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RecQ helicases and genome stability: lessons from model organisms and human disease

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Summary

Maintaining the integrity of genetic information is fundamental for the life of a cell and the survival of a species. Cells can encounter DNA damage as a consequence of normal cellular metabolism or as a result of exposure to chemical or physical agents. Eukaryotic cells have developed a network of responses in order to deal with DNA damage thereby preserving the integrity of their genetic information. In the presence of extensive genetic insult, a surveillance mechanism or "checkpoint" is activated [1]. The activation of this signal transduction pathway leads to an arrest of cell cycle progression to prevent replication and segregation of damaged DNA molecules and to induce transcription of several repair genes. Existing repair mechanisms are also mobilised, in a coordinated effort to restore the original DNA structure. Genes involved in either cell cycle checkpoints, DNA repair or genes that maintain the fidelity of chromosome segregation are often termed "antimutators" or "caretaker" genes, because they control the stability of the genome and prevent accumulation of mutations in so-called "gatekeeper" genes. This latter group of genes directly regulate the growth of tumours either by inhibiting growth or promoting death [2].

A fundamental requirement for many DNA metabolism processes is the separation of the complementary strands of the DNA duplex. This is promoted by DNA helicases, which unwind nucleic-acid duplexes in an ATP-dependent manner to provide access to the template for proteins of the replication, recombination, repair and transcription machineries [3]. Multiple DNA helicase families have been identified, all containing seven hallmark helicase motifs; members within each helicase family also share sequence homologies beyond and between these motifs. One example is the RecQ helicase family, named after the RecQ protein of *Escherichia coli*, which was identified during a search for mutants sensitive to thymine starvation [4]. Five members of the RecQ family have been identified in the human genome, and mutations in three of the genes are responsible for genetic diseases that are characterised by genomic instability and a high incidence of cancer [5]. Because mutants in RecQ family genes in other species also have unstable chromosomes, it was proposed that members of the RecQ helicase family play a central role in the maintenance of genomic stability and thereby the prevention of tumorigenesis.

Key words: RecQ helicases; checkpoints; recombination; replication

RecQ family helicases: structure and biochemical nature

Helicases of the RecQ family are found in prokaryotes, unicellular eukaryotes, as well as in vertebrates, where multiple homologues often exist [5]. A common structural characteristic in this family of enzymes is a central domain of 450 amino acids (aa), which contains the seven signature helicase motifs including a putative ATP binding sequence and a DexH-box. A 3´ to 5´ helicase activity has been reported for all family members analysed. As illustrated in figure 1, all of the RecQ-like proteins share a strong sequence similarity in a central domain, while subfamilies are defined by homology extending beyond this. One subfamily is represented by the bacterial RecQ enzyme itself, which is only 609 aa in length. Mutations in the E. coli recQ gene implicate the protein in homologous recombination and double-strand break repair (table 1). Human RECQL (for RecQ like) belongs to the same group, and contains only 649 aa. The second group is represented by larger RecQ helicases (generally around 1400 aa), which often share an area of extended sequence homology C-terminal to the helicase domain. This group includes the WRN, BLM and RECQ4 from man, Sgs1p and Rqh1p from budding and fission yeast, respectively (see fig. 1), as well as BLM homologues found in Drosophila and Xenopus. Mutations in each of the genes encoding these proteins result in

hyper-recombination and chromosome instability [6]. Furthermore, mutations in WRN, BLM or RECQ4 are linked to three recessive genetic diseases: Werner's, Bloom's and Rothmund-Thomson syndromes.

During the past years biochemical data have characterised the structure of the substrate DNA that these specialised RecQ helicases prefer *in vitro*. Recombinant BLM, WRN and Sgs1 proteins have been shown to have helicase activity *in vitro*, although they catalyse little or no unwinding of duplex DNA from blunt ends, from internal nicks or from partial duplex molecules with single-stranded 3´–5´tails [7–11]. They do, however, initiate unwinding from bubbles inserted internally into an otherwise blunt-ended duplex. Both BLM and WRN enzymes also efficiently unwind G-quadruplex DNA and synthetic X-junctions that model the Holliday junction recombination intermediate [12–14]. Finally, it was shown that Sgs1p can disrupt synthetic 3- and 4-way junctions *in vitro* [7]. These biochemical data suggest that the RecQ helicases share a substrate specificity that is very atypical amongst helicases. Their preference for substrates resembling Holliday junction recombination intermediates further indicates a role for these enzymes in the recombination pathways of eukaryotic cells, although such structures can also form at stalled replication forks [15]. This may be relevant to the genomic instability phenotype observed when cells lack a functional RecQ helicase.

Human syndromes associated with RecQ helicase deficiency

The *BLM* gene is mutated in Bloom's syndrome (BS), which is a rare recessive disorder associated with phenotypes such as short stature (proportional dwarfism), skin disorders (e.g., hyperpigmentation), male infertility, immunodeficiency, early onset of type 2 diabetes and a predisposition

Table 1

Phenotypes associated with mutants lacking a RecQ family helicase.

