

Recql5 and Blm RecQ DNA Helicases Have Nonredundant Roles in Suppressing Crossovers

Yiduo Hu,^{1,2} Xincheng Lu,^{1,2} Ellen Barnes,^{1,2} Min Yan,^{1,3} Hua Lou,^{1,2} and Guangbin Luo^{1,2*}

Department of Genetics¹ and Department of Molecular Biology and Microbiology,³ Case Western Reserve University, and Case Comprehensive Cancer Center, University Hospitals of Cleveland,² Cleveland, Ohio

Received 30 November 2004/Returned for modification 31 December 2004/Accepted 2 February 2005

In eukaryotes, crossovers in mitotic cells can have deleterious consequences and therefore must be suppressed. Mutations in *BLM* give rise to Bloom syndrome, a disease that is characterized by an elevated rate of crossovers and increased cancer susceptibility. However, simple eukaryotes such as *Saccharomyces cerevisiae* have multiple pathways for suppressing crossovers, suggesting that mammals also have multiple pathways for controlling crossovers in their mitotic cells. We show here that in mouse embryonic stem (ES) cells, mutations in either the Bloom syndrome homologue (*Blm*) or the *Recql5* genes result in a significant increase in the frequency of sister chromatid exchange (SCE), whereas deleting both *Blm* and *Recql5* lead to an even higher frequency of SCE. These data indicate that *Blm* and *Recql5* have nonredundant roles in suppressing crossovers in mouse ES cells. Furthermore, we show that mouse embryonic fibroblasts derived from *Recql5* knockout mice also exhibit a significantly increased frequency of SCE compared with the corresponding wild-type control. Thus, this study identifies a previously unknown *Recql5*-dependent, *Blm*-independent pathway for suppressing crossovers during mitosis in mice.

Homologous recombination is a basic molecular process that operates in both meiotic and mitotic cells in mammals. Meiotic recombination is necessary for the proper segregation of homologous chromosomes between daughter cells and for creating genetic diversity through the random mixing of the parental chromosomes and allele shuffling (23). Mitotic recombination facilitates the high-fidelity, nonmutagenic repair of DNA double-stranded breaks and damaged DNA replication forks (7, 21, 34), if the repair is mediated by gene conversion or by crossovers between identical sister chromatids. However, other types of crossovers can be mutagenic or oncogenic. For example, a reciprocal exchange between two nonhomologous chromosomes can lead to translocation, while crossovers between two homologous chromosomes can result in somatic loss of heterozygosity (LOH) (2). In mammals, translocations can lead to the activation of oncogenes or inactivation of tumor suppressor genes, while somatic loss of heterozygosity can convert an otherwise inconsequential heterozygous mutation in a tumor suppressor gene into a complete loss-of-function status (31, 32). In addition, reciprocal exchanges that result from intrachromosomal recombination can lead to deletions or inversions, which can also result in the activation of oncogenes or inactivation of tumor suppressor genes. Thus, while mitotic recombination plays critical roles in the repair of double-stranded breaks and damaged replication forks, crossovers resulting from these repair events can lead to oncogenic DNA rearrangements, increasing the risk of developing cancer (56). Therefore, in mammalian cells, mitotic recombination is highly regulated to favor gene conversion and minimize crossovers (27, 42, 48).

The mechanisms that regulate mitotic recombination remain poorly understood. In *Saccharomyces cerevisiae*, two DNA helicases, Sgs1p and Srs2p, are involved in different pathways that suppress crossovers (26, 33, 55). Sgs1p is a member of the RecQ family of DNA helicases (59). In mammals, homologues of the Bloom syndrome (BLM) helicase (9) have also been shown to play important roles in regulating mitotic recombination (37, 46, 54, 62). RecQ DNA helicases belong to a family of evolutionarily conserved enzymes that have been found in many organisms examined to date. The first *RecQ* gene was identified in *Escherichia coli* as a nonessential gene involved in the *RecF* pathway (44). The yeast RecQ homologue, *SGS1*, was identified in a genetic screen of suppressors for the slow-growth phenotype of *top3* mutant strains in *S. cerevisiae* (13). The *sgs1* mutant exhibits a range of phenotypes, including slow growth, hyperrecombination, and chromosome missegregation (59, 60). The corresponding *rqh1* mutant in *Schizosaccharomyces pombe* also exhibits similar phenotypes (43, 53). Unlike unicellular organisms, which have a single RecQ DNA helicase gene, both the mouse and humans have five genes (*Recql*, *Blm*, *Wrn*, *Recql4*, and *Recql5* for mice, and *RECQL*, *BLM*, *WRN*, *RECQL4*, and *RECQL5* for humans) encoding multiple RecQ homologues (9, 24, 29, 47, 50, 63). Mutations in *BLM*, *WRN*, and *RECQL4* give rise to three distinct syndromes: Bloom (9), Werner (63), and Rothmund-Thomson (30) syndromes, respectively.

Bloom syndrome is characterized by elevated frequencies of both sister chromatid exchange (SCE) and multiradial structures (4, 15) and increased susceptibility to a wide spectrum of malignancies (13). Likewise, cells from *Blm* knockout mice also exhibit elevated frequencies of SCE (5, 37, 41) and multiradial structure (41) and increased susceptibility to a wide variety of cancers (18, 37, 41). In contrast, cells from Werner and Rothmund-Thomson syndrome patients do not exhibit the SCE phenotype. Although patients of these two syndromes are

* Corresponding author. Mailing address: Department of Genetics, Case Western Reserve University, BRB, 7th floor, 10900 Euclid Ave., Cleveland, OH 44106. Phone: (216) 844-7050. Fax: (216) 368-3432. E-mail: GXL35@case.edu.

also cancer prone, they tend to develop specific types of cancers. These observations have indicated that both human *BLM* and its murine homologue play important roles in the suppression of crossovers in mitotic cells and have suggested that elevated crossovers in mitotic cells represent a common contributing factor in mammalian carcinogenesis.

The underlying mechanisms by which RecQ DNA helicases regulate mitotic recombination have not been well defined. However, it is clear that many RecQ helicases can recognize Holliday junctions or Holliday junction-like structures and unwind double-stranded DNA in a 3' to 5' direction (6, 14, 28). Genetic studies in yeast have implicated type IA topoisomerases in the RecQ helicase-mediated regulation of recombination (13). Biochemical studies have also shown that human *BLM* interacts with TOPOIII α , a type IA topoisomerase (25, 62); this interaction enhances the helicase activity of *BLM* (62). Therefore, it appears that the interaction between RecQ helicases and type IA topoisomerases plays an important role in the RecQ helicase-mediated regulation of mitotic recombination. Recently, it was shown that human *RECQL5 β* physically interacts with both TOPOIII α and III β (51), making it the only other human RecQ homologue (other than *BLM*) that physically interacts with type IA topoisomerases. This observation has led to the speculation that *BLM* and *RECQL5 β* might have similar roles in regulating mitotic recombination. In an attempt to address this question, Wang and colleagues deleted the chicken *RECQL5* homologue in DT40 cells. They detected no effect of *RECQL5* deletion on the frequency of spontaneous SCE. However, the same *RECQL5* deletion in a *BLM*^{-/-} background increased the frequency of SCE. These observations led them to conclude that *RECQL5* suppresses SCE but only under *BLM* function-impaired conditions (57).

Here, we report that inactivation of the *Recql5* gene alone is sufficient to cause a significant increase in the frequency of spontaneous SCE in both mouse ES cells and in differentiated mouse embryonic fibroblasts (MEF). Furthermore, deleting both *Blm* and *Recql5* in mouse ES cells resulted in an even higher frequency of SCE than mutating either one of these two genes alone. These results demonstrate that *Recql5*, like *Blm*, plays an essential role in the suppression of crossovers and it does so through a pathway that is distinct from that of *Blm*.

MATERIALS AND METHODS

Construction of *Recql5* targeting vectors. The recombinering technique (35, 64) was used to construct the *Recql5* targeting vectors. First, a 2.4-kb genomic sequence was amplified by PCR and used as a probe to screen the RPCI-22 129S6/SvEv bacterial artificial chromosome (BAC) library. The primers used were Q5e3f (5'-ATGGCGCCGAGATGGCAGCTTCAGCCTCCTTCCA-3') and Q5e5r (5'-ATGGCGCCCGGACATTAGCTTTGTCTACTCCCAT-3'). Clone RPCI-22-51D24 was found to contain the entire *Recql5* gene by PCR and Southern blot analysis (data not shown) and was chosen as the starting material for constructing *Recql5* targeting vectors. Subsequently, a targeting strategy and the first targeting vector were designed (Fig. 1a). In this strategy, a 7.3-kb genomic fragment was used to construct a targeting vector, with which a conditional *Recql5* knockout allele was created. In this allele, exon 4 of *Recql5* was replaced by a *loxP-exon4-loxP-PGKNeo-loxP* cassette. To construct this vector, an RPCI-22-51D24 BAC clone was introduced into DY380 cells by electroporation. In the meantime, a retrieval vector, pQ5RevTK, was constructed. In this vector, two small genomic fragments, 400 bp each and corresponding to the 5' and 3' ends of the 7.3-kb genomic fragment, respectively, were amplified by PCR and cloned into pMclTK. The primers used were as follows: Q5RevP1,

