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JB Commentary

RecQL4: a helicase linking formation and maintenance of a replication fork

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RecQ family helicases are conserved from bacteria to human. Across the species, they are known to be required for protecting genome from various genotoxic stresses. In human, five RecQ-related helicases have been identified and three of them, BLM, WRN and RecQL4, have been shown to be responsible for genetic disorders, Bloom, Werner and Rothmund-Thomson syndrome, respectively, which are characterized by cancer predisposition and premature ageing. RecQL4, the N-terminal portion of which shares similarity with Sld2 known to be required for assembly of a replication complex in yeasts, is unique in that it has been shown to be essential for the initiation phase of normal DNA replication. Recent biochemical characterization demonstrated the 3′–5′ DNA helicase activity associated with RecQL4. Understanding the molecular basis for how RecQ helicases are involved in generation and maintenance of normal and stalled DNA replication forks would be crucial to elucidation of the mechanisms of replication initiation as well as to that of how the loss of these conserved helicases leads to varieties of disease phenotypes.

Keywords: Cancer predisposition/DNA helicase/Genomic stability/RecQ/Replication fork.

Abbreviations: CMG, Cdc45-MCM-GINS; DSB, double-stranded DNA breaks; GINS, Go-Ichi-Ni-San for Sld5-Psf1-Psf2-Psf3; MCM, minichromosome maintenance.

DNA helicases play important roles in various nucleic acid transactions including DNA replication, transcription, splicing, repair, recombination and others. In eukaryotic DNA replication, the minichromosome maintenance (MCM) complex composed of six subunits of related structures plays a central role as a replicative helicase. This notion was first indicated by the discovery of DNA helicase activity associated with the purified MCM4-6-7 complex (*1*). At the fork, the MCM2∼7 complex may be associated with Cdc45 and Sld5-Psf1-Psf2-Psf3 (GINS), thus generating a bigger complex [CMG (Cdc45-MCM-GINS)] that is capable of efficient unwinding of duplex DNA (*2*) (Fig. 1A).

RecQ-related helicases are highly conserved through evolution. RecQ of *Escherichia coli* is known to be required for processing of stalled replication forks for genome stabilization (*3*). In human, five RecQ-related helicases have been identified and they have been shown to be required for maintenance of genomic integrity through their participation in DNA replication, recombination and repair (Fig. 1B). They are believed to play particularly important roles when replication forks are stalled by DNA damages or other genotoxic agents (*4*). Among them, mutations in BLM, WRN and RecQL4 were shown to cause cancer-predisposed genetic diseases, Bloom, Werner and Rothmund-Thomson syndrome, respectively. The patients suffering from these syndromes also exhibit premature ageing.

RecQL4, known to be frequently mutated in Rothmund-Thomson and Baller-Gerold syndromes (*5*), has a unique structural feature. Its N-terminal segment shares distinct similarity to Sld2 protein, known to be required for recruitment of DNA polymerases to the replication complex (*6*) (Fig. 1A). Indeed, immunodepletion of RecQL4 protein in *Xenopus* egg extracts lead to loss of DNA replication activity (*6*, *7*). RecQL4 was reported to be required for chromatin binding of DNA polymerase α in DNA replication in *Xenopus* egg extracts (*7*). In human cells, RecQL4 was shown to be required for assembly of CMG complex (*8*). RecQL4 was also reported to interact with MCM2∼7, MCM10, Cdc45 and GINS, and MCM10 is required for efficient interaction between MCM and RecQL4 (*9*) (Fig. 1B). Analyses in human cells, DT40 (a chicken B lymphocyte line), and *Xenopus* egg extracts indicated the roles of RecQL4 protein in various cellular responses to stalled replication fork including S-phase arrest (*10*), double-stranded DNA breaks (DSB) repair (*11*, *12*), nucleotide excision repair (*13*), base excision repair (*14*) and oxidative stress responses (*15*).

In *Xenopus* egg extracts, the N-terminal 596 amino acid segment devoid of DNA helicase domain is sufficient for efficient DNA replication (*7*). In DT40 cells, the cell viability can be maintained by the N-terminal domain lacking the helicase domain, although the cells expressing only the N-terminal domain are sensitive to genotoxic agents (*16*). On the other hand, in *Drosophila*, helicase-dead point mutant RecQL4 could not rescue the null mutant, indicating that

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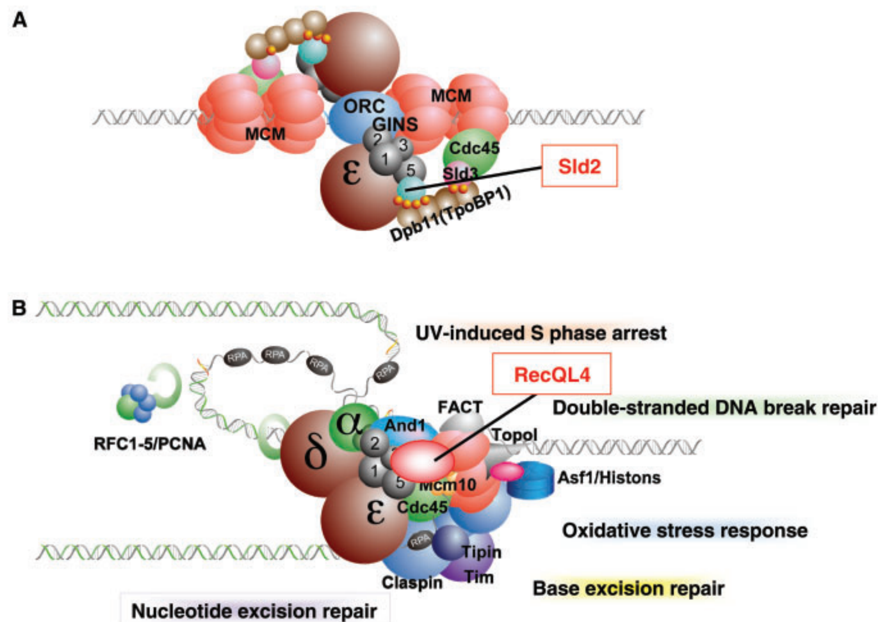

