

## SURVEY AND SUMMARY

# RTEL1: an essential helicase for telomere maintenance and the regulation of homologous recombination

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### ABSTRACT

**Telomere maintenance and DNA repair are crucial processes that protect the genome against instability. RTEL1, an essential iron–sulfur cluster-containing helicase, is a dominant factor that controls telomere length in mice and is required for telomere integrity. In addition, RTEL1 promotes synthesis-dependent strand annealing to direct DNA double-strand breaks into non-crossover outcomes during mitotic repair and in meiosis. Here, we review the role of RTEL1 in telomere maintenance and homologous recombination and discuss models linking RTEL1's enzymatic activity to its function in telomere maintenance and DNA repair.**

### INTRODUCTION

Telomeres are protective structures at the end of chromosomes, the maintenance of which is essential for genomic stability (1). In vertebrates, telomeres are composed of repetitive TTAGGG DNA (2) and associated proteins, which form a core complex known as the Shelterin complex (3). Telomeres protect chromosomes by distinguishing chromosomal ends from DNA double-strand breaks (DSBs), a function that is essential in avoiding chromosome end-to-end fusions and inappropriate recombination events (4).

Telomeres can form a protective lariat-like structure, referred to as the telomeric-loop, or T-loop (5). T-loops are created through strand invasion by the 3' single-stranded overhang of telomeric DNA into duplex telomere repeats. This strand invasion displaces the identical sequence strand of the duplex telomeric DNA and so forms a displacement-loop (D-loop) at the base of the T-loop. The D-loop is also an intermediate in the DNA repair pathway via homologous recombination (HR) (6). This repair pathway is the main method for repairing DSBs when sister chromatid templates are available and is also required for meiotic recombination. How the T-loop is resolved during replication or how the invaded strand in the D-loop structure is displaced to promote repair to a non-crossover (NCO) outcome is still largely unknown.

In addition to the T-loop configuration, the guanine (G)-rich nature of the telomere may also pose a challenge for telomere maintenance. *In vitro*, single-stranded G-rich telomeric sequences are capable of forming stable structures called G-quadruplex (or G4) DNA (7). *In vivo*, G4 DNA might form at telomeres during replication, repair and transcription. The resolution of T-loops and telomeric G4 DNA could be important for telomere maintenance, and therefore, genomic stability.

Rtel1 (for *Regulator of Telomere Length 1*) is an essential helicase that plays a crucial role in telomere maintenance and DNA repair (8,9). *Rtel1* was originally discovered as the dominant factor in setting telomere

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length in mice (9). In the absence of *Rtel1*, telomeres are not maintained and chromosome fusions are observed. In addition, *RTEL1* was found to be a key protein in the repair of DSBs (8,10). It disrupts D-loops *in vitro* and promotes synthesis-dependent strand annealing (SDSA) *in vivo*. Notably, *RTEL1* activity is not limited to mitotic cells, as *RTEL1* is required to regulate meiotic recombination in *Caenorhabditis elegans*. Here, we review the emerging role of *RTEL1* at telomeres and in DNA repair and introduce a model linking the anti-recombinase activity of *RTEL1* to its functions in telomere maintenance and DNA repair.

### **RTEL1 AND TELOMERE LENGTH REGULATION IN THE MOUSE**

Telomeres shorten with each round of DNA replication (11). Typical and sporadic losses of telomeric DNA are compensated for by the enzyme telomerase. In yeast (12), maize (13) and *Arabidopsis* (14), the telomere length set point seems to be determined by multiple genes. In mice, telomere length is controlled by genetic (9) as well as epigenetic factors (15). *Rtel1* was identified as a dominant genetic factor setting telomere length in mice (9,16). Most laboratory mice including *Mus musculus* have long telomeres, with lengths between 25 and 150 kb (17), but a related mouse species, *Mus spretus*, has telomeres between 5 and 15 kb (18), similar to the telomere length in human cells (19–21). Hodes and co-workers (16) found that telomeres of *Mus spretus*-derived chromosomes in the offspring of crosses between *M. spretus* with *M. musculus* were significantly longer than in the *M. spretus* parent, suggesting that a dominant genetic mechanism was elongating *M. spretus* telomeres during development. Genotype mapping pointed to a locus on distal chromosome 2 containing a dominant factor(s) determining telomere length setting in mice. This factor was shown to be *Rtel1* based on the finding that *Rtel1* expression from the *M. musculus* parent was required to elongate the telomeres of *M. spretus*-derived chromosomes (9). Mice lacking *Rtel1* die around Day 10–11 with defects in multiple organs. The average telomere length of *Rtel1*-deficient embryonic stem cells is around 68% of that in wild-type cells (9). It is unknown how *Rtel1* determines this telomere length equilibrium and how the difference between *M. musculus* and *M. spretus Rtel1* determines a long or short telomere phenotype in these mice. However, differences between *M. musculus* and *M. spretus Rtel1* are found in the promoter region, in the last four exons of the gene, and in mRNA splice variants (9). In addition, it is unknown if *RTEL1* also determines telomere length in humans. However, no association was found between telomere length and single-nucleotide polymorphisms (SNPs) in *RTEL1* (22).

