

## Review

# Molecular genetics of RecQ helicase disorders

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**Abstract.** The RecQ helicases belong to the Superfamily II group of DNA helicases, and are defined by amino acid motifs that show sequence similarity to the catalytic domain of *Escherichia coli* RecQ. RecQ helicases have crucial roles in the maintenance of genome stability. In humans, there are five RecQ helicases and deficiencies in three of them cause genetic disorders characterised by cancer predisposi-

tion, premature aging and/or developmental abnormalities. RecQ helicase-deficient cells exhibit aberrant genetic recombination and/or DNA replication, which result in chromosomal instability and a decreased potential for proliferation. Here, we review the current knowledge of the molecular genetics of RecQ helicases, focusing on the human RecQ helicase disorders and mouse models of these conditions.

**Keywords.** RecQ helicases, DNA repair, homologous recombination, chromosomal instability, cancer predisposition, premature aging, telomeres.

## Introduction

Helicases are the enzymes that separate the complementary strands of energetically stable, double-stranded, nucleic acid structures utilizing the energy derived from hydrolysis of ATP. The single-stranded (ss) RNA or DNA molecules thus produced are then used as templates or substrates in various biological processes, such as DNA replication, recombination, repair and transcription. One of the most highly conserved groups of DNA helicases is the RecQ family, which is named after the prototypical family member found in bacteria. RecQ was initially identified in *Escherichia coli* as a factor involved in modulating resistance to thymine starvation and in homologous recombination [1, 2]. The RecQ helicases contribute to the maintenance of genome stability across various species [3–7]. Interestingly, the number

of RecQ enzymes expressed by a particular organism is apparently correlated with their genome size. Some bacteria and archaea do not possess any *recQ* genes and, in most of these cases, these organisms possess a genome that is smaller than 2 Mbp; exceptions being *Mycobacterium tuberculosis* (4.40 Mbp) and *Mycobacterium leprae* (3.26 Mbp) [8]. Those bacteria and archaea that possess a large genome generally express one or two RecQ homologues [8]. To date, no eukaryotic organism has been found that lacks a 'RECQ' gene. *Saccharomyces cerevisiae* has one RecQ homolog (Sgs1) [4]; at least two are found in *Drosophila melanogaster*, and four homologues have been found in *Caenorhabditis elegans* [9]. Mammals and birds have five homologues, which are designated RECQ1, BLM, WRN, RECQ4 and RECQ5 in humans [10–14]. Currently, the intense interest in the RecQ helicase family is driven largely by connections with human genetic disease. Defects in at least three of five human RecQ homologues are responsible for defined genetic diseases, and we refer

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to these as Bloom's syndrome (BS, mutations in *BLM*), Werner's syndrome (WS, mutations in *WRN*) and the RECQ4 syndromes [Rothmund-Thomson syndrome (RTS), RAPADILINO, and Baller-Gerold syndrome (BGS)] [12, 13, 15–17]. These disorders display various features of premature aging, cancer predisposition and developmental abnormalities. In this review, we focus on recent molecular genetic studies of these RecQ helicase family diseases, with an emphasis on insights gleaned from analysis of mouse models of these conditions.

## Bloom's syndrome

### Clinical features and the molecular genetics of BS

BS is a rare genetic disease characterised by severe growth retardation and dramatic cancer predisposition [5, 18, 19]. The most common physical characteristics of affected individuals are a narrow face with a prominent nose, a butterfly-shaped facial rash that is induced by sunlight exposure, a high-pitched voice and abnormal skin pigmentation, especially on sun-exposed parts of the body. Some BS patients also show learning disability, mental retardation, immune deficiency, diabetes and/or mild anaemia. Men with BS cannot produce mature sperm and this results in infertility. Affected females are rarely infertile, and at least four pregnancies in BS females have been confirmed [5, 20]. However, menstruation is, in many cases, irregular and tends to cease at an unusually young age. One of the most characteristic symptoms of BS is cancer predisposition. Various kinds of malignancies appear early in life and within various tissues. With the possible exception of melanoma (perhaps because affected individuals avoid sunlight exposure) it would appear that BS individuals succumb to the full range of cancers seen in the normal population. However, they do so several decades earlier in life than is expected. Some patients also develop tumours that are normally very rare in the general population, such as osteosarcoma, Wilms tumour, and medulloblastoma. Moreover, multiple independent tumours are a feature in some cases.

Genomic instability is proposed to drive tumorigenesis in BS. Significantly, a higher frequency of somatic mutation is observed in cells from BS patients. The frequency of mutation at the hypoxanthine phosphoribosyltransferase (*HPRT*) gene locus in BS cells is elevated 10-fold and the frequency of mutation at *GPA* locus is more than 50-fold higher than that in control cells [21, 22]. BS cells also show an abnormally high rate of micronucleus formation [23], and homologous recombination [24]. The hallmark feature of BS is an increased rate (10-fold) of sister-chromatid

exchanges (SCEs), which is used as a molecular diagnosis of the disorder [25, 26]. SCEs are expected to have no functional consequence if the exchanges are precise, because sister chromatids have an identical sequence. However, if SCEs occur either unequally using identical sequences or between non-identical repeat sequences, they can lead to chromosomal rearrangements, such as duplications, deletions and translocations. Moreover, a likely source of functionally significant genetic exchanges in BS cells occurs when the homologous chromosome is utilised as a repair template and not the sister chromatid, because this can trigger loss of heterozygosity (LOH). This relationship between chromosomal instability, LOH and tumorigenesis was revealed in a mouse model of BS [27, 28], which we discuss below.

The mode of inheritance of BS is autosomal recessive. BS is extremely rare, but is somewhat more common in Jewish persons of Eastern European descent (Ashkenazi Jews) [29]. Ellis et al. [12] identified the gene responsible for BS by a positional cloning approach and named it *BLM*. The primary structure of *BLM* is homologous that of to *E. coli* RecQ. Most mutations in BS patients are either nonsense or frameshift mutations that cause truncation of the *BLM* protein. These truncated *BLM* proteins are expected to be non-functional because they either lack the essential catalytic helicase domain and/or the nuclear localization signal (NLS) located in the C-terminal region of *BLM* [30]. Missense mutations have also been found in *BLM* in BS cases. Analysis of these missense mutations has been performed in a limited number of cases and, where tested, these mutations destroy the enzymatic function of *BLM* [31, 32]. The most common mutation is the so-called *BLM*<sup>Ash</sup> allele, which contains a frameshift mutation (6-bp deletion and 7-bp insertion) in exon 10 that causes premature translation termination. It was estimated that approximately 1% of the Ashkenazi Jewish population carry this allele [33], but this mutation has also been found in individuals of non-Ashkenazi ancestry [34]. Taken together, these data indicate the BS is caused by loss of function mutations in *BLM*.

