RTEL1: Functions of a disease-associated helicase

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Review

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RTEL1: functions of a disease-associated helicase

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DNA secondary structures that arise during DNA replication, repair, and recombination (3R) must be processed correctly to prevent genetic instability. Regulator of telomere length 1 (RTEL1) is an essential DNA helicase that disassembles a variety of DNA secondary structures to facilitate 3R processes and to maintain telomere integrity. The past few years have witnessed the emergence of RTEL1 variants that confer increased susceptibility to high-grade glioma, astrocytomas, and glioblastomas. Mutations in RTEL1 have also been implicated in Hoyeraal–Hreidarsson syndrome, a severe form of the bone-marrow failure and cancer predisposition disorder, dyskeratosis congenita. We review these recent findings and highlight its crucial link between DNA secondary-structure metabolism and human disease.

DNA secondary-structure metabolism during DNA replication and repair

The formation and/or metabolism of DNA secondary structures is important for many physiological processes, and is particularly relevant during DNA replication and repair (reviewed in [1]). However, persistent or aberrantly processed DNA secondary structures can have pathological consequences and are an established source of genome instability [2,3]. DNA secondary structures can form from alternative DNA sequence motifs [e.g., trinucleotide repeats, or G-rich DNA that forms four-stranded DNA structures termed G-quadruplexes (G4)] or as intermediates generated during 3R processes.

Homologous recombination (HR) is a highly conserved mechanism of DNA double strand (ds) break (DSB) repair that is essential for DNA replication and for the establishment of crossovers during meiosis. HR is also required for the generation of the T-loop structure at telomeres, which forms when the 3′ single-stranded (ss) G-rich overhang (G-tail) invades into internal dsDNA telomeric repeats of the same chromosome end [4,5]. The T-loop structure is believed to play an important role in protecting the chromosome end from degradation and inappropriate repair (reviewed in [6]). HR is initiated by the 5′ end resection of the DSB to produce a 3′ ss overhang that serves as a substrate for the binding and nucleation of the strand-exchange protein Rad51 [7–9]. Once Rad51 is bound to the processed DSB as a nucleoprotein filament, it is able to catalyse invasion of the broken DNA end into an intact DNA duplex, and this serves as a template for DNA synthesis (Figure 1). The processing of specific DNA secondary structures, which form as intermediates during repair, directly impacts the outcome of HR. For example, the double Holliday junction structure, which forms late in the HR reaction, can be nucleolytically processed by resolvase enyzmes [e.g., GEN1 (Holliday junction 5′ flap endonuclease), SLX4 (synthetic lethal of unknown function; structure-specific endonuclease subunit) complex] or subjected to branch migration and decatenation by the BLM (Bloom syndrome, RecQ helicase-like)/RMI1 (RecQ mediated genome instability 1)/TOP3A (topoisomerase IIIα) complex, giving rise to crossover or non-crossover products [10–12].

The essential helicase, RTEL1, plays a crucial role in the metabolism of DNA secondary structures that arise during 3R processes [13]. In this review we highlight how RTEL1 is utilised in several distinct cellular contexts to affect repair outcome or subvert toxic repair activities. We also discuss recent discoveries implicating the *RTEL1* gene in a range of cancers and in the hereditary disorder, Hoyeraal–Hreidarsson syndrome [14–18].

RTEL1 in telomere homeostasis

RTEL1 belongs to the DEAH (named from the corresponding four amino acid motif in single-letter code) subfamily of the superfamily 2 (SF2) helicases, which contain a RAD3-related DNA helicase domain with 5′ to 3′ helicase activity (reviewed in [19,20]). RTEL1 is also a member of the iron–sulfur (Fe–S) cluster helicase family, which includes xeroderma pigmentosum group D (XPD), Fanconi anemia complementation group J (FANCJ), and DEAD/H box helicase 11 (DDX11; reviewed in [21]). The activity of these proteins is strictly dependent on their ability to coordinate an Fe–S cluster, which is assembled into the target protein by the cytosolic Fe–S protein assembly (CIA) machinery [22,23].

RTEL1 was first identified through genomic mapping of loci that control telomere length in *M. musculus* and *M. spretus* [24]. Although a definitive explanation of how RTEL1 controls telomere length remains elusive, the existence of different RTEL1 splice variants between these two