### **RTEL1 AND OTHER FeS CLUSTER-CONTAINING HELICASES**

*RTEL1* belongs within the DEAH subfamily of the Superfamily 2 (SF2) helicases and is classified as a

*RAD3*-related helicase with 5' to 3' directionality (23,24). An iron–sulfur (FeS) domain classifies *RTEL1* within a very small subclass of FeS cluster-containing DNA helicases (25). Xeroderma pigmentosum group D (XPD) is the founding member of this subclass, which also contains ChlR1 and FANCI, in addition to *RTEL1* (26). Mutations in XPD, FANCI and ChlR1 are responsible for the genetic disorders xeroderma pigmentosum (XP) (27), Fanconi anemia (FA) (28–31) and Warsaw breakage syndrome (32). Thus far, heritable mutations in *RTEL1* have not been linked to specific human genetic syndromes. However, two independent studies identified intronic SNPs in *RTEL1* associated with glioma susceptibility (33,34). Furthermore, *RTEL1* is located in a gene-rich cluster (20q13.3) that is amplified in several human cancers (35–38). It is unclear if *RTEL1* is directly implicated in these malignancies, since this cluster also contains other tumor susceptibility genes (38–40). The key characteristic of the XPD family of helicases is the conservation of four cysteine residues, which bind iron ions to form an FeS cluster (25). Removal of the archaeal XPD FeS domain abolishes its helicase activity and can destabilize its tertiary structure (25,41). The crystal structures of archaeal XPDs are consistent with a role of the FeS domain in separating the two strands of the DNA duplex (41–43). This is supported by the findings that the FeS cluster-containing domain of archaeal XPD recognizes the single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) junction and that the DNA duplex is opened near the FeS domain (44). Based on the sequence similarity of the helicase core domain, it is likely that other FeS cluster-containing helicases, including *RTEL1*, also recognize the ssDNA–dsDNA junction. Disruption of the FeS cluster in *RAD3* (yeast XPD) causes defective excision repair resulting in ultraviolet (UV) sensitivity *in vivo* (25). Clinically relevant mutations giving rise to syndromes in XPD and FANCI patients cause the destabilization of the FeS cluster in archaeal XPD (25,41,42).

The severity of FA, XP and Warsaw breakage syndrome emphasize the important roles of the helicases containing FeS clusters in the maintenance of genome stability. Changes in the availability of iron and in the biogenesis of FeS clusters will likely influence *RTEL1*, XPD, FANCI and ChlR1 protein levels. Interference with the packaging of FeS clusters into proteins was recently found to contribute to genomic instability (45). It is likely that compromised levels of FeS containing helicases are responsible for this phenotype.

In addition to its helicase motifs and FeS domain, a conserved eight amino acid PIP [proliferating cell nuclear antigen (PCNA)-interacting protein (46)] box is present at the C-terminus of the *RTEL1* protein. PCNA is a highly conserved eukaryotic protein that functions in DNA replication as a sliding clamp encircling the DNA and acts as a cofactor for DNA polymerases. Currently, the *RTEL1*–PCNA interaction is speculative, but the *RTEL1* PIP box could be necessary to properly orient the protein relative to DNA.