### Biochemical functions of the *BLM* helicase

*BLM* is a helicase that separates the complementary strands of duplex DNA. Although not shown formally, *BLM*, like other RecQ helicases, probably translocates unidirectionally (3'–5') along one strand of the duplex [35]. However, more recent studies suggest that *BLM* is a DNA structure-specific helicase [36–38]. *BLM* can unwind 3'-tailed duplexes, bubble structures, forked duplexes, G-quadruplex structures, DNA displacement loops (D-loops) and four-way junctions modelling the Holliday junction recombina-

nation intermediate [36, 38–40]. The eukaryotic ssDNA binding protein, RPA, stimulates the unwinding activity of BLM [41]. Interestingly, BLM possesses what appears to be the opposite activity to that of a helicase. BLM can catalyse the annealing of the complementary single strands of DNA [42, 43]. The annealing activity of BLM does not require ATP or  $Mg^{2+}$ , unlike its helicase activity, and annealing is actually inhibited by ATP. This suggests that ATP binding and hydrolysis triggers an alteration in the mode of action of BLM. The mechanism for this ATP-driven ‘switch’ is not clear, but it may be significant that DNA strand annealing-proficient forms of BLM bind ssDNA in a manner that is different from that of strand annealing-deficient variants [42].

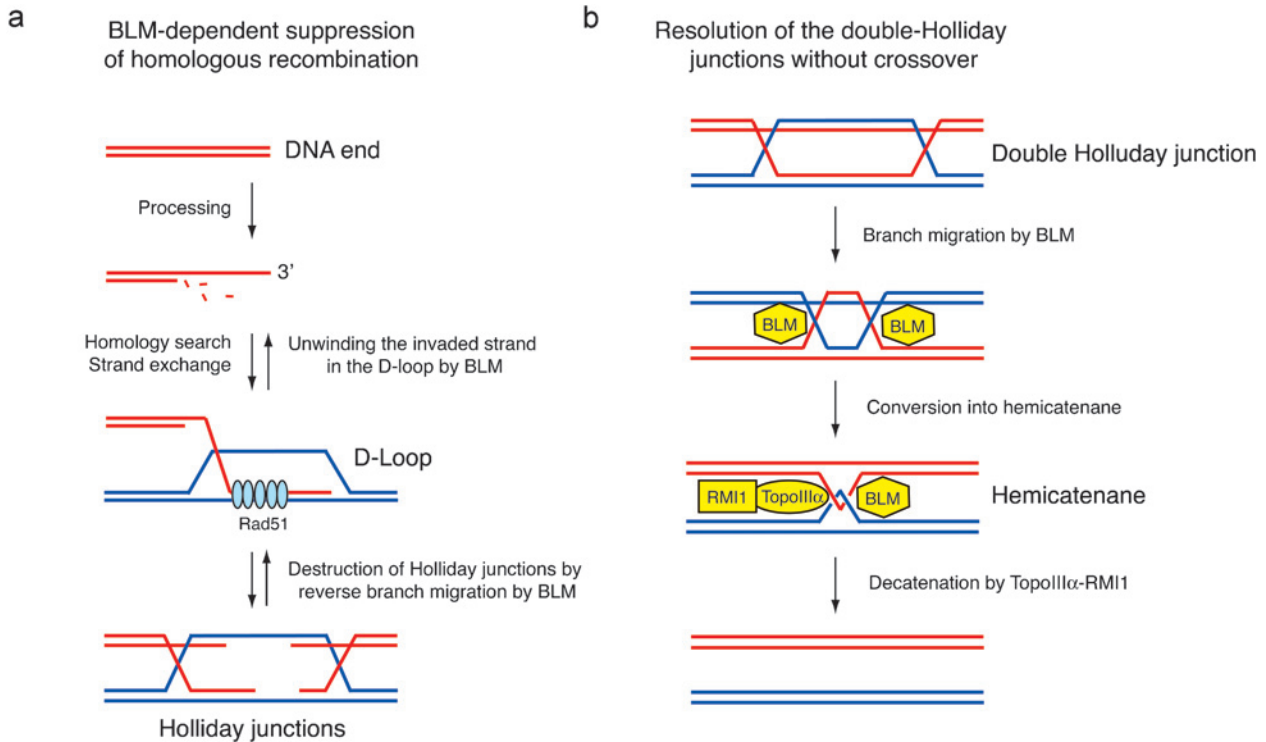
BLM can interact with topoisomerase III $\alpha$  (TopoIII $\alpha$ ) and can stimulate its DNA strand passage activity [44, 45]. TopoIII $\alpha$  is a type IA topoisomerase that can relax negative supercoiled DNA and can generate or resolve catenated structures [46]. Recently, BLAP75 was identified as a new factor that can interact with BLM-TopoIII $\alpha$  [47, 48]. The primary structure of BLAP75 is similar to that of the yeast Rmi1 protein. Yeast *rmi1* mutants show phenotypes similar to those of *top3* mutants, and yeast Rmi1p binds Sgs1p and Top3p in that organism [49, 50]. At this moment, the precise function of BLAP75/RMI1 is not known, and it is not clear if this protein possesses any catalytic functions. We describe a function of the BLM-TopoIII $\alpha$ -BLAP75/RMI1 complex in the next section.

### Roles of BLM in homologous recombination and in the DNA damage response

One of the characteristic phenotypes of BS cells is an elevated level of SCEs. The mechanism of SCE creation is not understood in detail, but at least some SCEs require homologous recombination for their formation [51, 52]. Biochemical studies showed that BLM preferentially disrupts two structures that typically form as intermediates in homologous recombination; D-loops and Holliday junctions [38, 40, 53]. One possible role for BLM is to negatively control homologous recombination reactions. Homologous recombination occurs *via* two major steps; strand exchange and resolution. RAD51, a highly conserved ‘recombinase’ protein, binds to ssDNA ends and exchanges strands between homologous regions (Fig. 1a). After strand exchange, a triple-stranded intermediate, termed a D-loop, is formed. D-loops can either be destroyed by an unwinding reaction, or be converted into four-way junctions (Holliday junctions). BLM may suppress homologous recombination by unwinding the invading strand from the D-loop. Indeed, this reaction can be catalysed very efficiently *in vitro* by BLM [38, 53]. If the D-loops are

