Cell

RTEL1 Maintains Genomic Stability by Suppressing Homologous Recombination

Louise J. Barber,[1] Jillian L. Youds,[1,2] Jordan D. Ward,[1] Michael J. McIlwraith,[3] Nigel J. O'Neil,[2] Mark I.R. Petalcorin,[1] Julie S. Martin,[1] Spencer J. Collis,[1] Sharon B. Cantor,[4] Melissa Auclair,[5] Heidi Tissenbaum,[5] Stephen C. West,[3] Ann M. Rose,[2] and Simon J. Boulton[1,*]

[1]DNA Damage Response Laboratory, London Research Institute, Cancer Research UK, Clare Hall, South Mimms EN6 3LD, UK
[2]Department of Medical Genetics, Faculty of Medicine, University of British Columbia, Vancouver, BC V6T 1Z4, Canada
[3]Genetic Recombination Laboratory, London Research Institute, Cancer Research UK, Clare Hall, South Mimms EN6 3LD, UK
[4]Program in Cancer Biology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA
[5]Program in Gene Function and Expression and Program in Molecular Medicine, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA
*Correspondence: simon.boulton@cancer.org.uk
DOI 10.1016/j.cell.2008.08.016

SUMMARY

Homologous recombination (HR) is an important conserved process for DNA repair and ensures maintenance of genome integrity. Inappropriate HR causes gross chromosomal rearrangements and tumorigenesis in mammals. In yeast, the Srs2 helicase eliminates inappropriate recombination events, but the functional equivalent of Srs2 in higher eukaryotes has been elusive. Here, we identify *C. elegans* RTEL-1 as a functional analog of Srs2 and describe its vertebrate counterpart, RTEL1, which is required for genome stability and tumor avoidance. We find that *rtel-1* mutant worms and *RTEL1*-depleted human cells share characteristic phenotypes with yeast *srs2* mutants: lethality upon deletion of the *sgs1/BLM* homolog, hyperrecombination, and DNA damage sensitivity. In vitro, purified human RTEL1 antagonizes HR by promoting the disassembly of D loop recombination intermediates in a reaction dependent upon ATP hydrolysis. We propose that loss of HR control after deregulation of RTEL1 may be a critical event that drives genome instability and cancer.

INTRODUCTION

Homologous recombination (HR) is an essential conserved process for dividing cells. In mitosis, HR is required not only for the accurate repair of DNA double-strand breaks (DSBs) but also for the restart of stalled replication forks. Furthermore, HR is crucial for meiotic DSB repair, which is required for accurate chromosome segregation at the first meiotic division. However, inappropriate HR can give rise to genome instability and cancer as a result of erroneous chromosomal rearrangements and the persistence of intermediate recombination structures that cannot be resolved. Hence, HR must be tightly regulated and temporally coordinated with cell-cycle progression and replication.

Current models of eukaryotic HR (Krogh and Symington, 2004) propose that a DSB is resected to produce 3′ single-stranded DNA tails that are bound by the DNA strand exchange protein RAD51 to form a nucleoprotein filament. These filaments are the catalyst for strand invasion into homologous duplex DNA, resulting in the formation of a D loop structure. The invading 3′ end provides a primer for DNA synthesis and D loop extension, which can be resolved either through displacement of the invading strand from the D loop and annealing to the other DSB end (synthesis-dependent strand annealing) or by the capture of the other resected end by the extruded strand of the D loop to form a double Holliday junction (dHJ). HR can be completed by endonucleolytic cleavage of the two HJs, which may result in a crossover.

In yeast, the initiation of strand invasion is antagonized by Srs2 to ensure that HR occurs at the appropriate time and place. Srs2, first identified 30 years ago (Lawrence and Christensen, 1979), is a 3′-5′ SF1 helicase related both by sequence and function to bacterial UvrD (Aboussekhra et al., 1989). *S. cerevisiae srs2* and *E. coli uvrD* mutants exhibit elevated rates of spontaneous recombination (Aguilera and Klein, 1988; Arthur and Lloyd, 1980; Bierne et al., 1997; Zieg et al., 1978). Yeast *srs2* mutants are synthetic lethal with deletion of the yeast RecQ helicase, *sgs1* (Lee et al., 1999; Wang et al., 2001). It was subsequently found that the inviability of *srs2 sgs1* mutants results from the accumulation of toxic HR intermediates because viability can be restored by loss of *RAD51* or *RAD54*, which are essential for the formation of the nucleoprotein filament and extension of the invading strand (Gangloff et al., 2000; Klein, 2001). Loss of *srs2* also results in significant sensitivity to a range of DNA-damaging agents, including IR and bleomycin, which directly cause DSBs (Bennett et al., 2001), and ultraviolet radiation (UV), camptothecin, and DNA interstrand crosslinks (ICLs), which lead to replication-blocking lesions (Aboussekhra et al., 1992; Birrell et al., 2002). Biochemical studies have shown that both UvrD and Srs2 act to inhibit strand exchange by disrupting RecA/Rad51 filaments (Krejci et al., 2003; Morel et al., 1993; Veaute et al., 2003). This has led to the model that UvrD and Srs2 negatively regulate HR by disassembling the nucleoprotein filament.

Sequence homologs of *SRS2* are not apparent in the genomes of higher eukaryotes. It has therefore been proposed that other helicases act in combination to substitute for Srs2 in order to negatively regulate HR and ensure genome stability. The function of an Srs2-related DNA helicase, Fbh1, overlaps with Srs2 in the processing of recombination intermediates in *Schizosaccharomyces pombe* (Morishita et al., 2005; Osman et al., 2005), and expression of human *FBH1* is able to rescue some recombination defects in yeast *srs2* mutants (Chiolo et al., 2007). Fbh1 is not conserved in budding yeast, *Caenorhabditis elegans*, *Drosophila*, or *Arabidopsis*. Although orthologs are found in humans, mice, and chickens (Kim et al., 2002; Kohzaki et al., 2007), *Fbh1* deletion mutants are viable and exhibit only a mild phenotype in DT40 cells (Kohzaki et al., 2007). Furthermore, the RecQ family helicases BLM and RECQL5 are able to disrupt RAD51 filaments in vitro and inhibit the initiation of HR (Bachrati et al., 2006; Bugreev et al., 2007; Hu et al., 2007).

In metazoans, there is a distinct family of helicases, defined by the discovery and characterization of *dog-1* (*d*eletion *o*f *g*uanine-rich DNA) in *C. elegans*, which is essential for the maintenance of polyG/C-tracts (Cheung et al., 2002). In mice, another member of this family, *Rtel*, is essential for telomere maintenance (Ding et al., 2004). Cells derived from the *Rtel*$^{-/-}$ null mice exhibit reduced proliferative capacity and chromosomal abnormalities (Ding et al., 2004), which may be phenotypes attributable to a more general role in the maintenance of genome stability. The human homolog of *Rtel* is amplified in gastric tumors (Bai et al., 2000), but how Rtel functions and how its deregulated expression promotes tumorigenesis remains unclear.

Here, we have utilized the genetic tractability of the nematode *C. elegans* to screen for a functional equivalent of Srs2 in a metazoan. We have identified a previously uncharacterized RAD3-like helicase, RTEL-1, which is the *C. elegans* homolog of murine Rtel. We show that *rtel-1* mutant worms and *RTEL1* knockdown human cells share a number of characteristic phenotypes with yeast *srs2* mutants, and demonstrate that recombinant human RTEL1 is a potent antagonist of HR that acts specifically to disrupt D loop recombination intermediates. Our results imply a role for RTEL1 as an antirecombinase and suggest that RTEL1 is a functional analog of Srs2 in metazoans.

RESULTS

Identification of *rtel-1*, the *C. elegans* Homolog of *Rtel*

One of the best-characterized antagonists of HR is the yeast helicase Srs2 (Krejci et al., 2003; Veaute et al., 2003), yet sequence analysis has failed to identify putative homologs of *SRS2* in higher eukaryotes. Because it would be detrimental for a cell to undergo inappropriate recombination, it is expected that mechanisms to restrain HR must exist in higher eukaryotes. We used a genetic approach to identify potential antagonists of recombination in the nematode *C. elegans*. One of the characteristic phenotypes of both budding and fission yeast *srs2* mutants is that growth is severely impaired by the additional loss of *sgs1*/*rqh1*, a homolog of the human Bloom's Syndrome helicase (*BLM*) (Lee et al., 1999; Wang et al., 2001). We therefore conducted a candidate-based synthetic lethal screen to look for helicase genes that result in significantly impaired viability when mutated in combination with the *C. elegans BLM* homolog, *him-6* (Wicky et al., 2004) (Supplemental Data available online). This screen identified *F25H2.13*, which we have named *rtel-1*. This gene is the *C. elegans* homolog of *Rtel* (Ding et al., 2004) (Figure S1A), which is essential for embryonic development, genome stability, and telomere maintenance in mice (Ding et al., 2004).

We obtained a nematode mutant allele of *rtel-1*, *tm1866*, in which a 1346 bp region comprising exons 2–5 is deleted. The deletion truncates the predicted protein and results in a premature stop codon downstream of the conserved IA helicase motif. *rtel-1 (tm1866)* animals are viable, although the brood size is reduced relative to the wild-type N2 strain (Table 1) and the life cycle is retarded (at 20°C, *rtel-1* worms take 24 hr longer to reach the gravid adult stage). The smaller brood size of *rtel-1* mutants may result from replicative stress because we observe a 3-fold increase in germline apoptosis (data not shown) and a high incidence (21%) of the protruding vulva phenotype, which is often associated with a persistence of unrepaired DNA damage during development (Weidhaas et al., 2006).