## RTEL1 ANTAGONIZES HOMOLOGOUS RECOMBINATION *IN VITRO*

Although RTEL1 was originally predicted to be a helicase involved in recombination and repair, a biochemical function had remained elusive. Recent studies have now shown that RTEL1 antagonizes homologous recombination events upon DSB formation (8,10). After a DSB is sensed, the two duplex ends are resected to give 3' ssDNA that is subsequently bound by Replication Protein A (RPA). RPA is then displaced by RAD51 recombinase to form a nucleoprotein filament, which searches for and invades the intact homologous dsDNA template (47), resulting in the formation of a D-loop structure (48). The invaded 3' end is extended by a DNA synthesis reaction, copying the information on the homologous DNA template. Subsequently, the strand is displaced and annealed to the other processed DNA end, and the repair reaction is completed by additional DNA synthesis and ligation. A DSB repaired via this SDSA pathway yields a NCO repair product (49,50). Alternatively, if the other processed DNA strand is also captured in the D-loop, a double Holliday junction intermediate will be formed. Resolution of a double Holliday junction can give rise to crossover (CO) repair products (51).

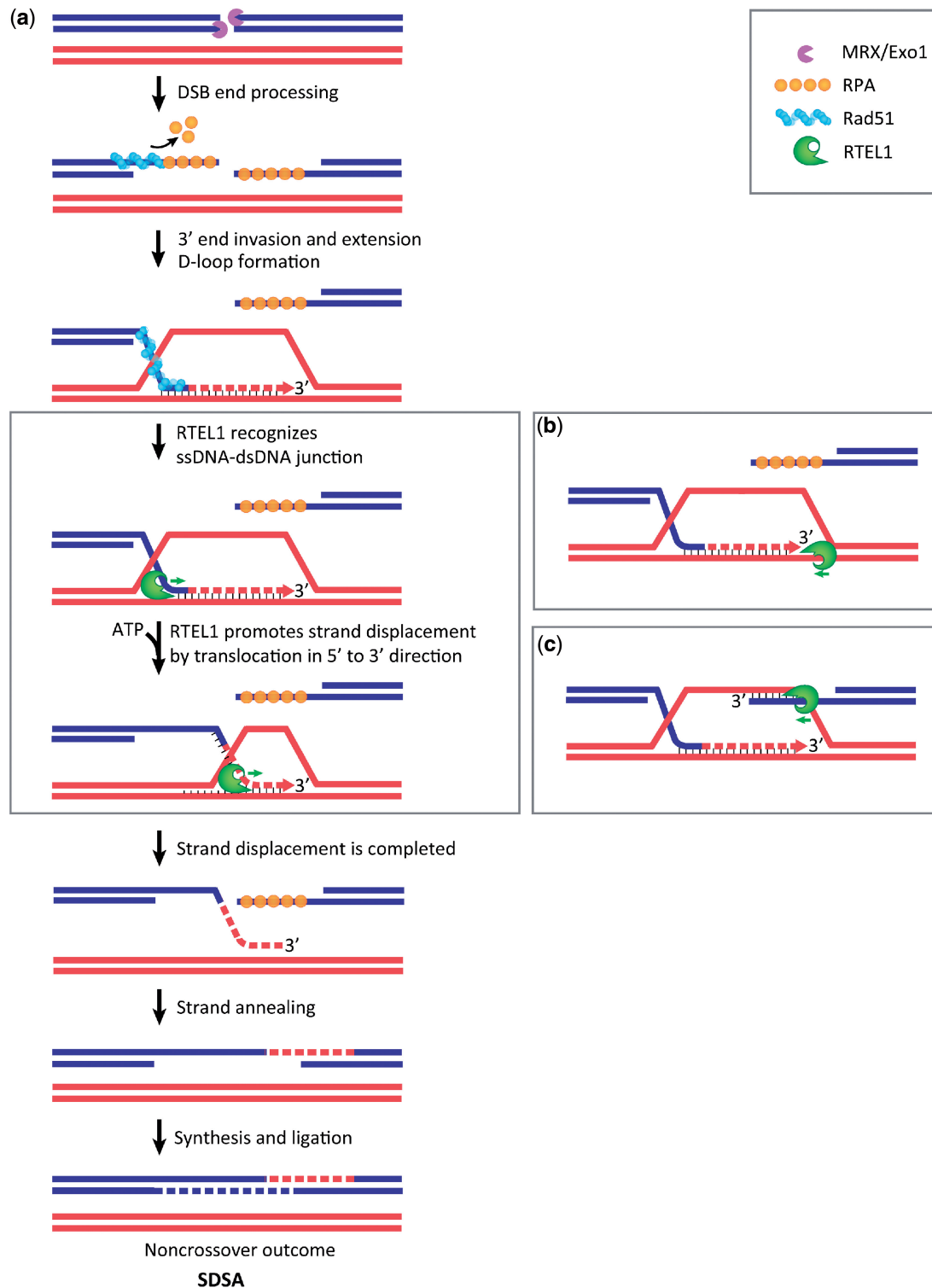
Human RTEL1 possesses biochemical properties consistent with a role in antagonizing HR by promoting SDSA (8,10,52). *In vitro*, purified RTEL1 inhibits the formation of D-loops in an ATP-dependent manner (Figure 1) (8). In addition, RTEL1 was found to actively reverse HR by disrupting preformed D-loops in the presence of