5'-GCGTGATCTAGAAACCCCAAAAAGATGATGAGG-3'; Q5RevP2, 5'-GCGGAATTCGCTAGCATTCCCTGAGGTAAGACA-3'; Q5RevP3, 5'-GCGGAATTCGGATCCAGTATTTGTCTGACCTC-3'; and Q5RevP4, 5'-CGTCGA GCGGCCGACTCCATAGTGGGCCAG-3'. These two genomic fragments provided the homologies for the recombination between the retrieval vector and the BAC clone, whereas the MclTK cassette was used as a negative selection marker in the gene targeting experiments in mouse ES cells. The retrieval vector was linearized by BamHI and electroporated into DY380 cells containing the RPCI-22-51D24 BAC clone. Induced recombination inside the DY380 cells resulted in the retrieval of a 7.3-kb genomic fragment from the BAC clone onto the retrieval vector, generating an intermediate vector, pQ5TK. Then, a modification cassette was created. First, a *loxP-exon4-loxP-PGKNeo-loxP* cassette was constructed. In the meantime, two small genomic fragments, 400 bp each and corresponding to the 5' and 3' ends of exon 4, respectively, were amplified by PCR and cloned into pBluescript (Stratagene). The primers used were Q5BMP1, 5'-CGGAATTCGCGCGCCCTTGGACAGAAGGCTGAGAAC GGG-3'; Q5BMP2, 5'-GCTCTAGAGATAAAGTTCGTATAATGTATGCTAT ACGAAGTTATCAGCCCACTACTAGAGCAGCA-3'; Q5BMP3, 5'-GC TCTAGAGCTGATTGTACCACCTTCCCTAG-3'; and Q5BMP4, 5'-GTG GCGGCCAAGGGTACCTGTTCTGTGTGATCCA-3'. Insertion of the *loxP-exon4-loxP-PGKNeo-loxP* cassette between the two genomic fragments then gave rise to the plasmid pQ5mod containing the 5' arm-*loxP-exon4-loxP-PGKNeo-loxP*-3' arm modification cassette. This cassette was then excised from pQ5mod and introduced into DY380 cells together with pQ5TK. Induced recombination between the homologous sequences in pQ5TK and the modification cassette led to the replacement of exon 4 with the *loxP-exon4-loxP-PGKNeo-loxP* cassette and gave rise to the first targeting vector, pRecql5tv1 (Fig. 1a). This vector was partially sequenced to confirm that no unintended mutations were created in exon 4 during these manipulations. Two other targeting vectors, pRecql5tv2 (Fig. 2a) and pRecql5tv3 (information available upon request) were generated by replacing the *PGKNeo* cassette of pRecql5tv1 with a *PGKHprt* minigene cassette and a *PGKPuro* cassette, respectively.

Gene targeting in mouse ES cells. Gene targeting experiments in the AB2.2 ES cells were conducted as described previously (37). For all the clones used in experiments, correct targeting events were verified by Southern hybridization. All ES clones were subsequently reseeded at low densities to obtain single-cell-derived clonal lines.

Mouse work. Two ES clones heterozygous for the M2 *Recql5* conditional knockout allele (+/M2) were used to generate chimeras and to obtain germ line transmission of the conditional knockout allele as previously described (38). *Zp3-Cre* transgenic mice (8) were used to excise exon 4 in the conditional allele of *Recql5* in mouse oocytes to generate heterozygous *Recql5* knockout mice (+/M3). *Recql5*-targeted alleles were maintained in an F2 mixed genetic background between 129/SvEv and C57BL/6J inbred strains.

Cell culture and analyses. ES cells were cultured in Iscove's modified Dulbecco's medium (Invitrogen) containing 15% fetal bovine serum as previously described (38). For cell growth analysis, 10⁴ cells were plated in each well of six-well feeder plates. The number of cells in each well was counted 48 h after plating and every 24 h afterwards. Standard clonogenic survival assays were used to assess sensitivities to gamma radiation. Briefly, ES cells were trypsinized into single-cell suspensions and then exposed to different doses (0 to 10 Gy) of radiation from a Cs-137 irradiator. After irradiation, cells were plated onto six-well feeder plates at 10⁴ cells/well to allow formation of single-cell-derived colonies. Eight days after plating, colonies that were at least 1 mm in diameter were counted. Experiments were carried out in triplicate and repeated twice. In each experiment, appropriate controls for evaluating plating efficiencies of individual cell lines were also included. Statistical analysis was performed using Prism software (GraphPad Software).

RT-PCR. Total RNA was isolated from ES cells grown in gelatinized plates using TRIzol (Invitrogen) according to the instructions provided by the manufacturer. All reverse transcription-PCRs (RT-PCRs) were carried out using Superscript II RT/Platinum *Taq* mixture (Invitrogen). Primer pair Q5e3f1 (5'-ATGGCGCCCGGACATTAGCTTTGTCTACTCCCAT-3')-Q5e5r1 (5'-ATG GCGGCCGCGGACATTAGCTTTGTCTACTCCCAT-3') was used in studying the expression of wild-type and targeted *Recql5* alleles. These primers are specific to exons 3 and 5, respectively. To quantify the expression of *Recql5* in BAC-rescued *Recql5* knockout ES cells by quantitative RT-PCR, primer pair Q5e3f2 (5'-GGCAATCTGAGGGACTTCTGCCT-3')-Q5e4r1 (5'-CTGCGTGGTAAG CCTTGGCGTTC-3') was used. Q5e4r1 is specific to exon 4, which is deleted in the *Recql5* knockout allele. Thus, RT-PCR using this primer pair results in the amplification of transcripts from only the wild-type *Recql5* and not the knockout alleles. The primers used for amplifying the internal control *Gapdh* are *Gapdh.f1* (5'-GTGCTGAGTATGTCGTGGAGT-3') and *Gapdh.r1* (5'-CACACACCA

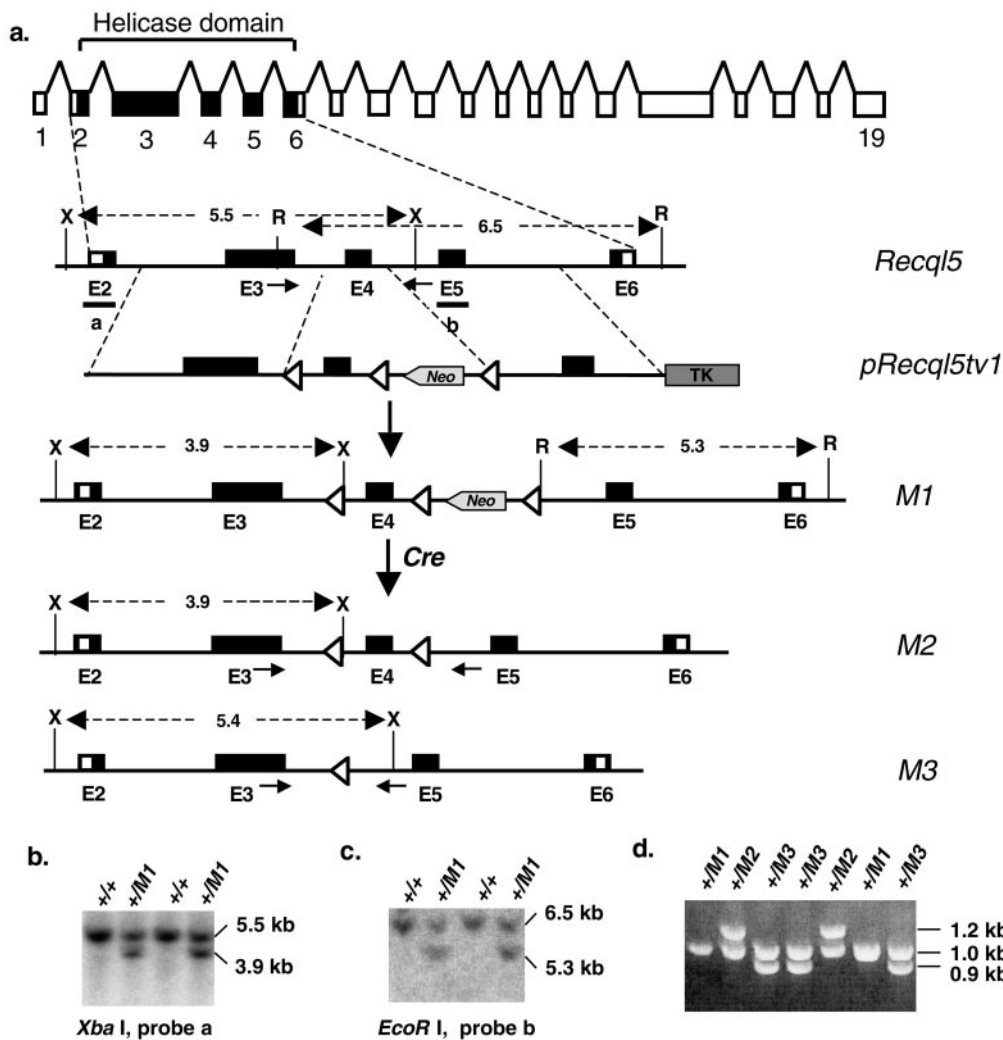


FIG. 1. Gene targeting at the *Recq15* locus. (a) The targeting strategy to delete exon 4 of the *Recq15* gene. The 19 exons encoding *Recq15* beta are shown as boxes, and the sequences encoding the conserved helicase domain are indicated by black boxes. With this strategy, a correct targeting event replaces exon 4 with a *LoxP*-exon4-*LoxP*-PGK*Neo*-*LoxP* cassette, giving rise to the first *Recq15* targeted allele, the M1 allele. Subsequently, a Cre-mediated deletion of sequences between the two *loxP* sites (open triangles) flanking the *Neo* cassette leads to a conditional *Recq15* knockout allele, the M2 allele, in which exon 4 is flanked by a pair of *loxP* sites. Alternatively, a Cre-mediated deletion of both exon 4 and the *Neo* cassette results in a true *Recq15* knockout allele, the M3 allele. Note the opposite directions of transcriptions for *PGKNeo* and *Recq15*. *Neo*, neomycin phosphotransferase gene; *TK*, thymidine kinase gene; X, *Xba*I; R, *EcoR* I. (b) Identifications of ES clones with correctly targeted events within the 5' homology by Southern using probe a. (c) Identification of ES clones with correctly targeted events within the 3' homology by Southern using probe b. (d) Identification of ES cell clones containing either the M2 allele or the M3 allele by PCR. The primers used in the PCR are shown in panel a as short arrows.