In our screen, we observed that the viability of *rtel-1; him-6* double mutants is severely compromised compared with either the *him-6* or *rtel-1* single mutants, with only 7% of progeny surviving to adulthood (Table 1). The synthetic lethality of *rtel-1; him-6* is in stark contrast to the 49% progeny viability observed for the *him-6* mutant, consistent with a previous report (Wicky et al., 2004). DOG-1 is the *C. elegans* homolog of FANCJ and is the helicase most closely related to RTEL-1 (Cheung et al., 2002; Youds et al., 2008). An earlier study by Youds et al. has shown that *dog-1; him-6* double mutants have fewer viable progeny than either single mutant (Youds et al., 2006) (Table 1). However, the reduced viability of later larval stages is far more severe in *rtel-1; him-6* animals (Table 1).

In addition to *him-6*/*BLM*, there are three other genes encoding RecQ family helicases in the *C. elegans* genome: *wrn-1*/*WRN*, *rcq-5*/*RECQL5*, and *K02F3.12*/*RECQL1* (Jeong et al., 2003; Lee et al., 2004). We found that *rtel-1* is also synthetic lethal with *rcq-5* (Table 1), with 100% lethality at the embryonic stage. Interestingly, although the eggs are inviable, the total number of eggs laid by *rtel-1; rcq-5* double-mutant worms is similar to the wild-type, suggesting that the *rcq-5* mutation is able to rescue the reduced brood size in *rtel-1* mutants (Table 1). In contrast to *rtel-1; him-6* and *rtel-1; rcq-5*, we did not observe synthetic lethality in the *rtel-1; wrn-1* double mutants, and the K02F3.12 helicase is essential, which precludes analysis. Therefore, the genetic relationship of *rtel-1* with RecQ helicases appears to be restricted to *him-6* and *rcq-5*.

It has been shown that the human homolog of *rcq-5* (*RECQL5*) is able to disrupt RAD51 nucleoprotein filaments in vitro (Hu et al., 2007); hence, we also considered the RecQ family genes in our primary screen for synthetic lethality with *him-6*. We observed that *wrn-1* and *rcq-5* single-mutant worms had brood sizes and viability similar to wild-type animals, whereas double mutants of each of these genes with *him-6* displayed viability similar to *him-6* single mutants (Table 1). Thus, *wrn-1* and *rcq-5* do not exhibit the characteristic synthetic lethality with *him-6*, which is predicted of a potential *SRS2* analog. In contrast,

Table 1. Genetic Interactions of *C. elegans rtel-1*

Equivalent Genotype						
Yeast	Human	*C. elegans* Genotype	Total Brood Size	Percent Embryonic Lethality	Percent Viable Progeny	Number Scored
wild-type	wild-type	N2 (wild-type)	257 ± 20	0.6 ± 0.05	99.4 ± 0.05	n = 5
–	*RTEL1*	*rtel-1*	68 ± 12	3.0 ± 0.9	86.0 ± 9.0	n = 20
sgs1	*BLM*	*him-6*	214 ± 14	49.7 ± 1.4	48.5 ± 1.6	n = 20
–	*FANCJ*	*dog-1*	229 ± 11	3.9 ± 1.7	96.1 ± 1.7	n = 15
mus81	*MUS81*	*mus-81*	153 ± 13	15.2 ± 6.0	84.8 ± 6.0	n = 20
–	*RECQ5*	*rcq-5*	209 ± 20	0.4 ± 0.1	99.6 ± 0.1	n = 20
–	*WRN1*	*wrn-1*	279 ± 13	0.3 ± 0.2	99.7 ± 0.1	n = 20
–	*RTEL1 BLM*	*rtel-1; him-6*	54 ± 11	72.1 ± 2.5	7.1 ± 1.6	n = 20
–	*RTEL1 FANCJ*	*rtel-1 dog-1*	0	0	0	n = 30
–	*MUS81 RTEL1*	*mus-81 rtel-1*	22 ± 7	100	0	n = 30
–	*RTEL1 RECQ5*	*rtel-1; rcq-5*	168 ± 19	100	0	n = 17
–	*RTEL1 WRN1*	*rtel-1; wrn-1*	47 ± 13	4.0 ± 6.3	84.5 ± 7.8	n = 20
–	*FANCJ BLM*	*dog-1; him-6**	185 ± 13	72.5 ± 1.8	27.6 ± 1.8	n = 41
–	*RECQ5 BLM*	*rcq-5; him-6*	111 ± 20	49.4 ± 4.2	50.6 ± 4.2	n = 10
–	*WRN1 BLM*	*wrn-1; him-6*	170 ± 23	56.4 ± 1.6	43.6 ± 1.6	n = 10
–	*FANCJ RECQ5*	*dog-1; rcq-5*	201 ± 19	1.3 ± 0.7	98.7 ± 0.7	n = 20
–	*FANCJ WRN1*	*dog-1; wrn-1*	247 ± 12	4.1 ± 2.4	95.9 ± 2.4	n = 15
–	*RECQ5 WRN1*	*rcq-5; wrn-1*	243 ± 16	ND	97.3 ± 1.4	n = 10

Analysis of brood size, embryonic lethality, and viable progeny reaching adulthood in single- and double gene-deletion mutants. Yeast and human homologs are indicated, and "–" denotes genes not found in yeast. All values are ± standard SEM, and "n" indicates the number of parent animals whose progeny were scored. *, data from (Youds et al., 2006).

the synthetic lethality of *rtel-1* in combination with *him-6* or *rcq-5* raised the possibility that RTEL-1 could be a candidate antagonist of homologous recombination.

rtel-1 Mutants Are Synthetic Lethal with Other Factors Required for DNA Metabolism

S. cerevisiae srs2 has been found to cause synthetic growth defects in a range of mutant backgrounds that impact on DNA repair and genome stability, in addition to *sgs1* (Chiolo et al., 2005; Klein, 2001; Palladino and Klein, 1992; Tong et al., 2004). These include mutants in genes encoding double-strand break repair factors such as Mre11, Rad50, Xrs2, and Rad54; the helicases Chl1 and Mph1; and nucleases such as the flap endonuclease Rad27 and the structure-specific heterodimeric nuclease Mus81-Mms4. Therefore, we investigated the genetic relationship between *rtel-1* and other factors involved in DNA metabolism. In the absence of viable *C. elegans* deletion mutants in *mre-11*, *rad-50*, *rad-54*, *rad-27*, *chl-1*, *mph-1*, or a putative homolog of *mms4*, we examined the interaction between *rtel-1* and *mus-81*. In *S. cerevisiae*, *mus81* shows a reduced growth rate in the *srs2* background (Fabre et al., 2002; Pan et al., 2006), and *mms4* displays a synthetic sick phenotype with *srs2* (Tong et al., 2004). The *C. elegans mus-81* strain is largely viable, whereas the *mus-81 rtel-1* double-mutant worms lay eggs that are completely inviable (Table 1). Furthermore, the brood size is severely reduced, beyond that of the *rtel-1* single mutant.

We also investigated how loss of the *dog-1* helicase might affect progeny viability in the *rtel-1* background. Strikingly, the *rtel-1 dog-1* double mutants produce no embryos, whereas both of the single-mutant strains produce progeny with 80% viability (Table 1). Cytological analysis of the parental *rtel-1 dog-1* animals revealed that the germline fails to develop appropriately. The mitotic tip contains only a few enlarged mitotic nuclei, which are indicative of replication stress (Ahmed et al., 2001), and these nuclei have fragmented chromosomes (Figure S2). Clearly, the mitotic nuclei do not progress into meiosis, resulting in sterility. Thus, loss of both *rtel-1* and the related helicase *dog-1* results in profound proliferation defects in the germline and synthetic sterility. Together, *rtel-1* exhibits synthetic sterility or lethality in combination with *dog-1*, *him-6*, *rcq-5*, or *mus-81*, reminiscent of the genetic interactions observed between *srs2* and factors required for DNA metabolism in yeast. These data reinforce the possibility that RTEL-1 may function to negatively regulate HR in metazoans in a similar manner to Srs2 in yeast.

Synthetic Lethality Correlates with a Massive Accumulation of Recombination Intermediates

It has been proposed that the inviability of yeast *srs2 sgs1* is caused by the accumulation of toxic recombination intermediates because viability can be partially restored by the additional loss of *RAD51* or *RAD54* (Gangloff et al., 2000; Klein, 2001). Unfortunately, *C. elegans* RAD-51 and RAD-54 are essential for meiotic DSB repair and subsequent progeny viability

