REVIEW ARTICLE

RecQ helicases: suppressors of tumorigenesis and premature aging

Csanád Z. BACHRATI and Ian D. HICKSON[1]

Cancer Research UK Laboratories, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, U.K.

The RecQ helicases represent a subfamily of DNA helicases that are highly conserved in evolution. Loss of RecQ helicase function leads to a breakdown in the maintenance of genome integrity, in particular hyper-recombination. Germ-line defects in three of the five known human RecQ helicases give rise to defined genetic disorders associated with cancer predisposition and/or premature aging. These are Bloom's syndrome, Werner's syndrome and Rothmund–Thomson syndrome, which are caused by defects in the genes *BLM*, *WRN* and *RECQ4* respectively. Here we review the properties of RecQ helicases in organisms from bacteria to humans, with an emphasis on the biochemical functions of these enzymes and the range of protein partners that they operate with. We will discuss models in which RecQ helicases are required to protect against replication fork demise, either through prevention of fork breakdown or restoration of productive DNA synthesis.

Key words: cancer predisposition, chromosomal instability, DNA repair, progeroid syndrome, RecQ helicase, topoisomerase.

INTRODUCTION

Helicases are enzymes that use the energy derived from the hydrolysis of ATP to separate the complementary strands of nucleic acid molecules. The majority of helicases unwind either DNA duplexes or RNA duplexes; however, some can disrupt dsDNA (double-stranded DNA) as well as dsRNA and DNA:RNA hybrids. Helicases are ubiquitous in Nature, and are essential for all aspects of DNA metabolism, including DNA replication, recombination, transcription and repair. The number of helicases expressed in higher organisms is strikingly high, with approx. 1 % of the genes in many eukaryotic genomes apparently encoding RNA or DNA helicases.

DNA helicases can be classified primarily in two ways, on the basis of either their mode of action or whether they contain particular amino acid 'signature' motifs. Most helicases translocate along DNA with a defined directionality, which is either $3' \rightarrow 5'$ or $5' \rightarrow 3'$ relative to the strand to which the enzyme is bound. However, this classification is really only applicable to those helicases that bind a ssDNA (single-stranded DNA) terminus and then translocate along that ssDNA 'tail' before commencing duplex unwinding from the ssDNA–dsDNA junction. Helicases that can unwind from a fully duplexed (blunt-ended) DNA terminus are much harder to classify on this basis. The second method of classification, based on sequence conservation, indicates that helicases comprise at least five superfamilies, of which superfamilies 1 and 2 (SF1 and SF2) contain the most members. A detailed review of helicases in general is outside the scope of this article, and we refer readers to other excellent recent review articles [1,2].

Given the requirement to separate the Watson and Crick strands of DNA to effect key steps in DNA replication or repair (etc.), it is not surprising that mutation of genes encoding helicases often has severe consequences. Indeed, many helicase genes are essential for cell viability, although this is not a universal feature. It is possible that redundancy exists in some helicase families (such as in the RecQ family, which is the subject of this article; see below), and this limits the severity of the phenotype in cases of loss of a single family member. Of great interest are those mutations in human helicase genes that lead to recognized hereditary disorders. The SF1 superfamily helicases form four helicase families, the Rad25, Rad3, SWI2/SNF2 and RecQ families, and includes members that, when defective, give rise to a variety of debilitating disorders.

This review focuses on the RecQ family of helicases (Figure 1), which was named after the *recQ* gene of *Escherichia coli*. RecQ is the sole member of the family in this organism. Lower eukaryotic species also generally only contain a single RecQ family representative; for example, Sgs1 in the budding yeast, *Saccharomyces cerevisiae*, and Rqh1 in the fission yeast, *Schizosaccharomyces pombe*. There are, however, multiple members in most higher organisms, with five being present in humans. Defects in three of these human RecQ helicases give rise to defined clinical disorders associated with cancer predisposition and variable aspects of premature aging. At the cellular level, all RecQ helicase-deficient mutants show genomic instability, although the detailed features of this instability can differ in different mutants and/or species. The major focus of our laboratory is on the relationship between a breakdown in the maintenance of chromosomal integrity and tumorigenesis, and as a result we will concentrate in this article on the human RecQ helicases encoded by the *BLM*, *WRN* and *RECQ4* genes, defects in which give rise to the cancer predisposition disorders BS (Bloom's syndrome), WS (Werner's syndrome) and RTS (Rothmund–Thomson syndrome) respectively. [Note that the approved name for *RECQ4* as recommended by the

Abbreviations used: ALT, alternative lengthening of telomeres; AT, ataxia telangiectasia; ATM, ataxia telangiectasia mutated; ATP[γS], adenosine 5′-[γ-thio]triphosphate; ATR, ataxia telangiectasia related; BS, Bloom's syndrome; DNA-PK, DNA-dependent protein kinase; DNA-PK$_{cs}$, catalytic subunit of DNA-PK; DSB, DNA double-strand break; dsDNA, double-stranded DNA; ES cells, embryonic stem cells; FA, Fanconi anaemia; FEN1, flap structure-specific endonuclease 1; FFA-1, foci forming activity-1; HR, homologous recombination; HRDC, helicase RNase D C-terminal; HU, hydroxyurea; MLH, mutL homologue; MMS, methyl methanesulphonate; MSH, mutS homologue; NHEJ, non-homologous end-joining; NLS, nuclear localization signal; 4NQO, 4-nitroquinoline-1-oxide; PCNA, proliferating-cell nuclear antigen; PML, promyelocytic leukaemia; Polδ, DNA polymerase δ; RAR, retinoic acid receptor; RFC, replication factor C; RNAi, RNA interference; RPA, replication protein A; RQC, RecQ family C-terminal; RTS, Rothmund–Thomson syndrome; SCE, sister-chromatid exchange; SDSA, synthesis-dependent strand annealing; SSB, single strand binding protein; ssDNA, single-stranded DNA; SUMO-1, small ubiquitin-related modifier-1; TRF, telomeric repeat binding factor; WS, Werner's syndrome.

[1] To whom correspondence should be addressed (e-mail ian.hickson@cancer.org.uk).

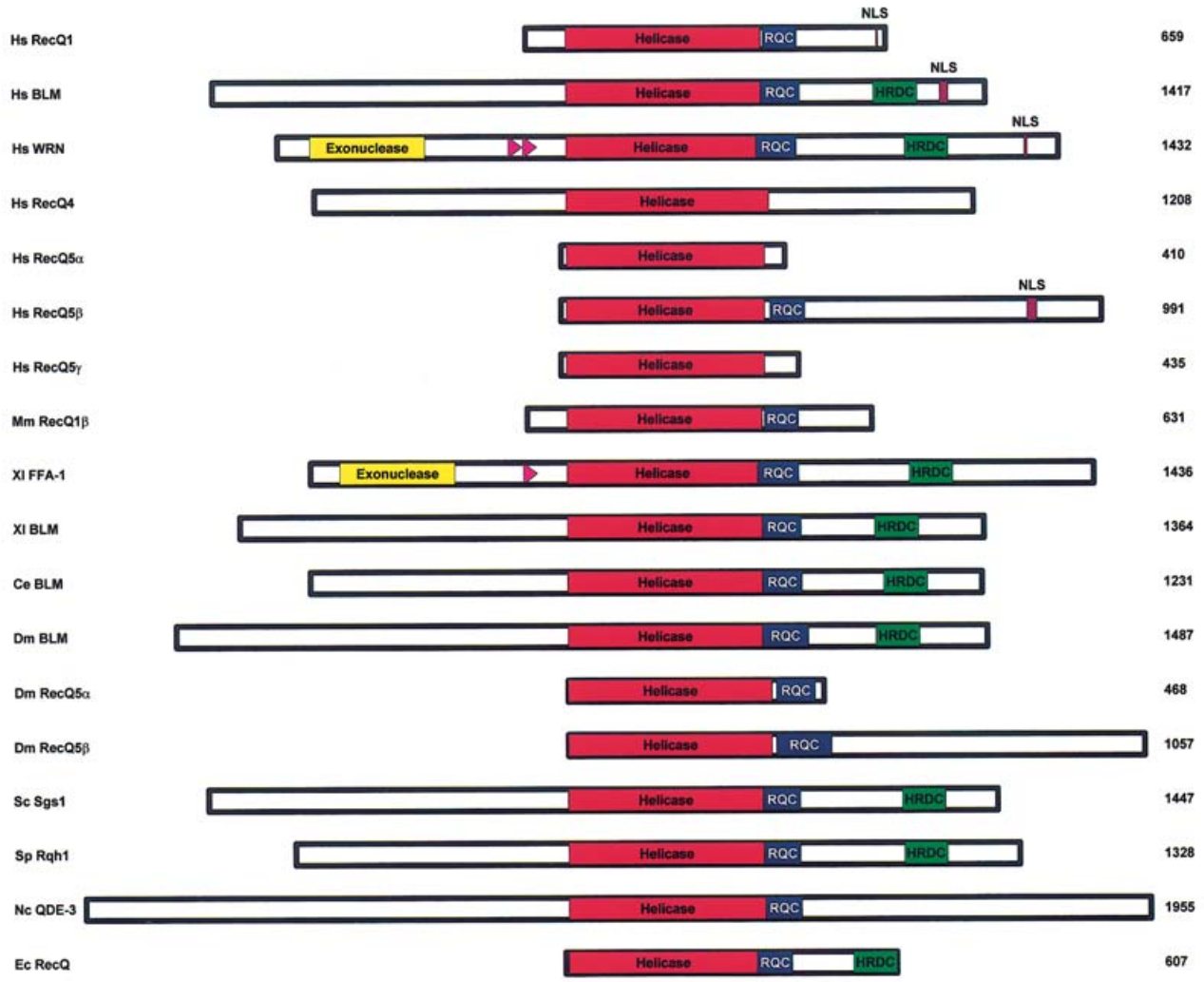

Figure 1 Schematic representation of RecQ helicases of various organisms

The names of the organisms (Hs, *Homo sapiens*; Mm, *Mus musculus*; Xl, *Xenopus laevis*; Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster*; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosacharomyces pombe*; Nc, *Neurospora crassa*; Ec, *Escherichia coli*) and the protein names are listed on the left. The sizes of the proteins (number of amino acids) are indicated on the right. The conserved helicase, RQC and HRDC domains are depicted in red, blue and green respectively, and labelled within the boxes. The conserved exonuclease domain of WRN and FFA-1 is indicated in yellow. The NLS sequences, where known, are shown in purple. The 27-amino-acid direct repeat of WRN, which can be found only in one copy in FFA-1, is indicated by pink triangles.

HUGO Gene Nomenclature Committee is *RECQL4* ('RecQ protein-like 4)'.] BS is of particular interest in this context, because affected individuals have an enormously elevated risk of cancer, and are susceptible to most, if not all, types of cancer. WS and RTS, although associated with an above average incidence of cancer, are associated primarily with features other than cancer predisposition – premature aging in WS, and skin and skeletal abnormalities in RTS. We will compare and contrast the biochemical properties of various RecQ helicases and discuss what information has been gleaned from the characterization of multienzyme complexes that include a RecQ helicase. Finally, we will give our personal view on probable roles of RecQ helicases in processes that contribute to the maintenance of genomic integrity.

COMMON FEATURES OF RECQ HELICASES

The RecQ family is defined by the presence of seven highly conserved amino acid sequence motifs (I, Ia, II–VI) in the so-called helicase domain, which is generally centrally located in RecQ family members (Figure 1). Just C-terminal to this domain is the RQC (RecQ family C-terminal) domain, which is also a characteristic of the RecQ family and appears to mediate protein–protein interactions (Figure 1; and see below). However, this domain is either absent from some family members or its sequence has diverged significantly enough to make its identification less straightforward. The C-terminal region of RecQ members also contains the HRDC (helicase RNase D C-terminal) domain (Figure 1), which has a probable role in DNA binding. Outside of these domains, there is little sequence similarity between family members. The WRN protein and its homologues (Figure 1) contain a domain near the N-terminus that is conserved in several nucleases, and biochemical studies have indicated that WRN possesses $3' \rightarrow 5'$ exonuclease activity (see below).

Structural models of two of the conserved domains have been provided. The three-dimensional structure of the helicase domain of the BLM protein was modelled based on the crystal structure of the helicase domain of PcrA. Based on this structural model, Rong

and co-workers [3] suggested that motifs Ia, III and V contain residues that establish direct contact with ssDNA. Motifs I, II–IV and VI form a pocket for binding ATP. Many of the point mutations identified in BS patients fall into these conserved helicase motifs, and eliminate the ATPase and helicase activities of the enzyme. The three-dimensional structure of the HRDC domain of *Sacch. cerevisiae* Sgs1 was determined directly, and used as a basis for establishing a general structural model of the HRDC domain. The HRDC domain exhibits structural similarity to domains of other helicases, recombinases and polymerases, in which it provides an additional contact to the DNA substrate, but expresses no enzymic activity. Indeed, the HRDC domain of Sgs1 has been shown to bind ssDNA *in vitro*, although with a low affinity, which suggests an auxiliary function in substrate recognition. The basic amino acids of the surface of the Sgs1 HRDC domain are not conserved in the human RecQ orthologues, which results in different characteristics: less positive charge and altered hydrophobicity to the surface [4,5]. These structural differences suggest pronounced functional diversity of the HRDC domain.

All members of the RecQ family of helicases express ATPase activity, which is dependent on DNA and Mg^{2+} cofactors. ssDNA gives a much better stimulation of ATPase activity than dsDNA in all cases, except with Sgs1 [6]. Longer ssDNA molecules are significantly better stimulators of the ATPase activity, which suggests that the RecQ helicases translocate along the ssDNA substrate processively [7–9]. The Mg^{2+} cofactor can be replaced by Ca^{2+} or Mn^{2+}, but Zn^{2+} ions are inhibitory. The ATPase activity of the RecQ helicases is not particularly selective. Although reports about the nucleotide selectivity of the various members are somewhat inconsistent, apart from ATP, all family members studied thus far accept and hydrolyse dATP. Less selective are the dmRecQ5α [10] and hBLM [11] enzymes, which can reportedly utilize GTP and dGTP.

The RecQ family of DNA helicases unwind DNA in the $3' \rightarrow 5'$ direction in relation to the DNA strand to which the enzyme is bound. The helicase activity depends on the hydrolysis of ATP, which itself is Mg^{2+}-dependent. Non-hydrolysable ATP analogues, such as ATP[γS] (adenosine 5′-[γ-thio]triphosphate) or adenosine 5′-[β,γ-imido]triphosphate, do not substitute for ATP; moreover, addition of ATP[γS] to a complete reaction mixture abolishes helicase activity, indicating competitive inhibition. The hydrolysis of ATP provides energy for the processive translocation of the enzyme [6,8,10,12–19]. There are major differences in substrate specificity and processivity among the different RecQ enzymes, and these are summarized in Table 1.

BLM

BS

BS is an extremely rare, autosomal recessive genetic disorder of humans. The gene mutated in BS, designated *BLM*, lies on chromosome 15q26.1 [20]. The positional cloning of *BLM* was facilitated largely by the existence, in some patients, of a population of revertant cells with low SCEs (sister-chromatid exchanges) (see below for a discussion of the cell phenotypes seen in BS). This phenomenon was predicted correctly to emerge from an intragenic somatic recombination event (crossing over) in those cases where the patient was a compound heterozygote [21,22].

BS is characterized by proportional dwarfism. Apart from the disproportionately small head and somewhat unusual skull and facial configuration, the body proportions of BS patients appear normal. The skin shows erythema, mostly on the nose and cheeks, and this can become chronic and more widespread following sun exposure. The level of intelligence is on the low side of average, often accompanied by learning disabilities and occasional moderate mental deficiency. Both sexes show impaired fertility. Men are sterile, have abnormally small testes, and their semen contains no sperm. Women, although fertile, have an irregular pattern of menstruation, which generally ceases at an unusually young age. A major clinical feature is immune deficiency; bacterial infections of the ear and lung are common, which can be life-threatening. Type II (non-insulin-dependent) diabetes mellitus has also been observed in young patients, which is rare in the general population.

BS patients also show a very high incidence of cancers of most types (although lung and prostate cancers are rare), with a very early average age of onset (mean of 24 years of age). Leukaemias predominate in childhood, and lymphomas and carcinomas appear during adolescence and early adulthood. Some normally rare tumours, such as osteosarcoma, Wilms tumour, medulloblastoma and meningioma, occur with a high prevalence in BS cases. The incidence of multiple neoplasms is also unusually high [23].

Most mutations in the *BLM* gene lead to severe truncation of the BLM protein due to either a nonsense mutation or a frameshift event leading to a premature termination codon. Interestingly, truncated protein species cannot generally be identified *in vivo*. Since the NLS (nuclear localization signal) of the BLM protein is located close to the C-terminus (Figure 1), prematurely terminated translation products would not be expected to be transported into the nucleus, and are presumably degraded rapidly in the cytoplasm. Post-transcriptional mechanisms must also be involved in the down-regulation of the mutated gene product in some cases, since the abundance of the mutant mRNA is also generally low in BS cells, probably due to nonsense-mediated mRNA decay. Approx. 15 % of BS cases are due to missense mutations in BLM [24], and these mostly map to exons encoding the helicase domain of the enzyme. Such mutations render the encoded enzyme catalytically inactive. Some of these single amino acid substitutions are denoted in Figure 2.

Recent data indicate that heterozygous carriers of a *BLM* mutation may be faced with a higher probability of developing colorectal cancer. Ashkenazi Jews with colorectal cancer were reported to be 2.76 times more likely to be carriers of the BLM^{Ash} mutation (a six-nucleotide deletion and a seven-nucleotide insertion at position 2281 of the cDNA, with a carrier frequency of $\sim$1 % among this ethnic population [25]) compared with a disease-free Ashkenazi Jewish control cohort matched for sex, age and geographical location [26]. Results from analysis of a transgenic mouse model seemingly also support the notion that carriers of a single defective *BLM* allele are cancer-prone [27]; however, given the number of mice studied, the statistical power of that study was somewhat low (see below).

Knockout mice

Given the severity and diversity of the symptoms displayed by human BS subjects, there was a great demand for the establishment of a mouse model of BS. This was achieved in several laboratories. The resulting BLM ‘knockout’ animals show strikingly different phenotypic features, which might be the consequence of the different strategies employed in their creation.