Species	*E. coli*	*S. cerevisiae*	*S. pombe*	*H. sapiens*	
Gene	recQ	SGS1	rqh1	BLM	WRN
nature of genomic instability	elevated illegitimate recombination	hyper recombination, extrachromosomal rDNA circles	HU induced hyper-recombination	chromosome breakage and rearrangements, sister chromatid exchanges	variegated translocation mosaicism, deletions
replication abberrancies	role in RecF pathway	sensitive to HU	hypersensitive to HU	abnormal replication intermediates, retarded fork progression	abnormal replication intermediates, sensitive to S-phase specific agents
response to DNA damage	UV sensitive	no UV sensitivity	UV sensitive	no sensitivity to UV	no sensitivity to UV
aging characteristics		reduced lifespan			reduced lifespan, telomere shortening
chromsome loss / non-disjunction		mitotic/meiotic chromosome non-disjunction	HU-stimulated chromosome loss	subfertility	subfertility

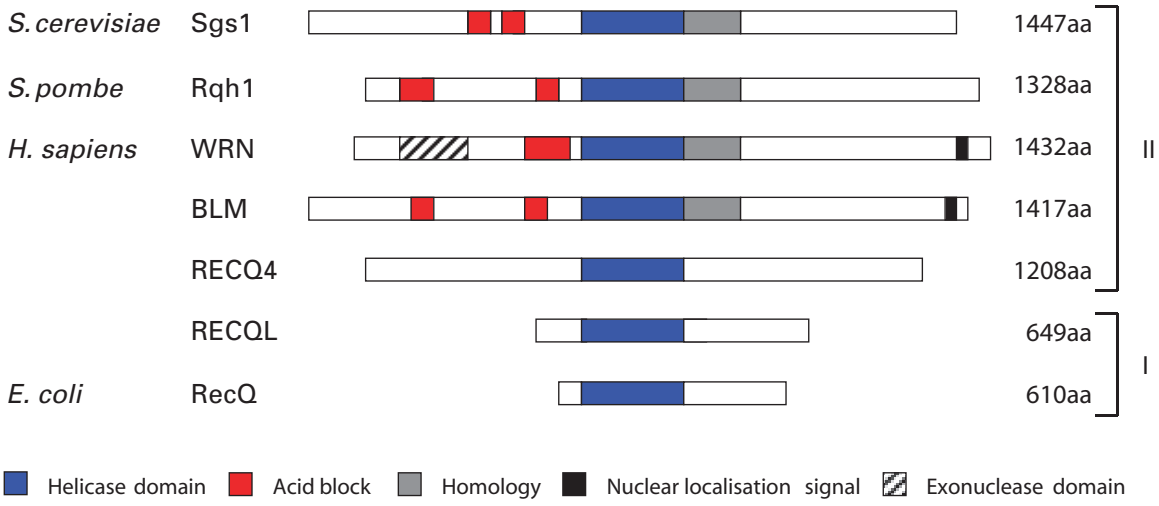


Figure 1

Schematic representation of members of the RecQ family of DNA helicases from *E. coli*, yeast and human. The size of each protein is shown on the right. Regions corresponding to the helicase domains, blocks of acidic amino acids, extended homology outside the core helicase domain, nuclear localisation signals and exonuclease domain are indicated and shown in the key below. The two subfamilies are indicated by I and II at the right.

to cancer with an onset between age 20 and 30 years [16, 17]. Interestingly, the affected individuals are susceptible to the full range of cancers seen in the normal population. BS cells show a high frequency of chromosome rearrangements and breakage, sister chromatid exchange and recombination [17–19], which may explain the predisposition to cancer. Such abnormal rearrangements tend to reveal haploinsufficiencies in tumour suppressor genes. Furthermore, studies on BS fibroblast cell lines have revealed retarded replication fork progression and the accumulation of abnormal replication intermediates [20, 21], suggesting an important function for the BLM helicase during the S phase of the cell cycle.

Werner's syndrome (WS) arises from mutations in the *WRN* gene and is characterised by the premature appearance of ageing phenotypes in young adults [22, 23]. These include loss and graying of hair, scleroderma-like skin, arteriosclerosis, osteroporosis, type 2 diabetes and an elevated incidence of rare sarcomas. Most patients with WS die between the ages of 40 and 50 years, primarily from malignant tumours or cardiovascular infarction. Cultured cells derived from patients with WS show an increased rate of somatic mutations, chromosome losses and deletions, as well as an attenuated division capacity [24, 25]. Although it is unclear how such chromosomal aberrations are linked to the complex phenotypes of ageing, they are expected to enhance neoplastic transformation.

Recently, mutations in the *RECQ4* gene were found in a subset of patients with the Rothmund-Thomson syndrome [26]. RTS-affected individuals display skin and skeletal abnormalities, growth deficiency, premature ageing (early graying and hair loss) and a predisposition to malignancy, especially osteogenic sarcomas [27]. Although the genomic instability of RTS cells still remains to be analysed in detail, an increased frequency of chromosome aberrations of RTS cells has been reported.