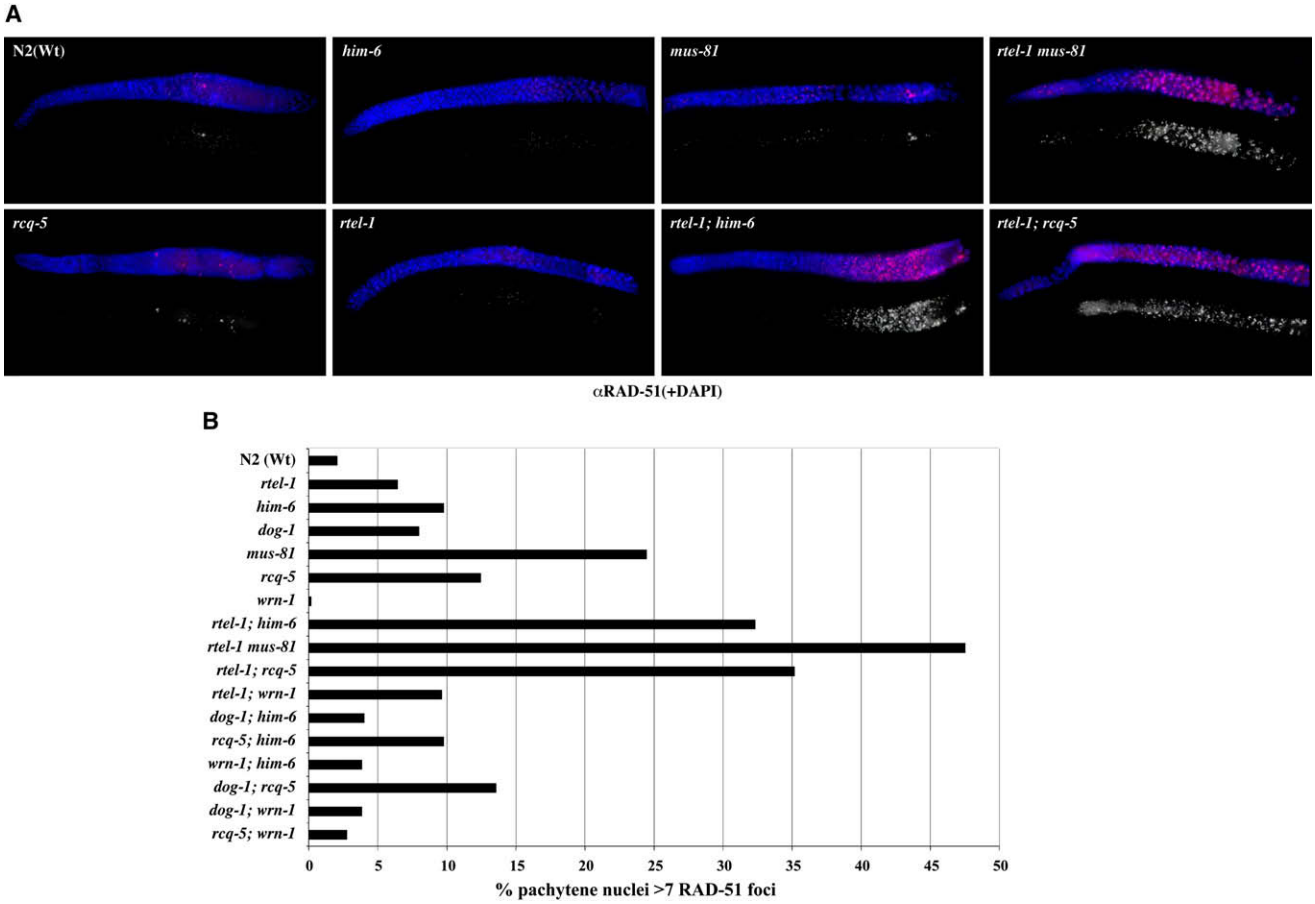

Figure 1. Synthetic Lethality Correlates with Elevated Levels of RAD-51 Foci

(A) Representative images of germlines (genotype as indicated) stained with α-RAD-51 (red) and DNA counterstained with DAPI (blue). Top: RAD-51 + DAPI merge. Bottom: RAD-51 in grayscale. The distal end of the germline is on the left.

(B) Quantification of nuclei containing more than seven RAD-51 foci in the pachytene region of the germline. At least five animals were scored for each genotype. A detailed quantification of total germline RAD-51 staining is shown in Figure S3.

(Alpi et al., 2003; Martin et al., 2005) (data not shown), and this precludes similar analyses of the synthetic lethal interactions observed with *rtel-1*. In view of this, we directly measured the occurrence of RAD-51 foci in the worm germline to determine whether the terminal phenotype associated with the synthetic lethality of *rtel-1* with *him-6*, *dog-1*, *rcq-5*, and *mus-81* correlates with persistent HR intermediates. Nuclei in the *C. elegans* germline are spatially ordered, progressing proximally from a region of mitosis in the distal tip through the different stages of meiotic prophase I, enabling the separate analysis of mitotic and meiotic RAD-51 foci (Alpi et al., 2003) (Figure S3).

In *rtel-1* mutants, normal levels of RAD-51 foci are observed in early meiotic prophase nuclei, corresponding to the initiation and repair of SPO-11-induced DSBs (Figure 1B and Figure S3). A normal incidence of RAD-51 foci is also seen in *him-6*, *dog-1*, *wrn-1*, and *rcq-5* worms, whereas *mus-81* germlines exhibit a slightly elevated level of RAD-51 foci suggestive of a reduced capacity to resolve mitotic and/or meiotic DSBs, or an increased incidence of spontaneous DNA damage (Boddy et al., 2001) (Figure 1A and Figure S3). In contrast, a massive accumulation of RAD-51 foci (up to 10-fold higher incidence of nuclei with more than six RAD-51 foci, relative to the wild-type) is observed in the pachytene region of all of the double-mutant germlines that give rise to progeny with severely impaired viability, namely (1) *rtel-1; him-6*, (2) *rtel-1; rcq-5*, and (3) *mus-81 rtel-1* (Table 1, Figures 1A and 1B). Elevated levels of RAD-51 foci were also observed in the *rtel-1 dog-1* double mutant. However, we were unable to quantify the occurrence of RAD-51 foci in the same manner as for the other strains because of the mitotic catastrophe and lack of an intact germline in these animals (Figure S2). Importantly, elevated levels of RAD-51 foci are not observed for double-mutant combinations that have been shown to be viable, including *rtel-1; wrn-1* and all possible double-mutant combinations of the RecQ family of genes and *dog-1* (Table 1, Figure 1B). We conclude that the terminal phenotype of the synthetic lethality of *rtel-1* with *dog-1*, *him-6*, *rcq-5*, and *mus-81* corresponds to a massive accumulation of recombination intermediates that persist and fail to be appropriately repaired.

Table 2. *rtel-1* Mutants Display Elevated Meiotic Recombination Frequencies

Genetic Interval Tested	Genotype	Total Progeny	Number of Recombinants	Map Distance in cM (95% CI)
dpy-11 to *unc-42*	wild-type	3135	114	3.71 (3.05–4.44)
dpy-11 to *unc-42*	*dog-1*	3273	84	2.60 (2.06–3.21)
dpy-11 to *unc-42*	*rcq-5*	3824	94	2.48 (1.99–3.05)
dpy-11 to *unc-42*	*rtel-1*	723	66	9.59 (7.37–12.25)
dpy-17 to *unc-36*	wild-type	3299	32	0.97 (0.65–1.35)
dpy-17 to *unc-36*	*dog-1*	3495	42	1.21 (0.86–1.60)
dpy-17 to *unc-36*	*rtel-1*	459	18	4.00 (2.47–6.24)

Meiotic recombination frequencies within the intervals defined by *dpy-11* to *unc-42*, and *dpy-17* to *unc-36*, were determined in different genetic backgrounds, as indicated. CI, confidence interval.

Meiotic Recombination Frequencies Are Elevated in *rtel-1* Mutants

In addition to the growth defects of yeast *srs2* mutants in combination with *sgs1*/*rqh1*, loss of *srs2* has also been shown to influence the frequency of recombination events (Ira et al., 2003; Rong et al., 1991). Therefore, we examined meiotic exchange in the *rtel-1* deletion strain. Using the visible mutant phenotypes "Dpy" (short, fat worms) and "Unc" (uncoordinated movement), we measured the frequency of crossing over for two intervals on two separate chromosomes. On chromosome V, crossing over in the interval between *dpy-11* and *unc-42* was increased by more than 2.5-fold in the *rtel-1* background compared to the wild-type (Table 2). Loss of either *dog-1* or *rcq-5* did not significantly alter the meiotic recombination frequency (Table 2). A similar result was observed between *dpy-17* and *unc-36* on chromosome III (Table 2). Crossover frequency was elevated 4-fold in *rtel-1* progeny relative to the wild-type, whereas deletion of *dog-1* did not significantly affect the recombination frequency (Table 2). We were unable to analyze recombination frequencies for *rcq-5* because of the close proximity of the *rcq-5* gene to the *dpy-17 unc-36* interval. Thus, RTEL-1 is the first example of a putative helicase that increases meiotic crossover frequencies when absent from *C. elegans*.

Loss of *rtel-1* Confers Sensitivity to a Range of DNA-Damaging Agents

Another characteristic phenotype of yeast *srs2* mutants is sensitivity to DNA-damaging agents (Aboussekhra et al., 1992; Bennett et al., 2001; Birrell et al., 2002). We investigated whether loss of *C. elegans rtel-1* had any effect on the capacity of *rtel-1* mutants to tolerate different types of DNA damage, using progeny viability as a measure of sensitivity. Embryonic survival after exposure to X-rays or UVC was not significantly affected in either the wild-type or *rtel-1* mutant worms (Figures 2A and 2B). However, the response to DNA ICLs was affected in the absence of *rtel-1*. Treatment with either UVA-activated trimethylpsoralen or nitrogen mustard led to a marked reduction in *rtel-1* progeny survival compared to the wild-type (Figures 2D and 2E). Furthermore, *rtel-1* worms were sensitive to treatment with the topoisomerase I inhibitor, camptothecin (Figure 2C). Hence, *rtel-1* is required for the *C. elegans* response to DNA damage, although this appears to be specific to lesions that affect replication fork progression.