TCACAAACATG-3'). Semiquantitative, low-cycle-number RT-PCR amplification with ³²P-labeled primers (40) was used to quantify the expression of *Recq15* transcripts. Specifically, for each RT-PCR, first-strand synthesis of cDNA was carried out using 1 μg of the total RNA. After that, ³²P-labeled 5' primers were added, and the PCRs were allowed to proceed for various numbers of cycles which were expected to be within the linear range of target amplification according to the results of trial experiments. The RT-PCR products were then fractionated on a nondenatured polyacrylamide gel. After electrophoresis, the gel was dried and analyzed with a PhosphorImager (Molecular Dynamics). Data from the RT-PCRs within the linear range were used to calculate the relative levels of *Recq15* expression.

SCE analysis. The SCE analysis was carried out using a classical 5-bromo-2-deoxyuridine (BrdU)-labeling protocol (17) with a minor modification: the concentrations of BrdU were reduced to 1.5 μg/ml for ES cells and 3 μg/ml for MEF cells, respectively. Also, to minimize the variations between experiments, all cell lines involved in the large-scale SCE analyses were processed simultaneously in a single experiment. Sister chromatids were visualized by staining with acridine

orange (Sigma) solution (0.1 mg/ml). Specifically, to count the number of SCE, images of metaphase spreads were captured, and SCE of individual cell lines were scored blind. Statistical analysis was performed with Prism software (GraphPad Software).

RESULTS

Inactivation of *Recq15* in mouse ES cells by gene targeting. Human *RECQL5* has been shown to express three different transcripts encoding three RecQ DNA helicase isoforms (α, β, and γ) (29, 51). The three isoforms share the conserved helicase domain encoded by exons 2 to 6 (45), but only *RECQL5*β, the longest isoform, contains a nuclear localization signal. The mouse *Recq15* gene is very similar to its human homologue;

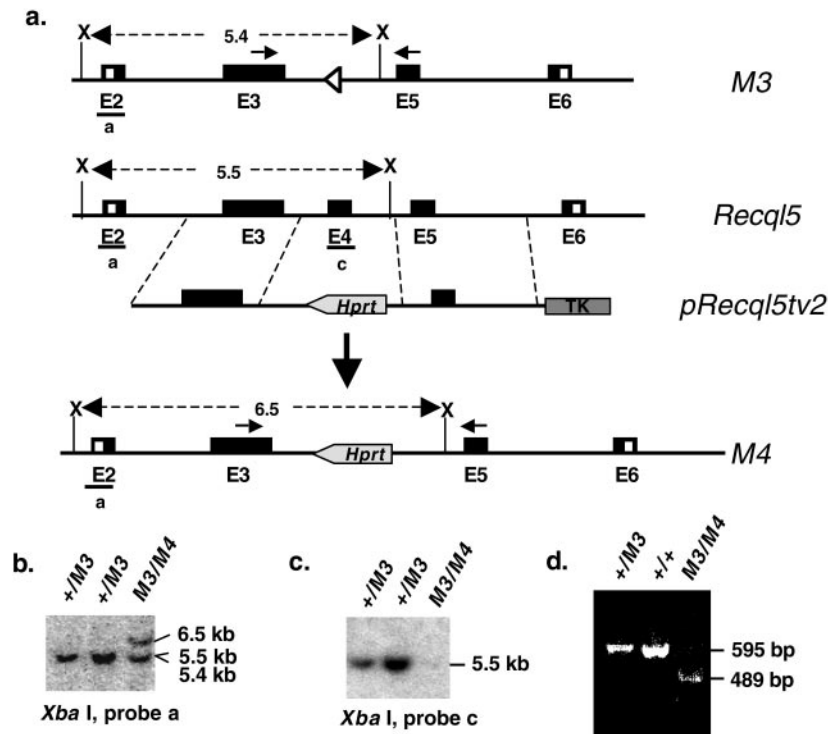


FIG. 2. Generation of *Recql5*^{-/-} ES cells. (a) A gene targeting strategy to replace exon 4 with a *PGKHprt* (*Hprt*) cassette. This strategy can be used to generate a true *Recql5* knockout allele, the M4 allele, in a single targeting experiment. Specifically, the targeting of the remaining wild-type *Recql5* allele in heterozygous *Recql5* knockout ES cells containing a M3 allele is shown. This resulted in the generation of homozygous *Recql5* knockout (*Recql5*^{-/-}) ES cells. Note the opposite directions of transcription for the *Hprt* cassette and *Recql5*. X, XbaI. (b) Identification of ES clones in which both *Recql5* alleles have been targeted. Clones in which the remaining wild-type *Recql5* allele was correctly targeted within the 5' homology were identified by Southern using probe a. (c) Confirmation of the complete deletion of exon 4 in clones identified in panel b by Southern with an exon 4-specific probe (probe c). (d) Results of an RT-PCR experiment showing the absence of the expected 595-bp product from the normal *Recql5* mRNA and the presence of a 489-bp product from an aberrant transcript in a homozygous *Recql5* knockout (M3/M4) ES cell clone. The primers used in the RT-PCRs are indicated in panel a as short arrows.

however, its expression has not been well characterized. Thus, to ensure the functional inactivation of the mouse *Recql5* gene, we decided to delete its 106-bp exon 4 to create a frameshift mutation at codon 260. Furthermore, in anticipation of the possibility that the deletion of *Recql5* might lead to lethality, we created a conditional *Recql5* knockout allele. Specifically, we replaced exon 4 of *Recql5* with a *loxP-exon4-loxP-PGKNeo-loxP* cassette to obtain a primary *Recql5*-targeted allele, the M1 allele (Fig. 1a). Following that, a conditional knockout allele (the M2 allele) and a true knockout allele (the M3 allele) were derived from this M1 allele by Cre-mediated deletions (Fig. 1a). ES cell clones carrying the desirable *Recql5* alleles were identified either by Southern (Fig. 1b and c) or by PCR (Fig. 1d) analysis. Two targeted ES cell clones with the M2 conditional knockout allele were used to generate conditional knockout mice (see below and Fig. 7). In the meantime, two clones with the M3 allele were used to generate homozygous *Recql5* knockout ES cells by deletion of the remaining wild-type *Recql5* allele with a different targeting vector, pRecql5tv2 (Fig. 2a). This vector was designed to replace exon 4 of *Recql5* with a *PGKHprt* minigene cassette, leading to another *Recql5* knockout allele, the M4 allele (Fig. 2a). Correctly targeted ES cell clones were identified by Southern analysis (Fig. 2b and c).

RT-PCR analysis showed that the homozygous *Recql5* knockout (M3/M4) ES cells no longer express the wild-type *Recql5* mRNA, but they do express an aberrant transcript (Fig. 2d). Sequencing analysis showed that this aberrant transcript originated from the M3 allele and is expected to encode a truncated polypeptide of 260 amino acids consisting of the first 259 amino acids of the 981-amino-acid Recql5 β helicase (45) plus an additional alanine residue. Thus, this M3 allele is expected to produce either an N-terminal truncated polypeptide without any discernible domain or no protein product at all because of a nonsense-mediated decay. Therefore, this allele likely represents a null allele without any dominant negative effect. These data also indicate that the M4 allele does not express any detectable transcripts. Thus, these *Recql5* knockout (M3/M4) ES cells represent *Recql5* null ES cells. They are referred to as *Recql5*^{-/-} ES cells hereafter.

***Recql5*^{-/-} ES cells have an elevated frequency of SCE and an increased incidence of multiradial structures.** *Recql5*^{-/-} ES cells have growth characteristics similar to those of their parental wild-type ES cells (Fig. 3a and data not shown). The major objective of this study was to investigate whether *Recql5* plays a role in suppressing crossovers in mitotic cells. Deletion of a gene can affect the frequency of crossovers by affecting either the recombination repair machinery or the regulation of

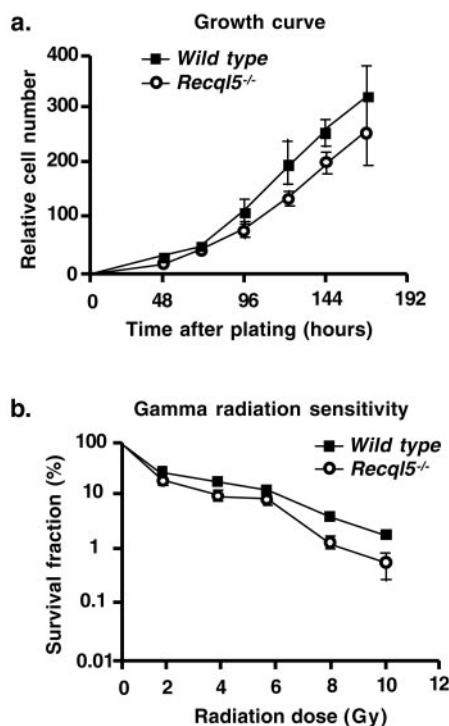


FIG. 3. Growth characteristics and responses to gamma radiation in mouse ES cells. (a) Growth curves of wild-type and *Recq15*^{-/-} ES cells. (b) Sensitivity to gamma radiation. The results of clonogenic survival assay experiments. Error bars represent standard deviations.

recombination. Thus, we first assessed the repair capacity of the recombination repair machinery in *Recq15*^{-/-} ES cells by comparing their sensitivity to gamma irradiation with that of its parental cell line. The result showed that *Recq15*^{-/-} ES cells and its parental line have similar sensitivities to gamma irradiation (Fig. 3b), indicating that deletion of *Recq15* does not significantly impair the recombination repair capacity in mouse ES cells.