At a cellular level these genetic disorders all reveal high levels of genomic instability. Such molecular defects are readily studied in model organisms, and particularly in a genetic model like yeast. Below we summarise the molecular characterisation of RecQ helicases, which is hoped to shed light on the molecular causes of these disease phenotypes.

RecQ helicases in yeast – yeast as a model system for human diseases

The unicellular budding and fission yeasts each contain but one RecQ helicase, Sgs1p and Rqh1p, respectively. This raises the question whether the yeast enzymes are less specialised than their mammalian counterparts, being able to perform multiple functions that were subsequently divided among multiple specialised RecQ helicase homologues later in evolution. Nonetheless, the cellular phenotypes associated with the lack of *SGS1* are surprisingly similar to the defects in cells from either BS or WS patients, including both hyper-recombination and enhanced cellular senescence.

In budding yeast, deletion of the *SGS1* gene gives rise to an elevated frequency of both meiotic and mitotic recombination (table 1). The hyper-recombination in *SGS1* cells is manifested as an increase in both intra- and inter-chromosomal recombination at more than one locus [28], especially at the repetitive ribosomal DNA [29]. Hyper-recombination has also been reported for *S. pombe* strains lacking the RecQ-like helicase Rqh1p, although the phenotype is most pronounced when cells have been treated with either UV irradiation or the DNA replication inhibitor hydroxyurea [30]. In *S. cerevisiae*, hyper-recombination at the rDNA results in an accumulation of extrachromsomal rDNA circles (ERCs) containing one or more rDNA repeats [31], a phenomenon that correlates with cellular senescence in yeast, or a limitation in the division capacity of a single cell. This lifespan reduction in *sgs1* mutants [28, 32], parallels the enhanced degree of cellular senescence observed in WS cells, although it is not clear whether the molecular basis of ageing is similar in the two organisms. In yeast, ageing correlates with the accumulation of the above mentioned ERCs, whereas enhanced telomere shortening correlates best with the limited division capacity of Werner's deficient fibroblasts [33].

The high degree of functional conservation within the RecQ helicase family is revealed by complementation analysis.

In fact, the expression of the human *BLM* or *WRN* gene in an *sgs1* deficient yeast cell suppresses some of the associated phenotypes, but not all. Either gene suppresses the hyper-recombination phenotype [34], whereas only *BLM* can suppress the slow growth phenotype of a DNA topoisomerase III mutant (see below) and the sensitivity to compounds that impair replication fork progression (hydroxyurea). This may argue that *BLM* function resembles that of Sgs1p more closely than that of *WRN*, at least when expressed in yeast. Surprisingly, *BLM* but not *WRN* suppresses the short-lived phenotype of the *sgs1* null alleles, although it does not repress ERC formation [35]. Thus the correlation between ERC formation and ageing may not hold under all situations; indeed, in mammalian cells rDNA instability does not appear to correlate with a limited division capacity [35].

A RecQ helicase partner: DNA topoisomerase III

DNA topoisomerase III is a ubiquitous enzyme, which is able to cleave single-stranded DNA and transfer another single-stranded DNA molecule through this break. This action allows the enzyme to manipulate the topology of a DNA molecule. Both yeast and human RecQ-like helicases interact with DNA topoisomerase III [29, 36, 37], and genetic studies reveal synergism between the gene products. In fact, the yeast *sgs1* mutation was isolated as a suppressor of the slow growth phenotype that is typical of a *top3* null allele [29]. Moreover, *top3* mutants also show hyperrecombination phenotypes and chromosome loss [38, 39]. Finally, the BLM helicase also interacts biochemically with DNA topoisomerase IIIα [37] and the two proteins co-localise by immunofluorescence [37, 40]. The paired action of RecQ-like helicases and DNA topoisomerase III enzymes is thought to facilitate aspects of DNA replication or recombination, either by introducing positive supercoils or helping to resolve intertwined strands where replication forks meet [41]. The similarity of the phenotypes of *sgs1* and *top3* mutants suggests that the two enzymes act together or co-operate in some reactions.

It is unlikely that topoIII controls the overall supercoiling homeostasis of the cell, yet, it is evident from the very severe phenotypes associated with the lack of this enzyme, that it performs functions which cannot be replaced by other classes of DNA topoisomerases. In *S. cerevisiae top3* null alleles have a very pronounced slow growth phenotype, while in both in *S. pombe* and mice, disruption of *top3* or *TOPOIIIα* is lethal [38, 42, 43]. In budding yeast, the observation that mutations in *SGS1* can suppress the hyper-recombination and slow growth of *top3* null alleles implies that the enzymes operate together. This genetic interaction is conserved since inactivation of *rqh1* is able to suppress the lethality of *top3* in *S. pombe* [42]. How these two enzymes co-operate to suppress hyper-recombination is still not understood, yet, one hypothesis is that in the absence of a RecQ helicase and Top3p, inappropriate strand invasion or pairing events may be converted into full recombination events or persist as concatenanes. The resulting intertwined chromosomes would result in chromosomal segregation defects, as observed in *sgs1*, *rqh1* and *top3* mutants [30, 36, 44].

