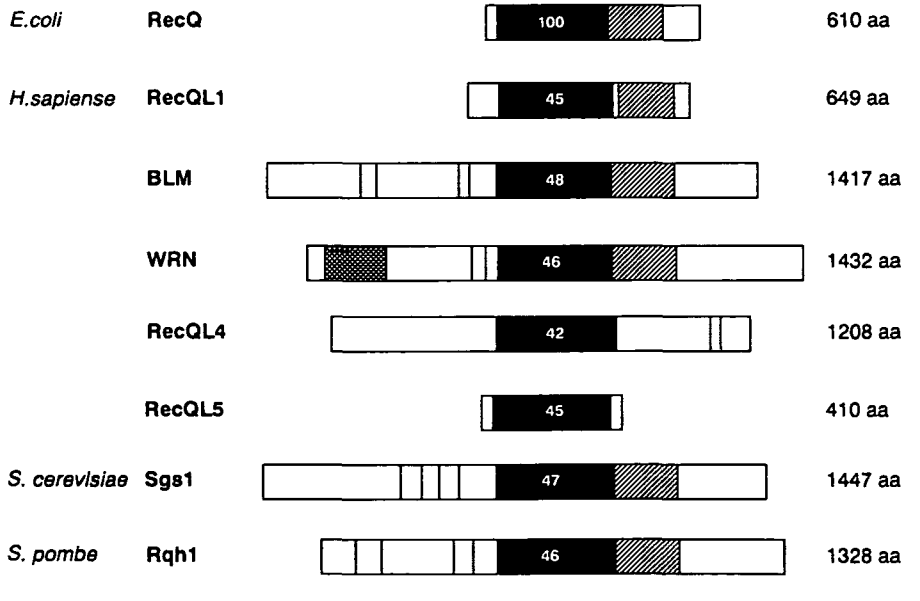

Fig. 1. **Schematic representation of RecQ family helicases.** The sequence identity in the helicase domain is expressed by comparing each domain with that of *E. coli* RecQ. ▨, exonuclease domain; □, acidic region; ■, helicase domain; ▨, conserved region outside the helicase domain.

In *sgs1* mutants, extrachromosomal ribosomal DNA circles (ERCs) accumulate more rapidly than in wild-type cells, and this chromosomal instability is associated with a reduction in life span (*15*). It has been suggested that the accumulation of ERCs results in a loss of viability by titrating out essential proteins necessary for DNA replication or transcription. The rDNA locus of *S. cerevisiae* contains hundreds of copies of a repeating unit in which there is an autonomously replicating sequence (ARS). DNA replication is initiated form a ARS in the repeating unit of rDNA, and replication forks move bidirectionally but the fork moving toward the 3′ end is halted by a replication fork barrier (*31*). It seems likely that replication fork block in the rDNA locus results in the formation of a recombinogenic DNA structure because the site of the replication fork barrier is a hot-spot for mitotic recombination. Thus, it is conceivable that Sgs1 functions to destroy the recombinogenic DNA structure.

It is indicated that Sgs1 interacts physically with DNA topoisomerase II and DNA topoisomerase III (*10, 27*) and genetically with DNA topoisomerase I and III (*27, 28*). In addition, it has been shown that the N-terminal region of Sgs1 is responsible for the direct interaction with topoisomerase III (*32*). Mullen *et al.* (*16*) reported the importance of the N-terminal region of Sgs1 for the suppression of several phenotypes of *sgs1* mutants, such as high sensitivity to MMS and hyper recombination. We recently observed that introduction of missense mutations within 13 amino acids from the N-terminus of Sgs1 resulted in a defect in the interaction with topoisomerase III as assayed with the yeast two-hybrid system and in the loss of ability to suppress MMS sensitivity and hyper recombination of *sgs1* mutants (unpublished data). Thus it seems likely that the N-terminal region of Sgs1 plays an important role in guiding topoisomerase III to the site where its function is required, and Sgs1 acts together with topoisomerase III to suppress MMS sensitivity and hyper recombination.