To investigate whether *Recq15* is involved in the suppression of crossovers in mouse ES cells, we first compared the frequencies of SCE among wild-type, *Recq15*^{+/-}, and *Recq15*^{-/-} ES cells. Initially, 20 metaphase spreads derived from wild-type, two *Recq15*^{+/-}, and two *Recq15*^{-/-} ES cell lines were examined. This experiment showed that wild-type and *Recq15*^{+/-} ES cells have similar SCE frequencies. However, the two *Recq15*^{-/-} ES cell lines have similarly higher frequencies of SCE that are about three times higher than those of wild-type and *Recq15*^{+/-} ES cells (data not shown). Subsequently, we conducted a large-scale experiment to compare the frequencies of SCE in wild-type and *Recq15*^{-/-} ES cells. As a positive control, we also included *Blm*^{-/-} ES cells (19, 37) in this experiment. Specifically, the *blm* (M3-M4) ES cells (19) were used. These ES cells express 12% of the wild-type Blm protein from the M3 hypomorphic allele (41). These were also the cells used to generate *Blm Recq15* double-knockout ES cells (see below). The results showed that *Recq15*^{-/-} ES cells indeed have a frequency of spontaneous SCE that is significantly higher than that of the wild-type control ($P < 0.001$) but is similar to that in *Blm*^{-/-} ES cells (Fig. 4a to d and Table 1). These data clearly indicate

TABLE 1. Spontaneous SCE in mouse ES cells^a

No. of SCE events per chromosome	Percentage of events in:			
	Wild type	<i>Recq15</i> ^{-/-}	<i>Blm</i> ^{-/-}	<i>Recq15</i> ^{-/-} <i>Blm</i> ^{-/-}
0	82.0	59.6	54.6	42.4
1	16.4	25.5	32.1	29.3
2	1.52	10.7	11.0	19.2
3	0.13	3.19	2.10	6.47
4	0	0.69	0.09	1.31
≥5	0	0.27	0.09	1.26

^a Total chromosomes were derived from 56, 80, 30, and 48 metaphase spreads of the wild type, *Recq15*^{-/-}, *Blm*^{-/-}, and *Blm*^{-/-} *Recq15*^{-/-} ES cells, respectively. Total chromosomes were 2,238 for the wild type, 2,913 for *Recq15*^{-/-}, 1,142 for *Blm*^{-/-}, and 1,902 for *Blm*^{-/-} *Recq15*^{-/-} ES cells. The total number of SCE events was 251 for the wild type, 1,769 for *Recq15*^{-/-}, 829 for *Blm*^{-/-}, and 1,884 for *Blm*^{-/-} *Recq15*^{-/-} ES cells. The average number of SCEs per chromosome (values in parentheses are standard errors) was 0.198 (0.009) for the wild type, 0.607 (0.016) for *Recq15*^{-/-}, 0.614 (0.023) for *Blm*^{-/-}, and 0.991 (0.025) for *Blm*^{-/-} *Recq15*^{-/-}.

that the deletion of the *Recq15* gene resulted in a significant increase in the rate of crossovers between sister chromatids.

In cells from both Bloom syndrome patients and *Blm* knockout mice, elevated frequencies of SCE are accompanied by an increased incidence of multiradial structures (16, 41), which are indicative of interchromosomal exchange. Consistent with these previous observations, we observed six such structures from 200 metaphase spreads derived from *Recq15*^{-/-} ES cells (Fig. 4e and f) but none from >500 spreads from wild-type ES cells. Thus, it appears that the increase in the frequency of SCE in *Recq15*^{-/-} ES cells is also accompanied by an increased frequency of interchromosomal exchange. However, the frequencies of other types of chromosome aberrations, such as chromosome-chromatid breaks, are not significantly different between *Recq15* knockout and wild-type ES cells (data not shown).

The SCE phenotype of *Recq15*^{-/-} ES cells is due to the inactivation of *Recq15*. In chicken DT40 cells, deletion of the *RECQL5* homologue does not affect the rate of spontaneous SCE (57). This raised the question as to whether the SCE phenotype seen in *Recq15*^{-/-} ES cells was indeed due to the loss of *Recq15* or some other unintended effects that were brought about when this *Recq15*^{-/-} ES cell line was generated. To address this issue, we attempted to rescue the SCE phenotype of *Recq15*^{-/-} ES cells by using a genetic complementation strategy. We argued that if the SCE phenotype of our *Recq15*^{-/-} ES cells was indeed caused by the loss of *Recq15* function, reintroduction of a single copy of a functional *Recq15* gene into *Recq15*^{-/-} ES cells would be sufficient to rescue the SCE phenotype, since *Recq15*^{+/-} ES cells have a normal frequency of SCE.

To ensure the proper expression of the *Recq15* gene, we introduced a *Recq15*-containing BAC vector that contains the coding region plus both 5' and 3' regulatory sequences of the *Recq15* gene into *Recq15*^{-/-} ES cells. We obtained a *Recq15*-containing BAC clone, RPCI-22-51D24, by screening a mouse BAC library. This BAC clone contains the entire *Recq15* coding sequence plus more than 10 kb of both 5' and 3' regulatory sequences (data not shown). A *PGKNeo* selection marker was introduced into this BAC clone by the recombinering technique (64). Then, this modified BAC clone was introduced into

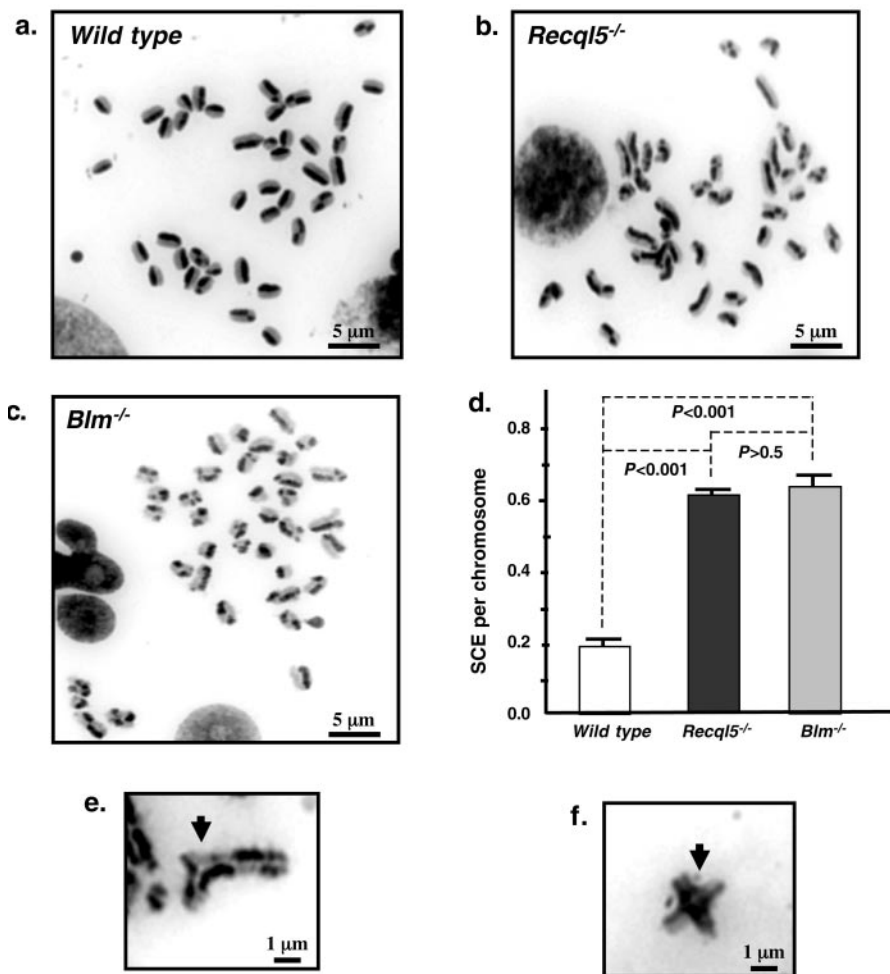


FIG. 4. Spontaneous crossovers in mouse ES cells. (a to c) Representative metaphase spreads from wild-type, *Recql5*^{-/-}, and *Blm*^{-/-} cells showing SCE. (d) The results of statistical analysis of SCE frequencies. Average number of SCE per chromosome in wild type, *Recql5*^{-/-}, and *Blm*^{-/-} cells were calculated and compared. Error bars indicate standard errors of the mean, and *P* values were calculated by one-way analysis of variance (ANOVA) with posttest. (e to f) Two examples of multiradial structures (indicated by arrows) observed in *Recql5*^{-/-} cells, indicative of interchromosomal exchanges.

Recql5^{-/-} ES cells to obtain stable transfected lines (designated *Recql5*^{-/-} *Res* cells) (data not shown). RT-PCR analysis using total RNA isolated from two of these *Recql5*^{-/-} *Res* clones showed that they both expressed wild-type *Recql5* transcript as expected (data not shown and see below).

Initial SCE analysis of 20 chromosome spreads from two of these clones showed that they have frequencies that are much lower than their parental *Recql5*^{-/-} ES cells, but similar to that of the wild-type control. Thus, a large-scale analysis was carried out to compare the frequencies of SCE among one of these two rescued (*Recql5*^{-/-} *Res*) ES cell line, the parental *Recql5*^{-/-} cells, and the wild-type ES cells. The results of this study confirmed that the SCE of the *Recql5*^{-/-} *Res* cell line is indeed rescued to the level of wild-type ES cells (Fig. 5b). However, it remained possible that the reduction of SCE frequency in the *Recql5*^{-/-} *Res* cell line was caused by a nonspecific effect, due to overexpression of *Recql5*. To address this issue, the expression of *Recql5* transcripts in this *Recql5*^{-/-} *Res* cell line and in wild-type ES cells were quantified and com-

pared. The results of semiquantitative RT-PCR experiments showed that *Recql5* was not overexpressed in *Recql5*^{-/-} *Res* cells; instead, it was expressed at only about 5% of that in the wild-type ES cells (Fig. 5c). Thus, these data provide the definitive proof that the SCE phenotype of *Recql5*^{-/-} ES cells is caused by the loss of *Recql5* function.