converted into Holliday junctions, these junctions have to be resolved otherwise the recombining molecules will remain covalently intertwined. The mechanism by which this occurs in eukaryotes is unknown. However, two possible mechanisms have been proposed. One mechanism, like that in bacteria, is for a specific endonuclease enzyme to resolve the Holliday junctions. In bacteria, the cleavage of Holliday junctions by these so-called resolvase enzymes generates one of two recombination products; a crossover or a non-crossover. The non-crossover class does not involve exchanging the flanking regions of chromosome, while the crossover class exchanges the flanking genetic markers. Since SCEs represent crossing-over events, the higher level of SCEs in BS cells can be explained by an elevated level of crossover recombination. BLM might suppress crossovers by destroying all forms of aberrant or unwanted strand invasion events through D-loop unwinding, as discussed above. Another possibility is that BLM promotes the process of synthesis-dependent strand annealing (SDSA) that only gives rise to non-crossovers (Fig. 1a). This has been proposed from studies in *Drosophila* [54], although recent evidence has argued against this being likely because BLM deficiency leads to structural alterations in both the template and the donor sequences, which would not be predicted from an SDSA model [55]. A third possibility is that BLM promotes resolution of Holliday junctions to generate exclusively or predominantly non-crossover products. In this regard, a new model for the resolution of Holliday junctions has been proposed recently. The BLM-TopoIII $\alpha$ -BLAP75/RMI1 complex was shown to resolve double-Holliday junctions without crossover (Fig. 1b) [56, 57]. Both BLM and TopoIII $\alpha$  are essential for this resolution [56] and BLAP75/RMI1 strongly stimulates the reaction [57, 58]. Unlike classical bacterial Holliday junction resolution, this new pathway does not require the endonucleolytic cleavage of Holliday junctions. It is proposed that BLM drives the convergence of the double Holliday junctions *via* branch migration, which converts the double Holliday junctions into a hemicatenane (Fig. 1b). Following this, the TopoIII $\alpha$ -BLAP75/RMI1 complex ‘resolves’ the hemicatenane structure using the ssDNA strand passage activity of TopoIII $\alpha$ . In this way, the double Holliday junction is removed without any crossing over (Fig. 1b) [56–59]. Because this reaction is mechanistically distinct from classical resolution, it has been termed Holliday junction dissolution [56]. Recent data indicate that the *Drosophila* orthologues of BLM and TopoIII $\alpha$  also catalyse dissolution [60].

Cell biological studies showed that BLM localises to promyelocytic leukaemia (PML) bodies in the ab-



**Figure 1.** Model of homologous recombination of BLM-dependent resolution without crossover. (a) Blunt DNA ends are first processed to generate 3' ssDNA tails. This tail is bound by Rad51, which then initiates a search of homology. Rad51 catalyses DNA strand invasion to create a D-loop. Some D-loops are converted into Holliday junctions. BLM could act to destroy D-loops or eliminate Holliday junctions by reverse branch migration. (b) A model for how BLM can act alongside its partners, topoisomerase III $\alpha$  and RMI1, to eliminate double Holliday junctions. BLM promotes convergent Holliday junction branch migration to create a hemicatenane. The hemicatenane is resolved by the strand passage activity of topoisomerase III $\alpha$  in conjunction with RMI1. This process is termed Holliday junction dissolution. See text for details.

sence of DNA damage [61–63]. The *PML* gene was originally identified as a site of translocation in a case of promyelocytic leukaemia, and the PML protein is required for the integrity of the PML body [64–66]. The function of PML bodies is still under investigation, but one role is suggested to be a storage site for proteins involved in the response to DNA damage. BLM can also form nuclear foci at sites of DNA replication, likely indicating a role for BLM at sites of damaged DNA replication forks (see below). Indeed, BLM dissociates from PML bodies and translocates to sites of DNA damage after treatment of cells with DNA damaging agents and replication inhibitors, such as hydroxyurea (HU),  $\gamma$ -rays and camptothecin [63, 67–69]. BLM foci induced by DNA damage colocalise with other DNA repair factors, especially with homologous recombination proteins such as RAD51, BRCA1, and the MRE11-RAD50-NBS1 complex [63, 70–72]. The dynamic behaviour of BLM has been well documented after the treatment of cells with HU. BLM dissociates from PML bodies quickly (<1 h) and localises to nuclear foci that represent sites of stalled replication forks. At 4 h after removal of HU, BLM has returned to the PML bodies [73]. Interestingly, the

rapid relocalisation of BLM from PML bodies does not occur in cells with a defect in the stress-activated kinase, ATR. A defect in the homologous kinase, ATM, required for the cellular response to DNA double strand breaks (DSBs), does not affect this function of BLM. However, the late response of BLM, in returning back to PML bodies, seems to require ATM. One way in which BLM might be controlled is through direct post-translation modification by these and other kinases. Indeed, BLM is known to be a phosphoprotein. Curiously, however, BLM phosphorylation peaks during mitosis, even in response to  $\gamma$ -irradiation and following exposure to DNA replication blocking agents such as HU. The Thr99 and Thr122 residues of BLM are phosphorylated by ATM kinase after  $\gamma$ -irradiation [74]. Indeed, a recent study confirmed that BLM is one of early responders to DSBs by the use of laser-induced DSBs [75]. The Thr99 and Thr122 residues are also phosphorylated in response to replication inhibition; but in this case, the phosphorylation is predominantly ATR dependent. Cells harbouring a T99A/T122A double substitution in BLM display hypersensitivity to HU and a greater degree of G2/M checkpoint arrest after release from

an S-phase blockade induced by HU than the corresponding cells expressing wild-type BLM [68]. These results suggest that BLM phosphorylation (on Thr99 and Thr122 and perhaps on other residues) has a crucial role in the recovery of cells from replicative stresses [68]. One possible role for BLM on the rescue of stalled forks is to promote replication forks regression, as has been shown biochemically [76].

BLM also seems to influence the signalling cascade that occurs following DNA damage. For example, p53BP1 accumulation after HU treatment is not observed in BS cells [73], and the formation of MRE11-RAD50-NBS1 foci is delayed [77]. Taken together, these studies strongly suggest that BLM is one of the early responders to DNA damage and likely plays a role, directly or indirectly, in the recruitment of other DNA repair factors to sites of DNA damage and stalled replication forks. A recent study showed that BLM is also phosphorylated by the MPS1 kinase, which plays a role in the spindle assembly checkpoint [78]. Ser144 on BLM is an important target for this kinase. Since cells carrying a *BLM* mutant with a S144A substitution cannot maintain a stable chromosome content, MPS1-dependent BLM phosphorylation seems to be important for ensuring that chromosome segregation is faithful. The role played by BLM in this process is not clear at this stage. However, our recent data indicate that BLM localises to anaphase bridges that represent delayed or failed sister-chromatid disjunction, suggesting a role for BLM directly in 'resolving' such structures (Kok Lung Chan and IDH, unpublished data).

There are conflicting reports concerning the sensitivity of BS cells to radiation and DNA damaging drugs. Some studies suggest a degree of  $\gamma$ -ray sensitivity, while others do not. Given that BS patients have been treated with therapeutic doses of x-rays for their malignancies without reports of adverse toxicity reactions, it seems unlikely that BS can be considered a radiosensitivity disorder, unlike ataxia telangiectasia caused by *ATM* deficiency. These conflicting reports may reflect the fact that many studies have not used isogenic cell lines. Our previous analyses using isogenic cells indicated clear sensitivity of BS cells to HU, in line with data from yeast and chicken models of BLM deficiency. Indeed, there have been consistent results from studies in chicken DT-40 cells lacking *blm*, which have indicated hypersensitivity to UV light, methyl methanesulphonate and 4-nitroquinoline-1-oxide, but not to  $\gamma$ -rays [52, 79].

#### A mouse model of BS

The generation of mouse models is an extremely useful way to study the function of a particular gene product at the organism level, because tissue culture

systems are limited to a study of cell autonomous functions. Moreover, studies on human patients with BS are complicated both by ethical issues and by the extreme rarity of the disorder. Currently, several groups have attempted to generate mice defective in *Blm*.