RecQ helicase and the S-phase checkpoint

There is emerging evidence that DNA replication does not proceed normally in the absence of RecQ helicase. As mentioned above, it had long been noted that cultured cells from BS and WS patients have minor defects in DNA synthesis. Cells lacking BLM have an abnormal profile of DNA replication intermediates [21], retarded DNA-chain growth [20]. The BLM protein itself accumulates at high levels in S phase, and its depletion from *Xenopus* egg extracts greatly retarded replication of *in vitro* assembled nuclei. Such data implicates BLM either in a DNA unwinding step or in overcoming barriers to fork progression [45]. WS cells are also impaired in S-phase progression, although this may be due to another pathway [46]. It has been shown that WRN helicase interacts with replication protein A [47], as well as co-fractionating on sucrose gradients with other replication proteins and co-precipitating with PCNA, a processivity factor for DNA polymerase δ [48].

In yeast, it was also shown that both *SGS1* transcript [49] and protein levels peak in S phase [50]. In yeast nuclei, Sgs1p has a focal distribution that overlaps significantly with sites of de novo DNA synthesis and with ORC, a six-protein complex essential for initiation of DNA replication [50]. Nonetheless, *sgs1*-deficient cells proceed normally through S phase, as long as damage is not induced. Finally, in fission yeast, *rqh1* mutants are defective in the recovery from S-phase arrest when exposed to the DNA synthesis inhibitor hydroxyurea [30]. Consistently, growth defects are greatly enhanced when temperature sensitive mutations in DNA polymerase δ subunits are combined with a *rqh1* mutation [51].

Based on studies in budding yeast, where fork progression in the absence of DNA damage proceeds normally in an *sgs1* null strain, it seems unlikely that this family of proteins will serve as a leading strand helicase. Rather, they are thought to help fork stability and resolve aberrant DNA structures that arise through the presence of impediments in chromatin, such as DNA damage or protein barriers. In addition, both genetic and biochemical evidence suggests that RecQ helicases may play a role in S-phase checkpoint control. The role of the S-phase checkpoint is to stabilise the replication fork, block late replication origins from firing when the genome is damaged, and promote the repair of damaged DNA [6, 52].

Genetic analysis of the DNA damage checkpoint pathways has allowed classification of its components into “sensors”, which detect different sorts of damage, “adaptors” which integrate and transmit the signal, and effector kinases, which promote downstream functions (see fig. 2), including the induction of repair genes, suppression of cell cycle progression, down-regulation of late origins, and the arrest of replication polymerases and of sister chromatid segregation [53]. Involved

as "sensors" for the replication checkpoint are members of the DNA-dependent protein kinase-like family (DNA-PK) that includes human ATM and ATR, *S. pombe* Rad3p, and *S. cerevisiae* Mec1p. These proteins are required for all DNA damage checkpoints and serve to activate effector kinases by phosphorylation (fig. 2). The effector kinase in fission yeast and mammalian cells is called Cds1p, and its homologue in budding yeast is called Rad53p. Rad53p is phosphorylated by Mec1p, an ATM homologue, in response to both DNA damage and replication fork arrest [54]. DNA damage checkpoint defects are linked on many levels to carcinogenesis, most strikingly in Ataxia telangiectasia or with respect to p53 mutations. We explore below the links between RecQ helicases and the checkpoint kinases mentioned above. We speculate that they have a common goal in S phase, which is to provide fork stability and to promote repair of stalled or broken replication forks during DNA replication.

Sgs1p contributes to Rad53p activation in budding yeast

DNA damage checkpoints during G1 and G2 are not affected by a loss of function mutant of *SGS1*, although *sgs1*-deficient yeast cells fail to fully activate the Rad53p-dependent response to stalled replication forks in S phase [50]. Genetic analyses indicate that *SGS1* forms an epistasis group with DNA polymerase ε and Replication Factor C, which also act upstream of Rad53p kinase activation to signal the arrest of the cell cycle in response to an unscheduled block in DNA replication [55]. The Pol-ε/Sgs1p-dependent checkpoint activation runs parallel to Rad17p and Rad24p, two components of a signalling cascade that leads to Mec1p and Rad53p kinase activation in response to DNA damage [6, 56]. Loss of both Sgs1p and Rad24p fully compromises Rad53p activation, and allows passage through the cell cycle prior to completing DNA replication [50]. Together with immunostaining studies that co-localise Sgs1p and Rad53p in S phase-specific foci, these data suggest that Sgs1p may play a role in recruiting Rad53p, although perhaps only transiently, to stalled replication forks. In agreement with this model, recent studies indicate that Sgs1p interacts *in vitro* with the FHA domains of Rad53p (C. Frei and J. A Cobb, unpublished data).