Five BS knockout alleles have been generated, four of which will be described in more detail below. Chester et al. [28] replaced part of the *blm* gene upstream of the helicase domain with a neomycin resistance cassette. Goss and co-workers [27] targeted exons 10–12 with an HPRT (hypoxanthine–guanine phosphoribosyltransferase) cassette. Luo et al. [29] targeted the first coding exon, exon 2, with a LoxP/neo cassette, but an unusual combination of insertion and amplification created three tandem copies

Table 1 Substrate specificity of various RecQ helicases

DNA substrates are shown diagrammatically in the left column. One tick in the column below an enzyme indicates robust unwinding, and three ticks indicate a preference for that particular substrate above others. No unwinding is indicated by ∅; detectable but very limited unwinding is indicated by ~. Respective references are given in the right column for each enzyme. The majority of these substrates were made up from synthetic oligonucleotides of various lengths. [a]This large Holliday junction substrate is made from a gapped double-stranded plasmid and a partially homologous linear duplex, and generated by RecA-mediated strand exchange. [b]G4 substrate. G-DNA can readily form *in vitro*, although its existence is still to be confirmed *in vivo*. In G-DNA, interactions between runs of guanines are stabilized by non-Watson–Crick bonding. Four guanines associate to form a G-quartet, a planar structure in which Hoogsteen pairing stabilizes interactions between adjacent guanines. The structure is stabilized by univalent cations. The presence of Na^+ favours the intermolecular interaction between four parallel DNA strands, referred to as G4 DNA, while in the presence of K^+ ions the G-quartet is formed between two antiparallel loops of DNA strands, referred to as G2′DNA. *E. coli* RecQ shows no preference for G4 substrates, which may reflect the fact that there are no characteristic G-rich domains in the *E. coli* genome [296].

		BLM		WRN		RecQ1		dmRecQ5 α		dmRecQ5 β		Rqh1		Sgs1		*E.coli* recQ	
		∅	[59, 60]	∅	[59, 148, 297]	∅	[19]	∅	[10]	∅	[228]					✓	[12, 13, 234, 236]
		∅	[59, 60]	∅	[297]	∅	[19]							∅	[256]	✓	[236]
		~	[59]	~	[59, 298]	✓	[19]	✓	[230]					✓	[256]	✓	[236]
		∅	[299]	∅	[297]												
		∅	[11]	∅	[11]												
	4 bp	∅	[59]					∅	[230]								
	8 bp and larger	✓	[59]	✓✓✓	[59, 148]	~	[19]	~	[230]								
		✓	[11, 59, 83, 299]	✓	[11, 59, 83, 157]			✓	[230]					✓	[15, 256, 300]	✓	[236, 296]
				✓	[301]			✓	[230]								
								✓	[230]					✓	[256]	✓	[236]
		✓✓✓	[59, 302]	✓	[59]			∅	[230]					✓	[256, 302]	✓	[236]
a	2.7 kb	✓	[61]	✓	[149]												
	17 – 30 nt	✓	[7, 285]	✓	[16, 160, 184, 303]	✓	[19]	✓	[10]	✓	[226, 228]			✓	[6]		
	33 – 40 nt			✓	[160]	∅	[19]					✓	[14]				
	42 – 100 nt	✓	[7, 8, 60, 88, 94, 285, 299]	∅	[16, 88, 184, 185]			✓	[10]	✓	[228]			✓	[6]	✓	[12, 234]
	109 – 343 nt	∅	[7]			∅	[19]							✓	[6]	✓	[234]
	558 nt and larger	∅	[7]											∅	[6]		
		✓	[60]	✓	[153]												
		✓	[11]	✓	[11]												
b	blunt	∅	[299]	∅	[59, 304]									∅	[15]		
	3' tail	✓✓✓	[59, 299, 302]	✓✓✓	[59]									✓ ✓ ✓	[15, 300, 302]	✓	[296]
	5' tail			✓	[304]												

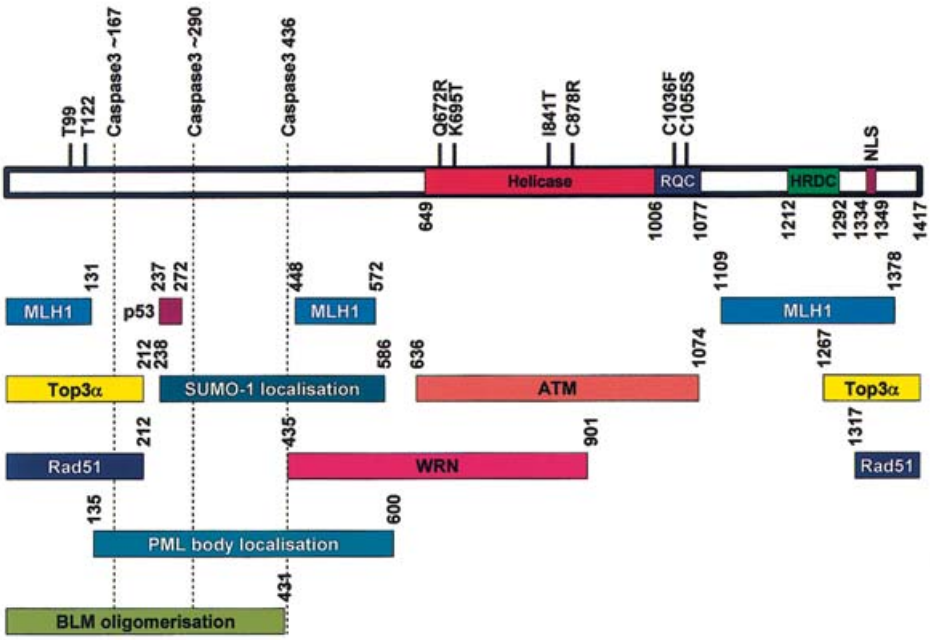

Figure 2 Domain structure and map of the protein interaction regions of BLM

Conserved domains of BLM are shown with the same colours as in Figure 1. Below this are shown regions of the protein where interactions with other proteins have been identified or which have defined functions. The amino acid positions given are the boundaries of the smallest peptide or recombinant fragment that harbours the appropriate interaction site. Selected missense mutations giving rise to single amino acid substitutions are indicated above the helicase and RQC domains. Removal of the N-terminal domain by caspase-3-mediated cleavage eliminates several interaction sites, as indicated by the vertical black broken lines. See the text for further details.

of the targeting cassette, each flanked by a copy of exon 3. A new allele was generated from this latter targeted allele by the excision of the intervening foreign sequences between the two most distal LoxP sites after crossing with a Cre recombinase-expressing mouse; this left behind a duplicated exon 3, but otherwise no foreign sequences [29]. The above alleles are all apparently null, since expression of the predicted truncated proteins was not detected in any of the studies. Hence these mice should be perfect models for the majority of BS cases. However, unlike in human BS, three of the cases showed embryonic lethality when the targeted allele was homozygous, and only one developed to maturity.

The knockout mouse model of Chester and co-workers [28] re-iterates some features of human BS. The short stature that is so characteristic of human BS is thought to be due to a uniform and proportional reduction of the number of cells constituting the body. In the early stages of the embryonic development of these homozygous mutant mice, the number of cells that constituted the embryo was shown to be greatly decreased compared with wild-type or heterozygote mice, due to an increased rate of apoptosis. At later stages, the increased rate of apoptosis was no longer evident; by then, however, the development of these embryos was delayed by 0.5–1 day compared with wild-type embryos. Nevertheless, these embryos appeared normal with regard to morphology, proportions and orientation. The death of the homozygous embryos was eventually caused by extreme anaemia at 13.5 days *post coitum*. Isolated red blood cells showed an increased frequency of micronuclei. These symptoms might also be common to human BS, as there is some indication of occasional embryonic lethality and mild anaemia in human BS [30]. Immortalized cell lines isolated from the mutant embryos are viable and show a high frequency of SCEs, as is seen in human BS cells.

The homozygous *blm* allele created by Goss et al. [27] also led to embryonic lethality. However, SCE analysis of primary embryonic lung fibroblasts showed no differences between homozygous mutant and wild-type cells, although the frequency of micronuclei was marginally increased (2 times). Heterozygous mice, which developed to term and appeared normal, showed an increased susceptibility to the development of cancers when either infected with murine leukaemia virus or crossed with mutant mice heterozygous for a mutation of the *APC* gene. The lack of the hallmark phenotype of BS, i.e. the high frequency of SCEs, suggests that the embryonic lethality of these mice may not be due to the homozygous disruption of *BLM*, but rather to a pleiotropic effect of the foreign sequences left behind in the genome after gene targeting.

Mice homozygous for the amplified LoxP/neo/exon 3 allele generated by Luo et al. [29] also showed embryonic lethality. However, this embryonic lethality was no longer evident when the integrated foreign sequences were excised with the Cre recombinase, leaving behind a *BLM* allele with a duplicated exon 3. ES (embryonic stem) cells and embryonic fibroblasts isolated from these mice show the hallmark elevated level of SCEs. The viable animals are apparently normal in many respects; they show no indication of a smaller than normal size or immunodeficiency, apart from a marginal decrease in the level of IgM. These mice, however, are cancer-prone. By the age of 20 months, nearly one-third (29%) of them had developed cancer, mainly lymphomas and carcinomas.

Although in many ways contradictory, the above results, taken together, support the notion that the murine *blm* locus is susceptible to an as yet uncharacterized pleiotropic effect of foreign sequences that remain in the locus following gene targeting. This is a phenomenon observed previously in mouse genetics. For

example, the foreign sequences might be influencing the expression of an adjacent essential gene. If this were the case, the viable mouse described by Luo et al. [29] would represent the true model of human BS. Clearly, it would be interesting to know, therefore, if these mice show the characteristic phenotypes of human BS other than those described, such as subfertility and immunodeficiency. Alternatively, the mice of Luo et al. [29] might be hypomorphic, and express a very low level of BLM protein that is not detectable with most available reagents, but is nevertheless sufficient to rescue the defective embryonic development. In this scenario, the true effect of *BLM* disruption in the mouse is embryonic lethality. At this stage it is difficult to distinguish between these possibilities.

Cellular phenotype

Cell lines established from BS patients generally show the same types of chromosomal aberrations as do normal cells, but the rate of occurrence of many types of these aberrations is higher. There is a striking tendency for the formation of spontaneous genetic exchanges, which are also apparently error-prone: gaps, breaks and structurally rearranged chromosomes occur at an elevated frequency [30–32]. However, the hallmark feature of BS cells, which is used in diagnosis in humans, is the approx. 10-fold increase in the frequency of SCEs [33,34]. Isolated ES cells, lymphocytes, primary or immortalized fibroblasts of *BLM* knockout mice [28,29] and *BLM*$^{-/-}$ DT40 cells [32,35] all show this increase in the frequency of SCEs. The SCEs are generally thought to represent HR (homologous recombination) events occurring between sister chromosomes during S phase or G_2. Moreover, BS cells [36,37], as well as fibroblasts isolated from heterozygous carriers, show a propensity to accumulate micronuclei, or to bud-out micronuclei at an unusually high rate [38].

ES cells isolated from *BLM*$^{-/-}$ mutant mice show an elevated frequency of gene targeting [29], which is also a characteristic phenotype of BLM-deficient chicken DT40 cells [32,35]. Increased loss of heterozygosity of the Gdf9/PGK-hprt locus in the *BLM*$^{-/-}$ mutant mice [29] is also indicative of an elevated level of HR in *BLM*$^{-/-}$ cells. This proposal is supported further by the constitutively high number of sites of ongoing HR repair (defined by the presence of the RAD51 protein in nuclear foci) in BLM-deficient cells, even outside of S phase when RAD51 foci are normally observed [39]. It appears that not only is HR elevated in BS cells, but NHEJ (non-homologous end-joining) activity is also abnormal. NHEJ is an error-prone pathway for repair of DSBs (DNA double-strand breaks) [40]. Using an *in vitro* plasmid rejoining assay [41], an increased frequency of inaccurate rejoining of DSBs was found in BS cell extracts compared with normal controls [42]. This error-prone repair was evident only when the assay plasmid was dephosphorylated after generating the DSB. When the 5′ phosphate groups were retained, and therefore the plasmid remained readily re-ligatable, there was no apparent difference between wild-type and BS cells [43].

Reviewing the sensitivity of BS or *BLM*$^{-/-}$ cell lines to treatments with different DNA-damaging agents is not straightforward, because the published results are somewhat contradictory, perhaps due to the experimental systems employed. BS cells show a cell cycle phase-specific increase in the number of chromosome aberrations induced by γ-irradiation, and they are prone to the induction of chromosome aberrations when irradiated in the late S or G_2 phases [44–46]. Some reports show increased [35,47,48] and some decreased [46] sensitivity to γ-irradiation or radiomimetic drugs, while others show no changes at all compared with wild-type cells [32,48]. Given the apparent lack of obvious adverse reactions in BS patients receiving radiotherapy, it seems unlikely that BS cells are significantly sensitive to γ-irradiation. Data on sensitivity to the topoisomerase I inhibitor camptothecin are also contradictory, even in studies using the same *BLM*$^{-/-}$ DT40 cells line [32,35]. The sensitivity of *Sacch. cerevisiae sgs1* and *Schiz. pombe rqh1* mutants to the ribonucleotide reductase inhibitor HU (hydroxyurea) is well established; however, inconclusive results have been reported on the sensitivity to HU of vertebrate BLM-deficient cells. Normal HU-induced S-phase arrest and recovery was reported to take place in BS [49] and *BLM*$^{-/-}$ DT40 [48] cells, while Beamish et al. [47] reported increased sensitivity, and Ababou and co-workers [49] decreased sensitivity, of BS cells to HU and HU-induced apoptosis respectively. Increased sensitivity of BS cells to treatment with ethyl methanesulphonate [51] or mitomicin C [52], and of *BLM*$^{-/-}$ DT40 cells to treatment with MMS (methyl methanesulphonate) or 4NQO (4-nitroquinoline-1-oxide) [32,35,48], has also been reported.

Consistent with a defect in some aspect of DNA replication (see below), chain elongation during DNA synthesis occurs at a significantly lower rate in BS cells than in normal control cells [53,54]. Moreover, BS cells accumulate abnormally sized DNA replication intermediates [55]. UV-irradiated BS cells show a significant decrease in the level of bromodeoxyuridine incorporation compared with control cells. This indicates that BS cells have a problem either repairing the photoadducts generated or dealing with replication forks stalled at UV lesions [49]. Consistent with this, *BLM*$^{-/-}$ DT40 cells that are UV-irradiated in S phase show an elevated frequency of chromatid gaps, breaks, quadriradials and chromatid exchanges [35,48].

Characteristics of the BLM protein

The BS protein is 1417 amino acids in length, with a predicted molecular mass of 159 kDa. Size-exclusion chromatography studies indicated that the native molecular mass of the human BLM enzyme is around 800 kDa, which presumably is the result of oligomerization. Consistent with this, electron-microscopic data obtained by negative staining of BLM particles showed a tetrameric or hexameric ring structure for BLM [56]. These results were supported further by size-exclusion chromatography studies using a recombinant N-terminal fragment of BLM, BLM$^{1–431}$, which suggested that this fragment forms oligomers (Figure 2) [57].

The mouse *BLM* orthologue encodes a 1416-amino-acid protein, which is 78 % identical/85 % similar at the amino acid level to human BLM [17,58]. The N-terminal regions of human and mouse BLM proteins differ in two short acidic stretches encoded by GAG and GAT repeats, the repeat number of which even differs between different mouse cDNA clones that have been isolated [17].

BLM is able to unwind a large array of substrates of different structure and length (Table 1) in a time- and enzyme concentration-dependent manner. There is an apparent preference for the unwinding of synthetic Holliday junctions, G4 DNA [59] and D-loop-containing [60] DNA substrates. BLM is also capable of branch-migrating RecA-generated Holliday junction recombination intermediates over distances of as much as 2.7 kb [61]. This apparently processive activity contrasts with the poorly processive helicase activity, although the latter is modulated by an association with RPA (replication protein A) (see below).

The expression of the BLM protein shows a discernible pattern of tissue-, cell type- and cell cycle phase-specific distribution. *In situ* hybridization studies indicated that BLM is expressed in all tissues where active cell proliferation takes place, and that it is restricted to the actively dividing cells. Most tumour cells also

express BLM [62]. Not surprisingly, therefore, the tissues/organs with the highest expression are the testis, ovary, thymus and spleen [28], which are also some of the organs that show major pathological features in BS cases.

Expression of BLM has been reported to be regulated during progression through the cell division cycle. However, some reports on the cell cycle regulation of BLM distribution should be viewed with some caution, as the synchronization methods employed involved the use of drugs that are thought to damage DNA either directly or indirectly [63,64] and this may have affected the results obtained. Data obtained using alternative methods, such as differential elutriation [64] or fluorescence-activated cell sorting [65] without blocking the cell cycle, reveal cell cycle stage-specific changes in BLM expression and subnuclear localization. BLM protein is barely detectable by Western blotting in G_1 cells, which is consistent with immunohistochemical analysis showing that quiescent or G_1 phase cells show only faint, diffuse nuclear staining. During S and G_2 phases, the amount of BLM increases greatly, and more cells can be observed with a focal distribution of BLM staining. In early and mid S phase, these foci sometimes overlap with sites of bromodeoxyuridine incorporation or the PCNA (proliferating-cell nuclear antigen) protein, which together define sites of DNA replication. In late S phase, however, co-localization of BLM with PCNA and sites of bromodeoxyuridine incorporation becomes more obvious [38,65]. The very low level of BLM in G_1 cells might be the result of proteolytic degradation after mitosis [65]. BLM has also been shown to be present in the nucleolus of several, although not all, cell lines [64].

The expression of BLM is induced by treatment of cells with γ-irradiation or various cytotoxic drugs, such as the radiomimetic bleomycin and the DNA replication inhibitors HU and aphidicolin. γ-Irradiation induces a rapid increase in the level of BLM mRNA, while the increase and subsequent decay in the amount of BLM protein follow much slower kinetics. The peak level of BLM protein coincides with arrest in G_2 phase. Abolishing the G_2 arrest with caffeine also abolishes induction of BLM, suggesting a causal link. Induction of the BLM protein following DNA damage coincides with the increased formation of nuclear foci containing BLM, which largely co-localize with nuclear bodies containing the PML (promyelocytic leukaemia) protein [also called PML oncogenic domains (PODs); see below]. These bodies also co-localize, especially in late S phase, with the RAD51 protein and RPA. The induction of BLM expression and the formation of BLM foci following DNA damage can also be induced in cells that are mutant for any of p53, DNA-PK_{cs} [the catalytic subunit of DNA-PK (DNA-dependent protein kinase)] and ATM (ataxia telangiectasia mutated); however, in cells expressing a dominant-negative form of PML, most of the RAD51 and BLM remains dispersed in the nucleoplasm [63,65,66].

The high level of expression in the testis, together with the known infertility of male BS patients, suggest an important function of BLM during spermatogenesis. In male mice, where spermatogenesis commences fairly synchronously, the mRNA for BLM begins to increase 12–14 days after birth, when early pachytene spermatids start to appear [58]. Fluorescence microscopy studies revealed that proteins required for meiotic HR appear in meiotic cells in a successive way in the consecutive stages of meiotic prophase. During the leptotene stage, RAD51/DMC1 foci are the most abundant, and are thought to indicate sites of active DNA homology searching and strand exchange. During late leptotene, RAD51 is replaced by RPA via a transitional phase at sites of interhomologous DNA interactions where RAD51 and RPA co-localize. In the zygotene stage, RPA apparently recruits BLM and MSH4 (MutS homologue 4), and the RAD51 foci then disappear. The BLM foci co-localize fully with RPA. The early meiotic DNA–DNA interactions are probably resolved without reciprocal recombination by this protein complex, which may include other proteins, such as Top3α (HUGO Gene Nomenclature Committee approved name TOP3A) [67]. Later, in the pachytene stage, the number of BLM and RPA foci declines, while MLH1 (MutL homologue 1) foci appear, and these co-localize with MSH4, but not with BLM [68–70]. ATM has also been reported to co-localize with RPA at sites where interhomologous DNA interactions occur during meiotic prophase [71].