The first *Blm*-knockout mouse study was reported by Chester et al. in 1998 [80]. The region of *Blm* upstream of the helicase domain was replaced by a neomycin-resistance cassette. The homozygote mice showed embryonic lethality. Homozygote embryos were smaller than *Blm*-proficient embryos, which is consistent with the small stature of human BS patients. Homozygote embryos also showed anaemia, which is a feature of some BS patients. Moreover, elevated apoptosis was detected from embryonic day (E) 6.5 to 11.5 in the *Blm*<sup>-/-</sup> embryos. Viable cells could, however, be isolated from the embryos. The proliferation rate of these *Blm*<sup>-/-</sup> mouse embryonic fibroblasts (MEFs) is reduced, and an elevated level of SCEs is evident. These phenotypes are also consistent with data from analysis of human BS cells. We have summarised the phenotypes of these and other *Blm*<sup>-/-</sup> mice and compared them to the human condition (Table 1).

Luo et al. [27] were able to generate a *Blm*-hypomorphic mouse model (called *Blm*<sup>m3/m3</sup> mice). The gene targeting strategy was designed to delete exon 2 of *Blm*, which they called the *Blm*<sup>m1</sup> allele. However, they discovered the clone contained three copies of the targeting construct that had inadvertently integrated into the intron between exons 3 and 4. They named this allele *Blm*<sup>m2</sup>. Since the targeting construct contained *loxP* sites flanking the neomycin-resistant cassette, they then removed all three neomycin-resistant genes from the *Blm*<sup>m2</sup> allele using the Cre site-specific recombinase. This resulted in the duplication of exon 3, generating the *Blm*<sup>m3</sup> allele. They found that *Blm*<sup>m2/m2</sup> mice were embryonic lethal. However, both *Blm*<sup>m2/m3</sup> mice and *Blm*<sup>m3/m3</sup> mice were viable and fertile. *Blm*<sup>m3/m3</sup> mice in the C57BL/6 $\times$ 129S5 genetic background did not show the expected small body size. Because human BS patients often show immune deficiency and a reduced level of IgM [81], the *Blm*<sup>m3/m3</sup> were analysed for immune deficiency and were found to express a reduced amount of IgM. *Blm*<sup>m3/m3</sup> mice showed a higher level of tumour formation than wild-type controls. *Blm*<sup>m3/m3</sup> mice were crossed with *Apc*<sup>Min/+</sup> mice, which is a model for familial adenomatous polyposis (FAP). *Blm*<sup>m3/m3</sup> *Apc*<sup>Min/+</sup> mice showed higher numbers of polyps in the small intestine than did *Blm*<sup>+/+</sup> *Apc*<sup>Min/+</sup> mice, confirming the role of *Blm* as a tumour suppressor gene. *Blm*<sup>m1/m3</sup> embryonic stem (ES) cells and MEFs showed elevated levels of SCEs, and mitotic homologous

**Table 1.** Symptoms of Bloom's syndrome and phenotypes of mouse models.

	Bloom's syndrome	Mouse model-1 ( <i>Blm</i> <sup>-/-</sup> ) 80	Mouse model-2 ( <i>Blm</i> <sup>m3/m3</sup> ) 27
Embryonic lethality	N.D.	+++	-
Small stature	+++	+++	-
Male sterility	+++	N.D.	-
Immune deficiency	+	N.D.	± (Less IgM)
Anaemia	±	+++	-
Cancer predisposition	+++	N.D.	+++
Elevated SCEs	+++	+++	+++
Chromosomal instability	+++	+++	+++
Elevated apoptosis	+++	+++	+++

+++; strong effect; +: mild effect or strong effect in limited cases; ±: very mild effect; -: not observed; N.D.: not determined; SCE: sister-chromatid exchange.

recombination, which in turn led to an abnormally high frequency of LOH. This is a plausible molecular defect for explaining the role of *Blm* as a tumour suppressor. We have summarised the phenotypes of *Blm*<sup>m3/m3</sup> mice in Table 1.

Approximately 1% of Ashkenazi Jews carry the *BLM*<sup>Ash</sup> mutation. This allele contains a frameshift mutation within exon 10 that causes premature translation termination. To simulate this allele, Goss et al. [82] deleted exons 10, 11 and 12 and replaced them with an *Hprt* gene cassette (to generate the *Blm*<sup>Cin</sup> allele). *Blm*<sup>Cin/Cin</sup> mice showed embryonic lethality. Primary lung fibroblasts from *Blm*<sup>Cin/+</sup> mice showed a higher number of micronuclei, although there was no detectable increase in SCEs. To investigate the haploinsufficiency of *Blm*, murine leukaemia virus (MLV) was infected into wild-type and *Blm*<sup>Cin/+</sup> mice. Although animals of both genotypes developed T cell leukaemia, wild-type mice had a longer average lifespan than the *Blm*<sup>Cin/+</sup> mice. In addition, *Blm*<sup>Cin/+</sup> *Apc*<sup>Min/+</sup> mice were analysed because the gastrointestinal tract is a common site of cancer in BS patients. *Blm*<sup>Cin/+</sup> *Apc*<sup>Min/+</sup> mice showed higher numbers of tumours than did *Blm*<sup>+/+</sup> *Apc*<sup>Min/+</sup> mice. This is similar to the findings discussed above with *Blm*<sup>m3/m3</sup> mice, but in this case, an effect was seen even with *Blm* heterozygous mice.

Chester et al. [83] generated a conditional knockout mouse model because *Blm* null mutant mice are not viable. In this conditional mouse model, exon 8 of the *Blm* gene can be excised using the Cre-*loxP* system. Using transgenic mice expressing Cre-recombinase, these authors could inactivate the *Blm* gene in selected tissues. These mice showed tissue-specific tumours and chromosome instabilities, which correlated with the inactivation of *Blm* gene, indicating that direct loss of *Blm* is associated with tumorigenesis.

Since *Blm*<sup>m3/m3</sup> mice are viable and show a cancer predisposition phenotype, these mice have been crossed with other mice modelling human disease, such as telomerase-deficient (*Terc*<sup>-/-</sup>) and tuberous sclerosis 1-deficient (*Tsc1*<sup>+/-</sup>) mice [84, 85]. The telomerase mice are discussed later in this review in the WS section. Tuberous sclerosis is an autosomal dominant disease characterised by the development of benign hamartomatous growths in various tissues, such as brain, skin, heart, lung and kidney. At least two genes, *TSC1* and *TSC2*, have been identified from human patients. Several groups have generated mouse models, and *Tsc1*<sup>+/-</sup> and *Tsc2*<sup>+/-</sup> heterozygous mice are each predisposed to the development of renal tumours. Wilson et al. [84, 85] reported that *Blm*<sup>m3/m3</sup> *Tsc1*<sup>+/-</sup> mice display a synergistically higher level of tumour formation compared to *Blm*<sup>+/m3</sup> *Tsc1*<sup>+/-</sup> mice. A higher frequency of LOH was also observed in the *Blm*<sup>m3</sup> genetic background [85]. These studies suggest that a *Blm* defect accelerates not only APC-dependent tumorigenesis (see above) but also Tsc-dependent tumorigenesis.