RTEL-1 Function Is Distinct from that of the Related Helicase DOG-1

In humans, sensitivity to ICL-inducing agents is a characteristic of cells deficient for any of the genes associated with the inherited cancer-associated disorder Fanconi anemia (FA) (Kennedy and D'Andrea, 2005). *C. elegans dog-1* is the homolog of human *FANCJ* and is epistatic to the *FANCD2* homolog, *fcd-2* (Youds et al., 2008). Given the considerable sensitivity of the *rtel-1* mutant to ICLs, we investigated whether *rtel-1* genetically interacts with the FA pathway in DNA repair. In contrast to *dog-1; fcd-2* double mutants (Youds et al., 2008), the progeny of *rtel-1; fcd-2* double mutants were significantly more sensitive to nitrogen mustard than either single mutant alone (Figure 2F). Hence, *rtel-1* is not epistatic with *fcd-2*, one of the key factors in the FA pathway. In response to replication stress, FCD-2 is recruited to nuclear foci (Collis et al., 2006). We found that loss of *rtel-1*/*RTEL1* has no effect on the relocation of FCD-2/FANCD2 to repair foci in either *C. elegans* or human cells (data not shown).

In *C. elegans*, *dog-1* mutants accumulate deletions at polyG/C-tracts; hence, DOG-1 is proposed to unwind secondary structures in these tracts during replication (Cheung et al., 2002). We investigated whether *RTEL-1* is also required for maintenance of polyG/C-tracts or alternative repetitive DNA sequences [polyA/T, $(CAG)_8$]. However, no deletions were identified in *rtel-1* mutants at any of the tracts investigated (data not shown). These results indicate that RTEL-1 has a role distinct from that of DOG-1 in DNA replication and repair in *C. elegans*.

Human RTEL1 Suppresses Homologous Recombination and Is Required for DNA Repair

To investigate whether or not the function of *C. elegans* RTEL-1 is conserved in humans, we depleted human *RTEL1* (*NHL*) by siRNA in HeLa cells and tested for phenotypes in common with the *C. elegans rtel-1* mutant (Figure S4). Delivery of human *RTEL1* siRNA to cells decreased the level of mRNA expression detected by quantitative real-time PCR by 90% relative to cells treated with control or *FANCJ* siRNA (Figure S4). We were unable to directly assess protein levels using the limited number of cells targeted in siRNA experiments because *RTEL1* is not sufficiently abundant to be detected in whole-cell or nuclear extracts. The low abundance of *RTEL1* is consistent with previous reports in mice (Ding et al., 2004). However, we were able to show that these *RTEL1*-specific siRNAs, but not the nontargeting control siRNA, can effectively repress the expression of *RTEL1* from a tetracycline-inducible promoter in a stable integrated HEK293 cell line (Figure S4B).

To investigate whether the human *RTEL1* homolog is involved in the regulation of HR repair, we employed an I-SceI-inducible DSB assay to quantify the frequencies of HR repair within an integrated SCneo substrate (Figure 3A) (Johnson and Jasin, 2001;

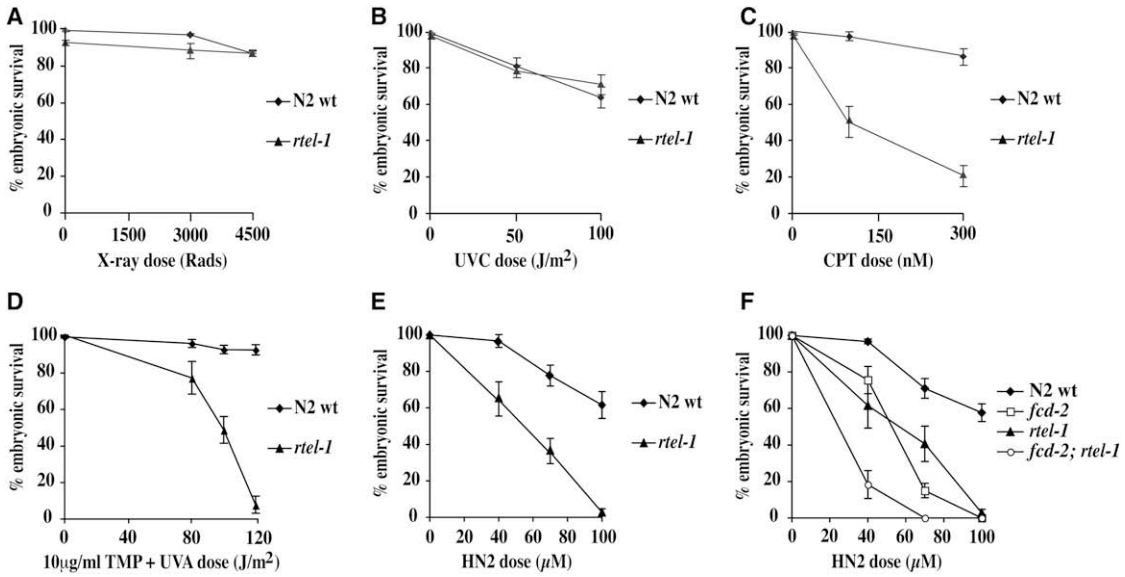

Figure 2. *rtel-1* Mutants Are Sensitive to Specific Types of DNA Damage

(A–E) Percentage progeny survival of worms treated with the indicated doses of X-rays (A), UVC (245 nm) (B), camptothecin (C), trimethylpsoralen activated with increasing doses of UVA (365 nm) (D), and nitrogen mustard (HN2) (E).

(F) Epistasis analysis of *rtel-1*, *fcd-2* and *fcd-2; rtel-1* mutants for sensitivity to HN2. Error bars indicate the SEM from at least 24 adult worms over three independent experiments.

Mohindra et al., 2002). As has been shown previously (Collis et al., 2007; Sorensen et al., 2005), depletion of *CHK1* leads to a reduction in the frequency of HR relative to the control (Figure 3B). In contrast, siRNA depletion of *RTEL1* consistently resulted in a 4-fold increase in the frequency of HR repair compared to the control (Figure 3B). Hence, as in *C. elegans*, human *RTEL1* suppresses the incidence of HR.

We next examined human *RTEL1*-depleted cells for sensitivity to different DNA-damaging agents. Knockdown of *RTEL1* in HeLa cells had no significant effect on survival after treatment with IR (Figure 3D). In contrast, cells depleted for human *RTEL1* exhibit a 50% reduction in cell survival after exposure to the ICL-inducing drug mitomycin C, relative to a nontargeting control siRNA treatment (Figure 3C). These data are consistent with the DNA damage sensitivity of *C. elegans rtel-1* mutants (Figure 2) and suggest that RTEL-1/RTEL1 function is conserved.

Human RTEL1 Inhibits the Formation of Recombination Intermediates In Vitro

Yeast Srs2 has been shown to suppress HR by disrupting RAD51 nucleoprotein filaments and preventing strand invasion (Krejci et al., 2003; Veaute et al., 2003). Our genetic data from both *C. elegans* and human cells suggest that RTEL1 may act to restrict the occurrence of HR, which we next sought to test biochemically. We utilized the baculovirus system to express and purify both wild-type human RTEL1 and a K48R mutant to near homogeneity (Figure S4C). As expected from comparison with mutations in the Walker A ATP-hydrolysing motif of other helicases, including Srs2 (Krejci et al., 2004), the K48R mutant was ATPase dead (Figure 4A).

An in vitro HR assay was used to assess whether RTEL1 is able to modulate recombination reactions catalyzed by RAD51 (McIlwraith et al., 2000). In this assay, a nucleoprotein filament is first formed between RAD51 and ^{32}P-labeled ssDNA. Upon addition of supercoiled (sc)DNA template, the nucleoprotein filament invades the supercoiled DNA (scDNA) duplex to form a joint molecule known as a D loop (Figures 4B and 4C). The heterotrimeric ssDNA-binding protein RPA is added to the reaction to stabilize the displaced strand of the D loop and also to prevent any reassociation of RAD51 with ssDNA if the nucleoprotein filament is disrupted (Krejci et al., 2003). It is clear that addition of wild-type RTEL1 purified from insect or human cells prior to scDNA severely impairs the formation of a D loop (Figures 4C and 4D, lanes 3–5). RTEL1 activity was dependent upon ATP hydrolysis because the K48R mutant protein was found to be incapable of blocking D loop formation (Figure 4D lane 6, Figure 4E).

RTEL1 Does Not Disrupt the RAD51-ssDNA Filament, but Can Disrupt Preformed D Loops

A number of proteins, including Srs2, BLM, and RECQL5, have been shown to disrupt the RAD51 nucleoprotein filament (Bugreev et al., 2007; Hu et al., 2007; Krejci et al., 2003; Veaute et al., 2003). To further investigate how RTEL1 antagonizes D loop formation, we performed gel shifts to examine whether RTEL1 is able to dissociate the RAD51 nucleoprotein filament (Figure 5A). Surprisingly, we were unable to detect any measurable effect of RTEL1 on the stability of the RAD51 nucleoprotein filament, either in the presence or absence of RPA or after addition of a 200-fold excess of unlabelled ϕX174 ssDNA competitor (Figure 5B and data not shown). This result suggested that RTEL1-dependent D loop inhibition occurs by a different mechanism to that exhibited by BLM and RECQL5. We next employed the D loop assay with a modified order of addition (Figure 5C) to determine whether RTEL1 instead acts on the D loop structure to