Physical and functional interactions of BLM with protein partners

BLM interacts physically and functionally with an array of proteins whose primary role is in the maintenance of genome integrity. We discuss some of these BLM-interacting partners below.

PML. The *PML* gene was identified originally as the site of translocations in cases of acute promyelocytic leukaemia. The PML protein functions as a tumour suppressor, and has been shown to be involved in numerous cellular processes. In some cases of acute promyelocytic leukaemia, which result from a (15:17) reciprocal translocation, the *PML* gene is fused to the RARα (retinoic acid receptor α) gene, resulting in the expression of a fusion protein, PML–RARα. PML has been shown to localize to nuclear foci, termed PML nuclear bodies, along with several other proteins. Many of these proteins are involved in DNA metabolism. Further information on PML protein and PML bodies can be found elsewhere [72,73]. BLM co-localizes with PML in most cells [38,46,64,67,74,75], but co-localization is never complete. This suggests a regulated association of the two proteins, and that PML bodies might, therefore, be temporary storage sites for BLM. It seems likely that BLM shuttles to and from PML bodies while effecting its function. Nevertheless, it remains a possibility that the PML body itself is the primary site of action of BLM. In PML–RARα-expressing cells, the nuclear distribution of PML and BLM has been shown to be severely impaired, but treatment of these cells with retinoic acid can lead to the apparent 'rebuilding' of PML nuclear bodies, and a restoration of the correct localization of BLM. In cells isolated from PML knockout mice, BLM does not localize to discrete foci, but rather is found in a uniform nucleoplasmic pattern [74]. Deletion derivatives of BLM, spanning the region encompassing residues 135–600, localize primarily to the nucleolus when expressed ectopically in BS cells, and not to PML bodies, which implies that a signal to direct PML localization resides in this segment (Figure 2). Consistent with this, expression of $BLM^{\Delta 133-237}$ (i.e. BLM lacking residues 133–237) in normal cells results in a failure to localize to PML bodies [46]. Internal deletions within BLM that disturb localization of the protein to PML bodies have been shown not to affect the ability of BLM to correct the unusually high SCE phenotype of BS cells, suggesting that localization to PML bodies is not vital for BLM function [75]. Apparently in contradiction to this, cells isolated from PML-deficient mice show a slightly higher number of SCEs than do wild-type cells, although whether this is a BLM-related phenomenon is not clear [74].

BRCA1. BRCA1 is defective in many cases of hereditary breast cancer [76,77]. The nuclear relocalization and phosphorylation of BRCA1 that occur in response to DNA damage/replication blockade are not impaired in BS cells [50]. However, BLM and BRCA1 have been shown to associate in human cells by the use of co-immunoprecipitation analyses. Moreover, BLM has been identified as a component of a multienzyme complex, termed BASC, that has been partially purified from human cells. BASC contains BRCA1 and several other DNA 'surveillance' proteins,

including MLH1, MSH6, MSH2, RFC (replication factor C), RAD50 and ATM. The BLM and BRCA1 proteins also co-localize in untreated cells to discrete nuclear foci. Treatment of cells with HU greatly increases the number of co-localizing BRCA1 and BLM foci [78].

p53. p53 is a tumour suppressor protein that acts to regulate gene expression under conditions of cellular stress, and appears to control decisions regarding whether cells either arrest the cell cycle and repair DNA damage or commit to apoptosis. BLM and p53 seem to be involved together in regulating cell growth and apoptosis. Ectopic expression of wild-type p53 induces apoptosis in normal cells and results in a retarded growth rate in p53-deficient cells. However, this p53-mediated apoptosis is attenuated in BS cell lines. Since BS cells display normal sensitivity to Fas-induced apoptosis, this resistance to apoptosis is not a general, but is rather a p53-dependent, defect [46].

Direct physical interaction between BLM and p53 has been demonstrated *in vivo*. p53 phosphorylated on Ser^{15} partially co-localizes with BLM and, to a lesser extent, with RAD51 following cell cycle arrest induced by aphidicolin [79]. BLM and p53 can also be co-immunoprecipitated from cells [46,79]. *In vitro* binding of purified p53 and BLM has been demonstrated, and the respective interaction segment has been mapped to the N-terminal domain of BLM (Figure 2) [80]. p53 can inhibit the unwinding of Holliday junctions catalysed by BLM, but has no effect on the unwinding of a standard helicase substrate. A fragment of p53, $p53^{373–383}$, can also inhibit the unwinding of Holliday junctions by BLM. p53 has been shown to bind to the Holliday junction structure preferentially at the crossover point, which might represent ssDNA arising due to 'breathing' of the junction. These data suggest that p53 may regulate HR through its modulation of interactions of BLM with recombination intermediates [79]. The functional interaction between BLM and p53 seems to be reciprocal. Co-expression of p53 and BLM in p53-deficient cells has been shown to result in a significant increase in the activity of the p21 promoter, a recognized target for p53-mediated transcriptional activation [80].

RAD51. The eukaryotic RAD51 proteins are structural and functional homologues of the bacterial RecA protein, which plays a central role in HR and DNA repair. These proteins catalyse pairing between a ssDNA tail and a homologous stretch of dsDNA, and promote DNA strand exchange, an important step in the initiation of HR. Direct physical interaction between BLM and human RAD51 has been shown *in vivo* and *in vitro*, and the interacting regions on BLM have been mapped to the N- and C-terminal parts of the protein (Figure 2). BLM and RAD51 co-localize in certain untreated cells, although this co-localization is most probably restricted to late-S-phase cells. γ-Irradiation or aphidicolin treatment of cells increases the extent of co-localization, as well as the number of cells showing co-localizing foci [39,63]. In early meiotic prophase cells, RAD51 and BLM co-localize to sites of interhomologue–DNA interactions (see above) [68]. The interaction between BLM and RAD51 is likely to be relevant both to the role of BLM in a HR repair reaction (see below) and to the known biochemical properties of BLM, such as its ability to promote Holliday junction branch migration.

MLH1. MLH1 is one of the eukaryotic homologues of the *E. coli* MutL protein, which participates in the DNA mismatch repair pathway. Direct physical interaction between MLH1 and BLM has been demonstrated *in vitro* and *in vivo* with various techniques, and the MLH1-interacting regions of BLM have been mapped (Figure 2). The BLM and MLH1 proteins co-localize *in vivo* in untreated as well as in aphidicolin-arrested cells [81]. Since the helicase component of mismatch repair in eukaryotic cells (i.e. the orthologue of bacterial MutU) remains to be identified, Langland et al. [82] and Pedrazzi et al. [81] tested whether BLM can exert any influence on the process of mismatch repair. However, addition of BLM to mismatch-repair-deficient extracts had no effect on mismatch repair activity [81,82]. As BS cells are also apparently proficient in mismatch repair, a direct involvement of BLM in mismatch repair can probably be ruled out.

RPA. RPA is a heterotrimeric ssDNA-binding protein that is required for all aspects of DNA metabolism. BLM interacts physically with human RPA; this is a direct protein–protein interaction with the 70 kDa RPA subunit and is not mediated by DNA [7,82]. BLM has also been shown to co-localize with the 34 and 70 kDa subunits of RPA in normal human fibroblasts [64], and in the meiotic prophase of mouse spermatocytes (see above) [68]. Importantly, a functional interaction has been shown between RPA and BLM *in vitro*, which depends on specific protein–protein interactions between the two proteins. In the presence of human RPA, BLM can unwind a standard M13 partial duplex substrate in which the duplex portion is as long as 259 nt (Table 1), while the upper limit of unwinding for BLM alone seems to be around 100 bp. However, M13 substrates of 851 bp cannot be unwound even in the presence of human RPA, and hence RPA is limited in its ability to increase the processivity or extent of DNA unwinding catalysed by the BLM helicase. The stimulation of BLM helicase does not appear to result from a change in the specific activity of the ATPase [7,83].

Top3α. Topoisomerases catalyse the passage of intact DNA strands through transient breaks in other DNA strands, and hence promote the interconversion of different topological isomers of DNA. According to their mode of action, topoisomerases are classified into two groups. Type I topoisomerases, such as topoisomerases I and III, generate ssDNA breaks, whereas type II topoisomerases, such as topoisomerases II and IV, make dsDNA breaks. Genetic and functional interactions between topoisomerase III and RecQ enzymes have been well documented from studies in several organisms. Here we concentrate on the functional interaction between BLM and human Top3α. For a recent comprehensive review, see [84].

BLM and Top3α co-localize to discrete nuclear foci in exponentially growing, normal [67] or transformed human fibroblasts [85]. These foci correspond to PML nuclear bodies. Further evidence of an *in vivo* association between BLM and Top3α came from the observation that Top3α is not localized correctly in BS cells lacking BLM. Far-Western analysis indicated that there may be two separate binding sites for Top3α on BLM, at least *in vitro*, near the N-terminus and the C-terminus (Figure 2) [85]. Hu and co-workers [86], however, suggested that the N-terminal 133 residues of BLM are necessary and sufficient for the interaction with Top3α. Indeed, expression of full-length BLM can restore Top3α focal staining in BS cells, while $BLM^{\Delta 1–133}$ cannot. A derivative of BLM with an internal deletion, $BLM^{\Delta 131–237}$, does not localize to PML bodies, and, as a consequence, cannot recruit Top3α to PML bodies. Hu et al. [86] concluded from this that the functions of residues 1–133 and 131–237 of BLM are distinct: residues 1–133 mediate an interaction with Top3α, while residues 131–237 are responsible for localization of BLM (and therefore Top3α) to PML bodies. This latter region also overlaps with the PML localization region mapped by Yankiwski and co-workers [75] (Figure 2). Of probably functional significance is the fact that $BLM^{\Delta 1–133}$ does not reverse the hyper-SCE phenotype when expressed in BS cells, which suggests that the interaction between

Top3α and BLM is important for the suppression of hyper-recombination in BS cells [86].

BLM interacts functionally with Top3α and has been shown to stimulate the relaxation of negatively supercoiled plasmid DNA catalysed by Top3α A truncated version of BLM, BLM$^{213–1266}$, which lacks both binding sites for Top3α, fails to effect this stimulation, indicating the functional importance of direct protein–protein interaction between BLM and Top3α. These authors also showed that BLM can recruit Top3α to DNA structures, which may be the mechanism by which the functions of BLM and Top3α are co-ordinated *in vivo* [87].

Taken together, the above data suggest that BLM targets Top3α to its sites of action on the DNA, where DNA structures generated by the BLM helicase are required to be 'resolved' by the topoisomerase. If these structures are not resolved, due to either the lack of Top3α or the lack of an interaction between the BLM and Top3α proteins, the consequence is detrimental to DNA metabolism. Consistent with this general hypothesis are the findings that the BLM–Top3α interaction is important for SCE suppression (see above) and that defects in *Sacch. cerevisiae*/*Schiz. pombe top3* mutants are suppressed by mutation of *SGS1* and *rqh1*$^+$ respectively (see below).

WRN. The bulk of the discussion in this and other articles concerning details of RecQ helicase function understandably assumes that in organisms such as humans, where there are multiple family members, the different RecQ enzymes act independently. The reported association between WRN and BLM [88] indicates that this assumption may not always be valid. A direct interaction between WRN and BLM has been shown *in vitro*, and the respective interaction sites for BLM and WRN have been mapped (Figures 2 and 3). This interaction apparently has functional consequences, since it was shown that BLM inhibits WRN exonuclease function in a concentration-dependent manner. Despite these findings, it seems unlikely that WRN and BLM always function in concert (they presumably do under certain circumstances) in DNA metabolism. This assertion is based on two findings. First, the proportion of WRN that can be co-immunoprecipitated with BLM is generally low [88], suggesting only a limited number of shared functions. Secondly, a double *WRN*$^{-/-}$ *BLM*$^{-/-}$chicken DT40 cell mutant has a more severe phenotype than is seen for either of the single mutants, indicating roles for BLM and WRN in separate biochemical pathways. However, the hyper-recombination and high SCE phenotype of the single *BLM*$^{-/-}$ mutant DT40 cells can be partially suppressed by the disruption of *WRN*, which indicates epistatic relationships, and a probable common involvement in the HR pathway [35].

TRF2 [HUGO Gene Nomenclature Committee approved name TERF2 ('telomeric repeat binding factor 2')]. Telomeres are the specialized structures that cap the ends of linear chromosomes in eukaryotes. TRF1 and TRF2 are homodimeric proteins that have been shown to bind exclusively to double-stranded telomeric DNA throughout the cell cycle, and are thought to be involved in the regulation of telomeric length [89]. Regulation of telomeres generally requires telomerase, a specialized reverse transcriptase enzyme (a recent review of telomeres and telomerase can be found elsewhere [90]). Some human cell lines and primary tumours maintain their telomeres by utilizing a recombination-dependent mechanism called ALT (alternative lengthening of telomeres). In a significant proportion of ALT cell populations, but not in telomerase-proficient or primary cells, TRF1 and TRF2 have been shown to co-localize with BLM. This co-localization occurs in atypical, telomere-associated PML bodies. Successful co-immunoprecipitation of TRF2 and BLM provided evidence for an *in vivo* interaction between the proteins [91]. Moreover, purified BLM and TRF2 have been shown to associate *in vitro*, suggesting that the interaction is direct [83]. TRF2 has also been found to regulate the helicase activity of BLM *in vitro*. TRF2 can stimulate BLM to unwind a non-telomeric forked DNA substrate, as well as a forked substrate containing two telomeric repeats (to which TRF2 does not bind). Hence this stimulation appears to depend on the physical interaction between BLM and TRF2, and not on the 'targeting' of BLM to its substrate by TRF2 [83]. Nevertheless, TRF2 may additionally play this latter role *in vivo* at telomeres.

FA (Fanconi anaemia) proteins. FA is a rare chromosomal instability disorder associated with a wide range of features, including a predisposition to certain cancers. Cells from individuals with FA are hypersensitive to DNA cross-linking agents. There are eight known genetic complementation groups of FA [76]. A recent report [92] indicates that BLM is present in a complex with five of the known FA proteins (termed FANC A, C, E, F and G). These data suggest that there may be a functional connection between BLM and the FANC proteins, and possibly that there is a defect in a common molecular pathway in these apparently distinct cancer predisposition disorders.

Post-translational modifications

Regulation by phosphorylation. BLM is a phosphoprotein *in vivo* and appears to be phosphorylated during mitosis [47,65] and in response to γ-irradiation [63,66], HU or UVC treatment of cells [49,50]. The phosphorylated form of BLM appears to lose its association with the nuclear matrix, and to be found preferentially in the nucleoplasmic fraction [65]. The induction of phosphorylation by DNA-damaging agents suggests the involvement of stress-responsive kinases such as ATM, ATR (ataxia telangiectasia related) and DNA-PK. Their possible involvement has been tested with mutant cell lines, and with the use of drugs to inhibit kinase activity. In cells from patients with the cancer-prone disorder AT (ataxia telangiectasia), which lack ATM, mitotic phosphorylation of BLM and induction of phosphorylation in response to γ-irradiation are absent [49,50,66], but phosphorylation in response to HU or UVC treatment is unaffected [49,50]. In contrast, in cells that express a kinase-dead version of ATR, ATR^{Kd}, BLM is phosphorylated following IR, but not in response to HU treatment [50]. No evidence for a role for DNA-PK in the modification of BLM has been found thus far [50]. The induction of BLM protein level and its translocation to nuclear foci in response to UVC, HU or γ-irradiation are apparently not dependent upon phosphorylation. These processes are also not affected in AT cells, although the basal level of BLM appears to be significantly lower in AT cells than in wild-type cells [49,63]. Taken together, these data indicate that BLM is probably phosphorylated by ATM in response to γ-irradiation and by ATR in response to inhibition of DNA replication, although this latter conclusion is currently tentative.

A physical interaction between BLM and ATM has been shown using standard *in vivo* and *in vitro* techniques. The region of BLM that interacts with ATM has been mapped to the central part of the protein, which covers the helicase and RQC domains (Figure 2). However, the putative target sites for ATM have been shown to lie outside this region, at Thr^{99} and Thr^{122}, within the motifs E^{98}TQR^{101} and T^{121}TQN^{124}. Phosphorylation of Thr^{99} has been shown to increase after γ-irradiation in a dose-dependent manner. Substitution of Thr^{99} resulted in decreased (but not abolished) phosphorylation *in vitro*, suggesting the involvement of other sites

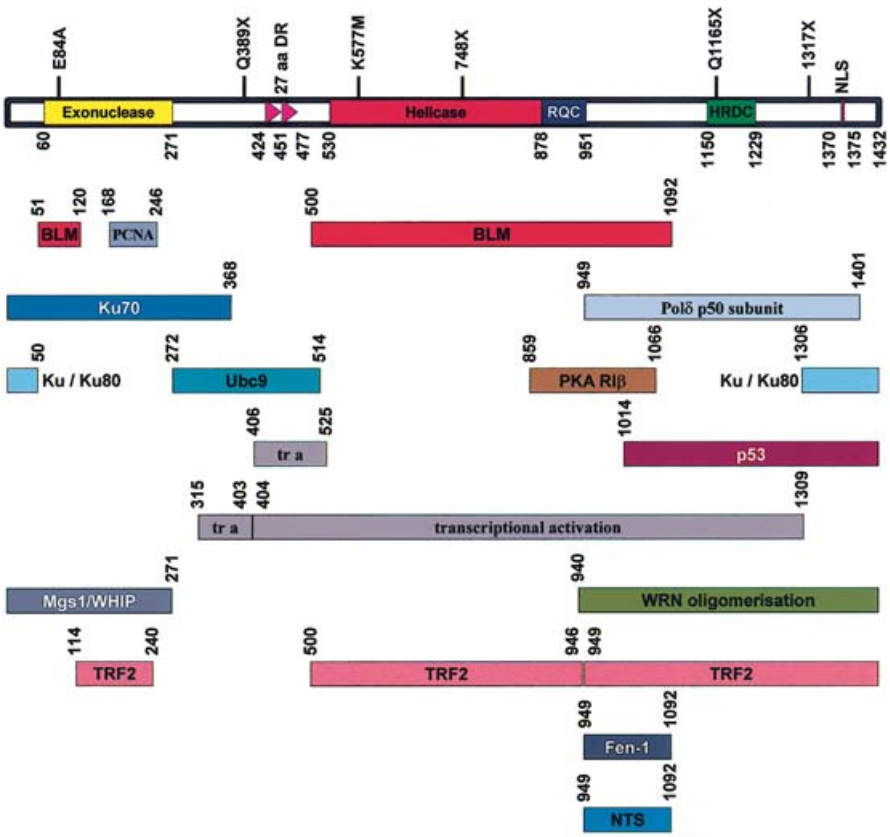

Figure 3 Domain structure and interacting protein partners of WRN

The conserved helicase, RQC and HRDC domains are shown as in Figure 1; the exonuclease domain is yellow. The 27-amino-acid direct repeat (27aa DR) is depicted by pink triangles. Protein interaction domains are shown as in Figure 2. The positions of the two 'laboratory' mutations, E84A and K577M, that eliminate the exonuclease and helicase activities of the enzyme respectively, as well as a few naturally occurring truncating mutations, are also shown. See the text for further details.