The role of BLM and WRN in the S-phase checkpoint is less clear in mammalian cells, but several studies show that the ATM protein kinase phosphorylates WRN *in vitro* and co-localises with the helicase and RPA in meiotic cells [57–59]. More recently, Werner protein was shown to co-localise with RPA in discrete nuclear foci upon replication arrest [12]. In addition, ATM functions upstream of the tumour suppressor protein p53, and loss of either protein results in a failure to respond properly to damaged DNA [60, 61]. Interestingly, the WRN helicase interacts biochemi-

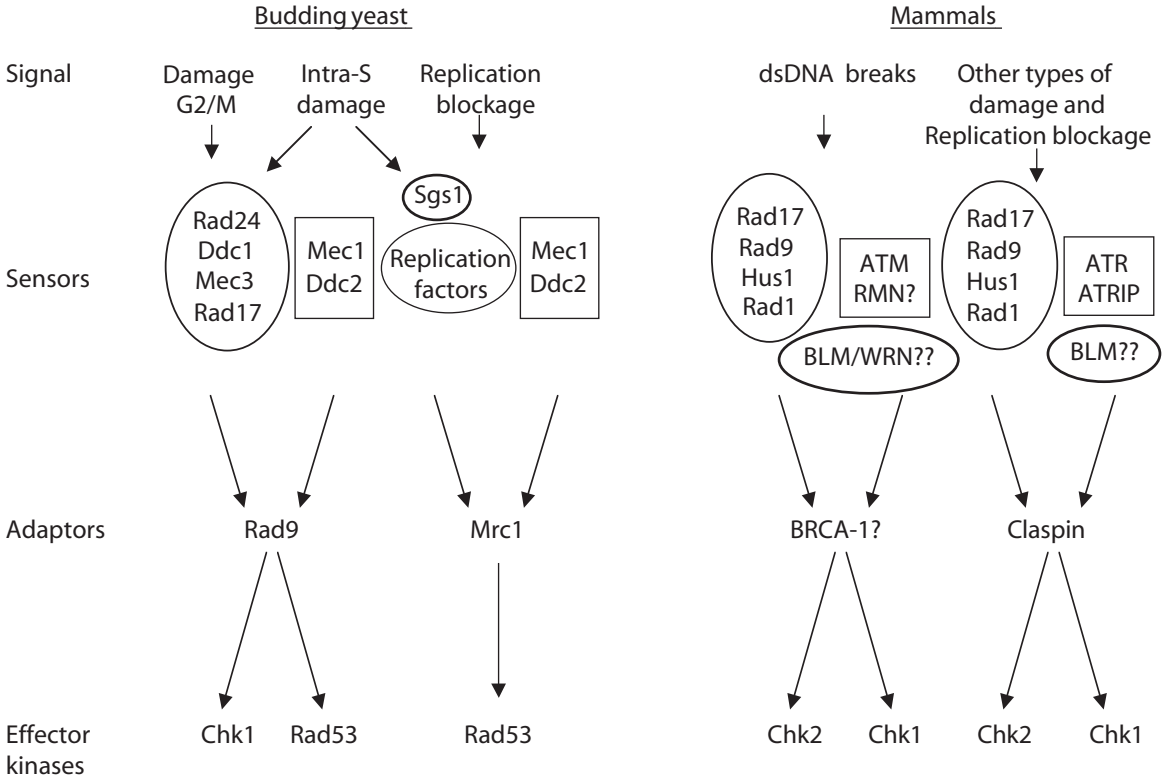


Figure 2

DNA damage checkpoint pathways are conserved from yeast to man.
Outline of the different DNA-damage and S phase checkpoint signalling pathways identified in budding yeast and mammals. Intra-S phase damage refers to DNA damage induced during S phase, often after treatment with MMS (methyl-methanesulphonate), which is known to affect the rate of S phase progression. A checkpoint signal activated due to replication block refers to the inhibition of DNA replication using either hydroxyurea or aphidocolin.