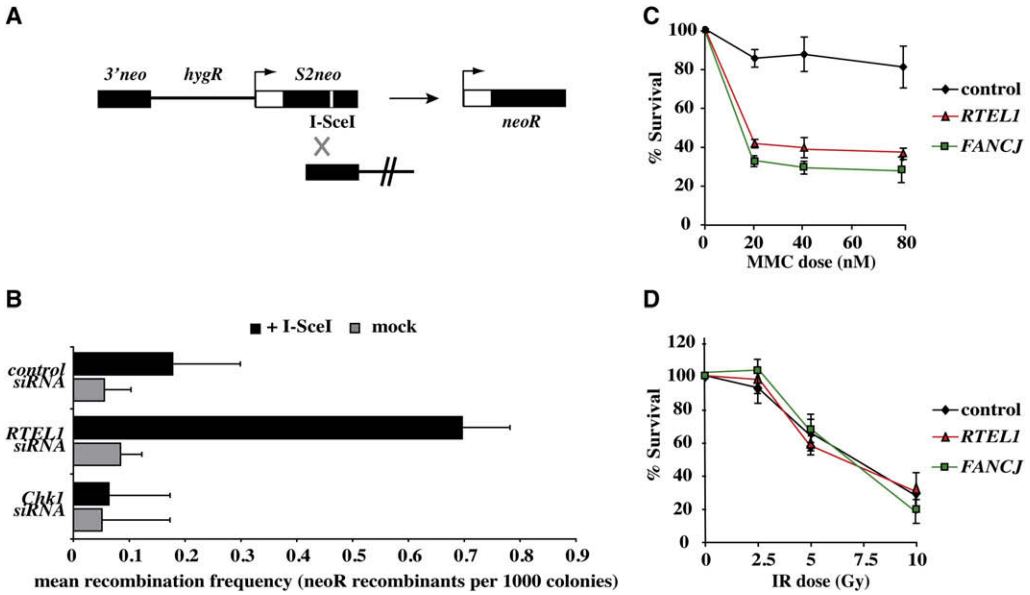

Figure 3. Human Cells Depleted for *RTEL1* Exhibit Similar Hyperrecombination and DNA Damage Sensitivity Phenotypes to *C. elegans rtel-1* Mutants

(A) Schematic of the integrated SCneo substrate used to measure recombination frequencies, comprising two nonfunctional alleles of the neomycin resistance gene. Initiation of a DSB at the I-SceI restriction site (white line) induces HR, restoring a functional neo^R cassette through gene conversion.

(B) Analysis of I-SceI-induced HR at the SCneo construct after nontargeting control, *RTEL1*, or *CHK1* siRNA depletion, and subsequent transfection of I-SceI expression vector in SW480/SN3 cells. Neomycin resistant colonies were scored at 10 days after transfection. Error bars indicate the SEM from three independent experiments.

(C and D) Sensitivity of siRNA-depleted cells to the indicated doses of mitomycin C (C) and IR (D). Error bars indicate the SEM from three independent experiments.

reverse strand invasion. Figure 5D (lanes 3–5) shows that RTEL1 disrupts preformed D loops to reverse the HR process in vitro. D loop disruption by RTEL1 is concentration dependent and is almost entirely inhibited at 80 nM RTEL1 (Figure 5D lane 5, Figure 5E). Furthermore, RTEL1 disrupts D loops in the presence of calcium (Figures 5D and 5E), which stabilizes RAD51 filaments (Bugreev and Mazin, 2004). In contrast, BLM cannot dissociate preformed D loops under these conditions (Figure 5D lanes 6–8, Figure 5E), consistent with previous findings (Bugreev et al., 2007). These results demonstrate that RTEL1 antagonizes recombination by disrupting D loop intermediates.

DISCUSSION

HR repair is an essential cellular process that must be tightly regulated to prevent genome instability through inappropriate recombination events. In this study, we have identified a putative helicase RTEL-1/RTEL1, conserved from *C. elegans* to humans, that demonstrates functional similarity to yeast Srs2. Although RTEL1 shows no sequence similarity to Srs2, loss of RTEL-1/RTEL1 either in the nematode or in human cells gives rise to a range of phenotypes analogous to *srs2* mutant yeast, including synthetic lethality with the *sgs1*/*BLM* homolog (Lee et al., 1999; Wang et al., 2001) that is associated with an accumulation of persistent recombination intermediates (Gangloff et al., 2000; Klein, 2001); hyperrecombination in human cell culture (Ira et al., 2003; Rong et al., 1991) and during *C. elegans* meiosis; and sensitivity to complex DNA damage such as ICLs (Birrell et al., 2002). Collectively, the phenotypes observed in the absence of RTEL-1/RTEL1 strongly suggest that it has a conserved function as an antirecombinase. Indeed, we demonstrate that human RTEL1 has potent D loop dissociation activity in vitro that is dependent upon ATP hydrolysis. D loop unwinding by RTEL1, unlike BLM, occurs in the presence of calcium, which is known to stabilize RAD51 filaments (Bachrati et al., 2006; Bugreev et al., 2007; van Brabant et al., 2000). This implies that RTEL1 can antagonize HR at an early stage, after strand invasion, and could promote synthesis-dependent strand annealing.

It should be noted that RTEL1 and Srs2 also differ in some respects—perhaps unsurprisingly, given the lack of sequence homology. Loss of RTEL-1/RTEL1 confers sensitivity to a more select range of DNA-damaging agents than for *Srs2*, and this could be indicative of a broader functional scope for Srs2, perhaps with different factors accountable for distinct roles in higher eukaryotes. Certainly our candidate screen was not exhaustive, and additional antirecombinases may subsequently be identified. Furthermore, the exact mechanisms by which RTEL1 and Srs2 antagonize recombination may not be identical. Srs2 has previously been shown to disrupt the Rad51 nucleoprotein filament (Krejci et al., 2003; Veaute et al., 2003), yet this was not observed for RTEL1. Also, although both RTEL1 and Srs2 can antagonize the formation of a D loop, it is not clear whether Srs2 can act on a preformed D loop structure. Previous work had suggested that Srs2 is unable to unwind D loop intermediates (Krejci et al., 2003; Veaute et al., 2003), although a recent study by Dupaigne and colleagues has demonstrated Srs2 activity

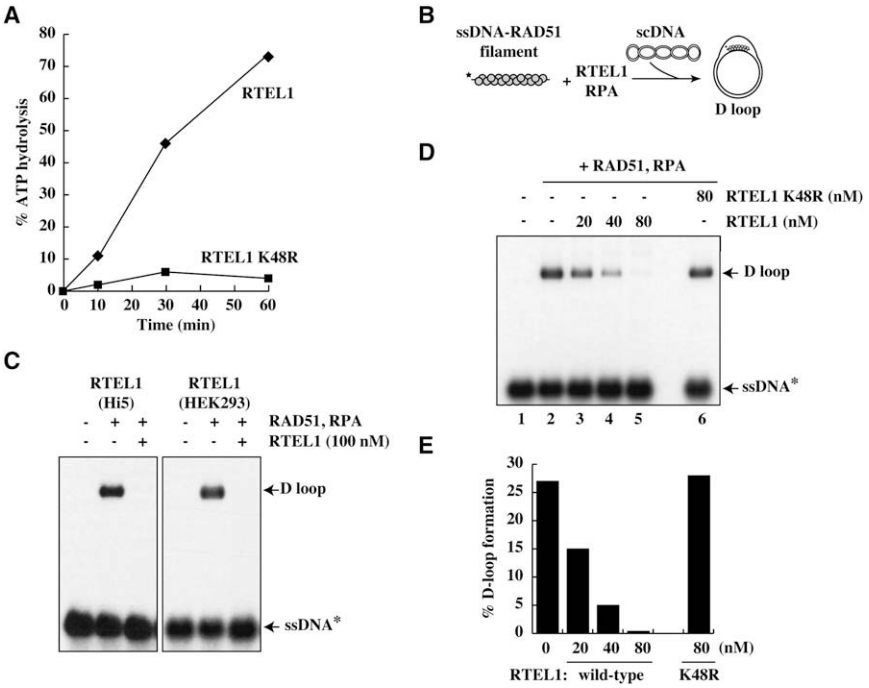

Figure 4. Human RTEL1 Inhibits D Loop Formation in an ATP-Dependent Manner

(A) ATP hydrolysis assay of human wild-type and mutant (K48R) RTEL1 performed in the presence of single-stranded DNA.

(B) Schematic of the D loop assay.

(C) D loop assay with 100 nM of wild-type RTEL1 purified from Hi5 insect cells or HEK293 cells, as indicated. The D loop species migrates slower than the ssDNA probe.

(D) D loop assay with wild-type or K48R RTEL1, as indicated.

(E) Quantification of D loop formation.

on the related PX junction (Dupaigne et al., 2008). Nevertheless, RTEL-1/RTEL1 is clearly a novel antirecombinase that shares some features with Srs2, and further study of RTEL-1/RTEL1 will facilitate the understanding of HR regulation in metazoans.

A role for RTEL-1/RTEL1 in the negative regulation of HR by disrupting D loop intermediates provides molecular insight into the phenotypes observed in this study and for the *Rtel* knockout mice. It is predicted that an inability of cells to antagonize HR would result in elevated levels of HR, as we have demonstrated in human cell culture and during *C. elegans* meiosis. The specific sensitivity to complex DNA damage such as ICLs but not IR or UV lesions may result from a failure to temporally regulate HR during the repair process. HR has been shown to be required as part of the composite pathway for the efficient repair of ICLs, most likely downstream of incision and processing of the lesion (Li and Heyer, 2008). However, if unrestricted, invocation of HR on an inappropriate intermediate of ICL repair could lead to persistent damage from irreparable intermediates. Furthermore, an inability of cells to reverse nonproductive HR intermediates provides a plausible explanation for the synthetic lethality of *rtel-1* in combination with *him-6*, *rcq-5*, *mus-81*, and *dog-1*. It is possible that the synthetic sterility observed in *rtel-1 dog-1* animals is due to a requirement for RTEL-1 function at replication forks stalled by polyG/C-tract secondary structure in *dog-1* mutants. However, it is equally possible that the phenotype of the *rtel-1 dog-1* double mutant is due to overlapping roles for RTEL-1 and DOG-1 at telomeres or other DNA structures in *C. elegans*.