The involvement of topoisomerase in conjugation with helicase in recombination has been suggested (*33*). They could act either to disrupt inadvertently paired DNA strands or promote branch migration, depending on the site of action. Harman *et al.* (*34*) found that, in the presence of RecQ and SSB, topoisomerase III could catalyze the linking of covalently closed circular plasmids to form catenanes and catalyze decatenation when the RecQ helicase activity was inhibited. In addition, in an *in vitro* replication system, bacterial topoisomerase III can catalyze decatenation of daughter molecules containing single-stranded DNA gaps (*35*). Thus, a possible function of eukaryotic topoisomerase III is to decatenate DNA molecules containing single-stranded DNA gaps at the late stage of DNA replication, and mutations in the *SGS1* gene should affect segregation. In fact, *sgs1* mutants show elevated levels of chromosome non-disjunction during mitosis.

Recently, we found that *SGS1* belongs to the *RAD52* recombinational repair pathway by an epistasis analysis of the MMS-sensitivity of *sgs1* disruptants (*36*). In addition, we found that the frequency of interchromosomal recombination, which increased in wild-type cells treated with MMS, was not increased in *sgs1* disruptants, indicating involvement of Sgs1 in the homologous recombination pathway. The involvement of Sgs1 in the recombination was also indicated upon exposure to UV light (*36, 37*). These results reveal dual functions of Sgs1; to suppress spontaneous recombination, and to promote recombination under damage-induced conditions. Epistasis analyses under DNA damage-induced conditions have also indicated that DNA polymerase ε and Sgs1 belong to the same epistasis group and act upstream of Rad53 to signal cell cycle arrest when DNA replication is perturbed (*38*). We also obtained results indicating that Sgs1 belongs to the same epistasis group along with Mec1 and Rad 53 when cells were treated with MMS (unpublished data). Thus, Sgs1 functions in a pathway from DNA replication check point to homologous recombination under DNA damage-induced conditions.

Bloom's syndrome gene product

Immunocytochemical studies indicated fluctuations in the amount of BLM during the cell cycle. BLM accumulates at high levels in S phase, persists in G2/M phase and sharply declines in G1 phase (*39*). BLM localizes primarily in the nuclear domain (ND) 10, also known as promyelocytic leukemia (PLM) nuclear bodies, except during S phase when it localizes in the nucleolus (*40*). ND10 is the site of early viral gene expression and DNA replication. PML is critical facto for ND10 formation, and in acute promyelocytic cells containing the PML-RARα fusion protein, ND10 disappears and BLM exists as microspeckles. A possible function of ND10 is to sequester various proteins such as helicases and topoisomerases which, when unregulated, may be deleterious. It was observed that in the late S phase, the distribution of BLM-containing foci partially coincided with that of DNA replication foci. Recently, it has been reported that *Xenopus laevis* BLM is absolutely necessary for DNA replication in *Xenopus laevis* egg extracts (*41*). Considering the localization of BLM during the cell cycle together with the slow replication fork progression and the accumulation of abnormal replication intermediates in BS cells, it seems likely that BLM is reserved in ND10s and is involved in a DNA surveillance mechanism operating during S phase.

Recombinant BLM has been shown to possess ATPase activity that is strongly stimulated by either single- or double-stranded DNA and 3′-5′ DNA helicase activity (*42*). Replication protein A (RPA) stimulates the helicase activity of BLM, but heterologous SSBs do not (*43*). Consistent with the functional interaction between RPA and BLM, a direct interaction between the two proteins is demonstrated. BLM is able to unwind G4DNA, and G4DNA is a preferred substrate of the BLM helicase, as measured both by efficiency of unwinding and by competition (*44*). In addition, BLM selectively binds Holliday junctions *in vitro* and acts on recombination intermediates containing a Holliday junction to promote branch migration over an extended length of DNA (*45*).