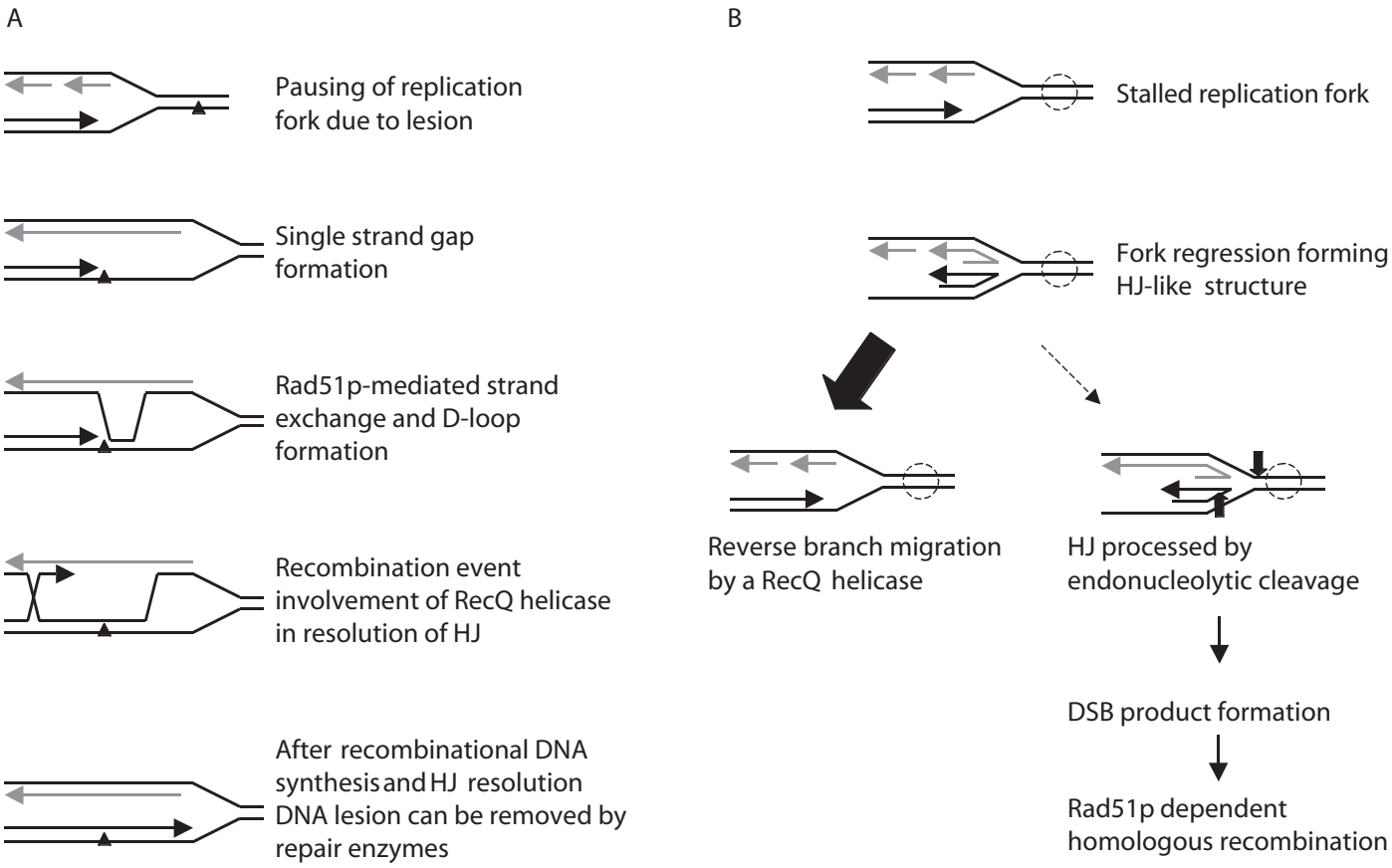
cally *in vitro* and *in vivo* with p53. This interaction may be physiologically significant, since p53-mediated apoptosis is attenuated in cultured WS cells [62, 63]. Moreover, this interaction requires the C-terminus of Werner's helicase, which contains a highly conserved domain [64], that is also important for Sgs1p checkpoint function in yeast [50, 65]. Finally, the BLM protein has been shown to co-localise with ATM and with other tumour suppressor and DNA damage repair proteins in a large complex called BASC (BRCA-1 associated genome surveillance complex), when cells are treated with agents that interfere with DNA synthesis [66].

Studies of *rqh1* mutations, suggested a slightly different S-phase function in fission yeast for the RecQ helicase. In brief, *rqh1* mutations decrease the viability of cells as they recover from an arrest provoked by HU but do not impair the arrest itself [30, 51, 67]. Thus, rather than a direct role in the checkpoint response, Rqh1p may be involved in the resumption of growth following genomic insult, perhaps in a pathway that allows the replication fork to bypass DNA damage [51]. Even this scenario, however, is consistent with the notion that the RecQ family serves as a specialised helicase associated with the replication fork. It is possible that in fission yeast the checkpoint signalling function is fulfilled by another protein.

RecQ helicases and recombination

The most pronounced phenotype associated with many RecQ helicase mutants is the loss of genomic stability. Specifically, genetic recombination seems to occur aberrantly and at an excessive frequency in cells lacking a functional RecQ helicase. Much of our knowledge concerning the involvement of RecQ helicases in the recombination process arises from studies in *E. coli* and yeast. Null mutations at the *E. coli recQ* locus, in conjunction with other mutations, result in a 100-fold reduc-

Figure 3
Dual roles for RecQ helicases in replication-associated DNA damage.
A. A model for the function of RecQ helicases in recombinational DNA repair. When a replication fork encounters a block (shown as a triangle), a single strand gap forms at the stalled fork. This region can be used for a Rad51p dependent strand exchange and D-loop formation to initiate a recombination event. A RecQ helicase can associate with Rad51p and might be involved in the ensuing branch migration and/or joint molecule resolution. After recombinational DNA synthesis and resolution of the Holliday junction, the DNA lesion resides in a duplex, which is the preferred substrate for repair enzymes.
B. A model for RecQ helicases as an anti-recombinase at sites of stalled replication. During replication pausing, the nascent DNA strands can dissociate from the template and anneal to each other, thereby forming a Holliday Junction-like structure. This structure might be reversed by RecQ helicases by branch migration or alternatively endonucleotlytic cleavage can occur, which will generate DSB products that can be repaired via homologous recombination.