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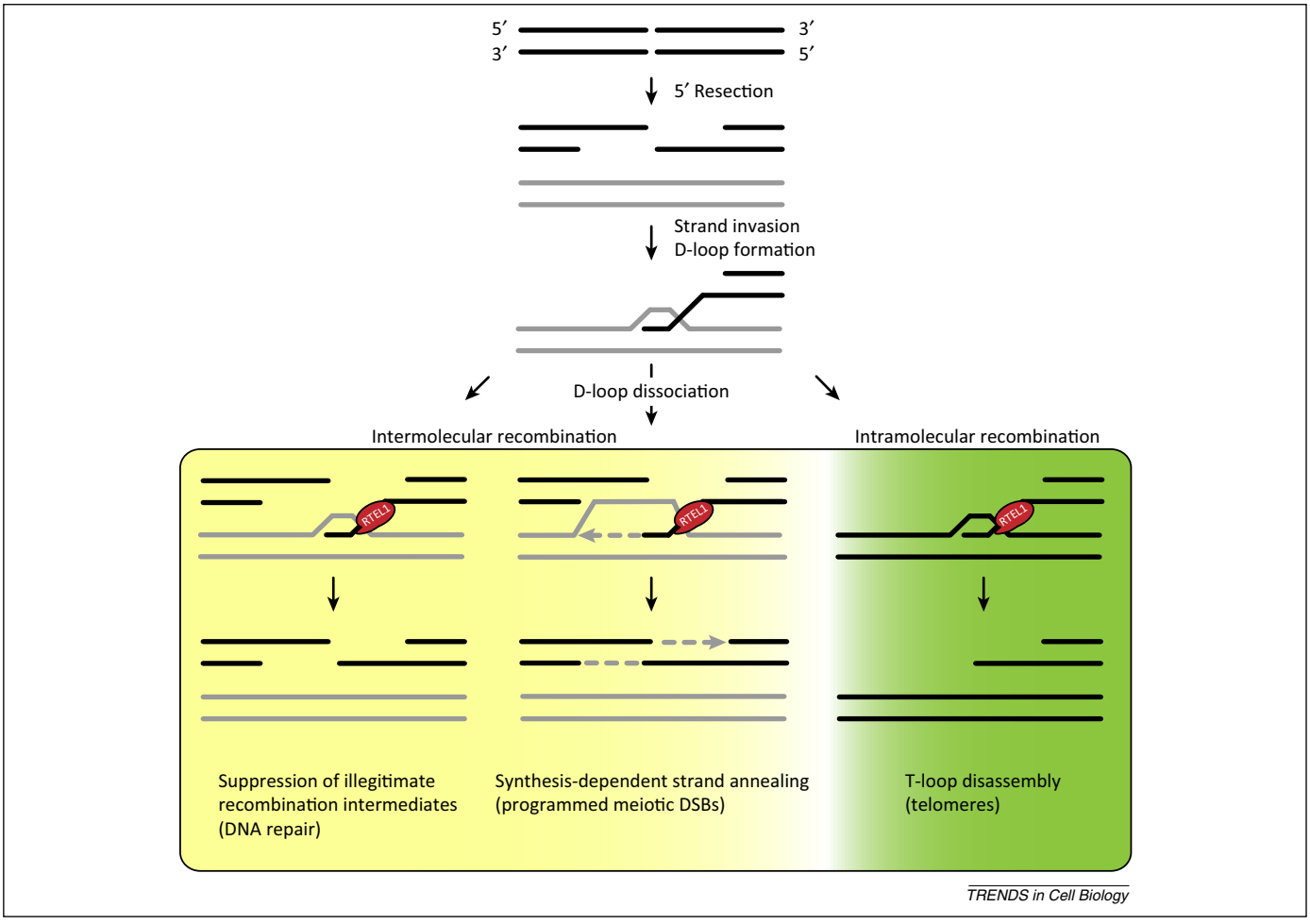

Figure 1. Toxic recombination intermediate substrates for RTEL1 (regulator of telomere length 1). Double-strand (ds) breaks (DSB) formed either from exogenous (ionising radiation) or endogenous sources (programmed meiotic breaks) or DSB naturally present at the end of the chromosome (telomeres) are highly recombinogenic structures. After processing of the 5′ end of the break, the 3′ single-stranded (ss) DNA is coated by Rad51 to form a nucleoprotein filament that invades homologous DNA template (inter- or intra-molecular, respectively grey and black molecules) yielding a strand exchange intermediate known as D-loop. RTEL1 is part of the superfamily 2 (SF2) helicases, which contain a RAD3-related DNA helicase and is also part of the iron–sulfur (Fe–S) cluster helicase family, which includes xeroderma pigmentosum group D (XPD), and Fanconi anemia complementation group J (FANCJ). Based on the ability of the Rad3 Fe–S cluster to recognise the DNA junction between ss and ds DNA [93,94], we propose that RTEL1 may recognize similar junctions in recombination intermediates (D-loops). RTEL1 DNA helicase activity towards D-loop structures is proposed to regulate homologous recombination based DSB repair, to promote repair by synthesis-dependent strand annealing (SDSA), and to facilitate replication through persistent DNA secondary structures at telomeres (T-loop).

mouse strains has been suggested as a possible reason. Indeed, the RTEL1 protein encoded by the most common transcript in *M. spretus* is lacking six amino acids at its C terminus [25].

A possible role for RTEL1 in telomere homeostasis was investigated using a constitutive *Rtel1* knockout mouse, which is embryonic lethal between days 10.5–11.5 of embryogenesis. Upon differentiation, embryonic stem cells derived from these mice exhibited reduced proliferation, chromosomal abnormalities and telomere length heterogeneity [25]. It remains unknown why this phenotype only manifests after differentiation, but it could reflect a change in replication timing that occurs as cells differentiate. Because RTEL1 is most related to human FANCJ and *C. elegans* DOG-1 (deletion of G-tracts), which unwind DNA secondary structures formed from G-rich DNA, it was proposed that the phenotype of RTEL1-deficient cells may reflect a problem in unwinding DNA secondary structures. Indeed, subsequent studies confirmed that at least a subset of RTEL1 null phenotypes are attributed to inefficient removal of specific DNA secondary structures [13,26,27].

RTEL1 controls recombination in mitotic and meiotic cells

RTEL1 was independently identified in *C. elegans* as a key regulator of HR in a genetic screen for synthetic lethality with mutation in the *sgs1*/*BLM* orthologue, which is associated with the accumulation of persistent recombination intermediates [28]. Consistent with a role for RTEL1 in suppressing HR (Figure 1), worms and human cells lacking RTEL1 exhibit hyper-recombination and sensitivity to DNA damaging agents. Moreover, *C. elegans rtel-1* mutants are also synthetic lethal when combined with mutations in the HR effectors *mus-81* and *rcq-5* due to the accumulation of persistent HR intermediates. Biochemical studies revealed that human RTEL1 acts by dismantling preformed displacement (D)-loop HR intermediates in an ATP-dependent manner (Figure 2). Based on these findings, it was proposed that loss of