calcium (8), which activates and stabilizes the nucleoprotein filament, making it capable of performing the strand exchange reaction (53). In contrast to RTEL1, BLM, the helicase defective in human Bloom's syndrome, and FANCI are unable to dissociate preformed D-loops in the presence of calcium (8,54,55), but can catalyze the disruption of a RAD51 nucleoprotein filament in an unstable state (54,55). RTEL1 has no detectable disruption activity toward single-stranded RAD51 nucleoprotein filaments (8), indicating a unique anti-recombinase mechanism of RTEL1. Thus, the biochemical activity of RTEL1 is distinct from other known anti-recombinases, including yeast Srs2 and mammalian FBH1, BLM, FANCI and RECQL5, all of which can disrupt single-stranded RAD51 nucleoprotein filaments (54–59). In the presence of RPA, RTEL1 was shown to preferentially disrupt a 3' ssDNA invasion D-loop with a 5' overhang but had almost no activity on a D-loop with a 5' ssDNA invasion and a 3' overhang, or a substrate with no overhang (10). The biochemical activity of RTEL1 is perhaps most similar to the yeast helicase Mph1, which is also able to disrupt D-loops; however, Mph1 was shown to have similar affinity for D-loops with a 5' ssDNA invasion and 3' overhang, with a 3' ssDNA invasion and 5' overhang and with no overhang (60). While Mph1 promotes NCO repair of mitotic DSBs (60), it is thus far unclear whether Mph1 might function to regulate meiotic recombination. The fact that RTEL1 can unwind a 3' end invaded D-loop supports the hypothesis that RTEL1 stimulates SDSA-mediated DSB repair, during which

