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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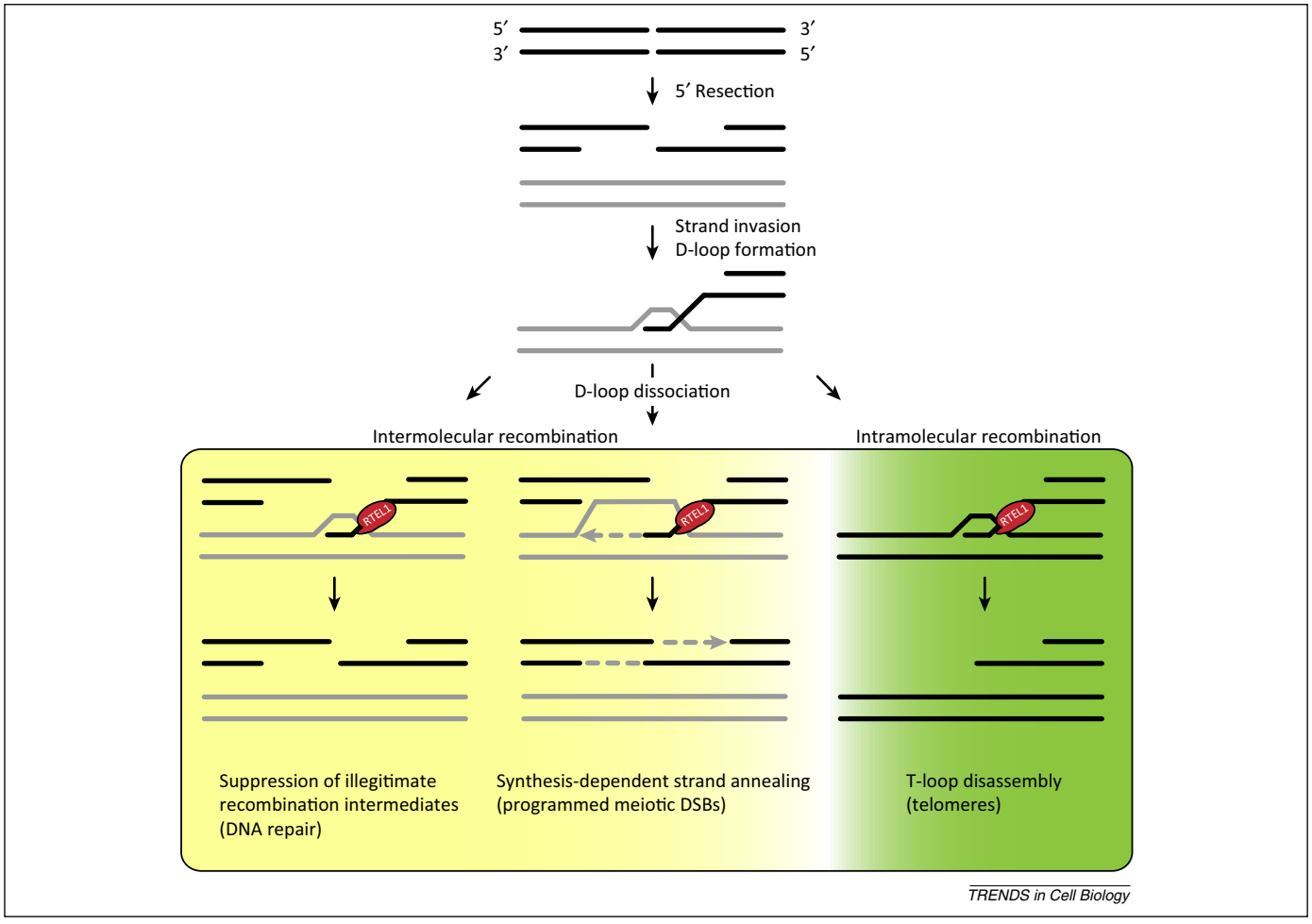

Figure 1. Toxic recombination intermediate substrates for RTEL1 (regulator of telomere length 1). Double-strand (ds) breaks (DSB) formed either from exogenous (ionising radiation) or endogenous sources (programmed meiotic breaks) or DSB naturally present at the end of the chromosome (telomeres) are highly 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