***Recql5* and *Blm* have nonredundant roles in the suppression of crossovers in mouse ES cells.** The observation that deletions of *Blm* or *Recql5* led to similar SCE phenotypes in mouse ES cells raised the question as to whether these two *RecQ* helicases are both required in the same genetic pathway or act independently through different pathways to suppress crossovers in mouse ES cells. To address this issue, we examined the effect of the deletion of *Recql5* on the frequency of SCE in *Blm* knockout ES cells.

To generate *Blm*^{-/-} *Recql5*^{-/-} ES cells, we inactivated the two copies of the *Recql5* gene in *Blm*^{-/-} ES cells, the *blm* (*M3/M4*) ES cells (19), by two consecutive rounds of gene targeting experiments. First, we generate a new *Recql5*-tar-

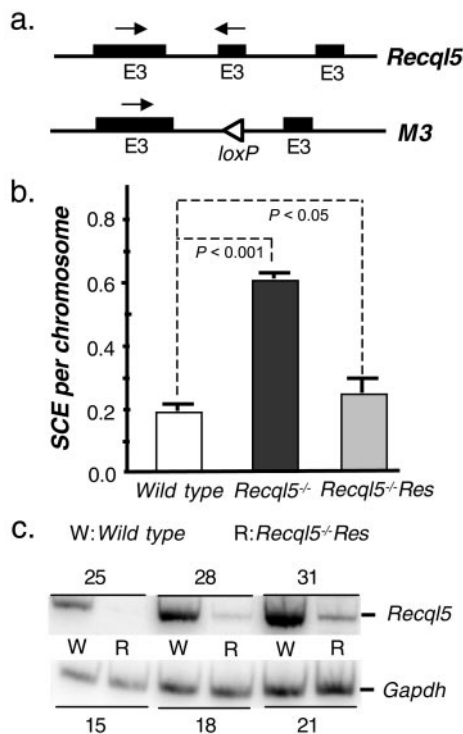


FIG. 5. Genetic rescue of the SCE phenotype in *Recql5*^{-/-} ES cells. (a) A schematic illustration of wild-type *Recql5* and M3 alleles, showing the locations of primers (arrows) used in the RT-PCRs. (b) The frequencies of SCE in wild-type, *Recql5*^{-/-}, and *Recql5*^{-/-} *Res* cells. Error bars indicate standard errors of the mean and P values were calculated by one-way ANOVA with posttest. (c) The results of semiquantitative RT-PCR analyses. Semiquantitative RT-PCRs were performed with total RNA extracted from wild-type (W) and *Recql5*^{-/-} *Res* (R) ES cells. Primers used in the RT-PCRs are indicated in panel a as arrows above the wild-type *Recql5* allele. The expression of the *Gapdh* gene was used as an internal control to compare the levels of *Recql5* expression among samples. The cycle numbers of RT-PCRs were indicated above or below the images, showing results for *Recql5* and *Gapdh*, respectively.

geted allele in these *Blm*^{-/-} ES cells, the M4 allele, by the same strategy described previously for a similar experiment with wild-type ES cells (Fig. 2a) but with inactivation of the other *Recql5* allele by another strategy which replaces exon 4 of *Recql5* with a *PGKPuro* cassette (information available upon request). These experiments resulted in the generation of several *Blm*^{-/-} *Recql5*^{-/-} ES cell lines. *Blm*^{-/-} *Recql5*^{-/-} ES cells showed growth characteristics similar to those of wild-type ES cells (data not shown). RT-PCR using a pair of primers specific for exon 3 and exon 5, respectively, failed to detect any *Recql5* transcript from these cells (information available upon request), indicating that neither mutant allele produces any detectable amount of *Recql5* transcripts.

We then examined the effect of the deletion of *Recql5* on the frequency of SCE in *Blm*^{-/-} ES cells. Specifically, we compared the frequencies of SCE among the *Blm*^{-/-}, *Recql5*^{-/-}, and *Blm*^{-/-} *Recql5*^{-/-} ES cells. To ensure the accuracy of the experiments, the SCE frequencies of all the cell lines described in this paper were derived from cells that were prepared simultaneously in one experiment. This study showed that *Blm*^{-/-} *Recql5*^{-/-} ES cells have a very high frequency of SCE.

In some cells, the presence of many SCE events on some chromosomes made it difficult to accurately measure the number of SCE (Fig. 6a). Nevertheless, even based on an anticipated underestimation, the overall frequency of SCE of the double-knockout ES cells was significantly higher than that of either *Blm*^{-/-} or *Recql5*^{-/-} ES cells ($P < 0.001$ in both cases) (Fig. 6b). In particular, the percentage of cells with more than 40 SCEs (e.g., an average of 1 SCE per chromosome) is much higher in the *Blm*^{-/-} *Recql5*^{-/-} ES cells (44%) than in *Blm*^{-/-} ES cells (9.3%), *Recql5*^{-/-} ES cells (10.7%), and wild-type ES cells (<0.1%) (Fig. 6c). Therefore, these data clearly showed that in the mouse ES cells, *Blm* and *Recql5* have nonredundant roles in the suppression of crossovers.

Primary embryonic fibroblast (MEF) cells derived from *Recql5* knockout mice exhibited an elevated SCE frequency. Our data from experiments using knockout ES cells clearly showed that *Recql5* plays an important role in the suppression of crossovers in mouse ES cells. However, mouse ES cells are unique in many aspects of their cell cycle checkpoint controls (1), as well as DNA repair and recombination (10, 11). Thus, a mutant phenotype observed in knockout ES cells might not indicate a similar phenotype in differentiated cells of the corresponding knockout mice. To examine whether *Recql5* also has a role in suppressing crossovers in differentiated mouse cells, we created *Recql5* knockout mice and studied the frequencies of SCE in differentiated cells derived from *Recql5* knockout mice.

We first used ES cells that carry the *Recql5* conditional knockout allele and the M2 allele (Fig. 1a) to generate a *Recql5* conditional knockout mouse model. Two M2 allele-containing ES cell lines, *Recql5*^{tm2Luo-1} and *Recql5*^{tm2Luo-2}, were used to generate mice carrying the M2 conditional knockout allele. Germ line transmission of the M2 conditional knockout allele was obtained from both cell lines (Fig. 7a and b and data not shown). We then introduced this *Recql5* conditional allele into female *Zp3-Cre* transgenic mice (8) to facilitate the deletion of exon 4 of *Recql5*. Removal of the exon 4 of the *Recql5* gene by Cre-mediated deletion in the oocytes of *Zp3-Cre* transgenic mice produced heterozygous *Recql5* knockout mutant mice carrying the M3 allele as designed (Fig. 7a and c). Heterozygous *Recql5* knockout mutant mice are viable, fertile, and indistinguishable from their wild-type littermates (data not shown). Intercrosses among these heterozygous mutants gave rise to progeny, including homozygous mutants, at the expected ratio (Fig. 7d and data not shown). To date, 50 homozygous *Recql5* knockout mice have been monitored for 3 to 7 months. These mice do not appear to have any gross developmental abnormalities. They also enjoy normal postnatal growth and development and have normal fertility (data not shown). Detailed phenotypic characterization of these mice is in progress. In the meantime, these knockout mice provide a source for differentiated *Recql5* knockout cells to study the effect of *Recql5* deficiency on the rates of crossovers in differentiated mouse cells.

Primary MEFs provide a readily available source of differentiated cells from knockout mouse models that are also compatible with SCE analysis. Thus, to examine whether *Recql5* plays a role in suppressing crossovers in differentiated mouse cells, we compared the frequencies of MEFs derived from three wild-type and four *Recql5* knockout embryos. The results

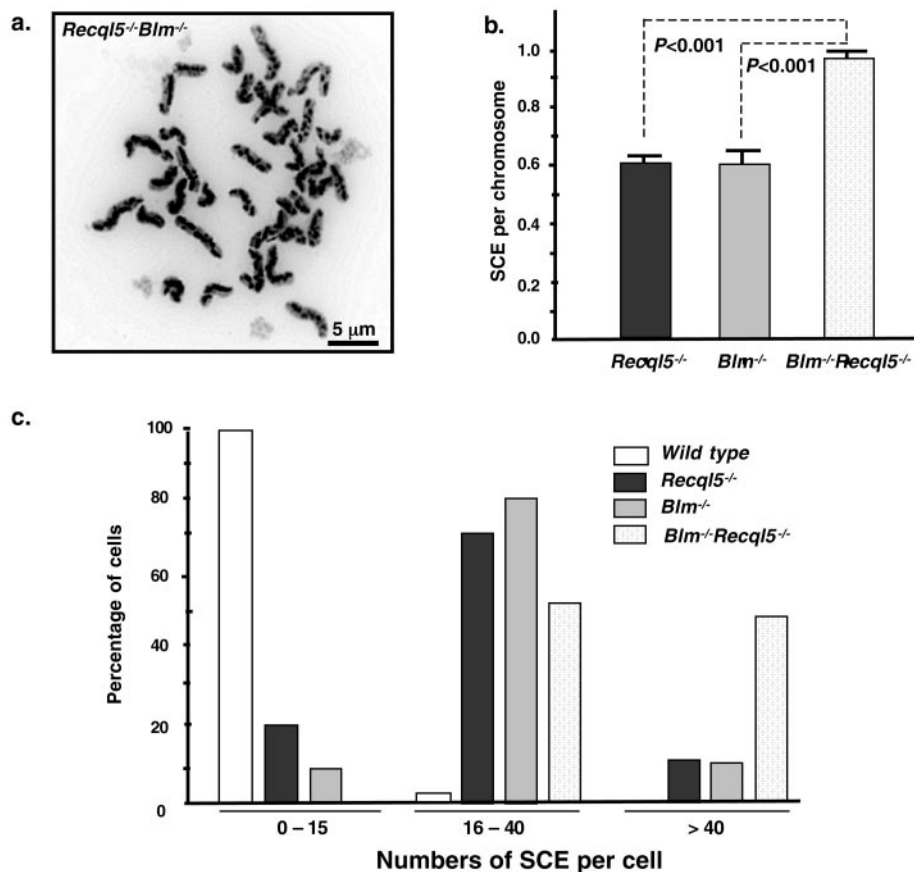


FIG. 6. Spontaneous SCE in *Recq15*^{-/-} *Blm*^{-/-} ES cells. (a) A representative metaphase spread with a high number of SCE. (b) The result of statistical analysis on the frequencies of SCE. The numbers of SCE per chromosome in *Recq15*^{-/-}, *Blm*^{-/-}, and *Recq15*^{-/-} *Blm*^{-/-} ES cells were compared. Error bars indicate the standard errors of the mean, and *P* values were calculated by one-way ANOVA with posttest. (c) The distributions of cells in categories of different ranges of SCE.