tion in homologous recombination proficiency [4], yet lack of *recQ* also gives rise to a 30-fold increase in illegitimate recombination [68]. *In vitro*, RecQ helicase initiates homologous recombination and unwinds a wide variety of DNA substrates, including homologous paring intermediates [69]. From these studies, it seems that *E. coli* RecQ helicase is a multifunctional enzyme capable of both initiating homologous recombination and suppressing illegitimate recombination. As mentioned previously, deletion of the *SGS1* gene gives rise to an elevated frequency of mitotic recombination manifested as an increase in both intra- and interchromosomal recombination [28]. Very early data reported as well that cell lines derived from BS individuals displayed an elevated rate of reciprocal exchanges both between sister chromatids and non-homologous chromosomes [70].

Genetic analyses in yeast provide a direct link between the RecQ helicase and recombination. Srs2p is another 3'–5' DNA helicase in yeast [71] that is implicated in the repair of damaged DNA especially during replication [72]. As for *sgs1* mutants, lack of *srs2* also results in increased rates of recombination [73, 74], and the expression of both genes peaks during S phase. These similarities raised the question whether the two helicases in yeast have a common function. Yeast cells lacking both *sgs1* and *srs2* were found to be inviable or extremely slow growing, which suggests that the two genes serve on parallel pathways redundant for an essential function [75, 76]. Importantly, however, the extreme slow growth of the double mutant could be suppressed by deleting *RAD51* [75], a highly conserved strand pairing protein that is essential for the initial stages of homologous recombination. Like the bacterial RecA, Rad51p catalyses homologous strand pairing and DNA exchange. Because the mutation of *rad51* suppresses the *sgs1 srs2* mutant phenotype, coincident with the elimination of homologous strand exchange, it is thought that very severe phenotype of the double mutant results from unresolved recombination events. These data indicate a role for Sgs1p downstream of Rad51p in the resolution of recombination structures, a role particularly important in the absence of Srs2p. This does not rule out that Sgs1p could also function as an anti-recombinase, preventing the occurrence of aberrant recombination events by acting upstream of Rad51p.

Further evidence that RecQ helicase family controls recombination efficiency, comes from studies in fission yeast. Like checkpoint proteins *rqh1* cells are hypersensitive to HU, however, this sensitivity is not due to a checkpoint failure, since the cells arrest the cell division in response to HU [30]. Nevertheless, *rqh1* cells show a chromosomal segregation defect upon release from S-phase arrest, indicating that *rqh1* cells are unable to recover from recombination intermediates that arise during S phase arrest. S phase arrest in *rqh1* cells elevates recombination consistent with the model that Rqh1p helps to prevent excessive recombination. This would also explain the segregation defect observed in *rqh1* cells after HU treatment. If cells enter mitosis with sister chromatids entangled due to unresolved recombination intermediates chromosome segregation becomes very difficult [30]. Interestingly, in another study, expression of an *E. coli* Holliday junction resolvase in *rqh1* cells was reported to partially complement the UV and HU hypersensitivity and the associated aberrant mitosis [77], suggesting that Holliday junctions accumulate in *rqh1* cells, which then impede the segregation of sister chromatids. This again leads to the hypothesis that Rqh1p is involved in either the prevention of Holliday Junction formation or in the processing of these structures.

In human cells a link between RecQ helicases and homologous recombination has also been suggested based on the finding that BLM protein and hRad51p interact [78]. Furthermore, γ radiation, which creates double strands breaks (DSB) in the DNA, increases the co-localisation of hRad51p and BLM, probably reflecting multiprotein complexes engaged in recombinational repair. It was proposed hRad51p may recruit BLM to sites of recombinational repair, where BLM would act to disrupt recombination intermediates by performing reverse branch migration [78]. On the other hand, it has also been proposed that WRN helicase may act to productively resolve recombination events. WS cells show an increase in apoptotic cell death in response to DNA damage. It was suggested that in the absence of WRN protein, hRad51p is able to promote recombination mediated repair, but that an aberrant resolution of the recombination events leads to cell death [79]. In support of this an additional study showed that WS cells can initiate mitotic recombination to the same extent as wild type cells, yet they fail to resolve recombinant products, and generating only a few viable gene conversion-type recombinants [80].