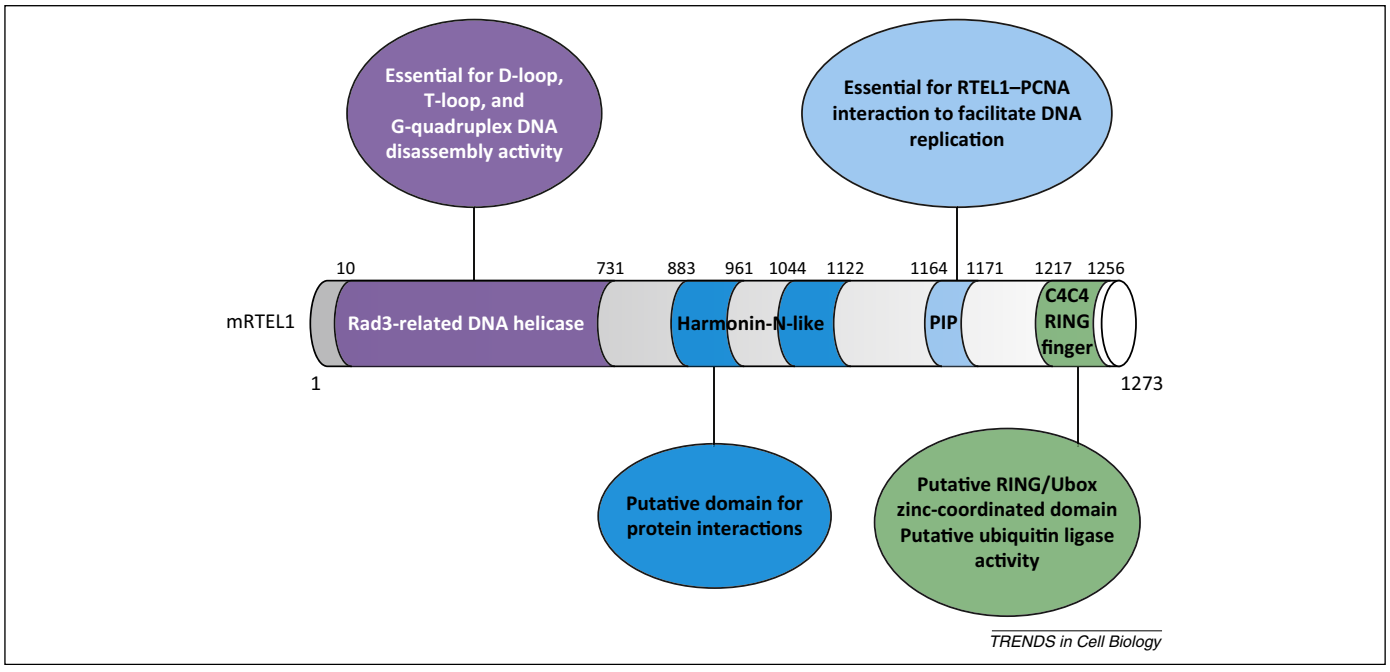

Figure 2. Mouse regulator of telomere length 1 (mRTEL1) structure of interaction and functional domains. RTEL1 is part of the subclass of Fe–S cluster-containing DNA helicase within the superfamily 2 (SF2) DEAH ('Asp-Glu-Ala-His') subfamily. RTEL1 contains a Rad3-related DNA helicase with 5′ to 3′ directionality. The helicase domain includes DEXDc2, DEAD_2, HELICx2, and DinG domains. Computational analysis of RTEL1 identifies harmonin-N-like domains that may play molecular scaffolds for protein–protein interactions to be determined, as well as the PCNA interacting protein motif (PIP) which was validated *in vivo* for RTEL1–PCNA interaction. The C terminus of the mouse protein may contain a C4C4 domain or RING-finger domain that is highly conserved between human, orangutan, cattle, and mouse (GenBank EDL07405) [14]; the domain may be important for protein–protein interactions, binding to ubiquitination enzymes, and hence for ligase function.

RTEL-1/*RTEL1* in *C. elegans* and in human cells results in a failure to regulate HR correctly [28].

Subsequent studies revealed that RTEL1 limits excessive cross over in meiosis and thus impacts on the outcome of the HR reaction. During meiosis, HR generates a single crossover (CO) per homologous chromosome pair and therefore HR is essential for correct chromosome segregation during the first meiotic division (reviewed in [29]). To ensure that at least one CO is generated between each homologous chromosome pair, an excess of meiotic DSBs are generated, of which the majority are repaired as non-crossovers [30]. Measurement of the CO frequency during meiosis in *C. elegans* revealed that *rtel-1* mutants generate multiple COs per homologous chromosome pair, and this contrasts with the single CO per homologous chromosome pair occurring in wild type [31]. The structure-specific endonuclease MUS-81 was shown to produce the excess COs in *rtel-1* mutants, likely by processing persistent D-loops that arise in the absence of RTEL-1 [31]. Taken together, these studies suggest that the D-loop disruption activity of RTEL1 may guide excess meiotic DSB towards a non-crossover (NCO) repair pathway, likely through synthesis-dependent strand annealing (Figure 1). Although important for limiting COs, RTEL-1 is not the only factor that promotes NCOs because NCO repair can still occur in *rtel-1* deficient worms [32].

RTEL1 controls recombination at telomeres

Visualization of vertebrate telomeres by electron microscopy and STORM (stochastic optical reconstruction microscopy) has revealed that some, if not all, chromosome ends adopt a lasso-like configuration called a T-loop [4,33]. The T-loop is proposed to form upon strand invasion of the 3′ ss TTAGGG telomeric repeats into an adjacent duplex of telomeric DNA, resulting in a D-loop intermediate at the site of strand invasion [34]. T-loops may protect the chromosome end from degradation and promiscuous DNA repair activities (reviewed in [6]), and until recently the mechanism controlling T-loop disassembly remained unclear [35].