#### New applications for *Blm*-deficient cells and mice

Since *Blm*<sup>m3/m1</sup> cells show an elevated level of LOH, these cells have been utilised for novel genetic screens. Such phenotype-driven genetic screening is not easy in diploid organisms because of a requirement for the creation of homozygous mutations. The elevated LOH activity in *Blm* mutant cells, however, makes it feasible to isolate homozygous mutants. For example, Guo et al. [86] identified known and novel factors of mismatch repair using *Blm*-mutated ES cells. In parallel, Yusa et al. [28] utilised a similar, but somewhat more sophisticated, system for related studies. Since *Blm* mutations cause various negative effects, such as increased mutation and unwanted LOH, these authors generated a system in which *Blm*-expression

can be turned on or off. Using a tetracycline-regulated system, they managed to inactivate and reactivate expression of the *Blm* gene. In the presence of doxycycline, an analogue of tetracycline, Blm protein was depleted from cells, but the expression of the *Blm* mRNA was recovered rapidly after the withdrawal of the doxycycline. They confirmed that this transient inactivation of Blm is sufficient to induce efficient LOH.

Suzuki et al. [87] have performed genome-wide screening in the mouse. Using *Blm*<sup>m3/m3</sup> mice, they identified several known, as well as novel, tumour suppressors. Since *Blm*<sup>m3/m3</sup> mice have an increased frequency of insertional mutagenesis, they were able to perform large scale screening of tumour suppressor genes relevant to retrovirally induced B cell lymphoma. Thus, *Blm*-deficient cells or mice have made it possible to perform genome-wide genetic screening in mammalian cells. This innovation will likely bring many new applications in mouse genetics.

### Werner's syndrome

WS is an autosomal recessive inherited condition that can be considered as a 'progeroid' disorder in that it is associated with the premature development of many of the typical symptoms of old age [88]. This disease is generally rare, but it should be noted that most of the existing patients (about two thirds of the total) are Japanese. Genetic studies indicate that 1 in 160 of the Japanese population is a carrier of the disorder. People with WS grow normally until around puberty, but then fail to undergo the normal growth spurt and consequently show short stature. Symptoms of aging then start to appear progressively. Epstein et al. [88] reported that people with WS start to show greying of hair (at a mean of 20 years of age), skin changes (25.3 years), loss of hair (25.8 years), voice changes (26.6 years), skin ulceration (33.0 years), and diabetes (34.2 years). The average age of death is around 47 years [88]. Later, Goto et al. [89] confirmed these data through an investigation of Japanese families. Men with WS are generally infertile. Most of them have small testes and show abnormal seminiferous tubules. Affected females, however, are not always infertile. At least 53 pregnancies have been confirmed in WS individuals and, in two cases, multiple siblings were conceived. However, in the most severe cases, affected females do not even menstruate. The cells isolated from WS patients show a reduced ability to proliferate, which may be one of the underlying reasons for premature aging. We discuss the possible role of cellular senescence in the aging process in the next section.

### Molecular genetics and biochemistry of WS

In 1996, Yu et al. [13] identified the gene responsible for WS and named it *WRN*. About 90% of patients diagnosed with WS carry mutations in *WRN*. So far, over 30 different classes of *WRN* mutation have been discovered [90–93]. Virtually all known *WRN* mutations cause truncation of the WRN protein, and these truncated WRN polypeptides cannot localise to the nucleus because the dominant nuclear localisation signal sequence in WRN is located near to the C terminus of the protein [94]. There are two common mutations. One is the *WRN* R367Stop, which is the most frequent mutation found in Caucasian patients, although some Japanese also carry this mutation. The other is a splice site mutation at exon 26, which leads to deletion of exon 26 coding information from the *WRN* mRNA. This mutation is common in patients of Japanese origin.

The WRN protein contains a central domain homologous to *E. coli* RecQ [13]. The unique aspect of WRN structure is that it also possesses an exonuclease motif in the N-terminal region. Indeed, the WRN protein exhibits both ATP-dependent 3'–5' helicase activity and 3'–5' exonuclease activity [95–99]. WRN preferentially unwinds particular types of DNA structures, such as bubble and forked structures [36, 100, 101]. In addition, WRN can also catalyse the branch migration of Holliday junctions [102]. The WRN exonuclease domain is homologous to the nuclease domain of *E. coli* DNA polymerase I. The structure of this domain of WRN has been recently determined [103]. The exonuclease activity of WRN is neither powerful nor processive, but this activity can be stimulated by the Ku protein complex [104, 105]. Interestingly, the exonuclease activity of WRN also has preferred DNA substrates [101, 106]. WRN is able to digest bubbles, stem loops, and three-way and four-way junctions (Holliday junctions). WRN is reported to interact physically with several proteins involved in DNA replication, recombination and repair. A detailed description of these interactions is beyond the scope of this article, and we refer readers to recent reviews that cover this topic [107–109].

### Cellular senescence and telomere maintenance in WS cells

Primary cells from WS patients show replicative senescence in culture at much earlier passages than is characteristic of control cells. This rapid senescence is not generally well correlated with the length of telomeres (the sequences that cap the ends of the linear chromosomes) in the WS cells. During the serial passage of primary WS cells, the average length of telomeres appears to decline faster than it does in control cells; nevertheless, at a single-cell level,

telomere erosion rates are normal [110]. It would appear that telomeres in cells from WS are 'unstable' rather than simply being prone to excessive shortening. These studies suggest that WRN has a crucial role for maintaining the structural integrity of the telomere. Indeed, WRN protein localises to telomeres and can interact with telomere binding proteins, including TRF2, Ku and POT1 [105, 111–117]. Recent studies have suggested that WRN may be involved in the telomerase-independent telomere lengthening mechanism called ALT (alternative lengthening of telomeres). The molecular mechanism of ALT is not yet understood, but involves recombination-mediated copying of telomeric sequences from intact telomeres or extra-chromosomal telomeric circles. This would be consistent with the known role of Sgs1p in an ALT-like process in yeast [118–121]. However, mouse genetic studies have indicated that, in the absence of WRN, telomeric recombination is elevated [122].

