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ATP Binding, ATP Hydrolysis, and Protein Dimerization Are Required for RecF to Catalyze an Early Step in the Processing and Recovery of Replication Forks Disrupted by DNA Damage

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In *Escherichia coli*, the recovery of replication following disruption by UV-induced DNA damage requires the RecF protein and occurs through a process that involves stabilization of replication fork DNA, resection of nascent DNA to allow the offending lesion to be repaired, and reestablishment of a productive replisome on the DNA. RecF forms a homodimer and contains an ATP binding cassette ATPase domain that is conserved among eukaryotic SMC (*s*tructural *m*aintenance of *c*hromosome) proteins, including cohesin, condensin, and Rad50. Here, we investigated the functions of RecF dimerization, ATP binding, and ATP hydrolysis in the progressive steps involved in recovering DNA synthesis following disruption by DNA damage. RecF point mutations with altered biochemical properties were constructed in the chromosome. We observed that protein dimerization, ATP binding, and ATP hydrolysis were essential for maintaining and processing the arrested replication fork, as well as for restoring DNA synthesis. In contrast, stabilization of the RecF protein dimer partially protected the DNA at the arrested fork from degradation, although overall processing and recovery remained severely impaired.

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Introduction

RecF is part of a ubiquitous family of recombination mediator proteins that includes Rad52, BRCA2, BLM, and WRN. These proteins are required to maintain genomic stability, but their precise cellular functions remain poorly understood.[1–7] The structure of the RecF protein of *Escherichia coli* reveals a strong similarity to the globular head domain of human Rad50, a protein involved in the detection and repair of double-strand DNA breaks.[8] Both RecF and Rad50 contain a conserved ATP binding cassette ABC-type ATPase, which is conserved among many structural maintenance of chromosome (SMC) proteins, DNA repair enzymes, and membrane transporters.[9–13]

In *E. coli*, RecF function, together with RecO and RecR functions, is required for replication to resume following disruption by DNA damage.[14–17] In the absence of any one of these genes, replication forks are not maintained following arrest, nascent DNA at the arrested fork is extensively degraded, and DNA synthesis fails to resume.[15,16,18] Either coordinately with or subsequent to RecF–RecO–RecR binding, the nascent lagging strand of the arrested fork is partially degraded by the combined action of RecQ, a 3′–5′ helicase, and RecJ, a 5′–3′ nuclease.[16,18] Nascent DNA degradation is thought to restore the lesion-containing region to a double-strand form that can be repaired by nucleotide excision repair, and it is essential for the rapid recovery of DNA synthesis.[19,20] In the absence of either processing or repair, the recovery of replication remains dependent on the RecF pathway, but occurs through the action of translesion synthesis polymerases.[19,20]

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Abbreviations used: ABC, ATP binding cassette; SSB, single-strand DNA binding protein.

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Once the lesion is removed or bypassed, an active and functional replisome must be restored to allow replication to resume.[19,20] The precise role of RecF in processing the disrupted replication forks remains unclear and could occur at any of several steps during the recovery process, including the initial binding and recognition of the disrupted forks, the processing or regression of the fork structure away from the offending lesion, or the reestablishment of an active replisome once the lesion has been removed or bypassed (Fig. 1).

The crystal structure and solution studies of the RecF protein revealed that the protein forms a dimeric clam-like structure and contains an ABC ATPase domain.[8] Similar to the ABC ATPase domains found in other SMC-like proteins, RecF contains three conserved motifs termed the Walker A motif, Walker B motif, and signature motif. On RecF, the Walker A and Walker B motifs are located in ATPase domain 1 at the N-terminus and the C-terminus, respectively, while the signature motif resides in domain 2 on the protein.[8] The signature motifs of ABC ATPases mediate ATP-dependent dimerization, with ATP bound at the interface of two opposing molecules.[25–28] RecF lacks the striking coiled-coil region between the N-terminus and the C-terminus of the globular head domain seen in other SMC-like proteins and Rad50, but is otherwise structurally similar to Rad50 and exhibits both ATP-dependent DNA binding and DNA-dependent ATP hydrolysis characteristic of SMC proteins.[8,29–31]