The realisation that RTEL1 can act as an anti-recombinase to promote D-loop disassembly raised the possibility that the telomere phenotypes associated with *RTEL1* deficiency might reflect a defect in dismantling recombination intermediates at telomeres, such as those formed by the T-loop. Indeed, such a role is supported by biochemical studies showing that RTEL1 disassembles a T-loop *in vitro* (Figures 1 and 2) [13]. A role for RTEL1 in promoting T-loop disassembly *in vivo* is supported by the observation that the acquisition of telomere length heterogeneity following loss of *RTEL1* coincides with the rapid accumulation of telomere circles (TCs). Strikingly, TC formation was dependent on the SLX1–SLX4 nuclease complex, which associates with telomeres and possesses Holliday junction resolution activity predicted to cleave a persistent T-loop resulting in the loss of the telomere as a circle [10]. Consistent with this observation, TCs induced by overexpression of a mutated form of TRF2 (telomeric repeat-binding factor 2), TRF2ΔB, or arising in ALT (alternative lengthening of telomeres) cells, depend on the presence of SLX1–SLX4 [13,36]. Furthermore, mouse cells deficient for SLX4 exhibit longer telomeres and reduced basal levels of TCs [37]. In addition, telomere length heterogeneity and TC formation in *RTEL1*-deficient cells requires active DNA

replication because these phenotypes are suppressed in cells treated with replication inhibitors [13]. It was suggested that a collision between the replisome and persistent T-loops might trigger SLX1–SLX4 to resolve the T-loop resulting in loss of the telomere as a circle.

Analysis of the telomere phenotype in *Rtel1*-deficient cells also revealed a second, distinct function in suppressing telomere fragility [13,38]. Fragile telomeres are defined as common fragile sites at telomeres due to there induction in response to replication stress (reviewed in [39]). It was proposed that telomere fragility reflects an intrinsic problem with replication through the repetitive TTAGGG sequence of the telomere, which can adopt G4 secondary structures that hinder DNA replication [1,38]. RTEL1 was found to unwind telomeric G4 DNA structures *in vitro*, suggesting that it plays a role in suppressing telomere fragility (Figure 2) [27]. Furthermore, telomere fragility is exacerbated in *RTEL1*-deficient cells treated with TMPyP4, a G4-stabilising drug that inhibited RTEL1-dependent telomeric G4 DNA unwinding *in vitro* [27]. Taken together, these observations suggest that RTEL1 performs two distinct functions essential for telomere integrity: it facilitates T-loop disassembly and telomeric G4 DNA unwinding [13]. How these distinct functions of RTEL1 are executed at telomeres remains an area of active investigation.

RTEL1 is essential to facilitate replication

Several observations suggested a possible role for RTEL1 during DNA replication. Mouse ES cells deficient for RTEL1 exhibit reduced proliferative capacity, and worms and mammalian cells lacking RTEL1 are particularly sensitive to DNA damaging agents that hinder DNA replication, such as inter-strand crosslinking agents [25,26,28]. Proteomic analysis of RTEL1 interacting proteins reinforced the link between RTEL1 and DNA replication, and identified multiple DNA replication factors, including mini-chromosome maintenance proteins (MCM), replication factor C (RFC) subunits, and proliferating cell nuclear antigen (PCNA) [27]. Computational analysis of the RTEL1 polypeptide identified a PCNA interacting protein motif (PIP box) in the C-terminus of the protein, which confers direct binding to PCNA (Figure 2). Strikingly, mouse cells in which the RTEL1–PCNA interaction was disrupted, exhibited accelerated senescence and reduced 5-bromo-2′-deoxyuridine (BrdU) incorporation, indicating a problem in S-phase. Indeed, examination of replication dynamics by DNA combing revealed that loss of the RTEL1–PCNA interaction leads to reduced replication-fork extension rates, increased origin usage, and replication fork instability, thus revealing a unexpected role for RTEL1 in facilitating global genome replication (Figure 2). Further analysis established that the RTEL1–PCNA interaction is primarily responsible for preventing fork stalling and/or collapse, which in turn impacts origin firing and fork extension rates. Under non-damaging conditions, RTEL1 colocalised with a subset of PCNA foci in a PIP box-dependent manner [27]. Whether this interaction is constitutive or represents a highly dynamic and regulated association remains to be clarified. Furthermore, the source of fork stalling/collapse in the absence of RTEL1 remains unclear; however, it is tempting to speculate that this may reflect a problem with regulating DNA secondary structures, in particular G4 and other DNA secondary structures that can arise at the replication fork (Figure 3).

RTEL1 in human diseases

Mutations in *XPD*, *FANCJ*, and *DDX11* have been shown to be an underlying cause of xeroderma pigmentosum (reviewed in [40]), Fanconi anemia [41–44], and Warsaw breakage syndrome [45,46], respectively. In the last few years, *RTEL1* variants have also been linked to several distinct human brain cancers and recently, *RTEL1* mutations have been shown to give rise to Hoyeraal–Hreidarsson syndrome, a severe form of the telomeropathy dyskeratosis congenita [14,16,17]. Below we discuss the relevance of *RTEL1* to the development of cancer and Hoyeraal–Hreidarsson syndrome.

RTEL1 and cancer predisposition

Genome-wide association studies (GWAS) established an association of single-nucleotide polymorphisms (SNPs) in *RTEL1* with increased susceptibility to brain tumours. A principal component-adjusted GWAS study, comprising over 275 000 autosomal variants among 692 adult glioma cases and 3992 controls, identified two SNPs within intron 12 (rs6010620) and intron 17 (rs4809324) of *RTEL1* that are significantly associated with glioma and astrocytoma predisposition [47]. Similarly, two further glioma GWAS studies, which genotyped over 500 000 tagged SNPs in a total of 1878 cases and 3670 healthy controls, revealed a significant association with SNP (rs6010620) in intron 12 of the *RTEL1* gene [48]. In this setting, amplification of the 20q13.33 region was observed in nearly 30% of gliomas, with copy-number changes correlating with *RTEL1* expression levels. Intriguingly, these SNPs primarily associate with high-grade gliomas [47,48] and have become a critical predictor of survival among patients with this type of brain tumour [49]. More recently, another *RTEL1* SNP within intron 12 was shown to associate strongly with glioblastomas, the most common and aggressive type of primary brain tumour [50,51]. In addition, several studies reported associations between this SNP and the risk of glioma, irrespective of family history [48,50,52]. Interestingly, SNPs in *TERT*, encoding the reverse transcriptase component of telomerase (which like RTEL1 is important for telomere maintenance), also strongly associate with glioma predisposition.