Several studies showed that TRF2, one of the telomere maintenance proteins, regulates the exonuclease activity of WRN. This regulation influences the ability of WRN to process telomeric ends that form into T-loops, a proposed loop-back structure in which the G-rich single-stranded end invades internal telomeric sequences to generate a three-stranded structure (a form of D-loop). Machwe et al. [115] showed that TRF2 recruits WRN to T-loop structures, and that telomeric ends can be released from T-loops by the exonuclease activity of WRN [116]. Opresko et al. [117] also demonstrated that WRN could release telomeric-ends from T-loops, but in this study the role of TRF2 appeared to be to limit the exonuclease activity of WRN. The POT1 protein (protection of telomeres-1 protein) can also interact with WRN. The function of POT1 is to bind the single-stranded G-rich portion of telomeres, and it plays a role in capping telomeric ends and preventing them being recognised as DNA damage (*i.e.* DSBs in DNA). Interestingly, POT1 strongly stimulates the unwinding of T-loops by WRN *in vitro* [117]. These studies suggest that WRN may have an important role in the release of telomeric ends from T-loops, presumably to permit DNA metabolic process such as DNA replication to occur. Importantly, Crabbe et al. [123] reported that the helicase activity of WRN is important for DNA synthesis on lagging strands at telomeres. At this time, it is not clear whether telomere instability in WS cells is correlated with rapid replicative senescence of those cells, but it is becoming clear that WRN plays an important role in the maintenance of telomeric structures in proliferating cells. Studies on the mouse WRN-knockout are consistent with this proposal (see below).

A simple proliferative defect likely would not be sufficient to explain the accelerated aging in WS, because BS cells also show poor proliferation and yet BS patients do not show widespread premature aging symptoms. In WS, many pathological aging features appear most dramatically in the skin. Histological studies have shown that the appendages and epidermis are atrophic and that the thickness of epidermis is reduced in WRN patients [88]. In addition, the numbers of hair follicles and sebaceous glands are reduced. These histological studies indicate that there are a reduced number of cells in the epidermis. According to recent studies concerning skin development, skin stem cells differentiate into transit-amplifying cells, which in turn differentiate into interfollicular epidermis, hair follicles and sebaceous glands [124, 125]. It is known that c-Myc promotes differentiation of stem cells to transit-amplifying cells for the sebaceous gland and interfollicular epidermis lineages [126, 127]. Interestingly, WRN can limit MYC-induced cellular senescence [128]. It is possible that MYC-dependent differentiation may be highly accelerated in the skin development of WS patients, which results in accelerated aging in this organ. Therefore, skin aging in WS patients may be due to the combined effect of a low proliferation potential and accelerated MYC-dependent differentiation. However, this remains mere speculation at this stage.

### Mouse models of WS

At least four examples of the generation of Wrn-knockout mice have been reported.

The first report of a Wrn-knockout mouse model was in 1998 [129]. The targeting construct was designed to replace helicase motifs II to IV with a neomycin-resistance cassette. Unexpectedly, the targeted allele expressed a mutant Wrn protein that contains deletion of motifs III and IV. Therefore, this allele is generally referred to as the *Wrn*<sup>Δhel/Δhel</sup> mutation. *Wrn*<sup>Δhel/Δhel</sup> mice were born with a reduced Mendelian ratio of 1 (+/+): 2.0 (+/-): 0.8 (-/-) on a NIH black Swiss×129/SvEv outbred background, and of 1 (+/+): 1.9 (+/-): 0.6 (-/-) on a 129/SvEv inbred background. However, the surviving homozygote mutant mice grew normally and displayed no clear phenotype. *Wrn*<sup>Δhel/Δhel</sup> ES cells showed a higher frequency of spontaneous mutation, and hypersensitivity to topoisomerase inhibitors such as etoposide and camptothecin. Moreover, a reduced growth rate was observed in the homozygote MEFs. The *Wrn*<sup>Δhel/Δhel</sup> mice have been crossed to so-called pink-eyed unstable mice, which have a 70-kb internal duplication at the pink-eyed (*p*) gene locus [130]. Using these mice, the frequency of deletion between the duplicated sequences was analysed. The *Wrn*<sup>Δhel/Δhel</sup> mutation led to a significantly higher frequency of



spontaneous and camptothecin-induced deletion mutation than that seen in the pink-eyed mice alone. This study suggests that Wrn protein suppresses abnormal recombination events. Moreover, *Wrn*<sup>Δhel/Δhel</sup> mice have been crossed to *Parp1*-knockout mice [131]. *Parp1* gene encodes poly(ADP-ribose) polymerase-1, and this gene product is involved in single-strand break repair as well as base excision repair. The double-mutant mice showed an increased level of tumorigenesis and chromosomal instability compared to controls.

Wang et al. [132] generated transgenic mice that express human WRN with a putative dominant-negative mutation (K577M). The K577M version of WRN causes an inactivation of helicase activity, but the exonuclease activity is unaffected. Fibroblast cells from the K577M transgenic mice showed hypersensitivity to 4-NQO and a reduced proliferative capacity. Wrn-truncation mutant mice were developed by Lombard et al. [133]. Since no Wrn protein could be detected in these mice, this allele is considered to be a loss of function *Wrn* mutation. Chimeric mice from targeted ES cells (129/SvJ background) were crossed to BALB/c mice. Unlike *Wrn*<sup>Δhel/Δhel</sup> mice, these mice did not show the abnormal Mendelian ratio following heterozygous crosses. The knockout mice were fertile, and no clear phenotype was observed. Homozygote MEFs did not show hypersensitivity to camptothecin or 4-NQO. Splenocytes from mutant mice could proliferate normally, but MEFs showed a degree of accelerated senescence. Lifespan was also normal in these mice.

Knock-in mice that carry a one base substitution at the splice acceptor site of exon 26 were generated by Ichikawa et al. [134]. This mutation, called Mut-4, is found frequently in human WS patients of Japanese origin. These mutant mice showed no clear abnormalities and were fertile.

Overall, the phenotypes of the *Wrn*-mutated mice described above are much milder than those seen in human patients. However, this seems contrary to several molecular/cell biology studies that indicate an important role for WRN in telomere maintenance. Because the telomeres of mouse chromosomes are much longer than those of human chromosomes, it seemed possible that the lack of phenotype of the *Wrn*<sup>-/-</sup> mice reflected a degree of 'protection' of the mice against genome instability due to their having long telomeres. To address this possibility, Chang et al. [135] established double-knockout mice lacking Wrn and telomerase (*Wrn*<sup>-/-</sup> *Terc*<sup>-/-</sup>) and these were bred for several generations and compared to similarly bred *Terc*<sup>-/-</sup> or *Wrn*<sup>-/-</sup> mice. A high proportion of the *Wrn*<sup>-/-</sup> *Terc*<sup>-/-</sup> double-knockout mice showed premature aging phenotypes between generation 4 and 6

(G4-G6), by which time the telomeric ends of chromosomes had eroded significantly. The double-mutant mice died significantly earlier and body mass was smaller than in the *Wrn*<sup>-/-</sup> or *Terc*<sup>-/-</sup> controls. Bone density was also lower and all of affected mice showed osteoporosis. Out of 12 affected double mutants, 10 also showed glucose intolerance and some affected mice showed insulin resistant by 4 months of age. Affected double-knockout mice also displayed alopecia by 8 months and the frequency of tumour formation was higher than that of controls. These phenotypes are typically observed in people with WS, strongly arguing for a key role for Wrn at telomeres. We have summarised the phenotypes of *Wrn*<sup>-/-</sup> *Terc*<sup>-/-</sup> double-knockout mice and compared them to the symptoms of human WS (Table 2).