In *S. cerevisiae*, physical and functional interaction between Sgs1 and topoisomerase III has been observed as described above. In human cells, BLM also colocalizes with topoisomerase IIIα, one of two topoisomerase III homologues, in the nucleus and can be immunoprecipitated with topoisomerase IIIα from cell extracts. The topoisomerase IIIα binding regions of BLM reside in the N-terminal 1–212 amino acids and the C-terminal 1,266–1,417 amino acids (*46*). Thus, it seems likely that BLM plays a role with topoisomerase IIIα like Sgs1 in recruiting topoisomerase IIIα to sites of action.

An effort to establish a mouse model for BS to gain insight into BLM function revealed that disruption of the m*BLM* gene causes embryonic lethality in mice (*47*). The growth retardation in mutant embryos is accounted for by increased apoptosis. Red blood cells and their precursors from mutant embryos are heterogeneous in appearance and have increased numbers of macrocytes and micronuclei, indicating the existence of defects in manipulating DNA.

The most characteristic feature of BS cells is a high incidence of SCE. Although the phenomenon of SCE has long been recognized, the molecular mechanism behind it, especially the SCE in BS cells, remained unclear. There are two major models to explain SCE. First, SCE is mediated by homologous recombination. Second, topoisomerase II causes transient double-stranded DNA breaks (DSBs) during replication, and the proximity of DNA breaks on sister chromatids may result in incorrect rejoining, causing an SCE.

To elucidate the molecular mechanism that increases SCEs in BS cells and to examine whether the increase is mediated *via* homologous recombination, we generated *BLM*$^{-/-}$ and *BLM*$^{-/-}$/*RAD54*$^{-/-}$ cells from the chicken B lymphocyte line DT40 and characterized their phenotypes (*48*). The *BLM*$^{-/-}$ DT40 cells showed elevated levels of SCE as expected and extremely high levels of targeted integration frequency. The increased frequency of SCE in *BLM*$^{-/-}$ cells was considerably reduced by disruption of the *RAD54* gene, indicating that a large portion of the SCE in *BLM*$^{-/-}$ cells occurs *via* homologous recombination. The *BLM*$^{-/-}$/*RAD54*$^{-/-}$ cells showed a slow growth phenotype and an increased incidence of chromosome-type breaks/gaps while each single mutant showed relatively small numbers of chromosome-type breaks/gaps. It is suggested that chromosome-type breaks are caused by DSBs generated prior to and during DNA replication. Zou and Rothstein (*49*) showed that Holliday junctions accumulate spontaneously during DNA replication in mitotically growing yeast. Recently, a model for the occurrence of DSBs at arrested replication forks in *E. coli* was proposed (*50*). According to this model, Holliday junctions are formed by the annealing of two newly synthesized DNA at arrested replication forks but not formed *via* DSBs, and DSBs are formed by cleavage of the Holliday junctions. Indeed, annealed molecules consisting of two newly synthesized DNA were detected in the cells from BS patients (*51*). If Holliday junctions are formed by the above mechanism, and one of the physiological functions of BLM is to disrupt these junctions, the results obtained with *BLM*$^{-/-}$ and *BLM*$^{-/-}$/*RAD54*$^{-/-}$ cells may be explained by the following scenario (Fig. 2). The defect of BLM will cause the formation of more Holliday junctions and more DSBs during DNA replication. The majority of DSBs formed during DNA replication due to the defect of BLM function are repaired by homologous recombination resulting in an increase in SCE in Rad54-proficient cells. In BLM- and Rad54-deficient cells, the majority of DSBs are repaired by a mechanism other than homologous recombination and unrepaired DSBs cause chromosome-type breaks.