Because these SNPs were mapped in non-coding regions of *RTEL1*, their impact at the molecular level in brain cancer development and pathogenesis is not entirely clear. Intronic SNPs that are located near to exon junctions may promote aberrant exon inclusion or deletion through altered mRNA splicing as well as formation and increase of nonsense transcripts [53,54]. Moreover, intronic SNPs that are deeper within introns have also been demonstrated to form cryptic splice sites [55,56]. However, none of these potential mechanisms were investigated in the abovementioned GWAS studies; therefore, a more in-depth comparative examination of *RTEL1* mRNA transcripts is required. Although the association of *RTEL1* SNPs with

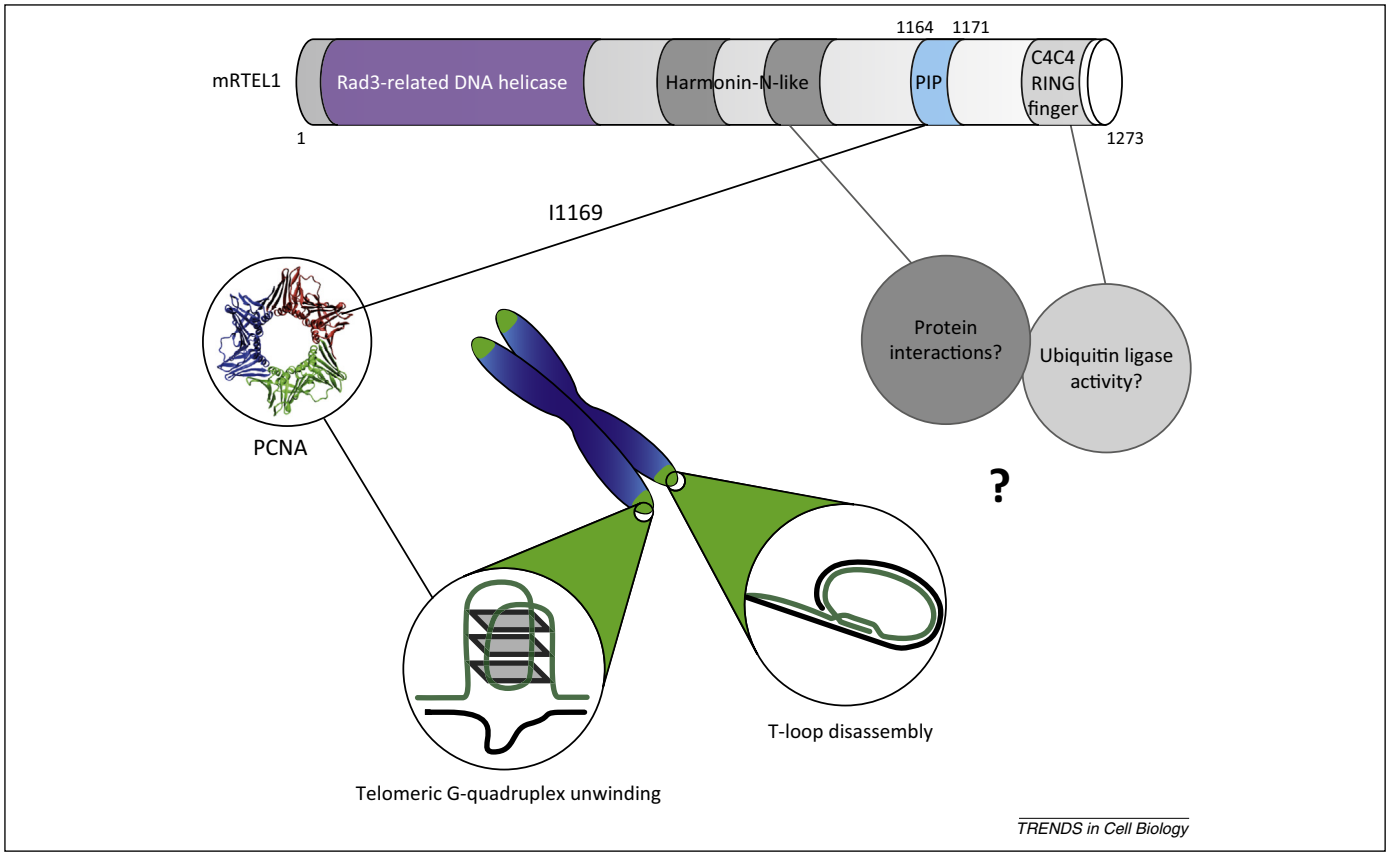

Figure 3. RTEL1 separation of function to facilitate DNA replication at telomeres. RTEL1 plays dual roles in facilitating DNA replication at telomeres through T-loop disassembly to prevent catastrophic telomere processing by SLX4–SLX1 complex, and through G-quadruplex (G4) structures to suppress telomere fragility, are executed separately. RTEL1 associates with the replisome via an interaction with PCNA mediated by the RTEL1 PIP box. Mouse cells disrupted for the RTEL1–PCNA interaction exhibit replication fork instability that affects genome-wide and telomere replication. Although telomere loop disassembly at telomeres was unaffected in the RTEL1 PIP mutant cells, telomere replication is compromised, leading to fragile sites at telomeres. RTEL1–PCNA interaction is required to counteract telomeric G4 DNA structures that arise at the replication fork, but is dispensable for T-loop unwinding, and thus execution of this latest function at telomeres remains unclear and might be mediated by another interaction with harmonin-N-like or RING finger domains.

human cancers is highly significant, it remains to be defined how these specific variants impact RTEL1 function during brain tumourigenesis.