**Table 2.** Symptoms of Werner's syndrome and phenotypes of mouse models.

	Werner's syndrome	Mouse model ( <i>Wrn</i> <sup>-/-</sup> <i>Terc</i> <sup>-/-</sup> )
Small body	+++	++
Low bone density	?	++
Greying hair	+++	++
Hair loss	+++	++
Skin ulceration	+++	++
Cataract	+++	++
Diabetes	+	++
Reduced male fertility	+	?
Chromosomal instability	+++	+++
Elevated apoptosis	+++	+++
Low proliferative capacity	+++	+++

+++ : strong effect in nearly all cases; ++ : strong effect but only in affected (39/62); + : mild effect or strong effect in limited cases; - : not observed.

Recently, Laud et al. [122] showed that telomere SCEs are elevated in *Wrn*-deficient cells, providing further evidence that Wrn suppresses telomere instability. In addition, Du et al. [136] established *Wrn*<sup>-/-</sup> *Blm*<sup>m3/m3</sup> *Terc*<sup>-/-</sup> triple mutated mice, which showed significant telomere length erosion even by G3. These triple mutant displayed several aging phenotypes that arose even earlier than was seen in the *Wrn*<sup>-/-</sup> *Terc*<sup>-/-</sup> mice, including loss of fertility, reduced testis mass and absence of spermatogenesis, reduced body mass, kyphosis (a curved spine), reduced bone density, and a reduced rate of wound healing. These observations strongly suggest that Wrn is involved in a telomere-dependent senescence pathway, at least in mouse, and that this phenotype is only revealed when telomeres become critically short. It would also seem that Blm is in some way able to partially compensate for loss of

Wrn function in mice, and that in the absence of both of these RecQ helicases, accelerated aging is observed.

### **RECQ4 syndromes (RTS, RAPADILINO syndrome and BGS)**

Unlike the *BLM* and *WRN* genes, which were isolated by positional cloning approaches, the *RECQ4* gene was cloned by virtue of its homology to RecQ helicases [14]. The same group later found that mutations in this gene are responsible for at least some cases of RTS [15, 137]. Unexpectedly, not only is RTS a *RECQ4* disorder, but RAPADILINO syndrome and BGS are also caused by the mutations in this gene [16, 17]. RTS is a rare autosomal recessive disorder characterised by poikiloderma, greying and loss of hair at an early age, cataracts, small stature, skeletal and dental abnormalities, and a predisposition to cancer (especially osteosarcoma). The skin is usually normal at birth, but red patches start to appear at between 3 and 6 months of age. Subsequently, poikiloderma develops. Some patients show photosensitivity, although this is highly variable. It is notable that any skin cancer that develops in RTS cases can occur on skin regions not normally exposed to the sun. RAPADILINO syndrome is also a rare autosomal recessive disorder that is characterised by radial hypoplasia or aplasia (RA), patellar hypoplasia or aplasia and cleft or high arched palate (PA), diarrhoea and dislocated joints (DI), little size and limb malformation (LI), and nose slender and normal intelligence (NO). This syndrome is largely found in Finland (14 individuals from 11 families) but non-Finnish cases have been reported. People with RAPADILINO syndrome do not display obvious cancer predisposition. The other distinct difference between RAPADILINO and RTS is the occurrence of poikiloderma. Poikiloderma is one of the hallmarks of RTS, but is only rarely observed in patients with RAPADILINO syndrome.

Recently, the gene responsible for BGS was identified. van Maldergem et al. [17] noticed clinical overlaps between RTS, RAPADILINO and BGS, and subsequently identified *RECQ4* mutations in two unrelated BGS families. Clinical symptoms of BGS are radial ray hypoplasia, skeletal dysplasia, short stature, and craniosynostosis. Craniosynostosis is not observed in either RTS or RAPADILINO, and, thus far, a predisposition to cancer has not been reported as a feature of BGS.

Table 3 summarises the clinical symptoms of the three *RECQ4* syndromes. Radial ray defects, short stature, and patellar abnormalities seems to be common to all

three syndromes. Poikiloderma is observed in both RTS and BGS. However, palatal abnormalities and joint dislocation are only seen in RAPADILINO syndrome. Cataracts, dental and nail abnormalities, sparse hair, and cancer predisposition are typically observed only in RTS patients. Although a range of different *RECQ4* mutations have been found in these syndromes, including nonsense mutations, missense mutations, frameshifts, splice site mutations, and intronic deletions, it is difficult to reconcile how these mutations lead to three distinct syndromes. Additional mouse genetic analysis will likely be necessary to explain this puzzling issue. Such phenotypic complexity associated with defects in a single gene is not unprecedented, since mutations in *XPD* in humans can give rise to xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy [138–142].

The enzymatic function of *RECQ4* is poorly understood. Recently, the biochemical activities of *RECQ4* were analysed using purified recombinant protein [143]. Although *RECQ4* has ATPase activity, as expected, no DNA helicase activity could be detected. However, *RECQ4* possesses an ATP-independent ssDNA annealing activity that is similar to that seen with *BLM*, *WRN*, *RECQ1* and *RECQ5β* [42, 54, 143–145]. *RECQ4* can interact with the ubiquitin ligases, *UBR1* and *UBR2*, but the biological role of these complexes is unknown. Petkovic et al. [146] showed that *RECQ4* foci form in the nucleus and co-localise with PML bodies in the absence of DNA damage. *RECQ4* relocalises within the nucleus after treatment with etoposide, and then co-localises with *RAD51* foci, a pattern that is similar to that seen with *BLM*.

### **Mouse model studies on Recq4**

Several *RECQ4*-knockout mice have been established in different laboratories. However, the phenotypes of these mice vary considerably.

Ichikawa et al. [134] reported the first *RecQ4*-knockout mice, in which the region spanning exons 5–8 was deleted. This region encodes a portion of *RECQ4* that is upstream of the conserved helicase domain. All of these knockout mice died in early embryonic stages (E3.5–E6.5) in both the 129/SvJ×C57BL/6J and the 129/SvJ×BALB/c genetic backgrounds.

Exon 13 deletion in mice was reported by Hoki et al. [147]. The expression level of *Recq4* in homozygotes was strongly reduced (to only 1% or 2%), but nevertheless was detectable, and therefore it is likely that a truncated *Recq4* protein may be expressed from this allele. About 40% of homozygote newborn mice died immediately after birth, and 80% of the surviving homozygote mice died within 2 days. Severe growth

**Table 3.** Symptoms of RecQ4 diseases and phenotypes of mouse models.

	Rothmund-Thomson syndrome	RAPADILINO syndrome	Baller-Gerold syndrome	<i>Recq4</i> mouse model-1 147	<i>Recq4</i> mouse model-2 149
Small stature	+++	+++	+	+++	-
Patellar abnormality	-	+	+++	N.D.	N.D.
Radial ray defect	+	+++	+	?	+++
Dental and nail abnormalities	+	-	-	+++	N.D.
Poikiloderma	+++	-	+++	-	+++
Cataract	+++	-	-	-	N.D.
Sparse hair	+	-	-	+	-
Cancer predisposition	+(Osteosarcoma)	± (only 1 case)	-	-	+
Palatal abnormalities	-	+++	-	N.D.	+++
Joint disjunction	-	+++	-	N.D.	N.D.
Craniosynostosis	-	-	+++	N.D.	N.D.