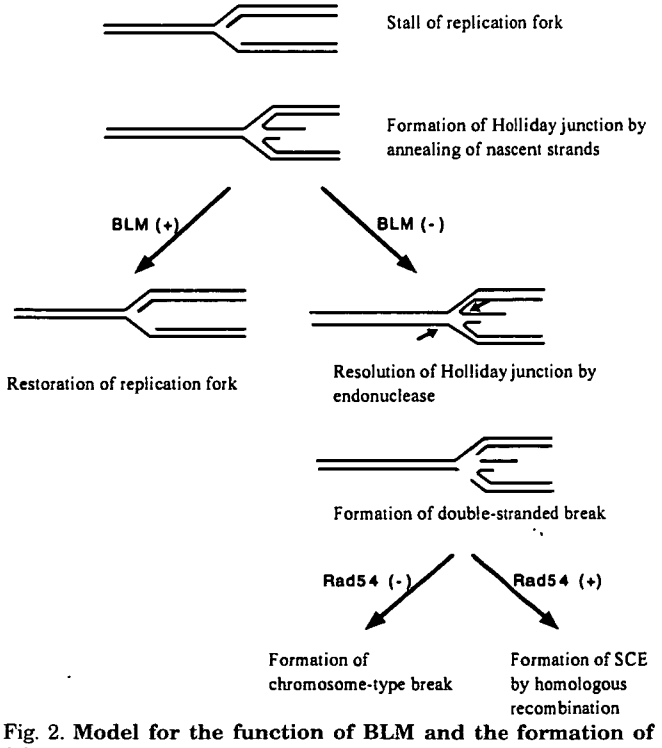

Fig. 2. **Model for the function of BLM and the formation of SCE deduced from the results obtained by using DT40 cells.**

Werner's syndrome gene product

WRN also possesses ATPase activity and 3′-5′ DNA helicase activity like BLM and Sgs1 (*52*). WRN efficiently unwinds the tetraplex structure of a specific sequence, $d(CGG)_7$ (*53*), and promotes the ATP-dependent translocation of Holliday junctions (*54*), as do BLM and Sgs1. WRN is the only RecQ homologue demonstrated to have exonuclease activity. WRN exonuclease digests DNA in a 3′-5′ directionality to generate 5′-dNMP products (*52*).

Recent studies have indicated physical and functional interactions of WRN with several proteins. Interaction of WRN with RPA was demonstrated by immunoprecipitation (*55*). RPA stimulates the helicase activity of WRN to unwind long DNA duplexes but *E. coli* SSB and T4 gene 32 protein fail to stimulate the helicase activity, indicating a specific functional interaction between WRN and RPA. Interaction of WRN with DNA polymerase δ was also indicated by immunoprecipitation. The interacting subunit of DNA polymerase δ is p50, and ectopically expressed WRN

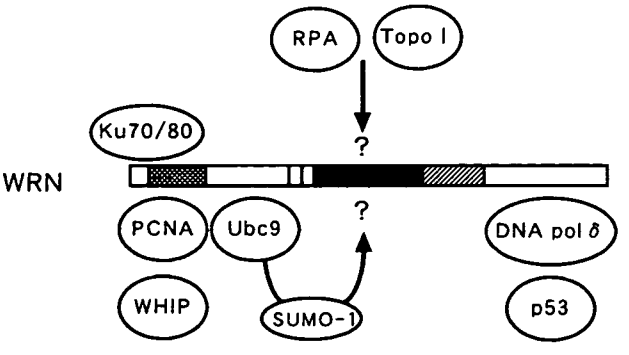

Fig. 3. **Interaction of WRN with various proteins.** The question marks indicate that the binding regions of WRN for RPA and topoisomerase I and the site of SUMO-1 modification have not been determined yet.

recruits p50 and p125, the catalytic subunit of DNA polymerase δ (*56*). The region of WRN which interacts with DNA polymerase δ is amino acids 949–1401 (Fig. 3). WRN increases the rate of incorporation by DNA polymerase δ in the absence of proliferating cell nuclear antigen (PCNA) but does not stimulate the activity of eukaryotic DNA polymerases α and ε (*57*). In addition to DNA polymerase δ, PCNA and DNA topoisomerase I have been indicated to interact with WRN (*58*). The region of WRN interacting with PCNA is the N-terminal portion containing a exonuclease domain. These proteins were found in the 17S multiprotein complex. Thus, the data seem to indicate that WRN functions at the replication fork.

Recently, it has been reported that WRN interacts with Ku80 through 50 amino acids at the N-terminus, and Ku70/80 stimulates the exonuclease activity of WRN (*59*). Since the Ku70/80 complex is known to participate in DNA double-stranded break repair along with DNA-dependent protein kinase, WRN may function in the repair of DNA double-stranded breaks occurring during DNA replication.