Rtel is the murine homolog of *C. elegans RTEL-1* and was originally identified by genomic mapping of loci that control telomere length differences between *M. musculus* and *M. spretus* (Ding et al., 2004). Rtel plays a critical role in genome stability because knockout mice are embryonic lethal and cells derived from these mice exhibit a rapid reduction in proliferative capacity upon differentiation, accompanied with an increased incidence of chromosomal abnormalities and telomere loss (Ding et al., 2004). On the basis of homology to *C. elegans* DOG-1, it was proposed that Rtel might function to unwind G-rich DNA secondary structures formed during DNA replication and at the telomere (Ding et al., 2004). However, the human homolog of *dog-1* has now been identified as *FANCJ* (Youds et al., 2008), and no mechanistic evidence has yet been provided to support this model. Although a role for Rtel in unwinding DNA secondary structures remains a possibility, *C. elegans rtel-1* mutants differ from *dog-1* mutants in that they do not exhibit instability at G-rich sequences. Our finding that RTEL-1/RTEL1 is an antirecombinase leads us to propose an alternative possibility: the inviability and severe genomic instability exhibited by the $Rtel^{-/-}$ null mouse results from an inability of cells to correctly regulate HR. HR has also been shown to cause deletion of the protective T loop structure formed by the sequestration of the 3′ telomeric end into a subtelomeric duplex TTAGGG repeat (Wang et al., 2004). The T loop structure requires both HR- and telomere-specific proteins for its assembly and has therefore been proposed to resemble a D loop HR intermediate (de Lange, 2004). It is therefore possible that in the absence of RTEL1/Rtel, the T loop structure may be erroneously resolved as a substrate by the HR machinery. Hence, the telomere deficiency in $Rtel^{-/-}$ mouse cells may result from the inability of cells to antagonize inappropriate HR at the T loop. Alternatively (or additionally), Rtel may function to unwind/disengage the T loop structure to allow telomerase access to complete chromosome end replication during each cell cycle.

The importance of correct restraint of HR is also highlighted by the observation that human *RTEL1* is located in a four-gene cluster that is overexpressed in some tumors of the gastrointestinal tract (Bai et al., 2000). It is likely that excessive RTEL1 activity could repress productive HR events and in this way mimic the loss of an essential HR factor, such as BRCA2, which is associated with familial breast and ovarian cancers (Venkitaraman, 2002). Amplification of RTEL1 may also drive genome instability and tumorigenesis by inappropriately disengaging the T loop structure, leading to telomere deprotection. It will now be imperative to screen human cancers for mutations, amplified

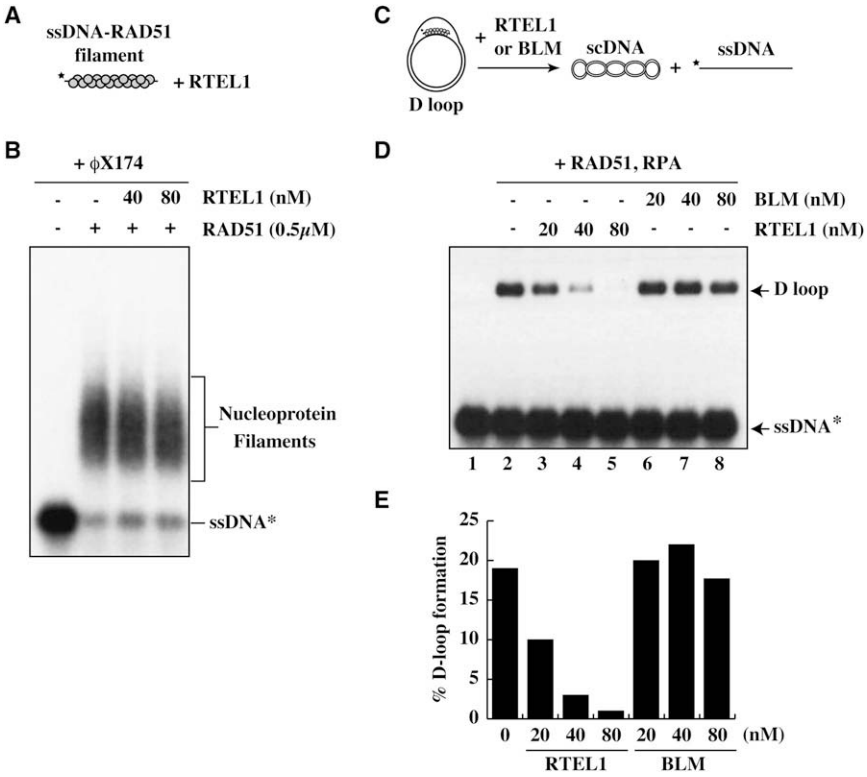

Figure 5. RTEL1 Specifically Disrupts Performed D Loops

(A) Schematic of the ssDNA-RAD51 filament disruption assay.

(B) Preformed ssDNA-RAD51 filaments were incubated with the indicated concentrations of RTEL1, followed by addition of a 200-fold excess of cold ϕX174 ssDNA competitor.

(C) Schematic of the modified D loop disruption assay.

(D) Preformed D loops were incubated with the indicated concentrations of RTEL1 and BLM.

(E) Quantification of D loop disruption.

expression, and copy number changes in *RTEL1*, especially given the association of other helicase regulators of HR with tumorigenesis (*BLM* [German, 1995], *RECQL5* [Hu et al., 2007]).

In summary, we have identified RTEL-1/RTEL1 as a novel suppressor of recombination that is conserved in metazoans. Our genetic and biochemical analysis reveal RTEL-1/RTEL1 as an anti-recombinase with some functional similarities to yeast Srs2. Our observations provide a possible mechanistic explanation for the genomic instability seen in *Rtel*$^{-/-}$ mice (Ding et al., 2004). Finally, the overexpression of human *RTEL1* in gastrointestinal tract tumors (Bai et al., 2000) raises the possibility that *RTEL1* may prove to be a valuable target for new anticancer therapeutics.

EXPERIMENTAL PROCEDURES

C. elegans Assays

Nematode strains were maintained as previously described (Brenner, 1974). *C. elegans* immunofluorescence microscopy was performed as previously described (Colaiacovo et al., 2003; Youds et al., 2008). The frequency of meiotic recombination was measured by scoring the number of recombinant progeny of a *cis*-heterozygote, as previously described (Rose and Baillie, 1979). *C. elegans* DNA damage sensitivity assays were performed as previously described (Collis et al., 2006; Youds et al., 2008).

Human Cell Culture Assays

Subconfluent cultures were transfected with 100 nM siRNA (Dharmacon ON-TARGET*plus* *RTEL1* and *FANCJ*; SMARTPool *Chk1*) with Dharmafect #1 reagent in antibiotic-free media. FLP-In T-Rex-293 pDEST-Flag/FRT/TO-RTEL1 cells were selected with 200 μg/ml Hygromycin (Invitrogen) and 15 μg/ml Blasticidin (Autogen Bioclear). Tetracycline (Sigma) was used at 1 μg/ml. Quantitative PCR analysis of mRNA levels was performed as previously described (Collis et al., 2007) with Superarray RT2 primer sets for *RTEL1*, *FANCJ*, and *ACTG1*. Homologous recombination frequencies were measured in SW480/SN3 cells that contain a single integrated copy of an *SCneo* substrate, as previously described (Collis et al., 2007; Johnson and Jasin, 2001; Mohindra et al., 2002). MMC (Sigma, Poole, UK) sensitivity was assayed 48 hr after siRNA treatment, as previously described (Collis et al., 2007).

Protein Purification and Antibodies

Wild-type and K48R mutant RTEL1 fused to a V5 epitope at the N terminus and MYC-6HIS at the C terminus were expressed in Hi5 insect cells with the baculovirus system. Five liters of Hi5 cells were infected at a concentration of 1 × 10^6 cells/ml and collected 72 hr after infection. The cell pellet was lysed in 50 mM Tris HCl (pH 8), 0.5 M NaCl, 4 mM MgCl2, 1 mM dithiothriotol (DTT), ethylene diamine tetra-acetic acid (EDTA)-free complete protease inhibitor cocktail, and 30 units/ml benzonase and sonicated three times on ice (30 s, max amplitude, 2 min rest interval). The soluble fraction was enriched for RTEL1 protein by 1.5 M ammonium sulfate precipitation, resuspended, and dialyzed in 20 mM Tris HCl (pH 7.5) and 0.5 M NaCl. The protein was purified to near homogeneity with anti-V5 agarose and cleaved from the affinity matrix by AcTEV protease digestion for 3 hr at 16°C. Purified protein was concentrated by serial passage over a Biomax ultrafree 5k NMWL spin column. Protein concentration was estimated by Bradford assay, and purity was confirmed by SDS-PAGE followed by Coomassie staining. Human RAD51 and RPA proteins were purified as published previously (Baumann et al., 1997; Henricksen et al., 1994). Human BLM protein was a kind gift from Ian Hickson (Oxford, UK). Primary antibodies used were FLAG-HRP (Sigma) and human Actin (Abcam).

ATPase Assay

Sixty microliter reactions contained 15 nM wild-type or K48R RTEL1 with 40 μM ϕX174 ssDNA in 70 mM Tris HCl (pH 7.6), 10 mM $MgCl_2$, 3 μCi of [γ-^{32}P]ATP (3000 Ci/mmol), 5 mM DTT, and 0.1 mg/ml bovine serum albumin (BSA) at 37°C. At the times indicated, 10 μl aliquots were removed and the reaction was terminated by the addition of 5 μl 0.5 M EDTA. Samples were analyzed by thin-layer chromatography on CEL 300 PEI/UV_{254} plates in 1 M formic acid and 0.5 M LiCl. ATP hydrolysis was quantified as the percentage of [γ-^{32}P]ATP hydrolyzed to [γ-^{32}P]ADP, with a Storm 860 Phosphorimager.