+++; nearly all cases; +: limited cases; -: not observed; N.D.: not determined.

retardation and skin abnormalities were observed in these *Recq4*<sup>-/-</sup> mice. In the rare survivors, hair loss was observed at around 6 weeks, and most mice showed dry skin at 3–4 months of age. This was correlated with smaller dermal papilla, lower hair follicle density, and thinner layers of inner and outer root sheaths. Bone dysplasia and dystrophic teeth were observed, and thymic and splenic development was retarded. These observations suggest that the *Recq4* mutation affects the development of highly proliferative cells. However, poikiloderma and tumour formation were not seen in these mice, perhaps because of their short lifespan. Later, Yang et al. [148] independently generated a knockout model with the same allele. However, these knockout mice showed embryonic lethality and even the heterozygote mice showed reduced bone mass. Variation in the genetic background or in environmental stresses might be responsible for these differences between apparently identical models.

Another helicase domain deletion allele was generated by Mann et al. [149]. In this, exon 9–13 of *Recq4* was replaced with *Hprt* gene cassette; 16% of the homozygous mutant mice died within 24 h of birth. Surviving homozygous mice grew normally and growth retardation was not observed. However, they displayed skin and skeletal abnormalities. All homozygous mice showed hypo- or hyper-pigmented skin by 12 months of age. Around 5% of knockout mice had skeletal defects of the limbs at birth, and all of analysed mice also showed a palatal patterning defect that was not seen in controls. Interestingly, this *Recq4* mutation enhanced the number and size of intestine adenoma in the *Apc*<sup>Min/+</sup> genetic background. At the

cellular level, there was some evidence of genome instability: 24% of *Recq4*-mutated MEFs showed hyperploidy, and premature centromere separation was a feature.

We have summarised the phenotypes of the different *Recq4*<sup>-/-</sup> mice and compared them to the human condition (Table 3). Although these mutant mouse models show different phenotypes, most of the phenotypes observed are typical of symptoms associated with the RTS and/or RAPADILINO syndromes.

### RECQ1 and RECQ5

RECQ1 and RECQ5 are RecQ family members that more closely resemble the prototypical *E. coli* RecQ protein in terms of size. At present, no human disease condition has been identified in which mutations in *RECQ1* or *RECQ5* are a feature. We therefore limit our discussion of the biology of these homologues to the phenotypes of mouse or other eukaryotic knockout derivatives that lack *RECQ1* or *RECQ5*. We refer readers to previous reviews for a discussion of the biochemical properties of these proteins [107, 150].

*recq1*-deficient chicken DT40 cells have been established, but no clear phenotype was observed except for a slow-growth phenotype that was present only in a *blm*-deficient genetic background [151]. Recently, a *Recq1*-knockout mouse model was reported [152]. The homozygote mice did not show any particular developmental abnormalities, but cells from these mice showed an elevated frequency of chromosomal instability and a role in the DNA damage response.

The *RECQ5* gene encodes three differentially spliced mRNAs, encoding *RECQ5* $\alpha$ ,  $\beta$  and  $\gamma$ . However, only *RECQ5* $\beta$  localises to the nucleus [153], and this isoform has been shown to interact with both topoisomerase III $\alpha$  and  $\beta$  [153]. *RECQ5* $\beta$  also has 3'–5' DNA helicase activity, ssDNA annealing activity, and can catalyse branch migration of Holliday junctions [144, 154]. These biochemical functions are very similar to those of BLM. Therefore, it was proposed that *RECQ5* $\beta$  might serve as a 'backup' system for BLM. To understand the cellular function of *RECQ5* and the functional redundancy between *RECQ5* and BLM, *Recq5* single and *Recq5 Blm* double-mutant cells were established in both mouse ES cells and chicken DT40 cells [151, 155]. *Recq5*<sup>-/-</sup> mouse ES cells show a mild slow-growth phenotype and a slightly higher level of sensitivity to  $\gamma$ -rays. In contrast, the *recq5*-knockout DT40 cells do not show a slow growth phenotype and are not hypersensitive to UV, MMS and MMC. *Recq5*<sup>-/-</sup> mouse ES cells and MEFs display a significantly higher number of SCEs, a phenotype similar to that of *Blm*-mutant cells. Interestingly, the rate of SCEs in the *Recq5 Blm* double-knockout cells is even higher than that in either single mutant. Again, however, this phenotype was not conserved in chicken cells, as the frequency of SCEs in *recq5* single mutant DT40 cells was comparable to that found in wild-type cells. Nevertheless, a conserved role for RecQ5 in SCE suppression was suggested by the finding that the rate of SCEs in the *blm recq5* double-knockout DT-40 cells is higher than that in *blm* single mutant. These studies indicate that *RECQ5* might possess a function involved in control of SCE levels that is redundant to that of BLM.

*Recq5*-knockout mice have been generated, but no striking phenotype has been observed thus far. These mice were reported to be viable, fertile and show normal postnatal growth and development [155].

## Perspective

More than 10 years has passed since the *BLM* and *WRN* genes were cloned and their gene products shown to be RecQ helicase family members. Shortly after this, the *RECQ4* and *RECQ5* genes were also cloned. Since then, biochemical and cell biological studies of the RecQ family have suggested various possibilities as to how these gene products serve to maintain chromosome stability. For instance, the BLM-TopoIII $\alpha$ -BLAP75/RMI1 complex can dissolve double-Holliday junctions without crossover, which provides a plausible mechanism by which BLM might suppress SCEs. However, the current situation is far from providing a clear molecular understanding of the

key cellular roles of the RecQ family. Recent studies strongly indicate that BLM is involved in repair of stalled DNA replication forks, and that WRN is required for telomere stability. However, the biochemical function of *RECQ4* is poorly understood because it lacks helicase activity, and the precise role that it might play in DNA replication remains to be defined. Clearly, further biochemical and cellular biological studies are needed to identify the role(s) played by *RECQ1* and *RECQ5* in DNA replication/repair. At this time, it is hard to explain the basis of the various symptoms that appear in those syndromes caused by loss of RecQ gene products. There are several clinical symptoms in common among the various RecQ syndromes, including growth retardation, premature aging and/or cancer predisposition. As things stand, there is such a paucity of information concerning the molecular basis of aging, that RecQ disorders should provide a vital link between DNA metabolism and the aging process. Lower proliferative capacity may be one factor in growth retardation and aging, but it is not clear why the lower proliferation abilities of cells isolated from the different RecQ helicase disorders produce such different outcomes. Instead, it seems likely that at least certain forms of genome instability can lead to increased cell death and senescence, which leads to accelerated aging phenotypes and developmental abnormalities. In the last ten years, we have learned a lot about the biochemistry of the RecQ helicases, but our knowledge of RecQ 'biology' is lagging some way behind. To plug this gap is a key challenge for the future.

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