Another aspect of the function of WRN was indicated by its interaction with p53. The interaction involves the carboxy terminal portion of WRN and the extreme carboxy terminus of p53, a region that plays an important role in regulating the functional state of p53 (*60*). Overexpression of WRN led to the stimulation of p53-dependent transcription and increased p21 protein expression. In addition, it was observed that WS fibroblasts showed an attenuated p53-mediated apoptotic response, and this deficiency was rescued by expression of wild-type WRN (*61*). Although the function of WRN in the p53-dependent apoptotic pathway is not clear at present, it may be as a downstream target of p53.

We also tried to identify proteins that interact with WRN by a yeast two-hybrid screening using cDNA encoding the mWRN as bait and identified three proteins: a novel protein which we designated as WHIP (Werner helicase interacting protein) and Ubc9 and small ubiquitin-related modifier-1 (SUMO-1) (*62*). Physical interaction of WRN with WHIP was confirmed by co-immunoprecipitation of these proteins. WHIP interacts with the N-terminal portion of WRN containing the exonuclease domain (Fig. 3). WHIP has homology to replication factor C (RFC) family proteins, which are required for loading PCNA on template-primer junctions, and is conserved from *E. coli* to human. Functional interaction of WHIP and WRN was indicated by the results of a genetic analysis of yeast cells in which disruption of the y*WHIP* gene alleviated the MMS sensitivity of *sgs1* disruptants. In addition, a functional interaction between yWHIP and DNA polymerase δ was also indicated genetically (unpublished results). These results further indicate an intimate association of WRN with DNA replication.

Ubc9 binds to the N-terminal region residing between the exonuclease domain and the acidic region (amino acid 272–514). Ubc9 and SUMO-1 are involved in the same biochemical pathway, in which Ubc9 conjugates SUMO-1 to target proteins. We confirmed that WRN was covalently attached by SUMO-1 (*62*). In addition to WRN, p53 and DNA topoisomerase I were shown to be conjugated with SUMO-1. Although the observation is suggestive that conjugation of p53 with SUMO-1 activated the transcriptional activity of p53, the physiological meaning of the SUMO-1 modification of these proteins is not clear at present. This point must be addressed in future studies.

Perspectives

The results obtained so far indicate an intimate association of RecQ homologues in DNA replication. The analyses of *sgs1* mutants revealed dual functions of Sgs1; to suppress spontaneous recombination of various types, and to promote homologous recombination under damage-induced conditions. It is attractive to expect that the former function is mainly performed by BLM and the latter by WRN in higher eukaryotic cells. This possibility will be probed in the near future. In addition, because of the physical interaction of BLM with DNA topoisomerase IIIα, how the function of BLM is mediated by DNA topoisomerase IIIα must be examined.

Genetic and biochemical analyses have indicated that Sgs1 functions in a pathway from the DNA replication check point, which includes Mec1 and Rad 53, to homologous recombination. In this context, the finding of a large complex containing the tumor suppressor, BRCA1, is encouraging. The complex named BASC (BRCA1-associated genome surveillance complex) includes BLM, RFC, the higher eukaryotic homologue for Mec1, ATM, proteins involved in mismatch repair, MSH2, MSH6, and MLH1, and proteins involved in double-stranded break repair, RAD50, MRE11, and NBS1 (*63*). In addition, BRCA1 is known to be associated with RAD51, which plays a major role in homologous recombination, and with p53. On the other hand, WRN interacts with DNA polymerase δ, PCNA, RPA, WHIP, Ku70/80 complex, DNA topoisomerase I, and p53. Thus, it seems likely that the WRN complex and BASC are linked by PCNA and RFC and that DNA replication is linked with check point control and various repair systems including recombination repair. The interaction of p53 with both BRCA1 and WRN also indicates communication between BASC and the WRN complex. Because of rapid progress in the study of the functions of RecQ homologues, we will soon obtain a more precise scenario concerning the functions of eukaryotic RecQ homologues.

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