(possibly Thr^{122}). However, a T122A substitution alone did not abolish phosphorylation following γ-irradiation [47].

Beamish and co-workers [47] found that the basal level of ATM kinase activity is elevated in BS cells, and that these cells are slightly radiation sensitive, which can be corrected by expression of the wild-type BLM cDNA. These authors showed that expression of substituted BLM proteins, BLM^{T99G} and BLM^{T122A}, in BS cells failed to correct radiation sensitivity, but restored the SCE frequency to normal. Interestingly, they also showed that transfection of the BLM phosphorylation site mutants into normal sells radiosensitized them slightly, and also increased the number of radiation-induced chromosomal aberrations [47]. The functional significance of this phosphorylation for the activity of BLM and for its involvement in various cellular processes remains to be elucidated in detail.

Proteolytic degradation of BLM. As outlined above, expression of BLM is regulated during the cell cycle, being high in late S and G_2/M cells, but barely detectable in early G_1 cells. This suggests mitosis-specific degradation, although the mechanism for this is not known. Degradation of BLM triggered by programmed cell death (apoptosis) is, however, a far better characterized phenomenon. During apoptosis, cells or groups of cells are destroyed in an organized and tightly regulated manner. Although the signals that trigger apoptosis are of a varied nature, the process eventually converges on a group of key 'execution' enzymes called caspases. Caspases are proteolytic enzymes with tight cleavage specificity. They cleave their target only at a limited number of sites, which in some cases results in the activation of the target protein, and in other cases in the inactivation of the target. Perhaps in order to permit this organized DNA degradation to proceed unhindered, enzymes normally involved in DNA repair need to be inactivated; indeed, several of them [e.g. poly(ADP-ribose) polymerase, ATM and DNA-PK] have been shown to be targets of caspase-mediated cleavage. During induction of apoptosis, specific cleavage of BLM has also been detected, which does not apparently depend on the means by which apoptosis is induced [65,93–95]. Cleavage of BLM occurs within 2 h, as rapidly as does the cleavage of poly(ADP-ribose) polymerase, a widely used 'early' marker of the process.

Using specific protease inhibitors, the caspases responsible for the cleavage of BLM have been identified as caspase-3 and -7. These caspases have the same cleavage site specificity, although only caspase-3 is localized in the nucleus. Indeed, recombinant caspase-3 has been shown to cleave BLM specifically into fragments of 112 and 47 kDa *in vitro*. Moreover, caspase-3-deficient cells fail to cleave BLM after apoptosis induction, strongly implicating this caspase in the cleavage of BLM *in vivo*. There is some dispute concerning the exact position of the main cleavage site within BLM, although it is agreed that this site lies in a narrow region of the N-terminal domain. Freire et al. [94] located the cleavage site after the $D^{433}SLD^{436}$ recognition motif, between amino acids 436 and 437, by microsequencing. Bischof et al. [93] initially identified a putative recognition motif, $T^{412}EVD^{415}$, for

caspase-3, and then found that a modified BLM, BLM^{D415G}, was not cleaved *in vitro*. Caspase-3-mediated cleavage does not appear to affect the helicase activity of BLM, as the unwinding of several helicase substrates has been shown not to be affected. The cleavage does, however, affect the binding of BLM to its interacting partner, Top3α [94]. BLM is also released from the nuclear matrix as a result of the apoptotic cleavage. It is possible that loss of the N-terminal region of BLM might also abolish oligomerization [93], as well as disrupting other important protein binding sites (Figure 2).

BLM orthologues in other organisms

xBLM, the BLM protein isolated from *Xenopus laevis*, comprises 1367 amino acids and shows 64% overall similarity and 50% identity with the human BLM protein. In reconstituted *Xenopus* egg nuclei, DNA replication is severely inhibited when xBLM is depleted from the extract; significantly, this defect can be overcome by re-introduction of purified xBLM. The residual replication that remains after the depletion of xBLM cannot be accounted for by FFA-1 (foci forming activity-1) (Figure 1). *Xenopus* eggs are programmed to execute extremely rapid DNA replication. Completion of S phase takes only 10 min, compared with more than 8 h in somatic cells. In *Xenopus* eggs, this extremely fast replication imposes a great demand on DNA helicases and other replication factors, and xBLM might be involved in facilitating the unwinding of template ahead of the replication fork (perhaps to effect removal of DNA secondary structures that slow fork progression; see models below) to permit this burst of replication to proceed. As human BS cells show only a mild DNA replication defect [53–55], it is unlikely that human BLM is involved in an essential step in this process [96], although the dramatic rapidity of *Xenopus* embryo replication may have revealed an auxiliary role for BLM in replication that can be satisfied by other proteins in more slowly replicating somatic human cells.

The *mus309* allele was originally isolated in a screen for *Drosophila* mutants that are sensitive to mutagens [97] and was wrongly identified as a mutant *Ku70* allele [98]. Kusano and coworkers [99] showed that *mus309* mutants are defective in the *dmBLM* gene. The cDNA for *dmBLM* is predicted to encode a protein comprising 1487 amino acids (Figure 1). *mus309* compound heterozygote mutant flies are sensitive to MMS and sterile. They show a marked increase in the number of chromosome-loss and non-disjunction events in the male sperm, compared with wild-type controls or heterozygotes, which can be partially rescued by the ectopic expression of human BLM. Recent results indicate a role for dmBLM in DSB repair via the SDSA (synthesis-dependent strand annealing) pathway [100].

The *Caenorhabditis elegans* genome project predicted four genes (K02F3.1, F18C5.2, E03A3.2 and T04A11.6) that potentially encode RecQ helicases. Him-6, encoded by the T04A11.6 gene, is 1231 amino acids long with a predicted molecular mass of 139.4 kDa. It shows the greatest identity with human BLM and is the only *C. elegans* RecQ helicase to be characterized in detail. Kim et al. [102] studied the possible genetic interactions between Him-6 and Top3α by silencing *TOP3*α gene expression with RNAi (RNA interference) in a wild-type or a *him-6* mutant background. The depletion of Top3α in a *him-6* background generated an increased frequency of males due to X chromosome non-disjunction. Half of the F1 generation *him-6*/*top3*α^{RNAi} adult worms became sterile, although they showed no signs of developmental problems. The sterile gonads expressed signs of excessive DNA damage and metaphase arrest. As neither of the above phenomena was seen in either of the single mutants, these data indicate that Him-6 and Top3α may participate in different pathways for the maintenance of chromosomal stability [101]. Nevertheless, the Him-6 and Top3α proteins have been shown to interact physically [102].

WRN

WS

WS is an autosomal recessive disorder with an estimated frequency of 1–22 cases per million of the population world-wide. Interestingly, the majority of WS cases have been described in Japan: of the approx. 1200 cases reported worldwide, around 850 are in Japan, where the prevalence of heterozygous carriers in the population is predicted to be greater than 6 in 1000. Hence there are approximately 35 WS patients per million of the Japanese population [103]. The *WRN* gene has been positionally cloned, and it encompasses 180 kb in the p11–p12 region of chromosome 8 [104,105].

All of the *WRN* mutations identified thus far in WS patients lead to premature termination of translation due to creation of a termination codon, a frameshift with premature termination or a splicing error with the generation of a frameshift and termination (http://www.pathology.washington.edu/research/werner/ws_wrn.html). The level of mutant transcripts is often also down-regulated, probably due to nonsense-mediated mRNA decay [106]. As the NLS resides near the C-terminus of the WRN protein (Figure 3), the nuclear import of any stably expressed truncated WRN protein is predicted to be impaired [107], and the defective translation products appear generally to be degraded in the cytoplasm [108]. Nevertheless, some of the C-terminally truncated WRN protein products can be detected in Epstein–Barr virus-transformed lymphoblastoid cell lines, particularly those that originate from the Japanese type 1, 5 and 8 mutations [109]. Significantly, WRN mRNA [106] and WRN protein [108] levels in cells from heterozygous carriers are approximately half those seen in normal individuals, and cells from these carriers show intermediate levels of sensitivity to DNA-damaging agents (see below). In spite of the above findings, cells from several diagnosed WS cases have been shown to express an apparently normal WRN protein [108]. Given the large array of interacting protein partners of WRN (see below), a plausible explanation for these cases is the existence of mutations in genes encoding any one of the WRN-interacting partners [110]. However, no such mutation has been described thus far, to our knowledge.

The *WRN* gene is transcribed from multiple transcription start sites. The promoter structure of the gene is reminiscent of that of several housekeeping genes, and contains multiple Sp1 and AP2 (activator protein 2) elements, but no TATA-box motif. Indeed, Sp1, the retinoblastoma protein Rb and p53 have been shown to be involved directly in the regulation of *WRN* gene expression [111]. The expression of WRN is up-regulated directly by the large-T antigen of simian virus 40 during cytological transformation with this virus [112].

WS was first described by Otto Werner in 1904 in his doctoral thesis from Kiel University [113]. As some of the clinical features which he described resemble those published by Rothmund [114] (see section on RECQ4 and RTS below), a clear distinction between WS and RTS was not made until the work of Thannhauser [115], who listed the principal characteristics of WS. Generally, the condition becomes apparent in adolescence due to a lack of the usual growth spurt, and WS patients are, consequently, of short stature. Other clinical features become apparent between the ages of 20 and 30 years. Changes are especially pronounced on the face, which include thinning and sharpening of the nose, which leads to

a 'bird-like' appearance. The skin is thin and dry, and is affected by areas of variable pigmentation. Hyperkeratoses of the skin occurs frequently over the bony areas of the limbs, which can become ulcerated. Cataracts appear at an unusually early age and are often bilateral. One of the earliest signs of the disease is premature greying, thinning and loss of hair. A high incidence of osteoporosis and soft tissue calcification has also been noted. Most patients have been reported to have abnormally small genitalia, although this is not always coupled with infertility. WS patients also have a high incidence of the 'late onset' (Type II) form of diabetes mellitus, but this arises earlier in life in WS than is seen in the general population. The similarity of several of the symptoms of WS and the normal aging process makes WS one of the most widely studied premature aging syndromes. A number of symptoms are common to normal aging and WS, including atherosclerosis, arteriosclerosis, greying of the hair, hypermelanosis, cerebral cortical atrophy, lymphoid depletion and thymic atrophy. Several other symptoms, however, differ significantly between the two 'conditions', the most important of which are the higher incidence of early-onset malignancies [116] and the lower incidence of Alzheimer-like neuropathologies [117] in patients with WS.

The age of death of WS patients varies between approximately 30 and 65 years, with a mean of 47 years. The two most common causes of death are malignancies and cardiovascular disease [116]. WS is associated with an excess of cancers of rare types, with an average age of onset of 44 years. The ratio of cancers of epithelial and mesenchymal origin is around 10:1 in the normal population, but is more like 1:1 in WS patients. Hence there is an excess of sarcomas, such as soft tissue sarcomas and osteosarcomas, as well as acral lentigenous melanomas, meningiomas and thyroid carcinomas [118].

Knockout mice

Two research groups established knockout mouse models for WS, but using different strategies and with a different outcome. Lebel and Leder [119] targeted the exons that encode motifs III and IV of the helicase domain of mouse WRN. The targeted gene, designated $WRN^{\Delta hel}$, is predicted to express a fully translated WRN protein that lacks 121 amino acids of the helicase domain, and is therefore expected to lack helicase activity (but possibly without affecting other putative functions of the protein). The knockout mouse generated by Lombard and co-workers [120] may superficially represent a better model of human WS: they targeted the 3′ most exon of the helicase domain, which results in the expression of a truncated protein, a situation similar to that seen in most human WS cases. Moreover, as is seen with many of the WS mutations in humans, these authors could not detect expression of the truncated protein. This mouse allele, therefore, can be considered as WRN^{-} [120].

Although the homozygous $WRN^{\Delta hel/\Delta hel}$ mice were born viable, they emerged in the F2 generation at a lower frequency than the expected Mendelian ratio, indicating that the mutation confers some developmental disadvantage [119]. During the first year, the $WRN^{\Delta hel/\Delta hel}$ mice appeared normal, although some of them showed signs reminiscent of human WS, such as myocardial fibrosis and T cell lymphoma. After 15 months, various tumours and other pathologies, such as myocardial fibrosis (59 %) and inflammations of the lung, gut and bladder, appeared with a higher than normal incidence. By 24 months, 62 % of the homozygous mice had developed some kind of hyperplasia or tumour [121]. Genetic crossing allowed further insight into the possible functional interactions of the mouse WRN protein. $WRN^{\Delta hel/\Delta hel}$ $p53^{-/-}$ double-knockout animals were shown to develop tumours more rapidly than the $p53^{-/-}$ parental line. Indeed, by 4–5 months, 50 % of the double-knockout population had developed tumours, the pathology of which was very unusual compared with that seen in either of the parental single-knockout mice, as they were mainly sarcomas (pericytoma, spindle-cell sarcoma, chondrosarcoma and hemangiosarcoma). Moreover, 17 % of the double-mutant mice developed multiple tumours [121]. Crossing the $WRN^{\Delta hel/\Delta hel}$ mice with a pink-eyed unstable indicator mouse strain ($p^{un/un}$) [122] revealed an elevated incidence of somatic mutations [123].

The $WRN^{-/+}$ heterozygous mice of Lombard and co-workers [120] gave rise to homozygous F2 animals in normal Mendelian ratios, which indicates a lack of embryonic lethality. The $WRN^{-/-}$ homozygous mutant animals were reported to be perfectly healthy. Also, $WRN^{-/-}$ embryonic fibroblasts from these animals showed no signs of sensitivity to 4NQO or camptothecin (see below for a discussion of cellular phenotypes), and neither was there a significant decrease in the replicative lifespan of these fibroblasts. However, $p53^{-/-}$ $WRN^{-/-}$ double-knockout mice died earlier, and the lack of p53 accelerated the mortality of $WRN^{-/-}$ or $WRN^{-/+}$ mice. Lombard et al. [120] suggested that the lack of an obvious phenotype in their $WRN^{-/-}$ mice might be explained by the fact that telomerase is expressed constitutively in rodent cells, effectively masking any effect of a loss of WRN function.

Cellular phenotype

Cultured primary cells isolated from human WS patients undergo replicative senescence much more rapidly than do normal cells, and this is considered to be one of the hallmark features of WS cells. The average lifespan of WS fibroblasts in culture is 27 % of that of normal cells, and the population doubling time is approximately double that of normal cells [124]. This rapid replicative decline cannot be accounted for by any accelerated shortening of telomeres, unlike in normal cells, as the telomeres of senescent WS fibroblasts are longer than those of senescent normal cells [125]. Indeed, the impaired growth characteristics of WS cells are retained even after cellular transformation [126]. Although one of the possible functions of WRN is in the maintenance of telomeric structures, WS cells can be immortalized by expression of the telomerase hTERT [127,128], apparently in the same way as occurs with normal fibroblasts.

WS cells have been reported by many groups to express increased sensitivity to 4NQO, and this has become one of the hallmark features of these cells [35,110,129]. Interestingly, expression of hTERT in transformed WS fibroblasts can revert this 4NQO sensitivity back to normal levels [130]. WS cells are also more sensitive to DNA cross-linking drugs (e.g. melphalan, chlorambucil, mitomycin C, cisplatin) [126,129,131]. However, ES cells isolated from $WRN^{\Delta hel/\Delta hel}$ mice do not show increased sensitivity to UV, γ-irradiation, MMS, dimethylbenzanthracene or mitomycin C treatment, but are more sensitive to the topoisomerase I inhibitor, camptothecin. Interestingly, heterozygous ES cells demonstrate a level of sensitivity to camptothecin that is intermediate between that of homozygous WS cells and wild-type cells, suggesting that haplo-insufficiency for WRN is important in determining sensitivity to this agent, which acts largely during S phase [119].

WS cells are slightly more sensitive to γ-irradiation than are wild-type cells. This sensitivity is not as dramatic as that of mice lacking, for example, the Ku complex required for NHEJ, but appears nevertheless to be significant [126,132]. Other investigators, however, found no unusual sensitivity of WS cells to H_2O_2, UV or γ-irradiation [35,110].

At the chromosomal level, the genomic instability of WS is manifested as a greater frequency of a variety of chromosomal rearrangements, translocations and inversions, as well as a high

frequency of extensive deletions [133]. This latter feature can even vary between different clonal cell lines derived from the same individual [134]. This phenomenon is often described as 'variegated translocation mosaicism' in the literature. Unlike BS cells, WS cells do not show an elevated frequency of SCEs [35,135,136]. The spontaneous level of DNA breaks has also been found to be higher in WS cells than in wild-type cells, and WS cells express a higher background level of RAD51 foci, which is indicative of high spontaneous level of unrepaired DSBs [137]. The increased genomic instability is discernible as a higher mutation frequency at several genomic loci [119,138,139]. The gene targeting frequency has been found to be slightly increased in *WRN*$^{-/-}$ chicken DT40 cells, possibly indicative of hyper-recombination [35].

There is evidence to suggest a role for WRN in HR. Prince et al. [140] provided evidence that WS cells lack the ability to faithfully complete the recombination reactions. Consistent with this, expression of the bacterial Holliday junction resolvase, RusA, or a dominant-negative form of RAD51 can significantly reduce the elevated spontaneous or DNA-damage-induced recombination rate in WS cells. A positive effect on the survival rate after cisplatin treatment is also evident in these cells [126]. Taken together, these data suggest a role for WRN in a late step of homologous recombinational repair, probably in the resolution of Holliday junction-containing DNA structures. Nevertheless, WS cells also show a dysregulation of NHEJ in plasmid rejoining assays, as suggested by the occurrence of large deletions at the rejoining ends, especially if the assay plasmid ends in 3′ protruding and blunt termini [141]. The role of WRN in NHEJ is not known at this stage, although the interaction between WRN and the Ku–DNA-PK complex required for NHEJ (see below) suggests that WRN may either act directly in NHEJ or act to regulate the process – perhaps the balance between NHEJ and HR?

Biochemical characteristics of the WRN protein

The human WRN protein is a 1432-amino-acid protein with a predicted molecular mass of 170 kDa. The domain structure of WRN is depicted in Figure 1. One unique feature of human WRN is the presence of a perfect direct repeat of 27 amino acids, which is present in only one copy in the mouse WRN protein and in FFA-1. Biochemical data obtained with recombinant N-terminal WRN fragments [142–144] and full-length WRN [144] suggest oligomerization (possibly trimeric) of WRN *in vivo*.