of this study showed that *Recq15* knockout MEFs have a significantly elevated frequency of SCE compared with that of wild type MEFs ($P < 0.0001$) (Fig. 8). Therefore, *Recq15* is also required for the suppression of crossovers in differentiated MEF cells.

DISCUSSION

Role of *Recq15* and its homologues in the suppression of crossovers in mitotic cells. The observation that human RECQL5 physically interacts with both TOPOIII α and TOPOIII β prompted the speculation that RECQL5 and its homologues might play important roles in the suppression of crossovers. This was challenged by the finding that deletion of the chicken *RECQL5* homologue in DT40 cells did not lead to an increase in the rate of spontaneous SCE (57). In contrast, we have shown here that the deletion of *Recq15* alone in mouse ES cells is sufficient to cause a significant increase in the frequency of spontaneous SCE, comparable to the increase caused by the deletion of *Blm*. Furthermore, this elevated SCE frequency is accompanied by an increased incidence of multi-radial structures, which are indicative of interchromosomal exchanges. The SCE phenotype observed in *Recq15* knockout ES cells does not appear to be caused by either a dominant negative effect or gain-of-function effect due to the targeted

mutation, since heterozygous knockout ES cells do not have this phenotype. Most importantly, the results from our genetic complementation experiment clearly demonstrated that the elevated SCE frequency is caused by the loss of *Recq15*. At present, we do not have a clear explanation for this apparent discrepancy between our findings and that based on DT40 cells (57). This could reflect either cell-type-specific or species-specific differences between chicken DT40 cells and mouse ES cells. However, our observations of similar SCE phenotypes in two different *Recq15* knockout cells, ES cells and MEFs, argue against a cell-type-specific difference. Further experiments are needed to resolve this issue. Importantly, our findings confirm the speculation that multiple pathways exist for suppressing crossovers in mitotic cells, which should be expected given the great complexity of the mammalian genome and the potential deadly consequences, i.e., carcinogenesis, due to crossovers. Mouse *Recq15* β and human RECQL5 β share 72% identities in amino acid composition and likely have similar functions. Thus, we believe that human RECQL5 is also required for the suppression of spontaneous crossovers in mitotic cells. Currently, it remains unclear whether these helicases play any roles in regulating meiotic crossovers.

Human *RECQL5* produces three mRNAs as the result of alternative RNA processing and hence was predicted to en-

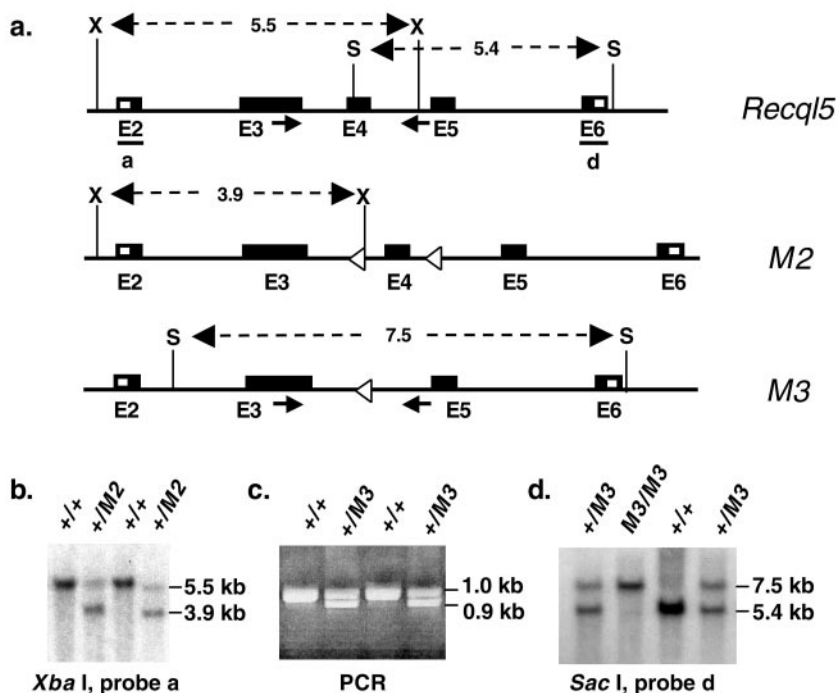


FIG. 7. Generation of *Recql5* knockout mice. (a) A schematic illustration of portions of the wild-type *Recql5* allele, the M2 conditional knockout allele, and the M3 knockout allele. Regions between exons 2 and 6 are shown. X, XbaI; S, SacI. (b) Identification of mice carrying the M2 allele (+/M2) by Southern analysis using probe a. (c) Identification of mice carrying the M3 allele (+/M3) by PCR. (d) Identification of homozygous *Recql5* knockout (M3/M3) mice among the progeny of a cross between heterozygous knockout (+/M3) mice by Southern analysis by using probe d. The positions of the primers (arrows) used in PCRs are indicated in panel a.

code three RecQ helicase isoforms (29, 49, 51). The expression of mouse *Recql5* has not been very well studied, but it is predicted to produce three isoforms similar to those of humans, since human *RECQL5* and mouse *Recql5* genes are highly conserved. In all the *Recql5* knockout alleles reported here, the targeted mutations were expected to delete the conserved helicase domain of all three predicted *Recql5* helicase isoforms. Thus, the SCE phenotype that we have observed could be due to the lack of any of these isoforms. However,

only RECQL5 β has been found to interact with TOPOIII α and III β (51), to have the 3'-to-5' helicase activity, and to have single-strand annealing activity (14). Although it is not yet clear whether the helicase activity or the single-strand annealing activity is related to the regulation of recombination, it has been shown that the interaction between BLM and TOPOIII α plays an important role in the suppression of crossovers (26). Furthermore, RECQL5 β is also the only isoform that contains a putative nuclear localization signal. Thus, we believe that the deletion of *Recql5* β is primarily responsible for the SCE phenotype observed in our *Recql5* knockout ES cells. But future experiments are needed to verify or refute this prediction.

The nonredundant roles of *Blm* and *Recql5* in the suppression of crossovers. In *Saccharomyces cerevisiae*, *SGS1* and *SRS2* are known to be involved in nonredundant pathways to control crossovers, indicating the importance of such regulatory mechanisms in the homeostasis of eukaryotes (26). This also suggests that crossovers can be initiated from different events that are subjected to the regulations of different pathways. We showed here that *Blm* and *Recql5* are apparently involved in independent, nonredundant pathways to suppress crossovers in mouse ES cells, confirming the speculation that mammals also have more than one pathway for suppressing crossovers in mitotic cells. Interestingly, to date, no mammalian homologues of *SRS2* have been found.

Our present data do not explain how these two helicases have different specificities towards specific types of recombination events or pathways. It is conceivable that the specificities lie in the unique structural motifs found within them.

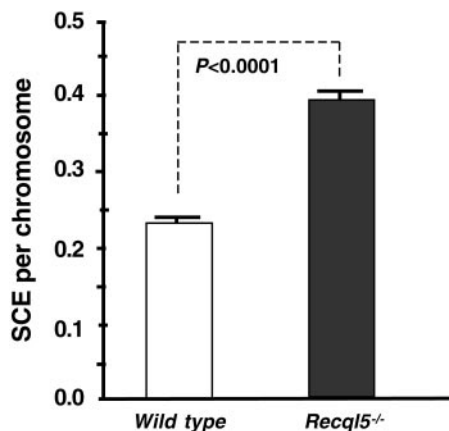


FIG. 8. The frequencies of spontaneous SCE in primary MEF cells. The numbers of SCE per chromosome in wild-type and *Recql5*^{-/-} MEF cells were compared. Error bars indicate the standard errors of the mean, and *P* values were calculated by *t* test.

Members of the RecQ helicase family all have a conserved helicase domain but also have unique motifs at their N or C termini, or both. Thus, the specificity of an individual RecQ helicase is likely dictated, at least in part, by its N- and/or C-terminal motifs. In this regard, it is noteworthy that BLM and RECQL5 β are structurally distinct. In particular, BLM has distinct structural motifs at both its N and C termini, whereas RECQL5 β lacks any discernible conserved motifs at its N terminus (51). BLM interacts with TOPOIII α and several other components of the replication and recombination machineries via both its unique N- and C-terminal motifs (3, 58, 61). To date, the only known interacting partners for RECQL5 are TOPOIII α and III β (51). But given the lack of any identifiable N-terminal motifs in all predicted isoforms encoded by RECQL5 (51), it is conceivable that their interacting partners differ from those of BLM. Functionally speaking, both BLM and RECQL5 β are able to unwind double-stranded DNA in a 3'-to-5' direction in vitro, but only RECQL5 β can stimulate the annealing of complementary single-stranded DNA molecules (14). Thus, it is clear that there exist both structural and functional differences between human BLM and RECQL5. Homologues of the RecQ family are highly conserved between mice and humans. In particular, human RECQL5 β and mouse Recql5 β share 72% identities. Thus, it is likely that mouse Blm and Recql5 β also have structural and functional properties similar to those of their human counterparts.