The precise catalytic function(s) of the conserved RecF motifs in processing and restoring arrested replication forks remains uncharacterized, although a number of biochemical characterizations are consistent with the idea that they could participate in any of the several progressive steps associated with the recovery process. Purified RecF, RecO, and RecR are able to displace single-strand DNA binding protein (SSB) and to enhance the nucleation of a RecA filament on DNA.[32–35] Although RecO and RecR proteins are sufficient to perform this reaction *in vitro*, RecF greatly stimulates the process in the presence of double-strand DNA fragments.[5,21,22] Furthermore, RecF appears to play an important role in targeting this nucleation to regions that contain a single-strand–double-strand DNA junction and have a 5′ DNA end.[5,21,29] These observations are consistent with the idea that RecF may act to recognize the arrested replication forks and to catalyze the loading of a RecA filament.

Other studies have suggested that RecF modulates both the ability of RecA filaments to form on single-strand regions and the RecA-mediated strand exchange reaction in a way that would enhance fork regression.[34,36,37] Consistent with this idea, *in vitro*, RecF cycles through a complex pathway that involves ATP-dependent dimerization, DNA binding, and repeated interactions with RecR in a DNA-substrate-dependent manner.[21,31,34]

Still other studies support the idea that RecF–RecO–RecR, along with RecA, may functionally interact with the replication machinery and may have a direct role in removing polymerase from its arrest site or in reestablishing the replisome after the

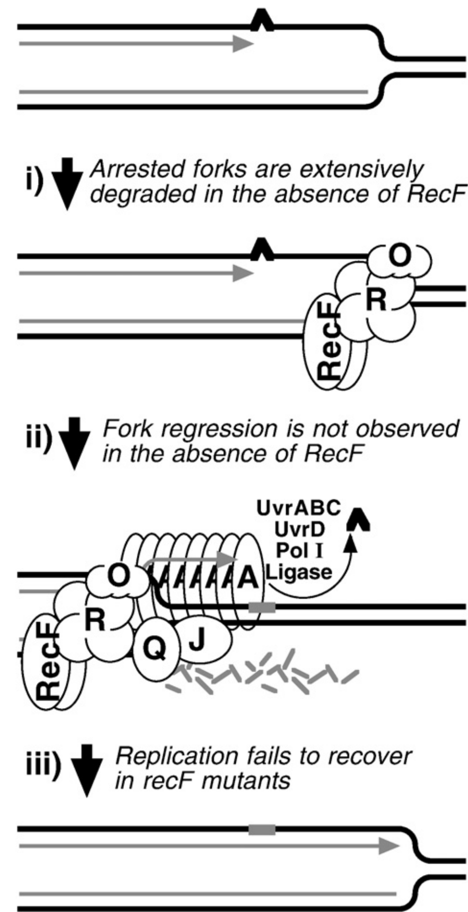

Fig. 1. Processing events involved in the recovery of replication following disruption by DNA damage and the phenotypes observed in the absence of RecF. (i) Replication is arrested following UV-induced DNA damage. *In vitro*, RecF is proposed to direct RecR and RecO to DNA junctions and to initiate the formation of a RecA filament at these sites, an activity that has been proposed to help target RecA to the arrested replication fork *in vivo*.[21,22] (ii) Either subsequent to or concurrent with this activity, the RecQ helicase and RecJ nuclease partially degrade the nascent lagging strand of the arrested DNA fork. The partial degradation is required for the rapid recovery of replication and is thought to restore the region containing the lesion to a double-strand form that allows nucleotide excision repair to remove the obstructing lesion.[18,20] In the absence of RecF, this degradation is much more extensive and eventually leads to the loss of the replication fork's integrity.[23] (iii) Once the lesion has been removed, an active replisome must be reestablished. It is not clear which components of the replisome are disrupted upon encountering a UV-induced lesion, although some evidence suggests that the replicative helicase remains bound,[24] suggesting that the replisome may remain at least partially intact. It is possible that RecF, along with RecO–RecR and RecA, may stabilize the nascent leading strand on the template to allow the replisome to resume from this structure. In the diagram, RecA, RecO, RecR, RecQ, and RecJ are denoted as A, O, R, Q, and J, respectively.

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