such enzymatic activity is required to disassemble the D-loop intermediate and thereby promote NCO repair products (52). Detailed analysis of the phenotypes of *C. elegans rtel-1* mutants and human *Rtel1*-deficient cells is consistent with the hypothesis that the biochemical activity of RTEL1 in disrupting D-loops is responsible for both mitotic repair and regulating meiotic recombination.

## TELOMERE LOSS IN *Rtel1*-DEFICIENT CELLS

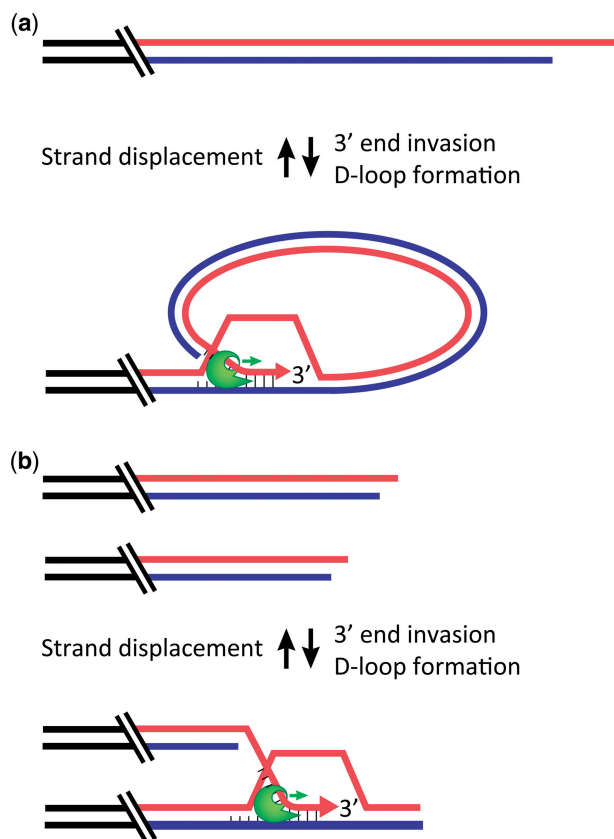
Chromosome ends with low or undetectable telomere repeats are abundant in *Rtel1* null ES cells (9), suggesting significant telomere loss. One possible explanation for this telomere phenotype might be that *Rtel1* is required to open the D-loop structure within the T-loop (Figure 2a). Importantly, the telomeric D-loop resembles the preferred RTEL1 substrate: a 3' ssDNA invaded D-loop with a 5' overhang. Potentially, telomeric D-loops could be inappropriately processed by the HR pathway since this structure resembles a strand-invasion intermediate in HR. This was observed in mammalian cells expressing a truncated form of TRF2 (telomere repeat-binding factor 2) in which HR dependent large T-loop-sized circular telomeric DNAs were generated (61). In the absence of RTEL1, T-loops might be less efficiently resolved. Failing to open the T-loop for replication and/or transcription could lead to large telomeric deletions causing the telomere phenotype seen in *Rtel1*-deficient cells. In addition, *Rtel1* could be required to prevent the 3' single-stranded telomere end from invading the telomeres of other chromosomes (Figure 2b). This activity might prevent telomere recombination events, chromosome entanglements and subsequent breakage when attached chromosomes attempt to segregate during mitosis. Two other helicases important for telomere maintenance are the human RecQ family members Werner syndrome protein (WRN) and BLM. Both helicases interact biochemically and functionally with TRF1, TRF2, and POT1 (protection of telomeres 1) (62–65). *In vitro*, WRN and BLM have been implicated in unwinding the telomeric D-loop (63,64,66). Future experiments will determine if RTEL1 similarly unwinds telomeric D-loop structures and if this activity is required *in vivo*.

An alternative explanation for the short telomere phenotype of *Rtel1* null cells could be that RTEL1, which shares sequence similarity with the helicase FANCI, may also be required to resolve G-quadruplex structures (67,68). G4 DNA could naturally be present at the telomere or arise during lagging strand replication of G-rich telomeric DNA, as was proposed (9). In addition, hybrid G4 DNA/RNA structures might form following transcription of telomeric DNA (69–71). *FANCI* patient cells present genomic deletions corresponding to a G4 DNA signature motif (67) and are hypersensitive to telomestatin (68), a compound which specifically binds G4 DNA (72,73). In *dog-1* (*C. elegans FANCI*) mutants, large G-tract deletions throughout the genome and mild telomeric instability was observed (74–76). By contrast, in *rtel-1* mutants genomic G-tract



**Figure 1.** Model for RTEL1 promoting synthesis-dependent strand annealing (SDSA). (a) After a DSB is sensed, the two duplex ends are resected to give 3'-ssDNA that is subsequently bound by RPA (orange). RPA is replaced by RAD51 recombinase (blue) to form a nucleoprotein filament that searches and invades the homologous dsDNA, forming a D-loop. The invaded strand serves as a primer for DNA synthesis, which copies the information on the homologous DNA strand. RTEL1 (green) promotes SDSA by the displacement of a 3'-end invaded D-loop. Although the mechanism by which RTEL1 promotes strand displacement is unknown, RTEL1 is proposed to recognize the ssDNA-dsDNA junction via its FeS domain, and act as a 5'- to 3'-helicase. The displaced strand can now anneal to the other processed DNA end and the repair reaction will be completed by DNA synthesis and ligation, resulting in a non-crossover outcome. Note that RTEL1 may displace invading strands from both of the processed DNA ends, however, only one invading strand is shown for clarity. Alternative models of RTEL1 action include: (b) RTEL1 translocation in a 5'- to 3'-direction along template strand, as opposed to the invading strand. (c) Although it is thought that SDSA occurs earlier than double Holliday junction formation, it is also possible, though less likely, that RTEL1 may act on the other processed DNA end. This activity might prevent the other processed DNA end from becoming part of the repair reaction, known as second end capture, and so prevent the formation of a double Holliday junction, which could be resolved as a crossover product.