The emergence of *RTEL1* variants associated with predisposition to some brain cancers has raised the possibility that *RTEL1* may function as a tumour-suppressor gene. Definitive proof of such a role has emerged from analysis of mice defective for the RTEL1–PCNA interaction. Despite genome-wide replication problems in primary cells, RTEL1-PIP box mutant mice are viable, which may be due to the firing of additional replication origins compensating for the replication defects in cells. Interestingly, loss of the RTEL1–PCNA interaction significantly accelerates the onset of tumourigenesis in mice also deficient for *p53*. Furthermore, unlike *p53* null mice, additional loss of the RTEL1–PCNA interaction results in predisposition to medulloblastomas [27]. Because *Rtel1* is expressed in cerebellar external granular layer (EGL) cells, the cell of origin for medulloblastomas [57], these data suggest that the RTEL1–PCNA interaction is important for protecting EGL cells from malignant transformation. Although the mechanisms underlying medulloblastoma initiation and possible aggressiveness are not well understood, tumourigenesis was associated with changes in telomeric homeostasis upon loss of RTEL1–PCNA interaction. Supporting this notion, analysis of tumours from RTEL1-PIP mutant mice revealed a high frequency of fragile telomeres when compared to non-tumour cells from the same mice, or tumour cells from *p53*-deficient controls [27]. Despite these findings, the relationship between medulloblastoma predisposition in mice and *RTEL1* variants associated with glioma and glioblastoma predisposition in humans remains unclear.

Interestingly, conditional targeting of mouse *Rtel1* enabled *in vivo* studies of biallelic *Rtel1* overexpression in tissues other than the brain. Ubiquitous overexpression of RTEL1 in the mouse specifically caused hepatocellular tumours that recapitulated a variety of malignant features of human hepatocellular carcinoma (HCC) [58]. The tissue specificity of tumour development is in contrast to the ubiquitous expression pattern of RTEL1 [25] and may reflect differences in expression of RTEL1 interactors and/or differential roles of RTEL1 in different tissues. These results are also interesting considering the complex histology of hepatocellular carcinoma in mice, which involves tumour cells possessing high numbers of mitotic figures (mitotic cells where chromosomes are aligned in metaphase, prior to separation) or multinucleated cells [58].

Because *RTEL1* can have both proliferative as well as growth-suppressive roles *in vivo* [27,58], it remains to be

clarified whether *RTEL1* can act as both an oncogene and a tumour suppressor. On the one hand, amplification of the *RTEL1* genomic locus is observed in variety of cancers such as breast cancer and gastrointestinal tract tumours [59–61]. In addition, the role of RTEL1 in supporting cell growth could be attributed to its potential participation in Wnt/β-catenin signalling [58], an 'oncogenic' pathway that promotes tumour progression (reviewed in [62]). It is possible that unrestrained RTEL1 activity resulting from its overexpression could lead to the promiscuous disassembly of productive recombination reactions, which could drive genome instability. On the other hand, the strong association of *RTEL1* SNPs with brain cancer susceptibility in humans together with the accelerated frequency of tumour formation and susceptibility to medulloblastomas conferred by the *Rtel1*-PIP mutation in the *p53* null background in mice establish *Rtel1* as a tumour-suppressor gene. Therefore, overexpression or downregulation of *RTEL1* may both contribute to tumourigenesis, depending on the cellular context.

RTEL1 and Hoyeraal–Hreidarsson syndrome

Telomere attrition, a natural biological process that arises from failure to maintain telomere homeostasis, has been linked to an elevated risk of a variety of age-related diseases (reviewed in [63–66]). Critically short telomeres signal the cell to terminate division and enter a stable cell cycle arrest termed senescence (reviewed in [67,68]). Mutations in genes encoding telomere-associated proteins give rise to premature aging disorders such as Werner Syndrome, Hoyeraal–Hreidarsson syndrome (HH), and dyskeratosis congenita (DC).

DC, also known as Zinsser–Engman–Cole syndrome [69], is a rare, inherited bone-marrow condition occurring in one out of 1 000 000 individuals [70]. Diagnosis is based on a triad of clinical symptoms such as mucosal leukoplakia, abnormal skin pigmentation, and nail dystrophy (reviewed in [71]). Other symptoms may include haematopoietic malignancies, thrombocytopenia, pulmonary fibrosis, liver disease, hair loss, and osteoporosis. DC patients live for an average of 30 years, although many die by the age of 15 as a result of bone-marrow failure, pulmonary complications, or cancer (reviewed in [72]). So far, three different forms of DC have been described depending on the pattern of inheritance: autosomal dominant (AD), autosomal recessive (AR) and the most common form, X-linked recessive (XLR) (reviewed in [72]). Importantly, germline mutations have been found in genes that regulate the activity of telomerase (*CTC1, DKC1, NOP10, NHP2, TCAB1, TERC, and TERT*) or the shelterin complex (*TINF2*) [73–79]. Thus, DC is predominantly a syndrome of defective telomere maintenance, whereby patients typically exhibit short telomeres.

HH syndrome is a multisystem disorder that shares many of the abovementioned DC clinical features. Symptoms usually present throughout the first year of life and a definitive diagnosis of HH can be made once a patient presents with 4 of the 6 common symptoms of HH: microcephaly, cerebellar hypoplasia, intrauterine growth retardation, developmental delay, immunodeficiency, or aplastic anemia resulting from bone-marrow failure [80]. Although, HH syndrome-causing germline mutations have been reported in *DKC1*, *TERT*, and *TINF2* genes [75,81,82], the genetic basis of HH is largely unclear and poorly understood. Indeed, 40–50% of DC/HH patients present in the DC registry (located at Barts and The London School of Medicine and Dentistry, UK) are genetically uncharacterised [83–85]. Of these, several recent studies have identified a complex array of *RTEL1* mutations responsible for the onset of DC/HH.