An elevated frequency of crossovers could either be due to an overall increase in the frequency of recombination or from a skewing in the resolution of recombination repair intermediates that favors crossovers. Based on our present data, we cannot determine the mechanism by which *Recql5* deficiency increases SCE frequency in mouse ES cells. It should be noted that this is also an unresolved issue with respect to *BLM*- and *Blm*-deficient cells. Future experiments are needed to address this issue.

RecQ DNA helicase-mediated suppression of crossovers and carcinogenesis. Mutations in the single RecQ DNA helicase-encoding gene in budding yeast cause a multitude of phenotypes, including hyperrecombination and chromosome missegregation (59, 60). In humans, mutations in three of five *RecQ*-encoding genes give rise to three distinct cancer-prone genetic diseases: Werner (63), Rothmund-Thomson (30), and Bloom (9) syndromes. These observations imply that RecQ helicases have multiple distinct roles and that in mammals, individual RecQ helicase has acquired specialized roles in maintaining the stability of the genome. Cells from Werner syndrome patients have been shown to exhibit extensive deletions (12). The nature of genomic instability associated with Rothmund-Thomson syndrome has not been well characterized. However, we very recently showed that *Recql4*-deficient cells derived from *Recql4* knockout mice exhibited a chromosomal instability phenotype as the result of defective sister chromatid cohesion (39). We showed here that two members of the mammalian RecQ gene family, *Blm* and *Recql5*, are involved in the suppression of crossovers. This new finding underscores both the essential role of recombination repair in mitotic cells and the importance of regulating this repair process to suppress crossovers, which can lead to deleterious consequences.

SCE, the outcome of crossovers between identical sister chromatids, results in high-fidelity recombination repair of

DNA lesions. Thus, SCE is not mutagenic or oncogenic. However, other types of crossovers in mitotic cells can lead to potential oncogenic events, such as translocations, deletion, inversion, and somatic LOH. In particular, recent data have shown that crossovers between homologous chromosomes can lead to crossover-mediated LOH (2, 36). This crossover-mediated LOH represents a major mechanism of LOH in noncancerous human cells (20). Importantly, such a mechanism also has been shown to play a critical role in the loss of function of tumor suppressor genes at the early stage of colorectal cancer (22, 37, 52). Consistent with this, both Bloom syndrome patients and *Blm* knockout mice exhibit striking cancer susceptibility phenotypes (16, 37, 41). Thus, our finding that *Blm* and *Recql5* are involved in nonredundant pathways to suppress crossovers in mouse ES cells suggests that *Recql5* also plays important roles in the suppression of crossovers and perhaps carcinogenesis. Currently, human RECQL5 mutations have not been linked to any cancer-prone disorders. However, given the viable nature of *Recql5* knockout mice and the anticipated functional conservation between human RECQL5 and mouse Recql5, it is reasonable to speculate that RECQL5 deficiency may be compatible with viability in humans. Thus, it remains possible that RECQL5 is the disease-causing gene for a cancer-prone syndrome. The mouse model reported here will provide a valuable tool for investigating the role of *Recql5* as well as the relative contributions of the *Blm*-dependent and *Recql5*-dependent pathways to tumor suppression in mice. The results of such studies may also aid in the future identification of the human disease(s) that is caused by RECQL5 mutations, should there be any.

ACKNOWLEDGMENTS

We thank Peter Harte, Patricia Hunt, and David Boothman for critical reading of the manuscript.

REFERENCES

- Aladjem, M. I., B. T. Spike, L. W. Rodewald, T. J. Hope, M. Klemm, R. Jaenisch, and G. M. Wahl. 1998. ES cells do not activate *p53*-dependent stress responses and undergo *p53*-independent apoptosis in response to DNA damage. *Curr. Biol.* **8**:145–155.
- Beumer, K. J., S. Pimpinelli, and K. G. Golic. 1998. Induced chromosomal exchange directs the segregation of recombinant chromatids in mitosis of *Drosophila*. *Genetics* **150**:173–188.
- Braybrooke, J. P., J. L. Li, L. Wu, F. E. Caple, F. E. Benson, and I. D. Hickson. 2003. Functional interaction between the Bloom's syndrome helicase and the RAD51 paralogs, RAD51L3 (RAD51D). *J. Biol. Chem.* **278**:48357–48366.
- Chaganti, R. S., S. Schonberg, and J. German. 1974. A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. *Proc. Natl. Acad. Sci. USA* **71**:4508–4512.
- Chester, N., F. Kuo, C. Kozak, C. D. O'Hara, and P. Leder. 1998. Stage-specific apoptosis, developmental delay, and embryonic lethality in mice homozygous for a targeted disruption in the murine Bloom's syndrome gene. *Genes Dev.* **12**:3382–3393.
- Constantinou, A., M. Tarsounas, J. K. Karow, R. M. Brosh, V. A. Bohr, I. D. Hickson, and S. C. West. 2000. Werner's syndrome protein (WRN) migrates Holliday junctions and co-localizes with RPA upon replication arrest. *EMBO Rep.* **1**:80–84.
- Cox, M. M. 2002. The nonmutagenic repair of broken replication forks via recombination. *Mutat. Res.* **510**:107–120.
- de Vries, W. N., L. T. Binns, K. S. Fancher, J. Dean, R. Moore, R. Kemler, and B. B. Knowles. 2000. Expression of Cre recombinase in mouse oocytes: a means to study maternal effect genes. *Genesis* **26**:110–112.
- Ellis, N. A., J. Groden, T. Z. Ye, J. Straughen, D. J. Lennon, S. Ciocci, M. Proytcheva, and J. German. 1995. The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* **83**:655–666.
- Essers, J., R. W. Hendriks, S. M. Swagemakers, C. Troelstra, J. de Wit, D. Bootsma, J. H. Hoeijmakers, and R. Kanaar. 1997. Disruption of mouse *RAD54* reduces ionizing radiation resistance and homologous recombination. *Cell* **89**:195–204.