D Loop Recombination Assay

Ten microliter reactions contained 1 μM 5′-^{32}P-end-labeled 100-mer ssDNA in recombination buffer (25 mM Tris-acetate [pH 7.5], 5 mM $CaCl_2$, 2 mM $MgCl_2$, 2 mM ATP, 1 mM DTT, and 100 μg/ml BSA). The following were then added sequentially, separated by 5 min incubations at 37°C: 0.5 μM RAD51, RTEL1 or BLM, 0.2 μM RPA, and 0.3 mM supercoiled pPB4.3 DNA. After a further 10 min incubation, the products were deproteinized by the addition of one-fifth volume stop buffer (0.1 M Tris-HCl [pH 7.5], 0.1 M $MgCl_2$, 3% SDS, and 10 mg/ml proteinase K) with 20 min incubation at 37°C. DNA products were

analyzed by 1% agarose gel electrophoresis, dried onto filter paper, visualized by autoradiography, and quantified with a Phosphorimager.

Nucleoprotein Filament Disruption Assay

Ten microliter reactions contained 1 μM 5′-^{32}P-end-labeled 100-mer ssDNA in recombination buffer. One-half micromolar RAD51 was added, then RTEL1, then 216 μM single-stranded ϕX174 virion DNA, each separated by 5 min incubations at 37°C. After a further 5 min, DNA products were analyzed by 1% agarose gel electrophoresis, dried onto filter paper, and visualized by autoradiography.

SUPPLEMENTAL DATA

Supplemental Data include Supplemental Experimental Procedures and four figures and can be found with this article online at http://www.cell.com/cgi/content/full/135/2/261/DC1/.

ACKNOWLEDGMENTS

We wish to thank Shohei Mitani (National Bioresource Project, Japan) and the *C. elegans* Knockout Consortium for providing nematode strains; Cancer Research UK Cell Services, Helen Bryant and Thomas Helleday for cells; Ian Hickson for BLM protein; and Helen Bryant, Thomas Helleday, and Janet Cronshaw for plasmids. H.A.T. is funded by the William Randolph Hearst Foundation; H.A.T. and M.A. are also funded by a grant from the National Institutes of Health (AG25891); A.M.R. is funded by the Natural Sciences and Engineering Research Council; J.L.Y. was funded by National Cancer Institute of Canada and Michael Smith Foundation for Health Research; S.C.W. is supported by the Louis-Jeantet Foundation and is part of the EU DNA Repair Consortium; and the labs of S.C.W and S.J.B. receive funding from Cancer Research UK.

Received: June 3, 2008
Revised: July 16, 2008
Accepted: August 11, 2008
Published: October 16, 2008

REFERENCES

Aboussekhra, A., Chanet, R., Zgaga, Z., Cassier-Chauvat, C., Heude, M., and Fabre, F. (1989). *RADH*, a gene of *Saccharomyces cerevisiae* encoding a putative DNA helicase involved in DNA repair. Characteristics of *radH* mutants and sequence of the gene. Nucleic Acids Res. *17*, 7211–7219.

Aboussekhra, A., Chanet, R., Adjiri, A., and Fabre, F. (1992). Semidominant suppressors of Srs2 helicase mutations of *Saccharomyces cerevisiae* map in the *RAD51* gene, whose sequence predicts a protein with similarities to procaryotic RecA proteins. Mol. Cell. Biol. *12*, 3224–3234.

Aguilera, A., and Klein, H.L. (1988). Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae*. I. Isolation and genetic characterization of hyper-recombination mutations. Genetics *119*, 779–790.

Ahmed, S., Alpi, A., Hengartner, M.O., and Gartner, A. (2001). *C. elegans* RAD-5/CLK-2 defines a new DNA damage checkpoint protein. Curr. Biol. *11*, 1934–1944.

Alpi, A., Pasierbek, P., Gartner, A., and Loidl, J. (2003). Genetic and cytological characterization of the recombination protein RAD-51 in *Caenorhabditis elegans*. Chromosoma *112*, 6–16.

Arthur, H.M., and Lloyd, R.G. (1980). Hyper-recombination in *uvrD* mutants of *Escherichia coli* K-12. Mol. Gen. Genet. *180*, 185–191.

Bachrati, C.Z., Borts, R.H., and Hickson, I.D. (2006). Mobile D-loops are a preferred substrate for the Bloom's syndrome helicase. Nucleic Acids Res. *34*, 2269–2279.

Bai, C., Connolly, B., Metzker, M.L., Hilliard, C.A., Liu, X., Sandig, V., Soderman, A., Galloway, S.M., Liu, Q., Austin, C.P., et al. (2000). Overexpression of *M68/DcR3* in human gastrointestinal tract tumors independent of gene amplification and its location in a four-gene cluster. Proc. Natl. Acad. Sci. USA *97*, 1230–1235.

Baumann, P., Benson, F.E., Hajibagheri, N., and West, S.C. (1997). Purification of human Rad51 protein by selective spermidine precipitation. Mutat. Res. *384*, 65–72.

Bennett, C.B., Lewis, L.K., Karthikeyan, G., Lobachev, K.S., Jin, Y.H., Sterling, J.F., Snipe, J.R., and Resnick, M.A. (2001). Genes required for ionizing radiation resistance in yeast. Nat. Genet. *29*, 426–434.

Bierne, H., Seigneur, M., Ehrlich, S.D., and Michel, B. (1997). *uvrD* mutations enhance tandem repeat deletion in the *Escherichia coli* chromosome via SOS induction of the RecF recombination pathway. Mol. Microbiol. *26*, 557–567.

Birrell, G.W., Brown, J.A., Wu, H.I., Giaever, G., Chu, A.M., Davis, R.W., and Brown, J.M. (2002). Transcriptional response of *Saccharomyces cerevisiae* to DNA-damaging agents does not identify the genes that protect against these agents. Proc. Natl. Acad. Sci. USA *99*, 8778–8783.

Boddy, M.N., Gaillard, P.H., McDonald, W.H., Shanahan, P., Yates, J.R., 3rd, and Russell, P. (2001). Mus81-Eme1 are essential components of a Holliday junction resolvase. Cell *107*, 537–548.

Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics *77*, 71–94.

Bugreev, D.V., and Mazin, A.V. (2004). Ca2+ activates human homologous recombination protein Rad51 by modulating its ATPase activity. Proc. Natl. Acad. Sci. USA *101*, 9988–9993.

Bugreev, D.V., Yu, X., Egelman, E.H., and Mazin, A.V. (2007). Novel pro- and anti-recombination activities of the Bloom's syndrome helicase. Genes Dev. *21*, 3085–3094.

Cheung, I., Schertzer, M., Rose, A., and Lansdorp, P.M. (2002). Disruption of *dog-1* in *Caenorhabditis elegans* triggers deletions upstream of guanine-rich DNA. Nat. Genet. *31*, 405–409.

Chiolo, I., Carotenuto, W., Maffioletti, G., Petrini, J.H., Foiani, M., and Liberi, G. (2005). Srs2 and Sgs1 DNA helicases associate with Mre11 in different subcomplexes following checkpoint activation and CDK1-mediated Srs2 phosphorylation. Mol. Cell. Biol. *25*, 5738–5751.

Chiolo, I., Saponaro, M., Baryshnikova, A., Kim, J.H., Seo, Y.S., and Liberi, G. (2007). The human F-Box DNA helicase FBH1 faces *Saccharomyces cerevisiae* Srs2 and postreplication repair pathway roles. Mol. Cell. Biol. *27*, 7439–7450.

Colaiacovo, M.P., MacQueen, A.J., Martinez-Perez, E., McDonald, K., Adamo, A., La Volpe, A., and Villeneuve, A.M. (2003). Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. Dev. Cell *5*, 463–474.

Collis, S.J., Barber, L.J., Ward, J.D., Martin, J.S., and Boulton, S.J. (2006). *C. elegans* FANCD2 responds to replication stress and functions in interstrand cross-link repair. DNA Repair (Amst.) *5*, 1398–1406.

Collis, S.J., Barber, L.J., Clark, A.J., Martin, J.S., Ward, J.D., and Boulton, S.J. (2007). HCLK2 is essential for the mammalian S-phase checkpoint and impacts on Chk1 stability. Nat. Cell Biol. *9*, 391–401.

de Lange, T. (2004). T-loops and the origin of telomeres. Nat. Rev. Mol. Cell Biol. *5*, 323–329.

Ding, H., Schertzer, M., Wu, X., Gertsenstein, M., Selig, S., Kammori, M., Pourvali, R., Poon, S., Vulto, I., Chavez, E., et al. (2004). Regulation of murine telomere length by *Rtel*: An essential gene encoding a helicase-like protein. Cell *117*, 873–886.

Dupaigne, P., Le Breton, C., Fabre, F., Gangloff, S., Le Cam, E., and Veaute, X. (2008). The Srs2 helicase activity is stimulated by Rad51 filaments on dsDNA: Implications for crossover incidence during mitotic recombination. Mol. Cell *29*, 243–254.

Fabre, F., Chan, A., Heyer, W.D., and Gangloff, S. (2002). Alternate pathways involving Sgs1/Top3, Mus81/ Mms4, and Srs2 prevent formation of toxic recombination intermediates from single-stranded gaps created by DNA replication. Proc. Natl. Acad. Sci. USA *99*, 16887–16892.