WRN is a bipartite and bifunctional enzyme. In addition to the ATP-dependent 3′ → 5′ helicase and DNA-dependent ATPase activities characteristic of all RecQ family helicases, it also possesses a functional 3′ → 5′ exonuclease domain, which is similar to the exonuclease domain of *E. coli* DNA polymerase I (Figure 1) [145]. The two functions of the enzyme are separable from each other: two amino acid substitutions, WRN^{D82A} and WRN^{E84A}, which disable exonuclease activity do not affect helicase activity [146]. Similarly, the ATPase and helicase activities are strongly diminished when the Lys^{577} residue in the ATPase domain is substituted, but this does not affect exonuclease activity [146,147]. Moreover, the helicase and exonuclease activities are physically separable: recombinant N-terminal fragments, such as WRN$^{1–368}$ [148], and the minimal exonuclease domain WRN$^{70–240}$ [142] display exonuclease activity, but no helicase activity. In contrast, N-terminal deletion derivatives, which lack the exonuclease domain, retain helicase activity. However, full function and regulation of catalytic activity may require the presence of other regions of the protein, which modify the activity of the minimal domains (see below).

The helicase activity of WRN shows very poor processivity and some differences in substrate specificity from those of other RecQ helicases (Table 1). WRN alone cannot unwind M13 partial duplexes of more than 40 bp in length. In comparative analyses utilizing a variety of different DNA substrates, it preferentially unwound bubble substrates, forked structures and G-quadruplex DNA [59]. WRN was also capable of branch-migrating Holliday junctions over distances as great as 2.7 kb [149].

WRN possesses a 3′ → 5′ exonuclease activity [144,146–148,150–152] that displays only low processivity. Exonucleolytic cleavage results in the production of 5′-deoxymonophosphates [150]. WRN exonuclease activity is generally analysed using substrates with 3′ recessed termini, since little or no activity is seen with fully double-stranded duplexes with blunt ends, partial duplexes with 3′ overhangs or ssDNA. However, this requirement is relaxed if the substrate can adopt certain defined structures, such as a bubble or a stem–loop. Under these circumstances, WRN can initiate digestion from blunt ends [148,153]. As a partial duplex with a single 3′ mismatch is a better substrate for the nuclease activity of WRN than is an otherwise identical non-mismatched molecule, WRN may play a role in 'proofreading' in a manner analogous to the proofreading exonuclease activity of some DNA polymerases [144,150]. The exonuclease activity of WRN is blocked by some damaged bases, including 8-oxoG, 8-oxoA, apurinic sites or cholesterol moieties, but not by others such as uracil, hypoxanthine or ethenoadenine [154,155]. Recombinant WRN$^{1–368}$, which contains only the exonuclease domain, exhibits a somewhat altered activity on substrates with modified bases, indicating that, although the exonuclease domain forms an independently folded and functional unit of WRN, other parts of the protein are able to influence its exonuclease activity [155]. Although the exonuclease activity of WRN is not ATP-dependent, several laboratories have reported that it is stimulated by ATP [148,152,156]. Interestingly, the N-terminal recombinant exonuclease fragment, WRN$^{1–368}$, is not able to digest a bubble substrate, which strongly supports the requirement for other regions of WRN for binding to this and other substrates [148]. Stimulation by hydrolysable NTPs is also much more pronounced on atypical substrates, such as a bubble, suggesting a coupling of unwinding to nuclease digestion [152]. Consistent with this, the helicase and exonuclease activities of WRN appear to act co-ordinately to disrupt and digest some substrates [157].

The expression of WRN shows tissue-specificity, and it is very highly expressed in the testis, ovary and pancreas. WRN is also expressed in the placenta, spleen and liver, but only weakly or not at all in prostate, thymus, leucocytes, lung, kidney, intestines, brain, heart and skeletal muscle [104,111,120,158–162]. The cellular level of WRN increases markedly during the cytological transformation of resting B cells and fibroblasts. Conversely, the expression of WRN decreases significantly when normal fibroblasts reach confluency, indicative of a primary role in proliferating cells [112]. Expression of WRN is also cell-cycle-regulated, being highest in the G_2/M phases [161].

WRN shows dynamic relocalization within the nucleus under different conditions of growth. Several laboratories have reported nucleolar localization of WRN in a large variety of exponentially growing cell lines. However, upon serum starvation or treatment with HU, aphidicolin, 4NQO, etoposide or camptothecin, WRN migrates from nucleoli to discrete nuclear foci [149,156, 163–166]. In cells treated with DNA synthesis inhibitors, these foci are coincident with sites of replication, as indicated by the presence of RPA. DNA damage also induces the formation of RPA and RAD51 foci, and these co-localize with WRN almost fully (RPA) or partially (RAD51) [166]. Of possible significance with regard to a role for WRN in recombinational repair during S phase, induction of RAD51 foci in response to HU or camptothecin treatment has been shown to be less marked in WS cells than

in wild-type cells, despite the elevated basal levels of these foci [137].

The tyrosine phosphatase inhibitor Na_2VO_4 can prevent the relocalization of WRN from nucleoli under certain stress conditions, indicating that phosphorylation plays an important role in regulation of the localization of WRN; the Ser/Thr phosphatase inhibitor okadaic acid apparently has no equivalent effect [165]. von Kobbe and Bohr [167] indicated that the nucleolar localization of WRN is directed by a nucleolar targeting sequence localized in the C-terminal domain of the protein (Figure 3).

Different types of post-translational modification of WRN have been demonstrated to influence the subnuclear localization of the protein under different growth and stress conditions. Co-localization of WRN with SUMO-1 (small ubiquitin-related modifier-1) has been demonstrated [168], which implies that it may be modified by SUMO-1. Indeed, sumoylated WRN has been detected in 293EBNA cell extracts [169]. Acetylation may also play an important role in the translocation of WRN from the nucleolus to discrete nuclear foci, as well as in its return to nucleoli. Direct acetylation of ectopically expressed WRN by p300 acetyltransferase has been demonstrated *in vivo*. The histone deacetylase inhibitor trichostatin A, as well as ectopically expressed p300, can modulate the transport of WRN to nuclear foci. Although these data demonstrate that the acetylation state of WRN is influenced by histone deacetylase(s) and by p300, a direct role for either enzyme *in vivo* is still to be proven [163]. The effects of the acetylation of WRN may be relevant to the dynamic changes in WRN localization seen following DNA damage and replication blockade.

A physical interaction between the regulatory subunit (RIβ) of protein kinase A and WRN has been demonstrated. The interaction site was mapped to a fragment including the RQC domain (Figure 3). Modulation of protein kinase A activity by treatment of cells with 8-bromo-cAMP or ectopic expression of the RIβ subunit has been shown to prevent the serum starvation-mediated relocalization of WRN from the nucleolus to the nucleoplasm [164].

Physical and functional interactions of WRN with protein partners

The bipartite and bifunctional nature of WRN, as well as the range of different symptoms seen in WS patients, suggest that WRN may be a versatile enzyme with an involvement in diverse cellular processes. Below we review evidence for roles in different aspects of DNA metabolism. Evidence to support a role in these processes is provided by the finding that WRN interacts physically and functionally with several other proteins required for DNA metabolism (Figure 3).

Proteins involved in transcription

Permeabilized WS cells exhibit a decreased rate of transcription (measured by UTP incorporation) compared with wild-type cells. As these permeabilized cells cannot initiate new rounds of transcription, this decrease in rate must reflect an impairment in the transcription elongation process. Analysis of different promoters indicated a considerable decrease in the rate of transcription from an RNA polymerase II-specific promoter in WS cells, but no difference in RNA polymerase I-mediated transcription [170]. Addition of purified WRN to an *in vitro* plasmid-based transcription system can stimulate transcription. This stimulation is dependent on the helicase/ATPase activity of WRN, and on the presence of the 27-amino-acid direct repeat between amino acids 424 and 477 (Figure 3). This direct repeat region has been found to act as a strong transcriptional activation domain in the yeast two-hybrid system [170]. Interestingly, this 27-amino-acid segment is present in only one copy in mouse WRN [158] or *Xenopus* FFA-1. Ye et al. [171] also reported the possible involvement of WRN in RNA polymerase II-mediated transcription. They mapped two regions that were critical for transcriptional activation by WRN (Figure 3). In contrast with these results, Shiratori and co-workers [172] demonstrated the involvement of WRN in rRNA transcription, which is mediated by RNA polymerase I. They purified the RNA polymerase I complex and showed that WRN was associated with it. They also showed that the level of rRNA transcription was markedly decreased in WS fibroblasts compared with normal cells [172]. Taken together, these data suggest that WRN may participate in either or both RNA polymerase I- and polymerase II-dependent transcription.

Proteins involved in replication

Lebel and co-workers [119,173] purified a 17 S multiprotein DNA replication complex that contained WRN as well as several *bona fide* replication factors, such as PCNA. They also demonstrated that, in addition to PCNA, topoisomerase I and RPA can form a physical complex with WRN [119,173]. Using atomic force microscopy, it has been shown that the minimal exonuclease fragment, $WRN^{70–240}$, binds to PCNA.

DNA polymerase δ (Polδ). Yeast DNA Polδ consists of three subunits: Pol3 (125 kDa), Pol31 (58 kDa) and Pol32 (55 kDa). Pol3 and Pol31 form a heterodimeric complex called Polδ*. The Pol32 subunit forms a complex with the Pol3–Pol31 heterodimer and induces oligomerization of the complex. *In vivo*, efficient replication catalysed by Polδ requires PCNA; the interaction with PCNA requires the Pol32 subunit.

Addition of increasing amounts of WRN to primer extension reactions containing yeast Polδ has been shown to increase both the amount of extended primer and the yield of the full-length product. This effect is exerted through an increased rate of initiation of DNA synthesis, and it does not require the helicase activity of WRN. In contrast, WRN has no stimulatory effect on the activity of a Polδ–PCNA complex, which suggests that WRN is not involved in normal processive DNA synthesis catalysed by Polδ. The efficient interaction between Polδ and WRN requires the Pol32 subunit, as WRN has no effect on the primer extension activity of the Polδ* complex [174]. A second role for WRN in Polδ-mediated DNA synthesis might be through the unwinding of DNA structures that impose a physical barrier to translocating replication complexes. For example, G-quadruplex structures can effectively block Polδ-catalysed DNA synthesis. The addition of WRN permits Polδ to replicate beyond G-quadruplex structures, implicating WRN in the removal of such atypical DNA secondary structures. Significantly, the Polδ* complex, which lacks the WRN-interacting partner Pol32, is not able to traverse through the G-quadruplex barrier even in the presence of WRN, suggesting that the direct WRN–Pol32 association is vital for this functional interaction. Consistent with this, WRN is not able to stimulate Polα or Polε to replicate beyond a G-quadruplex blockade [175]. A direct physical interaction between a C-terminal recombinant fragment (Figure 3) of WRN and the p50 subunit of the human Polδ has also been demonstrated; curiously, however, the p50 subunit is not the human homologue of yeast Pol32 [174,175].

WHIP. Mgs1/WHIP [HUGO Gene Nomenclature Committee approved name WRNIP1 (‘WRN-interacting protein 1’)] is a 660-amino-acid protein which shows similarity to subunits of RFC. *Sacch. cerevisiae* Mgs1 has ATPase and DNA-annealing activities *in vitro* [176]. It also shows remarkable evolutionary conservation. A functional interaction between Mgs1/WHIP and DNA Polδ has

been demonstrated in yeast [177]. A physical interaction between WRN and WHIP, but not between either BLM or RECQ1 [HUGO Gene Nomenclature Committee approved name RECQL ('RecQ protein-like')] and WHIP, has been shown by several independent methods, and the interacting region has been mapped (Figure 3) [162]. The functional consequences of this interaction remain to be elucidated, however.

FEN1 (flap structure-specific endonuclease 1). During Okazaki fragment processing, the RNA primer is digested and removed by RNase H1, with the exception of a single ribonucleotide at the RNA/DNA junction, which is removed subsequently by the so-called flap endonuclease FEN1. FEN1 is thought to bind to the 5′ end of the flap and translocate along it until it reaches the ssDNA/dsDNA junction, whereupon it makes an incision. Because of this putative mode of action, FEN1-mediated cleavage decreases significantly with increasing length of the 5′ ssDNA flap, and its activity is inhibited by secondary structures within the 5′ flap. FEN1 also removes the 5′ flap structures generated during strand displacement synthesis, such as in base excision repair [178–181].

The direct physical interaction between FEN1 and WRN has been demonstrated using several independent techniques. The interaction site maps to the C-terminal region of WRN (Figure 3). FEN1 has no apparent effect on the helicase and ATPase activities of WRN; however, WRN greatly stimulates the endonucleolytic activity of FEN1 on a flap substrate. The removal of a 1 nt ribonucleotide flap by FEN1 is greatly stimulated by WRN in a concentration-dependent manner. Moreover, WRN stimulates the cleavage of other substrates catalysed by FEN1, including some that either are poor substrates of FEN1 alone or are completely resistant to cleavage, such as long 5′ flap substrates and forks. A C-terminal fragment of WRN, $WRN^{940–1432}$, which encompasses the FEN1-interacting region, is able to promote FEN1 activity, but to a lesser extent than is seen with the full-length WRN protein [182,183]. These data suggest that WRN helicase function may not be required for stimulation of FEN1, which perhaps eliminates a variety of models for how WRN might improve access of FEN1 to flap structures containing, for example, secondary structures.

RPA. RPA is a heterotrimer composed of three subunits of 70, 32 and 14 kDa. It binds strongly to ssDNA and only weakly to dsDNA, and is required for DNA replication, recombination and repair. RPA markedly stimulates the DNA helicase activity of WRN. In the absence of RPA, WRN cannot unwind partial duplex substrates longer than 40 bp. However, in the presence of RPA, WRN can unwind substrates as long as 849 bp. This stimulatory activity is not exerted through increased ATPase activity, and RPA has no effect on the exonuclease activity of WRN [154]. Direct physical interaction between WRN and RPA has been demonstrated *in vitro* [184]. The stimulation of helicase activity is based on direct protein–protein interactions, and not on the mere coating of the displaced ssDNA strands created during DNA unwinding, since there is a lack of effect of other ssDNA binding factors, such as *E. coli* SSB (single-strand binding protein) or T4 gene 32 protein [16,185].

Proteins involved in the maintenance of genome integrity

TRF2. Possible involvement of WRN in the maintenance of telomeres was suggested by the demonstration of a physical interaction between TRF2 and WRN. Mapping of TRF2 interaction sites revealed three regions on WRN, although with different binding affinities (Figure 3). In telomerase-negative cells, in which telomeres are maintained by the ALT pathway, WRN co-localizes with TRF1, TRF2, PML, RAD52 and NBS1 in nuclear structures called ALT-specific, atypical PML bodies. In telomerase-positive HeLa cells, however, WRN localizes mainly to the nucleolus [186]. WRN-catalysed unwinding of a non-telomeric forked duplex or a forked duplex containing a telomeric repeat sequence, to which TRF2 does not bind, is stimulated by TRF2 in a concentration-dependent manner. The exonuclease activity of WRN is apparently not affected by TRF2 [83].

Interestingly, introduction of mouse WRN into *sgs1 est2 Sacch. cerevisiae* cells restores their ability to produce the type II class of survivors of telomere maintenance [187] (see below), which further supports the possible involvement of WRN in telomere maintenance.

p53. The tumour suppressor protein p53 has been demonstrated to interact physically with WRN *in vitro* and *in vivo*. The interacting segment of WRN has been mapped to the C-terminal domain (Figure 3). *In vivo*, this interaction may be limited, since only a small fraction of p53 can be co-immunoprecipitated with an anti-WRN antibody [79,156,188]. This physical interaction between p53 and WRN apparently modulates the activity of both partners. p53 inhibits the unwinding of a synthetic Holliday junction (four-way junction) by WRN. This regulatory activity is dependent on the phosphorylation state of p53: p53 phosphorylated on Ser^{378} does not inhibit unwinding by WRN. Indeed, a small peptide, $p53^{373–383}$, that harbours this phosphorylation site can also inhibit the unwinding of Holliday junctions by WRN, but when phosphorylated it cannot [79]. The exonuclease activity of WRN is also inhibited by p53 in a dose-dependent manner. The exonuclease activity of $WRN^{1–368}$, which harbours only the exonuclease domain but lacks the p53 interaction site (Figure 3), is unaffected by p53 [156]. The functional interaction between WRN and p53 is apparently reciprocal. WRN potentiates p53-mediated transcription from p53-responsive promoters in transient transfection assays. Exogenously expressed WRN also enhances the level of endogenous p21 (a p53-responsive gene) transcription [188].

DNA-PK, Ku. The physical and functional interaction between the DNA-PK complex (which comprises $DNA\text{-}PK_{cs}$ and the Ku70/Ku80 heterodimer) and WRN has been reported by several laboratories. Direct physical interaction between WRN and the Ku complex [143,154,189,190], Ku80 [189,191,192], Ku70 [191], $DNA\text{-}PK_{cs}$ [132] and the DNA-PK holoenzyme [190] has been demonstrated using various *in vitro* and *in vivo* techniques. There is some controversy, however, as to whether $DNA\text{-}PK_{cs}$ alone binds to WRN [132] or whether this interaction is mediated by Ku [190,193]. Ku70/Ku80 appears to bind both the N-terminal and C-terminal domains of WRN [189–192].

$DNA\text{-}PK_{cs}$ alone cannot bind to DNA; however, if the DNA is pre-bound by Ku, addition of $DNA\text{-}PK_{cs}$ results in the formation of a DNA–DNA-PK complex. There is some disagreement in the literature as to the role of WRN when associated with the DNA–DNA-PK complex. Yannone and co-workers [132] demonstrated that WRN stabilizes the DNA–DNA-PK complex, and that the WRN–DNA-PK–DNA complex is more stable than any of the subcomplexes. In contrast, Li and Comai [190] demonstrated that addition of WRN to the DNA–DNA-PK complex displaces $DNA\text{-}PK_{cs}$ from the complex, and that this displacement by WRN requires both Ku-binding sites on WRN. WRN is phosphorylated *in vivo* in untreated cells [132] and in response to bleomycin- or 4NQO-induced DNA damage [193]. The phosphoinositide 3-kinase inhibitor, wortmannin, can suppress this phosphorylation, and in the $DNA\text{-}PK_{cs}$-deficient M059J cell

line this phosphorylation is absent [132]. These data demonstrate clearly that, *in vivo*, WRN is phosphorylated by DNA-PK. DNA-PK can also phosphorylate WRN *in vitro*, in a DNA-dependent manner [132].