Mutational analysis by whole-exome sequencing performed on two families affected with HH syndrome revealed novel nonsense (p.Arg1010X, p.Arg998X) and missense (p.Glu615Asp, p.Ala645Thr) mutations in *RTEL1* [15]. Siblings affected with HH exhibited autosomal dominant inheritance of the p.Arg1010X biallelic mutation whereas compound heterozygous mutations, p.Glu615Asp and p.Arg998X, were inherited in an autosomal recessive manner. Both the mutations p.Arg1010X and p.Arg998X introduced a premature stop codon in exon 30 of *RTEL1*, which resulted in the deletion of the PIP motif. Although the cellular properties of these mutants have not been determined, it is reasonable to speculate that they may, to some extent, resemble the phenotypes recently reported for the RTEL1-PIP box mutant mice [27]. Interestingly, p.Glu615Asp and p.Ala645Thr mutations have been mapped to exon 22 and correspond with changes to highly conserved residues within the C-terminal helicase domain (helicase_C_2 domain) [15]. Along with the helicase ATP-binding domain, this region of the protein forms the catalytic core of RTEL1, which extends from amino acids 1 to 760 and is believed to be essential for the helicase activity of the protein. Impaired, altered, or loss of function of the C-terminal helicase domain might underlie the common phenotypic consequences observed in these patients, namely short telomeres [15].

Similarly, another study of 10 individuals from 7 families presenting with HH syndrome identified 11 biallelic *RTEL1* mutations with an autosomal recessive pattern of inheritance [18]. The patients harbouring *RTEL1* missense mutations displayed onset of severe bone-marrow failure and immunodeficiency in early childhood. Most patients also presented a similar clinical HH phenotype comprising microcephaly, immunodeficiency, and cerebellar hypoplasia. Interestingly, compound heterozygous substitutions of p.Lys921Glu or p.Arg981Trp were observed in unrelated HH individuals. Cells derived from these patients showed increased frequency of telomeric defects, including T-circle formation with corresponding telomere shortening. These results suggest that the abovementioned mutations impair the ability of RTEL1 to dismantle T-loops properly. Interestingly, these mutations have recently been localised to an ill-defined harmonin N-like/PAH helical fold domain (among other mutations reported in Figure 4) that could potentially form a hub for interaction with partner proteins [86].

In a third study, whole-genome linkage analysis combined with whole-exome sequencing revealed compound heterozygosity for two mutations in *RTEL1* [17]. The missense mutations occurred within generally conserved residues, whereas the p.Ile699Met substitution appeared within the catalytic core of RTEL1 at the helicase domain

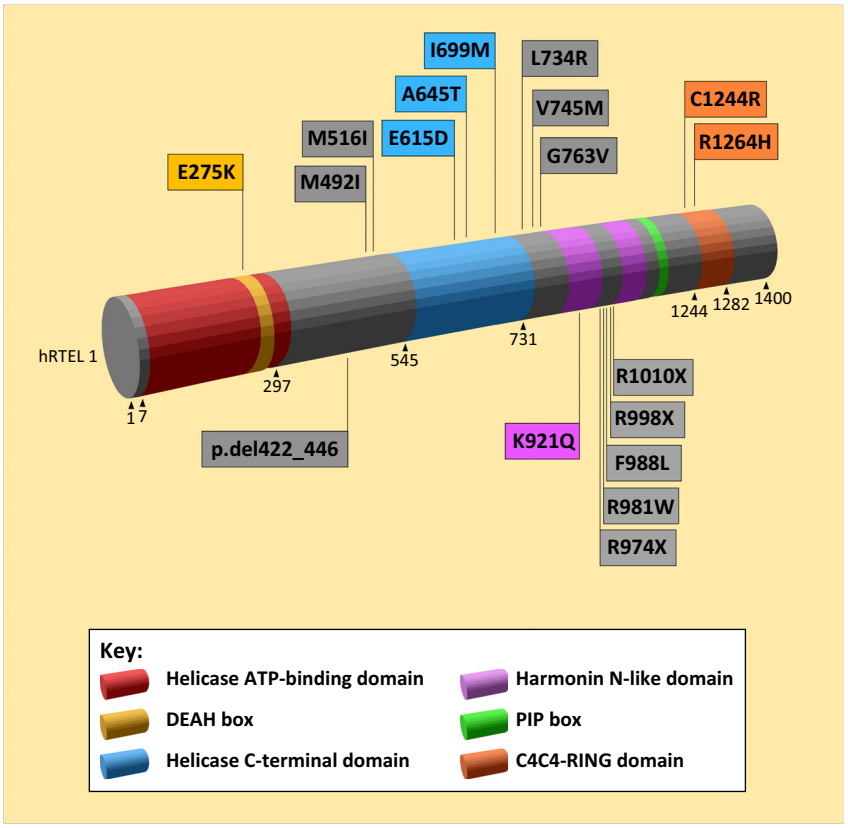

Figure 4. Schematic diagram representing human (h) RTEL1 mutations identified in dyskeratosis congenita (DC) and Hoyeraal–Hreidarsson syndrome (HH) individuals. The distribution of reported mutations along the sequence of hRTEL1 is depicted in coloured boxes. The domains and conserved motifs are colour-coded as follows: red, helicase ATP-binding domain; yellow, DEAH box; blue, helicase C-terminal domain (helicase_C_2_domain); purple, harmonin N-like domain; green, PCNA-interaction peptide (PIP); brown, C4C4-RING finger domain.

1 and 2 (HD1/HD2) boundary. Analogous to the related XPD helicase, HD1/HD2 in RTEL1 likely creates a composite ATP-binding edge that is essential for stability of the ATP-binding site and/or dynamics linked to an ATP-dependent conformational switch [87,88]. Structural changes due to mutations at the HD1/HD2 interface could impact conformational crosstalk between these two domains. Consequently, the noted alterations may either impact DNA-binding activity or disrupt interactions with binding partners, but this has yet to be tested. Another mutation within the C-terminal extension of the HD2 domain (p.Val745-Met) is also predicted to influence the stability of the HD2 structure [17]. Because this extension is important for protein–protein interactions in other SF2 helicases [89], p.Val745Met may also disrupt binding to key partners.