Gangloff, S., Soustelle, C., and Fabre, F. (2000). Homologous recombination is responsible for cell death in the absence of the Sgs1 and Srs2 helicases. Nat. Genet. *25*, 192–194.

German, J. (1995). Bloom's syndrome. Dermatol. Clin. *13*, 7–18.

Henricksen, L.A., Umbricht, C.B., and Wold, M.S. (1994). Recombinant replication protein A: Expression, complex formation, and functional characterization. J. Biol. Chem. *269*, 11121–11132.

Hu, Y., Raynard, S., Sehorn, M.G., Lu, X., Bussen, W., Zheng, L., Stark, J.M., Barnes, E.L., Chi, P., Janscak, P., et al. (2007). RECQL5/Recql5 helicase regulates homologous recombination and suppresses tumor formation via disruption of Rad51 presynaptic filaments. Genes Dev. *21*, 3073–3084.

Ira, G., Malkova, A., Liberi, G., Foiani, M., and Haber, J.E. (2003). Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. Cell *115*, 401–411.

Jeong, Y.S., Kang, Y., Lim, K.H., Lee, M.H., Lee, J., and Koo, H.S. (2003). Deficiency of *Caenorhabditis elegans RecQ5* homologue reduces life span and increases sensitivity to ionizing radiation. DNA Repair (Amst.) *2*, 1309–1319.

Johnson, R.D., and Jasin, M. (2001). Double-strand-break-induced homologous recombination in mammalian cells. Biochem. Soc. Trans. *29*, 196–201.

Kennedy, R.D., and D'Andrea, A.D. (2005). The Fanconi Anemia/BRCA pathway: New faces in the crowd. Genes Dev. *19*, 2925–2940.

Kim, J., Kim, J.H., Lee, S.H., Kim, D.H., Kang, H.Y., Bae, S.H., Pan, Z.Q., and Seo, Y.S. (2002). The novel human DNA helicase hFBH1 is an F-box protein. J. Biol. Chem. *277*, 24530–24537.

Klein, H.L. (2001). Mutations in recombinational repair and in checkpoint control genes suppress the lethal combination of *srs2*Delta with other DNA repair genes in *Saccharomyces cerevisiae*. Genetics *157*, 557–565.

Kohzaki, M., Hatanaka, A., Sonoda, E., Yamazoe, M., Kikuchi, K., Vu Trung, N., Szuts, D., Sale, J.E., Shinagawa, H., Watanabe, M., et al. (2007). Cooperative roles of vertebrate Fbh1 and Blm DNA helicases in avoidance of crossovers during recombination initiated by replication fork collapse. Mol. Cell. Biol. *27*, 2812–2820.

Krejci, L., Van Komen, S., Li, Y., Villemain, J., Reddy, M.S., Klein, H., Ellenberger, T., and Sung, P. (2003). DNA helicase Srs2 disrupts the Rad51 presynaptic filament. Nature *423*, 305–309.

Krejci, L., Macris, M., Li, Y., Van Komen, S., Villemain, J., Ellenberger, T., Klein, H., and Sung, P. (2004). Role of ATP hydrolysis in the antirecombinase function of *Saccharomyces cerevisiae* Srs2 protein. J. Biol. Chem. *279*, 23193–23199.

Krogh, B.O., and Symington, L.S. (2004). Recombination proteins in yeast. Annu. Rev. Genet. *38*, 233–271.

Lawrence, C.W., and Christensen, R.B. (1979). Metabolic suppressors of trimethoprim and ultraviolet light sensitivities of Saccharomyces cerevisiae rad6 mutants. J. Bacteriol. *139*, 866–876.

Lee, S.J., Yook, J.S., Han, S.M., and Koo, H.S. (2004). A Werner syndrome protein homolog affects *C. elegans* development, growth rate, life span and sensitivity to DNA damage by acting at a DNA damage checkpoint. Development *131*, 2565–2575.

Lee, S.K., Johnson, R.E., Yu, S.L., Prakash, L., and Prakash, S. (1999). Requirement of yeast *SGS1* and *SRS2* genes for replication and transcription. Science *286*, 2339–2342.

Li, X., and Heyer, W.D. (2008). Homologous recombination in DNA repair and DNA damage tolerance. Cell Res. *18*, 99–113.

Martin, J.S., Winkelmann, N., Petalcorin, M.I., McIlwraith, M.J., and Boulton, S.J. (2005). RAD-51-dependent and -independent roles of a *Caenorhabditis elegans* BRCA2-related protein during DNA double-strand break repair. Mol. Cell. Biol. *25*, 3127–3139.

McIlwraith, M.J., Van Dyck, E., Masson, J.Y., Stasiak, A.Z., Stasiak, A., and West, S.C. (2000). Reconstitution of the strand invasion step of double-strand break repair using human Rad51 Rad52 and RPA proteins. J. Mol. Biol. *304*, 151–164.

Mohindra, A., Hays, L.E., Phillips, E.N., Preston, B.D., Helleday, T., and Meuth, M. (2002). Defects in homologous recombination repair in mismatch-repair-deficient tumour cell lines. Hum. Mol. Genet. *11*, 2189–2200.

Morel, P., Hejna, J.A., Ehrlich, S.D., and Cassuto, E. (1993). Antipairing and strand transferase activities of *E. coli* helicase II (UvrD). Nucleic Acids Res. *21*, 3205–3209.

Morishita, T., Furukawa, F., Sakaguchi, C., Toda, T., Carr, A.M., Iwasaki, H., and Shinagawa, H. (2005). Role of the *Schizosaccharomyces pombe* F-Box DNA helicase in processing recombination intermediates. Mol. Cell. Biol. *25*, 8074–8083.

Osman, F., Dixon, J., Barr, A.R., and Whitby, M.C. (2005). The F-Box DNA helicase Fbh1 prevents Rhp51-dependent recombination without mediator proteins. Mol. Cell. Biol. *25*, 8084–8096.

Palladino, F., and Klein, H.L. (1992). Analysis of mitotic and meiotic defects in *Saccharomyces cerevisiae SRS2* DNA helicase mutants. Genetics *132*, 23–37.

Pan, X., Ye, P., Yuan, D.S., Wang, X., Bader, J.S., and Boeke, J.D. (2006). A DNA integrity network in the yeast *Saccharomyces cerevisiae*. Cell *124*, 1069–1081.

Rong, L., Palladino, F., Aguilera, A., and Klein, H.L. (1991). The hyper-gene conversion *hpr5–1* mutation of *Saccharomyces cerevisiae* is an allele of the *SRS2/RADH* gene. Genetics *127*, 75–85.

Rose, A.M., and Baillie, D.L. (1979). The effect of temperature and parental age on recombination and nondisjunction in Caenorhabditis elegans. Genetics *92*, 409–418.

Sorensen, C.S., Hansen, L.T., Dziegielewski, J., Syljuasen, R.G., Lundin, C., Bartek, J., and Helleday, T. (2005). The cell-cycle checkpoint kinase Chk1 is required for mammalian homologous recombination repair. Nat. Cell Biol. *7*, 195–201.

Tong, A.H., Lesage, G., Bader, G.D., Ding, H., Xu, H., Xin, X., Young, J., Berriz, G.F., Brost, R.L., Chang, M., et al. (2004). Global mapping of the yeast genetic interaction network. Science *303*, 808–813.

van Brabant, A.J., Ye, T., Sanz, M., German, I.J., Ellis, N.A., and Holloman, W.K. (2000). Binding and melting of D-loops by the Bloom syndrome helicase. Biochemistry *39*, 14617–14625.

Veaute, X., Jeusset, J., Soustelle, C., Kowalczykowski, S.C., Le Cam, E., and Fabre, F. (2003). The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. Nature *423*, 309–312.

Venkitaraman, A.R. (2002). Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell *108*, 171–182.

Wang, R.C., Smogorzewska, A., and de Lange, T. (2004). Homologous recombination generates T-loop-sized deletions at human telomeres. Cell *119*, 355–368.

Wang, S.W., Goodwin, A., Hickson, I.D., and Norbury, C.J. (2001). Involvement of *Schizosaccharomyces pombe* Srs2 in cellular responses to DNA damage. Nucleic Acids Res. *29*, 2963–2972.

Weidhaas, J.B., Eisenmann, D.M., Holub, J.M., and Nallur, S.V. (2006). A *Caenorhabditis elegans* tissue model of radiation-induced reproductive cell death. Proc. Natl. Acad. Sci. USA *103*, 9946–9951.

Wicky, C., Alpi, A., Passannante, M., Rose, A., Gartner, A., and Muller, F. (2004). Multiple genetic pathways involving the *Caenorhabditis elegans* Bloom's syndrome genes *him-6, rad-51*, and *top-3* are needed to maintain genome stability in the germ line. Mol. Cell. Biol. *24*, 5016–5027.

Youds, J.L., Barber, L.J., Ward, J.D., Collis, S.J., O'Neil, N.J., Boulton, S.J., and Rose, A.M. (2008). *DOG-1* is the *Caenorhabditis elegans BRIP1/FANCJ* homologue and functions in interstrand cross-link repair. Mol. Cell. Biol. *28*, 1470–1479.

Youds, J.L., O'Neil, N.J., and Rose, A.M. (2006). Homologous recombination is required for genome stability in the absence of *DOG-1* in *Caenorhabditis elegans*. Genetics *173*, 697–708.

Zieg, J., Maples, V.F., and Kushner, S.R. (1978). Recombinant levels of *Escherichia coli* K-12 mutants deficient in various replication, recombination, or repair genes. J. Bacteriol. *134*, 958–966.