The Ku complex stimulates the exonuclease activity of WRN significantly, but does not obviously affect its helicase or ATPase activities [132,193]. This stimulation of the exonuclease activity of WRN is seen with the helicase-dead K577M mutant protein [143] or with the N-terminal recombinant $WRN^{1–388}$ fragment that harbours the exonuclease domain [192], indicating that the helicase activity of WRN and the C-terminal Ku binding site in WRN are not involved in this functional interaction. Stimulation of the exonuclease activity depends on both subunits of Ku; neither Ku70 nor Ku80 alone is sufficient for activation [189]. In the presence of the Ku heterodimer, the substrate specificity of WRN is extended to include substrates with 3′ protruding or blunt ends [189]. Inhibition of the exonuclease activity of WRN by 8-oxoG and 8-oxoA adducts in the DNA [154,155] can be alleviated by the addition of Ku, allowing digestion to proceed beyond the damage [154].

Currently there are some conflicting data on the role of DNA-PK in the regulation of WRN function. In some studies, $DNA\text{-}PK_{cs}$ alone was shown not to influence the exonuclease activity of WRN in either the presence or the absence of ATP [190, 193]. However, Yannone and co-workers [132] reported that $DNA\text{-}PK_{cs}$ inhibited the helicase and exonuclease activities of WRN in a concentration-dependent manner, and that the presence of Ku alleviated the inhibition of both activities. The relieving effect of Ku was not phosphorylation dependent, as the presence of wortmannin or ATP did not affect the exonuclease activity significantly [132]. Karmakar and co-workers [193] reported a different result when $DNA\text{-}PK_{cs}$ was added to reactions containing WRN and Ku; in this case the exonuclease activity of WRN was markedly inhibited to a level lower than that displayed by WRN alone. Wortmannin alleviated this inhibition, suggesting that the protein kinase activity of DNA-PK is required for this effect [193]. In a third study, Li and Comai [190] found that the stimulatory effect of Ku on the exonuclease activity of WRN was not influenced by $DNA\text{-}PK_{cs}$, even in the presence of ATP, when phosphorylation of WRN was expected to occur. In the light of these rather contradictory reports, it is hard to draw a definitive conclusion regarding the effect of the DNA-PK holoenzyme on WRN function. Nevertheless, it is clear that Ku70/Ku80 has a stimulatory effect on the WRN exonuclease. By regulating WRN exonuclease activity, Ku and DNA-PK might limit the extent of DNA degradation during NHEJ or other repair processes, thereby preventing extensive deletions. The exonuclease activity of WRN might be involved directly in NHEJ, although, if so, this activity is probably redundant, since WS cells do not show an obvious DNA DSB repair defect. In WS cells, the exonuclease activity of WRN might be replaced by another, non-regulated, exonuclease activity that leads to the accumulation of extended deletions at sites of NHEJ-mediated repair.

FFA-1: the WRN homologue in *Xenopus laevis*

FFA-1, the *Xenopus* homologue of WRN, was identified as a factor essential for the formation of RPA foci associated with replication (FFA-1) [194]. A direct physical interaction between FFA-1 and RPA has been shown, and the interaction region has been mapped to between residues 197 and 587 of FFA-1 [195]. The two proteins also interact functionally: RPA stimulates the helicase activity of FFA-1 in a manner similar to that seen with the WRN–RPA interaction.

RECQ4: THE CAUSATIVE GENE OF RTS

The clinical features associated with mutation of the *RECQ4* gene have been known for decades, although only very recently were these recognized to be caused by a defect in *RECQ4*. Rothmund, a German ophthalmologist, described several patients from a German family with cataracts and unusual degeneration of the skin in 1868 [114]. Some 70 years later, Thomson, a British dermatologist, described a syndrome with similar skin abnormalities and skeletal malformations, but without cataracts, as ‘poikiloderma congenitale’ [196]. Taylor [197] later recognized that the symptoms described by Rothmund and Thomson were manifestations of the same disease, and hence he named the disorder RTS. In spite of its long history, RTS is not as well documented as BS or WS, and the molecular/cellular processes in which RECQ4 is involved remain unknown.

Patients with RTS show diverse skeletal abnormalities, among which are a decrease in bone density (osteopenia), as well as pathological fractures, dislocations and metaphyses, but the most striking feature is a ‘radial ray defect’. This can appear as appendages on the thumb, or the existence of small, missing or bifid thumbs. Most RTS patients are also proportionately small; apparently normal prenatal development is followed by retarded postnatal growth. Skin abnormalities are common, and mostly manifest as a characteristic poikilodermatous, sun-sensitive rash, which can be widespread. Most patients also have sparse and thin scalp hair and eyebrows, as well as dystrophic nails and teeth. Cataracts of the eyes are also very frequently reported, but other symptoms, such as mental disability or gonadal problems, are more variable features. Unlike BS, RTS is very frequently associated with one characteristic type of cancer, osteosarcoma, with a median age of onset of only 11.5 years. There are also reports of skin malignancies [198]. Because of the overlap in symptoms between BS and RTS, misdiagnoses have undoubtedly occurred.

Karyotypic analysis of RTS cells has revealed the existence of an unusually high frequency of chromosomal rearrangements, trisomies, translocations and deletions. These aberrations can even be of a different nature in different cells isolated from the same patient [199–201]. Several reports have documented chromosome 8 mosaicism or trisomy [199,200,202–204], which is probably a reflection of the fact that the *RECQ4* (*RTS*) gene has been mapped to chromosome 8. The above reports provide strong evidence for genomic instability in RTS cells. Increased sensitivity of RTS fibroblasts to ionizing radiation has also been found by several investigators [205–207], which is paralleled by a reduced level of DNA repair synthesis and an abnormally low rate of removal of radiation-induced DNA lesions [206]. Shinya et al. [208] described UV sensitivity and reduced unscheduled DNA synthesis after UVC exposure in RTS cells. On the other hand, RTS cells apparently show normal sensitivity to mitomicin C, bleomycin, vincristine, methotrexate [209], cisplatin and adriamycin [207]. Importantly, unlike in BS cells, the frequency of SCEs is apparently near normal in RTS cells [209].

The *RECQ4* gene was cloned before it was recognized to be defective in RTS. *RECQ4* lies on chromosome 8q24.3 [161] and it encodes a 1208-amino-acid protein. Outside of the helicase domain, RECQ4 shows no striking identity with the other RecQ family members. The block(s) of acidic amino acid residues usually located on the N-terminal domain of RecQ helicases is found in the C-terminal region of RECQ4. The *RECQ4* gene is unusually small (only 6.5 kb), and spans 21 exons. Its promoter structure suggests a ‘housekeeping’ nature, with multiple sites of transcription initiation [210]. The mouse *RECQ4* gene is similar in size to its human counterpart and encodes a 1216-amino-acid protein. The mouse RECQ4 protein is 86 % identical/95 % similar to the

human protein, which is an unusually high degree of conservation across mammalian species for a RecQ helicase [211].

The gene expression profile of *RECQ4* shows tissue and cell cycle regulation. RECQ4 mRNA levels are highest in testis, prostate and thymus, and in the S phase of the cell cycle. Although the discovery that a subset of RTS cases is caused by mutations in *RECQ4* was made in 1999 [212], the number of *RECQ4* mutations identified is still quite low, and most of them result in premature termination of protein translation [209,212,213]. Whether these mutations cause mislocalization of the truncated protein has not been assessed, and the NLS of the protein has not been identified to our knowledge.

RECQ1

Of the five human RecQ helicases, RECQ1 (also known in the literature as RECQL and helicase Q1) is the smallest, being only slightly larger in size than the founding member of the family, RecQ of *E. coli* (Figure 1). No human genetic disease has been attributed to RECQ1 deficiency thus far.

The cDNA encoding human RECQ1 was cloned by two groups independently [214,215]. It encodes a 649-amino-acid protein with a predicted molecular mass of 73 kDa. Some of the mRNA species encoded by *RECQ1* are very large, due to the presence of extensive 5′ or 3′ untranslated regions, which result from alternative splicing at the 5′ end and alternative usage of two 3′ poly(A) addition signals. More than one cDNA species, apparently generated by alternative mRNA splicing, have been described by Zhang and Xi [216]. Two isoforms of mouse RecQ1, generated by alternative splicing, have also been identified: RecQ1α is essentially equivalent to the human sequence, but RecQ1β is a little shorter (631 residues long) and differs at the C-terminal end. The human *RECQ1* gene is localized to chromosome 12p11-12 [214,217]. This chromosomal location has been shown to coincide with aberrations in testicular germ-cell tumours, in which an isochromosome, known as i(12p), as well as other cytogenic changes, have been found in many cases [218].

Mouse RecQ1 seems to be expressed ubiquitously, although the abundance of the mRNA differs between different tissues [111, 161,219]. During the synchronized spermatogenesis of young mice, RecQ1β expression increases dramatically from day 14, when most of the meiotic cells are in the pachytene stage of prophase I [219].

Biochemical characterization of purified RECQ1 began even before its cDNA was cloned. Yanagisawa and co-workers [220] purified DNA-dependent ATPases from HeLa and xeroderma pigmentosum cells, and found that the chromatographic properties of these ATPases were different in all of the xeroderma pigmentosum cells studied compared with that of HeLa cells. A 73 kDa ATPase, called ATPase Q1, was purified and shown to exhibit 3′ $\rightarrow$ 5′ helicase activity [18]. However, when the purified protein was microinjected into xeroderma pigmentosum cells, it failed to complement cell extracts for DNA repair defects [215]. The *bona fide XPC* gene was identified independently [221].

The purified RECQ1 protein probably exists as a dimer in solution. As determined using glycerol gradient and gel-filtration analyses, RECQ1 has a native molecular mass of $\sim$160 kDa, approximately double the predicted mass of the monomer. The protein concentration dependence of its helicase activity shows evidence of co-operativity under optimal reaction conditions, which is also suggestive of oligomerization [19]. RECQ1 is a helicase of low processivity, as it is incapable of unwinding M13 partial duplex substrates even of moderate length (Table 1). However, as with WRN and BLM, the presence of human RPA stimulates the helicase activity of RECQ1, permitting it to unwind longer partial duplexes. Interestingly, BSA seems to stimulate its helicase activity [19], perhaps reflecting stabilization of the protein in solution.

In a search for interacting protein partners for human RECQ1 using the yeast two-hybrid system, Seki and co-workers [222] identified two proteins, Qip1 and Rch1, that are two of the three human importin α homologues involved in nuclear–cytoplasmic transport. The interacting sequences reside in the C-terminal portion of RECQ1 [222], where a putative NLS sequence can be found at $K^{642}KRK^{645}$. Qip1 also binds to a peptide containing this sequence. The functional importance of this binding has been demonstrated in digitonin-permeabilized cell-free transport assays; Qip1 is able to mediate nuclear transport of BSA when this protein is conjugated to the full RECQ1 NLS [223].

RECQ5

As with *RECQ1*, no human genetic disease has been attributed thus far to a deficiency in the *RECQ5* gene [HUGO Gene Nomenclature Committee approved name *RECQL5* ('RecQ protein-like 5')]. RECQ5 protein is most obviously noteworthy because of the existence of several different isoforms of the protein that are generated by alternative mRNA splicing.

The 19 exons of the human *RECQ5* gene give rise to alternatively spliced mRNA species, which encode three isoforms, designated RECQ5α, RECQ5β and RECQ5γ (Figure 1). These are predicted to be proteins of 46 kDa, 108.9 kDa and 49 kDa respectively [224]. The *RECQ5* gene has been mapped to chromosome 17q25 [161], and loss of heterozygosity in this region has been found to be associated with ovarian cancers and familial breast carcinomas [225]. The mouse *RECQ5* gene spans over 40 Mb on chromosome 11. Only a cDNA encoding for mouse RecQ5β has been isolated thus far. The RecQ5 proteins of *Drosophila melanogaster* have been studied more extensively. Sekelsky et al. [225] reported the existence of different isoforms of dmRecQ5. The 54 kDa RecQ5b is similar to human RECQ5α. The 121 kDa RecQ5a is likely to be the *Drosophila* orthologue of human RECQ5β. The cDNA encoding the same isoform was also isolated by Jeong et al. [226], who named it RecQE. The *Drosophila* RecQ5c variant is only two amino acids larger than RecQ5b. Preliminary mutagenesis results showed that RecQ5 is probably not essential in *Drosophila* [225]. Two RecQ5 mutants, which are listed in FlyBase, are currently uncharacterized [227].

In human tissues, the different RECQ5 isoforms seem to be expressed ubiquitously, with a very high abundance in the testes. Similarly, the mRNAs for the three isoforms of dmRecQ5 are present during all developmental stages. Adult flies also express the small as well as the large isoforms [10]. dmRecQ5β possibly plays an important developmental role, as it has been shown to accumulate in early embryos [228]. In human cells, subcellular localization of the different isoforms has been studied using haemagglutinin-tagged proteins ectopically expressed in 293EBNA cells. The small isoforms, hsRECQ5α and hsRECQ5γ, are retained in the cytoplasm, while hsRECQ5β is transported to the nucleus, consistent with the presence of a putative NLS sequence between amino acids 854 and 872 (Figure 1) [224].

Thus far, only the small and the large isoforms of *Drosophila* RecQ5 have been characterized biochemically. Like the other RecQ helicases, *Drosophila* dmRecQ5β appears to have an oligomeric structure – probably tetrameric in this case. Apart from the standard ATPase activity (see above), the dmRecQ5β protein has also been shown to be a very efficient GTPase, although GTP cannot support helicase activity. However, GTP can stimulate ATP-mediated helicase activity in a concentration-dependent manner. The mechanism of this activation is not known. GTP

analogues do not appear to affect substrate preference or binding affinity, and also do not obviously stimulate the oligomerization of this enzyme. Based on sequence comparison of the ATPase/GTPase active centre of various RecQ helicases, Kawasaki et al. [228] suggested that such a GTPase activity might be a characteristic only of the RECQ5 orthologues and dmRTS, the RecQ4 orthologue of *Drosophila* [229].

As with other RecQ helicases, the extent of unwinding of long duplexes is strongly compromised (Table 1). However, unlike human WRN, BLM or RECQ1, the helicase activity of dmRecQ5α, when tested on longer substrates, is not obviously stimulated by RPA [10]. Detailed analysis of the substrate specificity of dmRecQ5α produced interesting results. In contrast with WRN and BLM, the preferred substrates for dmRecQ5α are forked structures, with no or minimal unwinding of the Holliday junction and bubbles (Table 1) [230]. The characteristics of dmRecQ5β helicase are apparently similar to those of dmRecQ5α [226,228].

RECQ HELICASES OF OTHER ORGANISMS

E. coli RecQ

The *recQ*$^+$ gene of *Escherichia coli* was originally identified in a screen for mutations that confer resistance to thymineless death. The *recQ*$^+$ gene encodes a 610-amino-acid protein (Figure 1). Mutations in *recQ* are also associated with UV sensitivity and largely abolish HR in a *recBC sbcB* background. This latter result indicates participation of RecQ in the so-called RecF recombination pathway [231,232]. Mutations in the *recQ* gene also cause a 20–300-fold increase in the frequency of illegitimate recombination. The sites of these illegitimate events are concentrated at specific hotspots, which show short stretches (4–10 bp) of identity between the recombining sequences [233].

E. coli RecQ protein shows a much wider substrate specificity and higher processivity than do the other RecQ family helicases; it is able to unwind longer partial duplexes, as well as fully blunt-ended duplexes of significant length (Table 1) [12,13,234,235]. Analysis of the ATP dependence of helicase activity suggests the existence of co-operativity, and that the enzyme may function as an oligomer. However, gel-filtration analyses failed to verify this association and showed that the enzyme exists as a monomer in solution. Also, an important feature of *E. coli* RecQ, but possibly not of the other RecQ helicases, is that it does not require free DNA ends to initiate unwinding of the substrate [13].

The presence of SSB significantly enhances the DNA unwinding activity of *E. coli* RecQ; it increases both the initial rate and the extent of unwinding, especially of longer substrates. SSB promotes the progression rather than the initiation of unwinding. The T4 gene 32 protein, which is also a ssDNA binding protein, is able to stimulate the helicase activity of *E. coli* RecQ to a similar extent to that achieved by SSB, suggesting a lack of any species-specific effect. This stimulation appears to be based on the sequestration of the unwound ssDNA product by SSB, rather than on a direct stimulation of the helicase activity [13,234].

Since *E. coli* RecQ is able to unwind fully duplexed DNA, it is also capable of producing ssDNA tails, which can be utilized by RecA in DNA strand exchange reactions. RecA alone cannot promote the generation of D-loops from fully duplexed DNA and supercoiled plasmid, but, in the presence of RecQ and SSB, joint molecules can be formed. Therefore, in common with RecBCD, RecQ is able to initiate recombination in concert with RecA and SSB. The joint molecules formed by these reactions are also substrates for unwinding (Table 1). However, as it does not express a greater level of helicase activity on Holliday junctions than on other substrates, unlike RuvAB and RecG, RecQ is not a branch migration-specific helicase. Nevertheless, it can act to dissociate certain recombination intermediates [236]. RecQ, in association with RecJ, has been shown to be involved in the degradation of nascent lagging strand DNA at stalled replication forks generated by UV irradiation. Deletion of either *recQ*$^+$ or *recJ*$^+$ abolishes this degradation. Courcelle and Hanawalt [237] proposed that this degradation might generate a substrate for RecA, which would then stabilize the stalled replication fork until such time that the UV lesion is repaired.

In the presence of SSB, *E. coli* RecQ can unwind covalently closed plasmids, generating a substrate for topoisomerase III. A combination of RecQ, Top3 and SSB has been shown to give rise to several forms of fully catenated, covalently closed plasmid multimers. This functional interaction between RecQ and Top3 is specific, as neither the combination of RecQ with Topo IV or DNA gyrase, nor the combination of helicase II with Topo II, can generate catenated plasmids. Top3 can decatenate the interlinked plasmid multimers if RecQ is inactivated in the reaction; however, the generation of the catenanes described above (essentially the reverse reaction to decatenation) requires the helicase activity of RecQ. Top3 alone apparently cannot decatenate purified plasmid multimers. These data indicate a requirement for the presence of either a nascent RecQ-generated DNA structure or RecQ itself for the dsDNA strand passage activity of Top3. This presumably reflects the fact that Top3 can only cut one DNA strand at a time, and must catalyse dsDNA strand passage through two sequential ssDNA strand passage events. The helicase could assist in this sequential reaction by generating a region of partially denatured DNA.