Intriguingly, p.Cys1244Arg and p.Arg1264His mutations map to a C-terminal cysteine-rich domain comprising a $Cys–X_2–Cys–X_9–Cys–X_2–Cys–X_4–Cys–X_2–Cys–X_{10}–Cys–X_2–Cys$ consensus sequence termed C4C4-type RING-finger motif [14,17,18], which is predicted to coordinate metal ions [90]. Patient cells harbouring the p.Arg1264His mutation exhibited a high frequency of heterogeneous telomeres, an increase in telomere sister chromatid exchanges, and elevated telomere loss, similar to the phenotype of cells maintaining their telomeres by alternative lengthening of telomeres (ALT) (reviewed in [91]). Moreover, patient-derived fibroblasts harbouring this mutation present with high levels of T-circles that are dependent on SLX4, similar to T-circle formation in RTEL1-deficient mouse cells [13]. Although the function of the C4C4 motif in RTEL1 remains unclear, it is intriguing that this domain is highly similar to the RING finger present in E3 ubiquitin ligases, raising the possibility that RTEL1 may possess both helicase and E3 ubiquitin ligase activity similar to FBH1 (F-box protein, helicase, 18; FBXO18) [92].

Concluding remarks

Although recent insights into the function of RTEL1 in controlling HR, telomere homeostasis and facilitating DNA replication have highlighted its importance in the maintenance of genome stability, many outstanding questions remain to be addressed. Currently, very little is known about how RTEL1 is regulated or how it is recruited to replication forks and telomeres to execute its functions. The association of RTEL1 with the replisome is evident from its ability to bind directly to PCNA, but the dynamics of this interaction and how it is regulated in response to replication stress remain unclear. RTEL1 appears to be recruited to telomeres to promote T-loop disassembly independently of its association with the replisome, but when and how this occurs is not known. Analogous to other DNA repair helicases, RTEL1 is likely to be subject to extensive post-translational modifications (PTMs) that could restrain or promote RTEL1 activities at specific cell cycle stages. The identification of PTMs of RTEL1 will be a

Table 1. Clinical and molecular features of DC/HH individuals with germline *RTEL1* mutations

RTEL1 mutation	G763V R1264H	F988L		M516I R998X			K921E L734R	del422_446 R981W	E275KR981W	R981W c.102+2T>C	R1010X	
		I	II	I	II	III					I	II
Mode of inheritance	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AD	AD
Zygosity	CHT	HZ	HZ	CHT	CHT	CHT	CHT	CHT	CHT	CHT	HZ	HZ
Clinical features												
BMF	+	+	+	+	+	+	+	+	+	+	+	+
Microcephaly	–	–	+	+	+	+	+	+	–	+	+	+
Cerebellar hypoplasia	–	–	+	+	+	+	+	+	+	+	+	+
Immunodeficiency	+	–	?	+	+	+	+	+	–	+	+	+
IUGR	–	–	+	–	+	+	+	+	–	+	+	?
Developmental delay	–	–	+	–	+	+	+	+	+	–	+	+
Classic DC triad	–/–/–	+/+/–	–/–/–	+/+/–	–/–/–	+/+/–	+/+/–	–/+/–	+/–/–	–/–/–	–/–/–	–/–/–
Telomeric defects												
T-circle formation	NA	NA	NA	NA	NA	+	+	NA	NA	NA	NA	NA
Telomere heterogeneity	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Telomere loss	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Refs	[18]	[18]		[18]			[18]	[18]	[18]	[18]	[15]	

RTEL1 mutation	E615D R998X	A645T	I699M C1244R		V745M c.IVS24+5G	M492I R974X	R1264H	
			I	II			I	II
Mode of inheritance	AR	AR	AR	AR	AR	AR	AR	AR
Zygosity	CHT	HZ	CHT	CHT	CHT	CHT	HZ	HZ
Clinical features								
BMF	+	+	+	+	+	+	+	+
Microcephaly	+	–	+	+	+	NA	+	+
Cerebellar hypoplasia	+	–	+	+	+	NA	+	NA
Immunodeficiency	+	+	+	+	+	NA	+	+
IUGR	?	–	+	+	+	NA	+	+
Developmental delay	+	+	+	+	+	NA	+	+
Classic DC triad	+/+/+	–/–/–	+/+/NA	–/–/–	+/+/NA	NA	–	NA
Telomeric defects								
T-circle formation	NA	NA	NA	NA	NA	–	NA	+
Telomere heterogeneity	NA	NA	NA	NA	NA	NA	NA	+
Telomere loss	NA	NA	+	+	+	+	NA	+
Refs	[15]	[15]	[17]		[17]	[16]	[14]	

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; BMF, bone marrow failure; CHT, compound heterozygous; HT, heterozygous; HZ, homozygous; IUGR, intrauterine growth retardation; NA, data not available.
The classic DC triad is as follows: leukoplakia/nail dystrophy/abnormal skin pigmentation.

considerable challenge given its very low expression levels and the toxicity to cells when RTEL1 is even moderately overexpressed. It is now clear that mutations in RTEL1 are causal for HH/DC and possibly for some brain cancers, but the impact of specific disease-associated mutations/variants on the known functions and regulation of RTEL1 remain to be determined (Table 1). A precise genotype–phenotype assessment of patients carrying *RTEL1* mutations together with comprehensive biochemical and functional analysis of RTEL1 mutant activities is clearly required to elucidate why a particular amino acid substitution leads to a particular defect in the patient. It is also important to consider that mutations occurring in neighbouring amino acids may cause an unrelated disease or phenotype, as is the case for XPD helicase mutations, which can give rise to three different disorders: xeroderma pigmentosum, trichothiodystrophy, or Cockaynes syndrome. In conclusion, it is likely that a detailed mechanistic understanding of the function and regulation of RTEL1 will offer further clues into the impact of disease-causing mutants/variants, and this could be exploited in affected patients.

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