deletions were not detected and a telomere phenotype was not reported (8,74). It is interesting to note that *C. elegans rtel-1 dog-1* double mutant animals display a synthetic lethal phenotype in which sterility results from



**Figure 2.** Model for RTEL1 anti-recombinase function in telomere maintenance. (a) A telomere end is protected from being recognized as a DSB by folding back onto itself in a structure known as the T-loop. At the base of the T-loop, the naturally occurring 3'-ssDNA overhang invades the double-stranded repetitive telomere DNA forming a D-loop. The similarity of D-loop intermediates during DNA repair and telomere maintenance have led to the proposal that RTEL1 (green) may reverse T-loops. (b) D-loops can also form at telomeres by invasion of the 3'-ssDNA telomere overhang into sister or non-sister telomeres, leading to telomere strand-exchange and chromosome entanglements. RTEL1 may reverse inappropriate T-loop formation and thereby prevent telomere strand exchange.

replication-associated problems in the germline prior to meiosis [(8); Youds and Boulton, unpublished data]. Thus, some overlapping function for *rtel-1* and *dog-1* during replication is likely, at least in *C. elegans*. Experiments to conditionally knock out both *Rtel1* and *FancJ* might reveal partially overlapping roles with regard to G4 DNA maintenance, particularly at telomeres. In addition to FANCI, the 3' to 5' helicases WRN and BLM are able to unwind G4 DNA *in vitro* (77–79). Table 1 compares the existing data on RTEL1 and FANCI.

A recent study revealed that mammalian telomeres are difficult regions to replicate and resemble fragile sites (80,81). In the absence of Trf1, the replication fork has a greater tendency to stall when encountering telomeric DNA. It was proposed that Trf1 might act by recruiting or activating helicases like Rtel1 and Blm to telomeres (80). *Rtel1*-deficient ES cells and single *Rtel1* and *Blm* knockdown mouse embryonic fibroblasts (MEFs) both show a mild fragile-telomere phenotype, which in MEFs is epistatic to the deletion of *Trf1* (80). These results indicate that Rtel1 and Trf1 act in the same pathway to suppress telomere fragility. Additional functional studies of the biochemical and cellular activities of RTEL1, FANCI, WRN and BLM may reveal more specific and redundant roles of these proteins in the regulation of the telomeric D-loop, G-quadruplexes, and other biological structures.

### RTEL1 REGULATES MITOTIC AND MEIOTIC RECOMBINATION *IN VIVO*

Besides the requirement of Rtel1 for telomere maintenance, a role in DNA repair was envisioned upon studying differentiating *Rtel1*-deficient ES cells (9). Interestingly, within hours of the induction of differentiation, *Rtel1*-deficient ES cells showed a variety of chromosomal abnormalities, including broken chromosomes and chromatid gaps, pointing to a more general role of Rtel1 in maintaining genomic stability. Subsequently, a role for RTEL1/RTEL1 in regulating HR was elucidated from work in *C. elegans* and human cell lines (8).

In the budding yeast *Saccharomyces cerevisiae*, the DNA helicase Srs2 is an anti-recombinase that regulates

**Table 1.** Summary of the relationship of RTEL1 and FANCI

	<i>dog-1 FancJ FANCI</i>	<i>rtel-1 Rtel1 RTEL1</i>
Best BLAST hit	<i>rtel-1 Rtel1 RTEL1</i>	<i>dog-1 FancJ FANCI</i>
Domains of interest in human protein	Helicase domains including DEXDC, HELICc2, DEAD_2 and DinG, BRCA1-interaction site, Fe-S motif	Helicase domains including DEXDC2, DEAD_2, HELICx2 and DinG, PIP box, Fe-S motif
Synthetic lethal in <i>C. elegans</i> when combined with mutation in	<i>rtel-1</i>	<i>dog-1, mus-81, him-6 BLM, rcq-5 RECQ5</i>
DNA damage sensitivity?	Yes: ICL-inducing agents	Yes: ICL-inducing agents, camptothecin
Telomere phenotype?	<i>dog-1</i> : mild instability	<i>Rtel1 RTEL1</i> : dramatic loss
Elevated recombination?	None identified	Yes
Activity on <i>in vitro</i> biochemical substrate	G4 DNA, forked duplex DNA, D-loop	D-loop recombination intermediate
Activity with respect to recombination	Inhibits RAD51 strand exchange	Disassembles D-loop intermediate
Human patients identified?	Yes: Fanconi anemia subgroup J	None identified