As mentioned above, the BLM protein has been found to be a part of a multiprotein complex called BASC (BRCA1-associated genome surveillance complex). This complex contains at least 15 subunits, which all have functions in DNA repair mechanisms, either post-replicational repair, mismatch repair or transcriptional coupled repair [66]. Very recent data suggest that the BLM protein is necessary for the correct relocalisation of the Rad50-Mre11-Nbs1 complex (RMN) to sites of stalled replication fork after HU treatment [81]. The precise function of the RMN complex is still not fully elucidated, yet in yeast the analogous Rad50/Mre11/Xrs2 complex is known to provide nuclease activity for the processing of DSBs, ensuring the 3´ssDNA tail necessary for the initiation of strand exchange [82, 83].

A physical and functional interaction, has been reported for the WRN protein and the Ku heterodimer complex, suggesting that WRN protein is also involved in DSB repair [84, 85]. It was shown that this interaction stimulates the exonuclease activity of WRN to remove certain damaged

nucleotides that would not be removed by WRN alone [86]. The Ku complex is involved in DSB repair via the nonhomologous end-joining pathway as opposed to the Rad50-Mre11-Nbs1/Xrs2 proteins which is involved in DSB repair via the recombination. This raises the possibility that different RecQ helicases in higher eukaryotes operate in both end-joining and recombinational pathways for DSB repair.

The connection between RecQ homologues and Ku appears to be relevant for telomere maintenance as well. Recent work has shown that primary fibroblasts from Werner's syndrome individuals, like Ku-deficient murine cells, display excessive telomere shortening and premature replicative senescence, which is thought to contribute to the early onset of aging seen in Werner's syndrome patients [87]. Indeed, expression of telomerase suppresses the accelerated telomere shortening and premature senescence of Werner's syndrome cells [88]. Yeast Sgs1p is also required for a telomere maintenance pathway that is independent of telomerase and dependent on recombination [89, 90] and this telomere defect in *sgs1* mutants can be partially overcome by overexpression of WRN [91]. Thus the RecQ helicases help to define two mechanistically distinct telomere maintenance pathways that are both telomerase-independent and recombination-dependent.

Conclusion

The data described above clearly indicate a role for the RecQ helicase family in controlling recombination, both helping to signal to the machinery in response to stalled forks and by resolving structures that lead to chromosome catenation. Control of recombination during DNA replication is particularly important since stalling or collapse of the replication fork caused either by collisions with DNA lesions or nucleoprotein complexes or limiting concentrations of nucleotides will expose both single-stranded DNA and DNA strand breaks to the recombination machinery of the cell [56]. A key intermediate in homologous recombination is the Holliday Junction, which resembles structures formed by the regression of stalled replication forks [92, 93]. Formation of the regressed fork may not require Rad51p function, as it may form spontaneously to relieve the superhelical tension that builds up in front of the replication fork.

In our summary figure we show two sites at which RecQ helicases could play important roles in controlling the outcome of recombination. In the first one (fig. 3a), RecQ helicase would serve during recombinational repair of sequences already replicated, ie, behind the fork (see figure legends for further details). In the second scenario (fig. 3b) RecQ helicases would perform a function at the stalled fork itself. The Holliday Junction-like structure formed by fork regression can be resolved in two ways. Preferably, a RecQ helicase (i.e., Sgs1p) would catalyse a reverse branch migration allowing re-initiation of DNA synthesis through stabilisation of DNA polymerase (see bold arrow fig. 3b). Alternatively, in the absence of this helicase, the structure could be processed by endonucleolytic cleavage, generating recombinogenic DNA ends and a collapse of the fork (see dotted arrow fig. 3b)

Although careful genetic studies and further biochemical analyses are needed to understand fully how RecQ DNA helicases influence the processing and/or the prevention of recombination intermediates, it is striking that these functions are universally required to maintain genome integrity. Furthermore, transcription analysis has shown that the human RECQL4 is the 6th most highly over-expressed gene in a large array of human tumour types [94]. Certainly genome instability is at the heart of neoplastic transformation, and RecQ helicases at the heart of genome stability.

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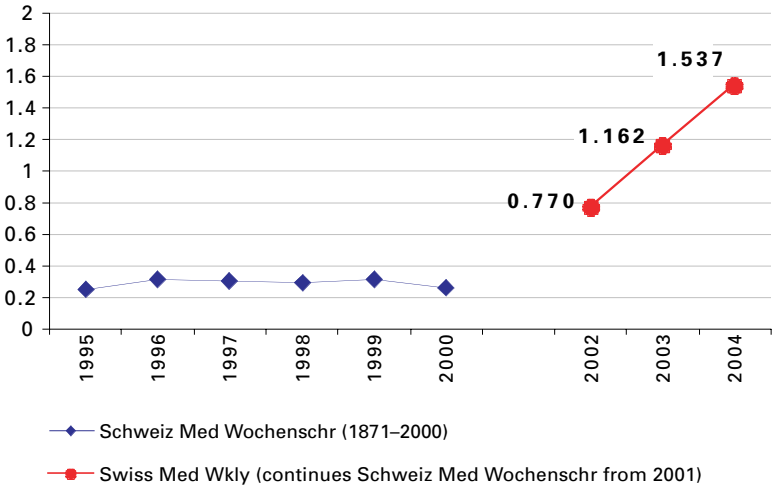

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