Fig. 1 Sld2/ RecQL4 in initiation and replication fork complexes. (A) A putative initiation complex at replication origin. The model is based on yeast results and nomenclatures use those in budding yeast. Sld2, bound to Dpb11 in a CDK-phosphorylation-dependent manner, recruits GINS and DNA polymerase ε to origins (*22*). Similarly, RecQL4 facilitates the assembly of CMG complex at origins. Although Dpb11 is essential for bringing GINS and DNA polymerase ε to the origin complex, it is not known whether TopBP1 plays similar roles in mammalian cells. (B) A putative replication fork complex. Nomenclatures are those in mammalian cells. RecQL4 interacts with MCM2∼7, MCM10, Cdc45 and GINS and may stabilize the replication fork complex. Upon replication fork stalling, RecQL4 may facilitate various checkpoint/repair processes including S-phase arrest (*10*), DSB repair (*11*, *12*) and nucleotide/base excision repair (*13*, *14*). DNA helicase activity of RecQL4 is likely to play a crucial role at least in this process of stalled fork response.

DNA helicase activity is essential for viability of higher eukaryotes (*17*).

Early report on biochemical characterization of RecQL4 showed DNA-dependent ATPase activity and strand annealing activity, but helicase activity was not detected (*18*). However, more recently, DNA helicase activity was detected in RecQL4 in reactions containing excess single-stranded DNA to prevent reannealing (*19*). Furthermore, two distinct segments of the protein, the conserved helicase domain and the Sld2-like N-terminal domain, independently exhibited DNA helicase activity (*19*). However, Ishimi's group at Ibaraki University discovered that the purified RecQL4 could displace the annealing single-stranded DNA without added single-stranded DNA (*20*). Interestingly, requirement for ATP and magnesium concentrations was different between helicase and ATPase measurements. Higher concentration of ATP was required for helicase assays. It was suggested that previous failure in detecting helicase activity may be due to the low concentration of ATP used in the assays (*20*). RecQL4 could displace 17-mer but not 37- or 53-mers, indicative of its low processivity. The movement of the helicase was from 3′ to 5′, consistent with other RecQ helicases (*20*). The helicase activity of RecQL4 was confirmed later by another report (*21*). In this report, helicase activity was shown to be inactivated by a mutation in the conserved helicase domain, suggesting that the helicase domain is responsible for the observed helicase activity. RecQL4 from *Drosophila* was also shown to possess 3′–5′ helicase activity as well as single-strand DNA annealing activity (*17*). A point mutation replacing a conserved lysine with asparagine leads to complete loss of helicase activity, but not single-strand DNA annealing activity. This also indicates the presence of a unique DNA helicase domain in RecQL4. The discrepancy from the other report (*19*) needs to be clarified by future studies.

Crucial questions remaining include the precise roles of the N-terminal Sld2-releated domain and the C-terminal helicase domain and how the helicase activity of RecQL4 may contribute to the initiation event as well as to the regulation of stalled replication fork. In yeast, Cdk-dependent phosphorylation of Sld2 facilitates its binding to Dpb11, which then recruits DNA polymerase ε (*22*). In *Xenous* egg extracts, RecQL4 is required for chromatin loading of DNA polymerase α (*7*). In human cells, RecQL4 appears to be required for assembly of the helicase complex containing MCM, Cdc45 and GINS (*8*). MCM10 also plays important roles in this process by directly interacting with RecQL4 (*9*). It remains to be seen whether RecQL4 is a target of CDK for loading of other factors.

These results indicate that at least two active helicases, MCM and RecQL4, are involved in initiation of DNA replication in higher eukaryotes. It is an intriguing possibility that both are involved at the replication fork for unwinding of duplex DNA. Indeed, a putative helicase-defective mutant (D605A) of RecQL4 could not restore the replication in RecQL4-depleted Xenopus egg extracts (*6*). However, at the moment, there is no strong evidence that shows the

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constitutive localization of RecQL4 at the active replication fork. The distributive nature of RecQL4 helicase (*20*) is also unsuitable for a replicative helicase at the fork. Furthermore, some reports show that initiation and repair functions could be separable in RecQL4 and only the N-terminal domain lacking the helicase domain could be sufficient for DNA replication (*7*, *16*). Thus, it may be more likely that the helicase activity of RecQL4 may be specialized in the rescue of stalled replication fork. Alternatively, it may also contribute to the initial unwinding step at the replication origin. In either case, the joining of two functionally independent domains in RecQL4 may facilitate the coupling of initiation events to the fork maintenance activities in the DNA chain elongation phase.

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Conflict of interest
None declared.

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