HR (56,57). Yeast *SRS2* is synthetic lethal with *SGS1*, the sole *S. cerevisiae* RecQ helicase that bears homology to five human RecQ helicases, particularly to human BLM (82). This synthetic lethality is due to an accumulation of toxic recombination intermediates (82). No sequence homologs of *SRS2* exist in higher organisms, but the *SGS1* homolog in *C. elegans* is *him-6/BLM* (83). Therefore, based on the synthetic lethality of *SRS2* and *SGS1* in yeast, a screen to find a candidate for a *C. elegans* gene with an Srs2-like function was conducted by searching for genes that showed synthetic lethality when knocked-down in a *him-6/BLM* mutant strain. *rtel-1* was identified as being synthetic lethal with *him-6/BLM*, making it a candidate for the *SRS2* analog in *C. elegans*. Furthermore, *rtel-1* showed synthetic lethal phenotypes when combined with mutations in *mus-81*, *dog-1* and *rcq-5* (8). The synthetic lethality in these strains correlated with an accumulation of RAD-51 foci in the germline, a marker of persistent HR intermediates. Furthermore, *rtel-1* mutation causes synthetic sick phenotypes when combined with deletions in either *rfs-1* or *helq-1*, two genes that encode proteins functioning late in HR to disassemble RAD-51 from double-stranded DNA filaments (84).

*Caenorhabditis elegans rtel-1* and *dog-1* mutants and human cells deficient for *RTEL1* and *FANCD2* are sensitive to DNA interstrand crosslinks (ICLs) (8,28–31,75). Based on epistasis experiments in *C. elegans*, *dog-1* acts in the *fcd-2/FANCD2* pathway, while *rtel-1* appears to act in a separate pathway for ICL repair. *rtel-1* mutants are also sensitive to camptothecin (8). Surprisingly, *C. elegans rtel-1* mutants and *RTEL1* depleted cells are not sensitive to ionizing radiation (IR). However, it is possible that in the absence of *RTEL1*, all DSBs are efficiently repaired with a CO outcome. In accordance with a role of *RTEL1* in antagonizing recombination, recombination frequencies were increased in *RTEL1*-depleted HeLa cells (8).

In addition to mitotic DNA repair, *RTEL1* is also required to promote NCO repair during meiosis (10). Meiotic DSBs are not randomly distributed along chromosomes, but tend to occur in specific regions (85). Through homologous recombination, DSBs can be repaired by either a CO or a NCO event (86). The number of DSBs created exceeds the number of final CO events, in some organisms by more than ten times (87,88). How specific DSBs are selected to become COs is unknown, but the mechanism behind this ensures that each pair of homologs gets at least one CO (known as the obligate CO). In addition, a mechanism called CO interference regulates the distribution of COs along the chromosome in such a way that COs tend to occur further apart from each other than expected by chance (89). When the number of meiotic DSBs is reduced, the number of COs is maintained at the expense of NCOs; this is called CO homeostasis (90).

Recently, *C. elegans RTEL1* was shown to enforce both meiotic CO interference and homeostasis (10). Wild-type *C. elegans* only ever have a single CO per chromosome (91,92). *rtel-1* mutants showed up to three COs per chromosome that were randomly distributed, suggesting a lack of CO interference. Furthermore, the