11. Essers, J., H. van Steeg, J. de Wit, S. M. Swagemakers, M. Vermeij, J. H. Hoeijmakers, and R. Kanaar. 2000. Homologous and non-homologous recombination differentially affect DNA damage repair in mice. *EMBO J.* **19**:1703–1710.
12. Fukuchi, K., G. M. Martin, and R. J. Monnat, Jr. 1989. Mutator phenotype of Werner syndrome is characterized by extensive deletions. *Proc. Natl. Acad. Sci. USA* **86**:5893–5897.
13. Gangloff, S., J. P. McDonald, C. Bendixen, L. Arthur, and R. Rothstein. 1994. The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Mol. Cell. Biol.* **14**:8391–8398.
14. Garcia, P. L., Y. Liu, J. Jiricny, S. C. West, and P. Janscak. 2004. Hum. RECQ5 β , a protein with DNA helicase and strand-annealing activities in a single polypeptide. *EMBO J.* **23**:2882–2891.
15. German, J. 1993. Bloom syndrome: a mendelian prototype of somatic mutational disease. *Medicine (Baltimore)* **72**:393–406.
16. German, J. 1995. Bloom's syndrome. *Dermatol. Clin.* **13**:7–18.
17. German, J., and B. Alhadeff. 1994. Sister chromatid exchange (SCE) analysis, p. 8.6.1–8.6.10. *In* N. C. Dracopoli, J. L. Haines, B. R. Korf, D. T. Moir, C. C. Morton, C. E. Seidman, J. A. Seidman and D. R. Smith (ed.), *Current protocols in human genetics*. John Wiley & Sons, New York, N.Y.
18. Goss, K. H., M. A. Risinger, J. J. Kordich, M. M. Sanz, J. E. Straughen, L. E. Slovek, A. J. Capobianco, J. German, G. P. Boivin, and J. Groden. 2002. Enhanced tumor formation in mice heterozygous for *Blm* mutation. *Science* **297**:2051–2053.
19. Guo, G., W. Wang, and A. Bradley. 2004. Mismatch repair genes identified using genetic screens in *Blm*-deficient embryonic stem cells. *Nature* **429**:891–895.
20. Gupta, P. K., A. Sahota, S. A. Boyadjiev, S. Bye, C. Shao, J. P. O'Neill, T. C. Hunter, R. J. Albertini, P. J. Stambrook, and J. A. Tischfield. 1997. High frequency in vivo loss of heterozygosity is primarily a consequence of mitotic recombination. *Cancer Res.* **57**:1188–1193.
21. Haber, J. E. 1999. DNA recombination: the replication connection. *Trends Biochem. Sci.* **24**:271–275.
22. Haigs, K. M., and W. F. Dove. 2003. A Robertsonian translocation suppresses a somatic recombination pathway to loss of heterozygosity. *Nat. Genet.* **33**:33–39.
23. Hassold, T., S. Sherman, and P. Hunt. 2000. Counting cross-overs: characterizing meiotic recombination in mammals. *Hum. Mol. Genet.* **9**:2409–2419.
24. Hickson, I. D. 2003. RecQ helicases: caretakers of the genome. *Nat. Rev. Cancer* **3**:169–178.
25. Hu, P., S. F. Beresten, A. J. van Brabant, T. Z. Ye, P. P. Pandolfi, F. B. Johnson, L. Guarente, and N. A. Ellis. 2001. Evidence for BLM and topoisomerase III α interaction in genomic stability. *Hum. Mol. Genet.* **10**:1287–1298.
26. Ira, G., A. Malkova, G. Liberi, M. Foiani, and J. E. Haber. 2003. Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell* **115**:401–411.
27. Johnson, R. D., and M. Jasin. 2000. Sister chromatid gene conversion is a prominent double-strand break repair pathway in mammalian cells. *EMBO J.* **19**:3398–3407.
28. Karow, J. K., A. Constantinou, J. L. Li, S. C. West, and I. D. Hickson. 2000. The Bloom's syndrome gene product promotes branch migration of Holliday junctions. *Proc. Natl. Acad. Sci. USA* **97**:6504–6508.
29. Kitao, S., I. Ohsugi, K. Ichikawa, M. Goto, Y. Furuichi, and A. Shimamoto. 1998. Cloning of two new human helicase genes of the RecQ family: biological significance of multiple species in higher eukaryotes. *Genomics* **54**:443–452.
30. Kitao, S., A. Shimamoto, M. Goto, R. W. Miller, W. A. Smithson, N. M. Lindor, and Y. Furuichi. 1999. Mutations in *RECQL4* cause a subset of cases of Rothmund-Thomson syndrome. *Nat. Genet.* **22**:82–84.
31. Knudson, A. G., Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl. Acad. Sci. USA* **68**:820–823.
32. Knudson, A. G., Jr. 1978. Retinoblastoma: a prototypic hereditary neoplasm. *Semin. Oncol.* **5**:57–60.
33. Krejci, L., S. Van Komen, Y. Li, J. Villemain, M. S. Reddy, H. Klein, T. Ellenberger, and P. Sung. 2003. DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature* **423**:305–309.
34. Kuzminov, A. 2001. DNA replication meets genetic exchange: chromosomal damage and its repair by homologous recombination. *Proc. Natl. Acad. Sci. USA* **98**:8461–8468.
35. Lee, E. C., D. Yu, J. Martinez de Velasco, L. Tessarollo, D. A. Swing, D. L. Court, N. A. Jenkins, and N. G. Copeland. 2001. A highly efficient *Escherichia coli*-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. *Genetics* **73**:56–65.
36. Liu, P., N. A. Jenkins, and N. G. Copeland. 2002. Efficient Cre-loxP-induced mitotic recombination in mouse embryonic stem cells. *Nat. Genet.* **30**:66–72.
37. Luo, G., I. M. Santoro, L. D. McDaniel, I. Nishijima, M. Mills, H. Youssoufian, H. Vogel, R. A. Schultz, and A. Bradley. 2000. Cancer predisposition caused by elevated mitotic recombination in Bloom mice. *Nat. Genet.* **26**:424–429.
38. Luo, G., M. S. Yao, C. F. Bender, M. Mills, A. R. Bladl, A. Bradley, and J. H. Petrini. 1999. Disruption of *mRad50* causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc. Natl. Acad. Sci. USA* **96**:7376–7381.
39. Mann, M. B., C. A. Hodges, E. Barnes, H. Vogel, T. J. Hassold, and G. Luo. 2005. Defective sister-chromatid cohesion, aneuploidy and cancer predisposition in a mouse model of type II Rothmund-Thomson syndrome. *Hum. Mol. Genet.* **14**:813–825.
40. McCullough, A. J., and S. M. Berget. 1997. G triplets located throughout a class of small vertebrate introns enforce intron borders and regulate splice site selection. *Mol. Cell. Biol.* **17**:4562–4571.
41. McDaniel, L. D., N. Chester, M. Watson, A. D. Borowsky, P. Leder, and R. A. Schultz. 2003. Chromosome instability and tumor predisposition inversely correlate with BLM protein levels. *DNA Repair (Amsterdam)* **2**:1387–1404.
42. Moynahan, M. E., and M. Jasin. 1997. Loss of heterozygosity induced by a chromosomal double-strand break. *Proc. Natl. Acad. Sci. USA* **94**:8988–8993.
43. Murray, J. M., H. D. Lindsay, C. A. Munday, and A. M. Carr. 1997. Role of *Schizosaccharomyces pombe* RecQ homolog, recombination, and checkpoint genes in UV damage tolerance. *Mol. Cell. Biol.* **17**:6868–6875.
44. Nakayama, H., K. Nakayama, R. Nakayama, N. Irino, Y. Nakayama, and P. C. Hanawalt. 1984. Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichia coli* K12: identification of a new mutation (*recQ1*) that blocks the RecF recombination pathway. *Mol. Gen. Genet.* **195**:474–480.
45. Ohhata, T., R. Araki, R. Fukumura, A. Kuroiwa, Y. Matsuda, and M. Abe. 2001. Cloning, genomic structure and chromosomal localization of the gene encoding mouse DNA helicase RECQL5 β . *Gene* **280**:59–66.
46. Onclercq-Delic, R., P. Calsou, C. Delteil, B. Salles, D. Papadopoulo, and M. Amor-Gueret. 2003. Possible anti-recombinogenic role of Bloom's syndrome helicase in double-strand break processing. *Nucleic Acids Res.* **31**:6272–6282.
47. Puranam, K. L., and P. J. Blackshear. 1994. Cloning and characterization of RECQL, a potential human homologue of the *Escherichia coli* DNA helicase RecQ. *J. Biol. Chem.* **269**:29838–29845.
48. Richardson, C., M. E. Moynahan, and M. Jasin. 1998. Double-strand break repair by interchromosomal recombination: suppression of chromosomal translocations. *Genes Dev.* **12**:3831–3842.
49. Sekelsky, J. J., M. H. Brodsky, G. M. Rubin, and R. S. Hawley. 1999. *Drosophila* and human RecQ5 exist in different isoforms generated by alternative splicing. *Nucleic Acids Res.* **27**:3762–3769.
50. Seki, M., H. Miyazawa, S. Tada, J. Yanagisawa, T. Yamaoka, S. Hoshino, K. Ozawa, T. Eki, M. Nogami, K. Okumura, et al. 1994. Molecular cloning of cDNA encoding human DNA helicase Q1 which has homology to *Escherichia coli* Rec Q helicase and localization of the gene at chromosome 12p12. *Nucleic Acids Res.* **22**:4566–4573.
51. Shimamoto, A., K. Nishikawa, S. Kitao, and Y. Furuichi. 2000. Human RecQ5 β , a large isomer of RecQ5 DNA helicase, localizes in the nucleoplasm and interacts with topoisomerases 3 α and 3 β . *Nucleic Acids Res.* **28**:1647–1655.
52. Sieber, O. M., H. Lamlum, M. D. Crabtree, A. J. Rowan, E. Barclay, L. Lipton, S. Hodgson, H. J. Thomas, K. Neale, R. K. Phillips, S. M. Farrington, M. G. Dunlop, H. J. Mueller, M. L. Bisgaard, S. Bulow, P. Fidalgo, C. Albuquerque, M. I. Scarano, W. Bodmer, I. P. Tomlinson, and K. Heinemann. 2002. Whole-gene *APC* deletions cause classical familial adenomatous polyposis, but not attenuated polyposis or "multiple" colorectal adenomas. *Proc. Natl. Acad. Sci. USA* **99**:2954–2958.
53. Stewart, E., C. R. Chapman, F. Al-Khodairy, A. M. Carr, and T. Enoch. 1997. *rgh1+*, a fission yeast gene related to the Bloom's and Werner's syndrome genes, is required for reversible S phase arrest. *EMBO J.* **16**:2682–2692.
54. Traverso, G., C. Bettgowda, J. Kraus, M. R. Speicher, K. W. Kinzler, B. Vogelstein, and C. Lengauer. 2003. Hyper-recombination and genetic instability in *BLM*-deficient epithelial cells. *Cancer Res.* **63**:8578–8581.
55. Veaute, X., J. Jeusset, C. Soustelle, S. C. Kowalczykowski, E. Le Cam, and F. Fabre. 2003. The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature* **423**:309–312.
56. Vilenchik, M. M., and A. G. Knudson. 2003. Endogenous DNA double-strand breaks: production, fidelity of repair, and induction of cancer. *Proc. Natl. Acad. Sci. USA* **100**:12871–12876.
57. Wang, W., M. Seki, Y. Narita, T. Nakagawa, A. Yoshimura, M. Otsuki, Y. Kawabe, S. Tada, H. Yagi, Y. Ishii, and T. Enomoto. 2003. Functional relation among RecQ family helicases RecQL1, RecQL5, and BLM in cell growth and sister chromatid exchange formation. *Mol. Cell. Biol.* **23**:3527–3535.
58. Wang, Y., D. Cortez, P. Yazdi, N. Neff, S. J. Elledge, and J. Qin. 2000. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.* **14**:927–939.
59. Watt, P. M., I. D. Hickson, R. H. Borts, and E. J. Louis. 1996. *SGS1*, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. *Genetics* **144**:935–945.
60. Watt, P. M., E. J. Louis, R. H. Borts, and I. D. Hickson. 1995. Sgs1: a

- eukaryotic homolog of *E. coli* RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. *Cell* **81**:253–260.
61. **Wu, L., S. L. Davies, N. C. Levitt, and I. D. Hickson.** 2001. Potential role for the BLM helicase in recombinational repair via a conserved interaction with RAD51. *J. Biol. Chem.* **276**:19375–19381.
62. **Wu, L., and I. D. Hickson.** 2003. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* **426**:870–874.
63. **Yu, C. E., J. Oshima, Y. H. Fu, E. M. Wijsman, F. Hisama, R. Alisch, S. Matthews, J. Nakura, T. Miki, S. Ouais, G. M. Martin, J. Mulligan, and G. D. Schellenberg.** 1996. Positional cloning of the Werner's syndrome gene. *Science* **272**:258–262.
64. **Yu, D., H. M. Ellis, E. C. Lee, N. A. Jenkins, N. G. Copeland, and D. L. Court.** 2000. An efficient recombination system for chromosome engineering in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **97**:5978–5983.