Schizosaccharomyces pombe Rqh1

The *Schiz. pombe* orthologue of RecQ helicases was isolated in different laboratories using independent approaches. Enoch et al. [238] screened for mutants that show a defect in the S phase checkpoint and undergo aberrant mitosis following treatment with HU. One of these HU-sensitive mutants, *hus2*, was found to encode the *Schiz. pombe* orthologue of RecQ helicases. Freyer and co-workers [239] isolated a UV-sensitive mutant, *rad12.502*, which was deficient in nucleotide excision repair, while Murray et al. [240] identified the gene mutated in the *rad12* mutants as the *Schiz. pombe* homologue of *E. coli* RecQ. Because of its similarity to *E. coli* RecQ, the gene was renamed *rqh1*$^+$, and the two alleles described above designated *rqh1.h2* and *rqh1.r12*. The *rqh1.h2* mutant harbours a stop codon at amino acid position 790, and results in a truncated protein, while the protein encoded by *rqh1.r12* contains a single amino acid substitution, T543I, at a conserved position of the Walker A box ATP binding motif, and is considered to be a helicase-defective, but otherwise intact, protein [240]. The *rqh1*$^+$ gene encodes a 1328-amino-acid protein with a predicted molecular mass of 149.6 kDa (Figure 1).

rqh1 mutants show a retarded growth rate and decreased viability. Moreover, they exhibit a greatly increased sensitivity to HU, MMS, bleomycin [241], camptothecin [242], and UV and γ-irradiation [243]. It may be significant that *rqh1* mutants, unlike *Sacch. cerevisiae sgs1* mutants, are sensitive to ionizing radiation. There is also a difference between the phenotype of *Schiz. pombe* strains that harbour these different mutant alleles. The survival of an *rqh1* helicase-dead point mutant is greater than that of a *rqh1* deletion mutant following exposure to HU, MMS, UV light or γ-irradiation [243], but no difference is evident in growth parameters in the absence of genotoxic insults [14]. Indeed, Maftahi et al. [244] found that a deletion mutant showed better viability than a helicase-dead mutant under normal growth

conditions. These data indicate that, for some functions of Rqh1, the helicase activity may be at least partially dispensible. This is consistent with results obtained using helicase-dead versions of Sgs1 [245].

rqh1 cells are able to arrest the cell cycle following inhibition of DNA synthesis (with HU) and are also able to complete DNA synthesis upon removal of such a synthesis blockade. However, following this, chromosome segregation cannot take place normally in many cells, leading to the appearance of the so-called 'cut' phenotype, where the septum often bisects the nuclear DNA. These chromosome segregation problems can also account for the elevated rate of chromosome loss in *rqh1* mutants, which is seen even under normal growth conditions, but is dramatically enhanced following HU treatment [246]. These chromosome segregation problems appear to be caused, at least partially, by the persistence of unresolved Holliday junctions, since ectopic expression of a recombinant bacterial Holliday junction resolvase, RusA, is able to partially complement the HU, UV and MMS sensitivity of *rqh1* mutants, and reduce the proportion of cells that exhibit the cut phenotype. However, RusA expression has no effect on the elevated levels of both spontaneous and UV-induced mitotic recombination, or on hypersensitivity to camptothecin and γ-irradiation in *rqh1* mutants [242,247]. Mus81/Eme1 (the *Schiz. pombe* orthologues of *Sacch. cerevisiae* Mms4/Mus81), a heterodimeric endonuclease that can resolve Holliday junctions as well as cleave several other branched DNA substrates, is essential for viability in the absence of Rqh1 [248], which may be relevant to the proposed impaired ability of *rqh1* cells to process Holliday junctions.

Unlike the situation with *Sacch. cerevisiae sgs1* strains (see below), the level of mitotic recombination under normal growth conditions is unaltered in *rqh1* cells; however, it is increased dramatically in these mutants following HU treatment. This indicates that Rqh1 operates in the suppression of recombination arising following arrest of DNA replication [246]. This is consistent with a primary role in the 'repair' of damaged or arrested replication forks (see models below).

Genetic data indicate that *rqh1*$^+$ is epistatic to *rhp51*$^+$, *rhp54*$^+$ and *rad22*$^+$ (the *Schiz. pombe* orthologues of Rad51, Rad54 and Rad52 respectively) [239–241] with respect to UV sensitivity, but is not epistatic with the genes encoding proteins that participate in the nucleotide excision repair system. These data suggest that Rqh1 participates in some aspects of the recombinational repair pathway [240]. The checkpoint Rad genes (*rad1*$^+$, *rad3*$^+$, *rad9*$^+$, *rad17*$^+$, *rad26*$^+$, *hus1*$^+$), as well as *cds1*$^+$, also exhibit genetic interactions with *rqh1*. Deletion mutant alleles of *rqh1* show synthetic lethality with mutations in *rad3*$^+$ and *rad26*$^+$, i.e. these double mutants are non-viable. The helicase-dead *rqh1.r12* allele, however, is not synthetically lethal with *rad3*$^+$ and *rad26*$^+$, which emphasizes further the probable importance of functions of Rqh1 other than the helicase activity in cell-cycle checkpoint responses to DNA damage. These data also indicated that Rqh1 is involved in the Cds1-mediated, checkpoint Rad-dependent DNA damage recovery pathway. Since Rqh1 is apparently not required for the generation of a checkpoint-dependent signal, Murray and co-workers [240] placed Rqh1 downstream of the checkpoint Rad proteins and Cds1. Davey et al. [243] and Marchetti et al. [249], however, placed Rqh1 upstream of Rad9 and proposed roles in maintaining normal cell morphology and in ensuring maximal slowing of DNA synthesis after MMS treatment respectively.

Topoisomerase III is a type I topoisomerase, and is related structurally to topoisomerases I and III of *E. coli*. It has poor DNA relaxation activity, and requires access to ssDNA regions for activity (also discussed above) [250]. Several lines of evidence suggest a role for topoisomerase III in some aspects of genetic recombination, rather than in overcoming DNA superhelical stress [251]. It has also been suggested that Top3 might act at arrested or converging replication forks to unlink the parental duplexes [252]. A *top3* null mutation is lethal in *Schiz. pombe*; mutant spores stop dividing after between four and eight cell divisions. The lethality of *top3* mutations can be suppressed by deletion of *rqh1*$^+$ and also by a helicase-dead mutant version *rqh1*^{K547I}. Since this genetic interaction is conserved in *Sacch. cerevisiae* (see below), it is likely to be highly relevant to the role of the RecQ helicases/topoisomerase III in DNA metabolism.

Saccharomyces cerevisiae Sgs1

The RecQ orthologue of *Sacch. cerevisiae* was identified by three different groups using different approaches, but in all cases the approaches involved topoisomerase genes/proteins. Gangloff et al. [253] isolated *SGS1* in a screen for suppressors of the *top3* slow growth phenotype. Watt and co-workers [254] identified the same gene in a yeast two-hybrid screen for interaction partners of topoisomerase II. Lu and co-workers [255] isolated the *SGS1* gene based on its genetic interaction with mutations affecting topoisomerase I.

Sgs1 is 1447 amino acids in length, with a predicted molecular mass of 164 kDa (Figure 1). As expression and purification of full-length Sgs1 have proved unsuccessful, enzymic analyses have been carried out to date on a C- and N-terminally truncated protein designated Sgs1$^{400–1268}$. The enzymic properties of this recombinant protein resemble those of the RecQ helicases generally (see above). The recombinant Sgs1$^{400–1268}$ protein has been shown to bind specifically to the single-stranded–double-stranded junction of DNA substrates, and this binding requires a 3′ overhanging DNA tail of at least 3–4 nt. The recombinant Sgs1$^{400–1268}$ protein makes specific contacts within the first 4 nt of a ssDNA region, and can also distinguish between 3′ → 5′ and 5′ → 3′ backbones [256]. Sgs1 helicase is also able to unwind RNA/DNA hybrid molecules.

The intracellular level of Sgs1 shows cell-cycle-dependent changes. It is very low in the M and G_1 phases, peaks in S phase and decreases again in G_2. Similarly, Sgs1 is almost undetectable in cells during M and G_1, but forms nuclear foci in S-phase cells, which partially co-localize with Rad53 and the origin recognition complex found within replication foci. These foci are then lost when the cells traverse G_2. Sinclair et al. [257] also reported a significant enrichment of Sgs1 in the nucleolus; however, other investigators could not confirm this finding [258].

Cellular phenotypes

sgs1 mutants show an approx. 40 % decrease in average lifespan and a greater than 50 % decrease in maximum lifespan compared with wild-type cells [257,259]. Detailed lifespan analyses revealed two classes of senescent cells. Some of the *sgs1* cells stop dividing early, and accumulate as large budded cells, which is a characteristic of G_2-arrested cells. In contrast, those cells that cease cell division at later times are similar to senescent wild-type cells in being bud-free (G_1 cells). The G_2 arrest was reported to be an age-independent stochastic event, while the second class of cells arresting in G_1 was suggested to represent prematurely aged cells. The lifespan of *sgs1* cells that escape the stochastic G_2 arrest can be extended by mutations in other genes that also extend the lifespan of wild-type cells, such as a *fob1* mutation or the overexpression of Sir2. The stochastic G_2 arrest was shown to be dependent, at least partially, on *RAD9* and therefore on the DNA damage checkpoint; however, deletion of *RAD9* cannot restore a normal lifespan in *sgs1* cells [260].

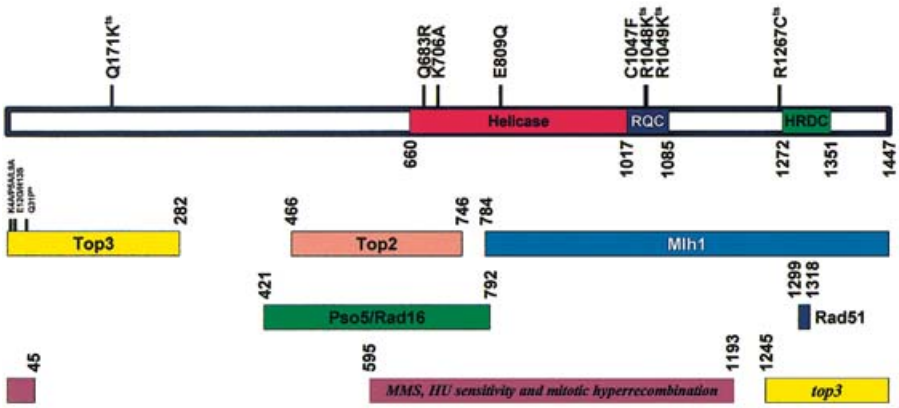

Figure 4 Domain structure and interaction map of Sgs1

The conserved RecQ helicase domains are indicated as in Figure 1. Point mutations that eliminate Sgs1 enzymic activity, and amino acids shown to be important for binding to Top3α, are indicated above the structure and on the Top3α binding fragment respectively. Protein interaction regions are indicated as in Figure 2. Regions of the protein involved in genetic interactions, and the corresponding functions, are depicted at the bottom of the Figure, in *italics*. See the text for further details.

Sacch. cerevisiae cells that lack either the telomerase RNA component, *TLC1*, or *EST1*/*EST2*, which encode subunits of telomerase, are defective in the major pathway for the maintenance of telomeres. These mutants senesce much more rapidly than do wild-type cells. Some cells can escape this premature senescence and maintain their telomeres in the absence of telomerase. Two different classes of survivors have been identified: the telomeres of so-called type I survivors consist of amplified units of Y′ subtelomeric elements, but only short telomeric $TG_{1–3}$ repeats. In contrast, type II survivors contain massively amplified $TG_{1–3}$ repeats. The emergence of type I survivors requires Rad52 and Rad51, while the type II survivors require functional Rad52 and Rad50. The type II survivors grow faster and generally predominate in surviving clones [261].

sgs1 mutants do not show differences in telomere length from wild-type cells [262], indicating that increased telomere erosion cannot be the overriding reason for their reduced lifespan. However, *sgs1 tlc1* and *sgs1 est2* double mutants show an increased rate of telomere shortening compared with *tlc1* single mutants, and also a higher proportion of cells arrest in G_2/M. Both of these double mutants generate survivors with similar efficiency to *tlc1* or *est2* mutants, although only after a protracted delay. The emergence of type II survivors, however, is blocked by an *sgs1* mutation, indicating that Sgs1 is involved in the generation of type II survivors together with Rad50. Re-introduction of Sgs1 into established type I survivors is not apparently sufficient to induce the cells to convert into a type II phenotype. These data suggest that Sgs1 is not required for the maintenance of the characteristic chromosomal end structure, but may be required for the transition from the type I to the type II mode of the telomerase maintenance process [186,187,263]. Putative roles of Sgs1 in telomere maintenance have been discussed elsewhere [264].

sgs1 mutants are not dramatically sensitive to any DNA-damaging agents [258,262], but consistently show some increase in sensitivity to MMS [265,266] and UV light [267,268]. Similarly, *sgs1* mutants are sensitive to the replication inhibitor HU [265]. There seem to be significant strain differences in the level of sensitivity of *sgs1* mutants to these agents.

sgs1 mutants display elevated rates of several different types of mitotic recombination events, including marker loss [262,269], unequal SCE [270], gross chromosomal rearrangements [271], and all types of loss-of-heterozygosity events [272]. They also show an increased rate of illegitimate recombination [273]. Interestingly, the frequency of UV-induced heteroallelic recombination has been shown to be reduced in diploid *sgs1* cells compared with wild-type cells. [267]. Similarly, MMS-induced interchromosomal recombination was found to be significantly reduced in *sgs1* compared with wild-type cells, and little induction is observed in response to UV [269].

In yeast cells, a major pathway for the repair of DSBs is Rad52-dependent HR. A second pathway is NHEJ, which is dependent upon DNA ligase IV. An epistatic relationship has been demonstrated between mutations in *SGS1* and *RAD51*. This, coupled with the demonstration of a direct physical interaction between Sgs1 and Rad51 (Figure 4) [39], implies that Sgs1 plays a role in HR. The elevated sensitivity to genotoxic agents, as well as the checkpoint-dependent reduction in lifespan, certainly suggest that *sgs1* mutants are impaired in one of the DNA repair pathways. We and others have suggested that this role is in a recombinational repair pathway that is operational particularly at stalled replication forks. Recent genetic data suggest that this role may be in the repair of single-stranded gaps at the sites of stalled forks [274].

Genetic and biochemical interactions

Topoisomerases. Sgs1 shows physical and genetic interactions with all three topoisomerases expressed in *Sacch. cerevisiae*. *sgs1 top1* double mutants grow very poorly, but the reason for this synthetic defect is not known. Sgs1 and Top2 have been shown to interact physically [254,275] (Figure 4). Moreover, genetic analyses indicate that mutations in *SGS1* and *TOP2* are epistatic with regard to reducing chromosome non-disjunction, suggesting that Sgs1 and Top2 act on the same chromosome segregation pathway [254]. Mutations in the *SGS1* gene suppress most of the phenotypic abnormalities displayed by *top3* mutants, including G_2 delay, elevated frequency of marker loss and altered morphology. [253,276]. Sgs1 and Top3 also interact physically. The region of Sgs1 that interacts with Top3 has been mapped using different biochemical techniques to the extreme N-terminal region of the protein (Figure 4). Studies with Sgs1–Top3 fusion proteins that

lack the Top3 interaction segment on Sgs1 indicate that the N-terminal Top3 interaction domain probably has no function other than to recruit Top3 to sites of action [277]. The molecular and genetic studies outlined above suggest that Top3 and Sgs1 function mainly as a complex. It seems likely that Sgs1 helicase creates a DNA structure that is resolved by the ssDNA strand passing activity of Top3. Possible structures are discussed in the general discussion presented below. Top3 is highly specific for ssDNA, but probably lacks additional substrate specificity. Nevertheless, as the MMS sensitivity of *sgs1 top3* cells is greater than that of *sgs1* cells, some lesions produced by MMS may be processed by Top3 independently of Sgs1 [276].

The *SLX* genes. Mullen and co-workers [278,279] have identified a panel of genes in a screen for mutations that show synthetic lethality in an *sgs1* background. These genes were designated *SLX*. They also found synthetic lethality between the *slx* mutants and *top3*, suggesting that the Slx proteins function in pathways parallel to that of Sgs1/Top3. *SLX6* was shown to be allelic to *SRS2* (see below). Slx5 and Slx8 are both putative RING-finger domain proteins, which exist as a complex in the cell and are required for efficient sporulation. Slx1, which contains a rare C-terminal C_4HC_3-type zinc binding domain, and Slx4 also form a complex. *SLX2* and *SLX3* have been identified as the two subunits of the Mus81–Mms4 heterodimeric complex that acts as a nuclease [278,279]. There is some controversy about the key substrate(s) for this complex, but these could include branched/forked structures in DNA (such as recombination intermediates), as well as Holliday junctions [248,280].

sgs1 srs2 double mutants are synthetically lethal or very slow growing [260,267,278]. Following sporulation of heterozygous diploids, approx. 40% of the *sgs1 srs2* double mutant spores form microcolonies of 2–100 cells. Lifespan analyses of cells from these colonies revealed that they survive on average for only three generations. The microcolonies contain a high percentage of large budded cells, which are probably arrested at the G_2/M checkpoint [260]. Triple mutants having the combination of *sgs1 srs2* with *rad51*, *rad55* or *rad57* are viable, however, indicating that disabling HR rescues the *sgs1 srs2* inviability [267]. The synthetic phenotype is likely, therefore, to be a consequence of unconstrained recombination or the accumulation of recombination structures that cannot be resolved adequately in the absence of these helicases. There are, however, apparently some overlapping functions of Sgs1 and Srs2. The *SGS1* gene has been identified as a multicopy suppressor of the sensitivity of *srs2* mutants to MMS and HU [281].

Mutation of *RAD51*, *RAD52*, *RAD54*, *RAD55* or *RAD57* can also suppress the synthetic lethality of *mus81 sgs1* double mutants. Similarly, *rad51* suppresses the synthetic lethality of *mms4 sgs1* double mutants. These data suggest that the Mus81–Mms4 complex acts in the HR pathway, but in a branch different from that in which Sgs1 is required. However, as *mus81 sgs1 rad51* triple mutants still grow more slowly than the corresponding single *rad* mutants, the effect of *mus81 sgs1* is unlikely to be restricted to HR [274].

MGS1/WHIP. *MGS1* encodes a protein related to the subunits of RFC. The central region of Mgs1 is also similar to the corresponding region of *E. coli* RuvB, and contains motifs characteristic of ATPases [176]. Its human orthologue (WHIP) has been found to interact physically with WRN (see above) [162]. The Mgs1 protein has ssDNA-dependent ATPase and DNA annealing activities.

mgs1 top3 double mutants show a very severe growth defect, which can be partially suppressed by mutation of *SGS1* [176]. *sgs1 mgs1* double mutants also grow abnormally slowly, and show dramatic decreases in lifespan and plating efficiency [162]. The frequency of recombination is also increased in the double mutant. However, unlike *sgs1 srs2* and *sgs1 mus81* double mutants, deletion of *RAD52* does not rescue the slow growth of *sgs1 mgs1* mutants. Mgs1 and Sgs1 have been shown to interact with the Pol3 and Pol31 subunits of DNA Polδ in a yeast two-hybrid screen [282]. Although the synthetic genetic interactions outlined above suggest the involvement of Sgs1 and Mgs1 in different biochemical pathways, the physical interaction between the two proteins would point to some common functions, which are also conserved between WHIP and WRN in human cells.