CO increase was not due to increased formation of meiotic DSBs (10), distinguishing the mechanism of *RTEL1* function from that of condensin complex components (92,93). When DSBs were generated using IR, wild-type animals showed a small increase in COs, while *rtel-1* mutants displayed a large, dose-dependent increase in COs, supporting a function for *RTEL1* in maintaining CO homeostasis. In yeast, it was shown that NCOs and COs appear with different kinetics during meiosis, suggesting that they are regulated by different mechanisms (49,94). In addition, NCOs are primarily repaired by SDSA in yeast meiosis (94). Thus, it is likely that NCOs occur via meiotic SDSA and that the D-loop disassembly ability of *RTEL1* is required for this pathway. Interestingly, the increase in the number of ZHP-3 foci, a protein restricted to recombination foci (95), was relatively small compared with the increase in CO events in *rtel-1* mutants (10). Furthermore, after *rtel-1* mutants were treated with IR, the number of COs increased, but ZHP-3 foci did not. These results indicate that two classes of meiotic recombination events are elevated in *rtel-1* mutants: (i) ZHP-3-associated, obligate-type COs (small increase) and (ii) COs produced by recombination events not associated with ZHP-3 (predominant). Like in *S. cerevisiae* (86,96), this second class of COs requires MUS-81 and is likely interference-independent (10). This finding indicates that meiotic HR intermediates not reversed by *RTEL1* during SDSA will be MUS-81 substrates resulting in COs, which is consistent with the embryonic lethality of *rtel-1 mus-81* double mutants (8).

Mets and Meyer (92) have shown that the average number of DSBs per *C. elegans* chromosome in wild-type meiosis is 2.1, while just one of these is resolved into a CO. How *RTEL1* is recruited and regulated in a way that just half of the DSBs induced during wild-type meiosis result in a CO is an intriguing question. Designated COs may be protected from *RTEL1* activity by certain proteins; alternatively, *RTEL1* activity may be carefully regulated such that *RTEL1* only becomes active after the obligate COs have been completed.

It will be of interest to determine if *RTEL1* is also required for CO interference and homeostasis in mammals. Human *RTEL1* could have similar functions in meiosis since it was shown to disassemble D-loop recombination intermediates *in vitro* (8,10). In mice, the highest *Rtel1* expression is detected in spermatogonia and meiotic spermatocytes within the testes (9). Although full knockout of *Rtel1* function is lethal, the requirement of mouse *Rtel1* in meiosis could potentially be studied if a (pre)meiotic-Cre transgenic line were available, since mice with conditional knock-out alleles have been established (97).

## CONCLUDING REMARKS

*RTEL1* is an essential helicase, a dominant factor in setting telomere length in mice and is required for telomere and genome maintenance. Furthermore, *RTEL1* is a key protein in mitotic and meiotic DSB

repair and promotes NCO repair outcomes through SDSA. As it is currently unknown how RTEL1 is recruited and regulated during DSB repair, in both mitotic and meiotic cells, determining its post-translational modifications and interacting proteins may shed light on these questions. Live-cell imaging of fluorescent-tagged RTEL1 and co-localization studies with other DNA repair proteins will enhance our understanding of the biological function and dynamics of RTEL1.

The data available on RTEL1 so far do not exclude the possibility that RTEL1 is preferentially recruited to, or has higher enzymatic activity at G-rich regions in the genome. In humans, 25 618 meiotic recombination hot spots have been identified (98). Interestingly, potential G4 DNA-forming sequences were found to be significantly enriched within recombination hot spots (99). Also, G4 DNA was predicted to be formed within a 50-bp window around all of the top seven previously reported short recombination enriched sequences (98,99). It is possible that RTEL1 is recruited to meiotic DSBs by G4 DNA structures at recombination hot spots to prevent COs from occurring. Possible recruitment to G4 DNA could be direct or indirect via binding to other G4 DNA-binding proteins. In yeast, Mre11 and the meiosis-specific protein Hop1 bind G4 DNA, and Hop1 itself promotes G4 DNA formation (100,101); thus, homologs and binding partners of these proteins are potential recruiters of RTEL1 to G4 DNA.

Finally, given that RTEL1 has already been shown to play multiple roles in maintaining genome stability, both at telomeres and more generally at sites of mitotic DNA damage, as well as regulating meiotic recombination, RTEL1 could be classified as having a tumor suppressive function. Certainly, *Rtel1*-deficient cells will undergo uncontrolled HR and that may result in telomere loss and/or gain and chromosomal rearrangements and translocations, all of which are characteristic of cancer cells. Conversely, upregulated *RTEL1* function might prevent HR when it is needed as a legitimate means for repair. *RTEL1* mutation has already been associated with risk of glioma (33,34), and *RTEL1* was shown to be overexpressed in gastrointestinal tract tumors (35). A challenge ahead will be to determine whether RTEL1 dysfunction plays a causative role in these and other cancers.

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