Neurospora QDE-3, and its involvement in RNAi

In post-transcriptional gene silencing, or 'quelling' as it is usually called in *Neurospora crassa*, three classes of defective mutants (*qde-1*, *qde-2* and *qde-3*) have been isolated. The *QDE-3* gene, identified by Cogoni and Macino [283], encodes a 1955-amino-acid protein with a predicted molecular mass of 216.6 kDa (Figure 1). To date, QDE-3 represents the largest member of the RecQ family. Apart from being deficient in quelling, *qde-3* mutants show increased sensitivity to the topoisomerase I inhibitor camptothecin compared with wild-type cells. However, wild-type and *qde-3 N. crassa* cells show the same capacity to repair DNA damage induced by MMS, HU, mitomycin C and UV [283].

CONCLUDING REMARKS AND MODELS FOR THE ROLE(S) OF RECQ HELICASES

RecQ helicases are apparently expressed in every organism, ranging from bacteria to humans; however, the number of RecQ family members present in each organism differs. *E. coli* and yeasts express a single RecQ helicase, while humans have at least five. The different RecQ helicases of higher eukaryotes seem to be involved in at least some distinct cellular processes, a contention based on the diversity of clinical symptoms associated with the three RecQ helicase-related human genetic diseases. Some of these processes will be evolutionarily conserved and present in lower organisms, while others might be related more specifically to the complexity of the genomes of the multicellular organisms. The conserved functions, however diverse they are, must be carried out by the sole RecQ helicase of *E. coli* and yeasts.

Ectopic expression of human BLM or WRN can, at least partially, rescue the elevated rates of spontaneous recombination and illegitimate recombination of *sgs1* mutants [273,284], which suggest evolutionary conservation of the anti-recombinogenic role of Sgs1, BLM and WRN. However, complementation of the reduced lifespan [284] and HU sensitivity [273] of *sgs1* mutants can be accomplished only by BLM, and not by WRN. BLM, but not WRN, can also restore the slow growth phenotype reminiscent of single *top3* mutants when expressed in an *sgs1 top3* double mutant [273]. Restoration of growth [85,285] and HU sensitivity [285] to the level of the single *top3* mutants by BLM in *sgs1 top3* double mutants have also been reported independently. Human Top3β has also been reported to interact with Sgs1 [286], although there is no evidence that BLM interacts with this isoform. Instead, Top3β may form a complex with RECQ5 in humans [224]. As discussed above, BLM is associated *in vivo* with Top3α.

WRN or WRN-like proteins have hitherto been isolated only from vertebrates. However, proteins containing a nuclease domain similar to that of WRN have been identified as AtWRNexo from *Arabidopsis thaliana* [287], CG7670 from *Drosophila* [288] and

Mut-7 from *Caenorhabditis elegans* [289]. Most interestingly, AtWRNexo has been shown to interact with AtRecQL2, one of the five RecQ homologues of *Arabidopsis* [287], which indicates that the fusion between the exonuclease and helicase parts of a WRN prototype is likely to have been a relatively late evolutionary event. Hitherto, no orthologue of the helicase domain of WRN has been identified in *Drosophila*. The *Caenorhabditis* F18C5.2 gene product, which lacks an exonuclease domain, shows strong identity with WRN, and this protein may form a heteromeric complex with Mut-7 to give a functional WRN-like activity. Mut-7 has been described as a mutator gene causing transposon mobilization, and its involvement in RNAi is also documented [289]. There is, however, currently no evidence for a role for any of the human RecQ helicases in these processes [290].

The most obvious cellular manifestation of a deficiency in a RecQ helicase is inherent genomic instability. This generally takes the form of an increase in the frequency of various classes of chromosomal aberrations – but the hallmark feature is hyper-recombination (or at least what is scored as hyper-recombination; see below). These hyper-recombination events occur both between identical sequences (such as between sister chromatids) and between non-identical sequences, such as the elevated illegitimate recombination seen in *recQ* mutants and the elevated homeologous (similar, but not identical) recombination seen in *sgs1* mutants. Despite the above, it seems very likely that RecQ helicases act primarily during DNA replication to prevent replication fork demise and/or to assist in the repair of damaged/arrested forks. In this scenario, the apparent hyper-recombination results from an inability of the cell to utilize the preferred, RecQ helicase-dependent, replication fork 'repair' pathway. Alternatively, or in addition, RecQ helicases may act directly as 'anti-recombinases' to prevent and/or reverse the process of DNA strand invasion/exchange, etc., during recombination reactions. In this latter scenario, RecQ helicases act primarily as quality controllers of recombination processes – perhaps those occurring both during and outside of DNA replication. We shall discuss these general ideas in more detail below.

Based on the biochemical properties of RecQ helicases, and on the phenotypic consequences of loss of RecQ function in different organisms, three general models can be proposed for the role of these enzymes in DNA metabolism. Model 1 (Figure 5) is derived from the striking and possibly unique ability of members of the RecQ family to unwind DNA molecules that adopt a secondary structure distinct from regular B-form DNA. The most widely studied of these 'alternative' DNA structures is G-quadruplex (G4) DNA, a highly stable DNA structure that can form in guanine-rich loci such as within the c-*myc* gene promoter, where it seems to regulate gene expression, and within the G-rich strand of telomeres. In this model, RecQ helicases act as 'roadblock removers', eliminating DNA secondary structures or other obstacles that would otherwise impede the progress of translocating protein complexes. The most obvious context for RecQ enzymes to effect this role would be during DNA replication, where they would act to smooth progression of the advancing replisome. In the absence of a RecQ helicase, forks would stall or be disrupted, and this would lead to genomic instability during the attempted rescue of the blockade. This putative role may be more critical if the obstacle is located in the leading strand template. It is conceivable that the discontinuously synthesized lagging strand could continue to be produced in the face of such an obstacle, because it could be synthesized to include daughter strand gaps that would require subsequent filling via a post-replicative, recombination-mediated process.

In model 2 (Figure 6), RecQ helicases act as regulators of the fidelity of genetic recombination reactions. This putative 'quality control' function could act at any one of a number of stages during the multi-step recombination process. For example, the RecQ helicases are able to disrupt D-loop structures that are an essential early step in the formation of joint molecules during DNA recombination. The conserved interaction between RecQ helicases and RAD51 may be relevant in this context, since RecQ enzymes might be targeted to recombination intermediates and then serve to 'reject' those that are in some way inappropriate, such as those in which DNA mismatches are present. The conserved interaction between RecQ helicases and mismatch repair factors may also, therefore, be relevant. This role for RecQ enzymes would act to suppress homeologous and other forms of unscheduled DNA recombination. RecQ helicases also selectively recognize Holliday junction recombination intermediates and catalyse efficient and extensive branch migration of these structures. Hence they could act at a later step during recombination reactions, after Holliday junction formation, and serve to reverse the reaction and hence prevent completion of recombination.

The third model, which has been discussed extensively elsewhere [291], is really a collection of inter-related models that seek to explain the various ways in which HR processes can facilitate the restart of stalled replication forks or the repair of damaged forks. In these models, RecQ helicases may act at one or more

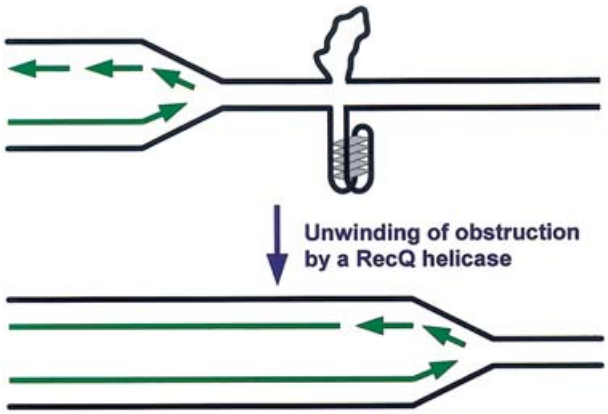

Figure 5 Model for a role for RecQ helicases as 'roadblock removers'

The progression of the replication fork could be impeded by the presence of DNA secondary structures or other roadblocks. An intrastrand G-quadruplex is shown as an example. A RecQ helicase operating ahead of the fork might disrupt the structure, permitting smooth replisome progression.

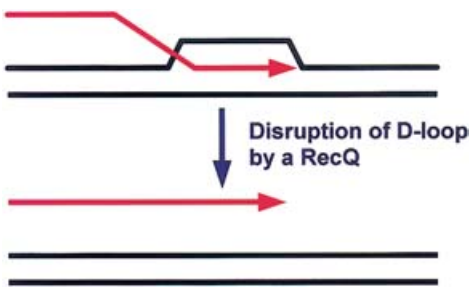

Figure 6 Model for a role for RecQ helicases as 'anti-recombinases'

In recombination, a single-stranded tail (red) invades a homologous duplex (black) to generate a D-loop. RecQ helicases could act to suppress recombination by disruption of the D-loop structure.

than one stage. In their absence (i.e. in RecQ helicase-deficient mutants), there is a 'pathway switch' such that the preferred, non-recombinogenic pathway is eliminated and instead restoration of a productive replication fork depends on processes that involve exchanges between homologous sequences. In this scenario, RecQ helicase deficiency does not actually lead to an increase in the number of 'lesions' (or fork blockages – unlike the roadblock remover model) that arise and hence there is no increase in the intrinsic rate of recombination. Instead, the pathway-switch model proposes that the 'read-out' or outcome of the repair reactions differs depending on whether RecQ helicases act or not, i.e. no exchanges (cross-overs) between sequences in the presence of RecQ compared with exchanges in their absence. This would manifest in a RecQ helicase-deficient cell as hyper-recombination/hyper-SCE formation etc.

One such pathway-switch model has been proposed recently on the basis of studies by Adams et al. [100] on *Drosophila blm* mutants lacking the BS homologue in that organism. These authors proposed that BLM acts in a DNA strand break repair pathway known as SDSA, in which no Holliday junctions are generated and no reciprocal exchanges occur between the donor and recipient DNA molecules (Figure 7). Hence SCEs never form with this mode of repair. These authors argue that, in *blm* mutants, where SDSA is either absent or impaired, lesions are forced down alternative pathways, such as the classic DSB repair pathway involving Holliday junction formation [100], and that it is this that leads to SCEs when the junctions are resolved in an orientation that generates crossing over. The role, if any, of BLM in SDSA has not been defined, but one possibility is that the migration of the D-loop structure during the DNA synthesis phase is promoted by BLM.

A conceptually related model, which has been outlined extensively elsewhere and which we proposed previously to account for the excess of SCEs in BS cells, is the so-called 'chicken foot' model (Figure 8). This model has been developed from studies in bacteria and eukaryotes, and proposes that forks stalled at lesions in the leading strand template can cause fork regression and the annealing of the nascent DNA strands to generate a four-armed structure analogous to a Holliday junction. It is known that, at least under certain circumstances, the lagging strand is extended beyond the point where the leading strand is arrested at the lesion. If this were to occur, the regressed fork would be asymmetrical, and the longer lagging strand could then act as a template for extension of the leading strand. Potentially, all that would be required to re-set the fork is to have Holliday junction branch migration and reloading of the replisome. The extension of the leading strand prior to branch migration means that the lesion in the template would have been bypassed and could be removed later by excision repair pathways. Clearly, there is the potential for the 'reverse' Holliday junction branch migration proposed in this model to be catalysed by a RecQ helicase, given the known ability of these enzymes to promote this reaction. Assuming a role for RecQ helicases in such a non-recombinogenic lesion bypass/fork restoration pathway, it seems likely that the excessive recombination seen in RecQ helicase-deficient cells would result from cleavage of the Holliday junction at the regressed fork when reverse branch migration is not possible. Several potential routes for replication restart after junction cleavage have been proposed. For a more extensive discussion of this area, we refer readers to some excellent recent review articles [291–295].

If, as we argue above, many RecQ helicases act in concert with topoisomerase III, it is worth considering how the ssDNA strand passage activity of topoisomerase III might fit into any of these models. It is not immediately obvious (at least not to us) what role topoisomerase III might play in the roadblock remover model or the chicken foot model. Given the apparent selective association between topoisomerase III and D-loop structures, it is possible to envisage roles in the SDSA process (perhaps termination?) or in an anti-recombination model. Moreover, it has long been recognized, and has been discussed by us and others elsewhere, that topoisomerase III has the potential to be involved in the resolution of Holliday junctions – perhaps in combination with the branch migration activity of RecQ helicase. We are currently testing this idea biochemically using human BLM and its partner topoisomerase IIIα. There is certainly indirect evidence that unresolved (or presumably toxic) recombination intermediates, presumably including Holliday junctions, accumulate in RecQ helicase-deficient cells, because ectopic expression of the bacterial RusA resolvase can suppress defects in, for example, *rqh1* mutants and WS cell lines.

Although the models described above are derived from the known biochemical properties of RecQ helicase, and are consistent with the phenotypic consequences of loss of RecQ helicase function, there is really very little conclusive evidence available that points specifically to one clearly defined role for these enzymes. Even in bacteria, the details of replication fork repair processes are only just emerging, and the situation in eukaryotes is

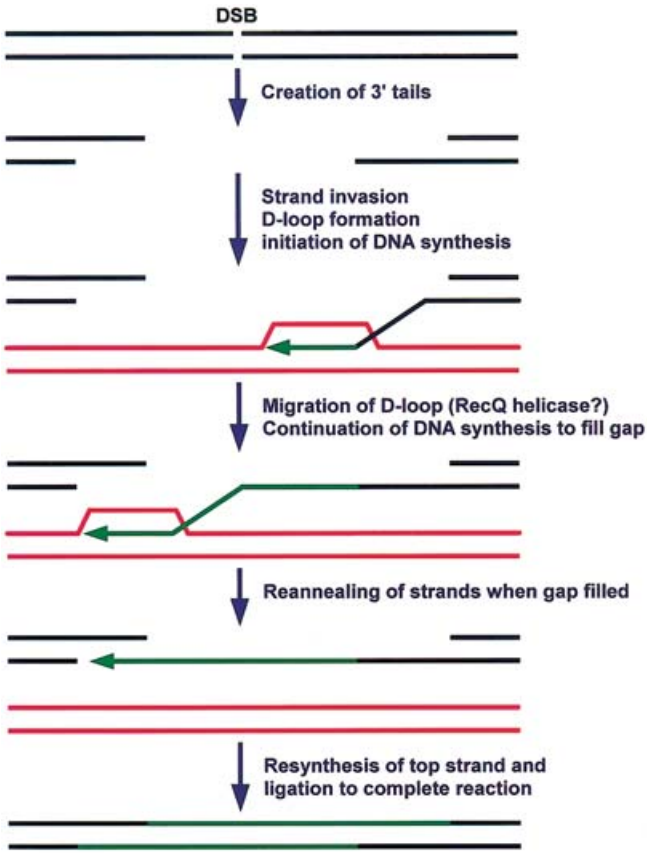

Figure 7 Model for a role for RecQ helicases in the SDSA pathway for DNA repair

A DSB is shown in one duplex (black), and this is resected to generate 3′ ssDNA tails. A 3′ end invades a homologous duplex (red) to create a D-loop. The 3′-hydroxy terminus primes new DNA synthesis (green). The D-loop migrates along the intact template accompanied by continued DNA synthesis. RecQ helicases could catalyse this migration of the D-loop; when the D-loop reaches the end of the gap, the newly synthesized strand is displaced from the D-loop and re-anneals with the opposite of the broken duplex. Repair is completed by gap-filling DNA synthesis of the 'top' strand.

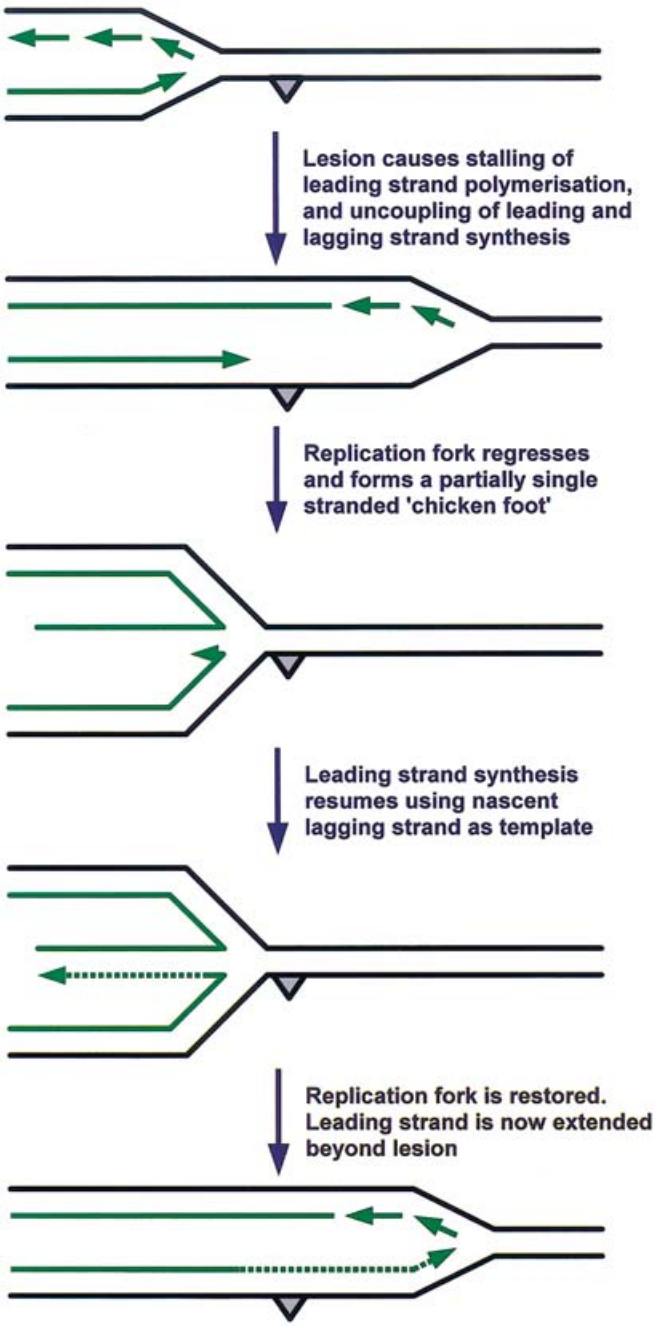

Figure 8 'Chicken foot' model for a role for RecQ helicases in replication restart

A lesion in the leading strand template (grey triangle) blocks leading strand synthesis. Lagging strand synthesis continues for a short period. The fork regresses, allowing the nascent strands to anneal, creating a 'chicken foot' structure (four-way junction). The leading strand is then extended (broken green line) using the longer lagging strand as a template. The fork is reset (by a RecQ helicase?) by branch migration of the four-way junction, and now the leading strand has been extended beyond the lesion. Replication can recommence.

even less well defined. Of course, these models are just that – models, and may require extensive reworking in the light of new data that may emerge in the coming years. It is certainly possible that these models are not mutually exclusive and that RecQ helicases play several different roles in different pathways to assist in the smooth progression of the replisome. This is perhaps a more likely scenario in mammals, where the multiple RecQ family members may each play specialized roles that are all performed by the single RecQ helicase in bacteria and yeasts. If we are to understand how RecQ helicases suppress neoplastic transformation and delay the aging process in humans, it is essential that we devise new genetic and biochemical approaches in order to improve our currently poor level of understanding of the functions of these highly conserved enzymes.

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