span as does tRNA. Termination chemistry is built into the peptidyl transferase (see Kisselev and Buckingham, 2000), itself an ancient ribosomal ribozyme (Nissen et al., 2000) likely present in similar form since the RNA world (Yarus and Welch, 2000). Presumably only prodding of peptidyl transferase by another molecule that stretched to the termination codon would be required for primordial termination. A somewhat similar idea has actually been around for some time but with another emphasis. It is said that eRF1 (and other translational proteins) are "tRNA mimics," meaning that they take the shape of tRNA (Nakamura et al., 1996). We may perhaps take this notion more literally, as an indication of origins. Class I protein RFs may be ghosts of a vanished tRNA mechanism. Does some trace of these release RNAs still exist somewhere?

A possibility immediately presents itself. The tmRNAs act as both tRNA and mRNA, providing an alternative termination-and-peptide-tagging mechanism for messages without stop codons (Keiler et al., 1996). The tmRNAs are most of what we seek: tRNA analogs, responsible for special translational termination. These molecules are usually assumed to use protein RFs for termination at their own termination codons. However, it does not seem to be known whether they necessarily employ protein RFs or alternatively can stimulate the hydrolytic activity of peptidyl transferase on their own. This now needs clarification. More speculatively, one cannot help but wonder if tmRNAs have yet unrecognized relatives that would appear if RNAs that simply terminated translation were widely sought.

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A Step Backward in Advancing DNA Replication: Rescue of Stalled Replication Forks by RecG

In the October 5 issue of *Cell*, Singleton et al. report the crystal structure of RecG protein bound to an analog of a stalled DNA replication fork. This structure shows how RecG can recognize branched DNA structures and suggests a mechanism for fork reversal, an early event in recombination-dependent reinitiation of DNA replication.

Under normal growth conditions, most bacterial DNA replication forks encounter roadblocks, in the form of DNA damage, secondary structure, or protein-DNA complexes, that halt their progress prior to completion of chromosomal replication. Incomplete replication would prove lethal for at least one daughter cell, were it not for recombination-dependent mechanisms that rescue replication by reinitiating DNA synthesis in an origin-independent manner (Kogoma, 1996). Various pathways have been discussed for recombination-dependent replication restart (Kowalczykowski, 2000; McGlynn and Lloyd, 2000, 2001; Michel et al., 2001). If the fork stalls and the template strands remain intact, the critical first step in this process is to drive the fork backward to form a Holliday junction (Figure 1). Thereafter, the nature of the restart process will depend on the initial cause of the stalling and the resulting local DNA structure formed. In one suggestion of McGlynn and Lloyd, if leading-strand synthesis stalls prematurely due to a base lesion, reversal of the fork to a point beyond the damage in the template strand would allow priming of leading-strand replication by template switching (Figure 1). Holliday junction migration would then restore the fork for assembly of the replisome.

A growing body of genetic and biochemical evidence from the Lloyd laboratory suggests that *E. coli* RecG protein catalyzes the fork reversal reaction and that this constitutes a major pathway for replication restart. RecG is a monomeric, 76 kDa protein containing Superfamily 2 (SF2) helicase motifs. Although primary structure analyses suggest that RecG homologs are not ubiquitous, genetic complementation and biochemical studies identify phage T4 UvsW, a protein whose in vivo function is to displace R loops, as a functional homolog (Dudas and Kreuzer, 2001). Therefore, a RecG-like function may be present in many organisms. Like UvsW, RecG's unwinding activity is specific for branched DNA structures. It is capable of displacing R and D loops and drives Holliday junction migration. More recently, it was shown that the enzyme promotes conversion of a replication fork into a Holliday junction in vitro and in vivo (McGlynn and Lloyd, 2000, 2001). While RecA protein and positive supercoiling also promote replication fork reversal, RecG promotes fork reversal even under the inhibitory conditions of negative supercoiling (McGlynn et al., 2001). Despite the considerable progress of the Lloyd laboratory, the molecular details of these remarkable DNA manipulations have remained unclear.

In this month's issue of *Cell*, Wigley and coworkers describe the crystal structure of *Thermatoga maritima* RecG in complex with an analog of a stalled replication fork (Singleton et al., 2001). The DNA substrate is a three-way junction consisting of a 10 bp template arm,

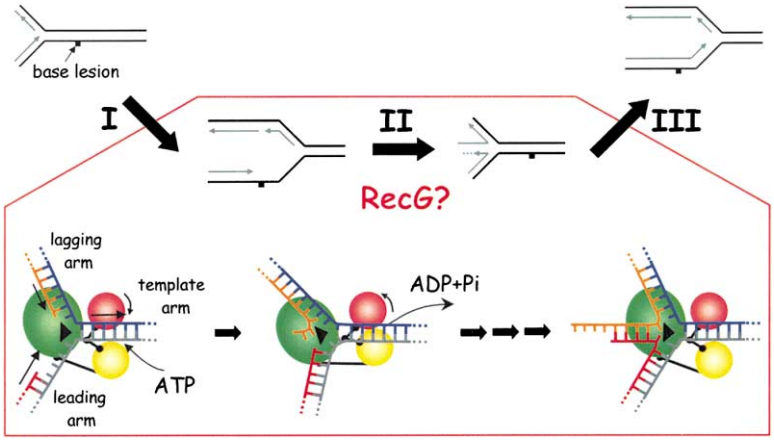

Figure 1. A Suggested Role for RecG in the Rescue of a Stalled Replication Fork

(Top) Template switching mechanism for replication fork rescue. Step I, leading strand synthesis stalls prematurely due to a base lesion in the template. Step II, replication fork reversal catalyzed by RecG protein. Step III, leading strand synthesis (primed by the nascent lagging strand) is followed by branch migration to recover the fork.

(Bottom) Fork reversal mechanism proposed by Singleton et al. Domains 1, 2, and 3 of RecG are green, yellow, and magenta, respectively. The triangle represents the wedge of domain 1. ATP hydrolysis-dependent motor activity pulls the template arm (blue/gray) over domains 2 and 3. This reseals the parental duplex and breaks apart the lagging duplex at the wedge domain. After multiple rounds of hydrolysis, the nascent leading strand (red) is encountered and a fourth duplex arm (orange/red) forms at the wedge domain. Figure modified from Singleton et al.

a single base gap followed by 9 bp in the lagging arm, and a 10 base single-stranded leading arm. It was designed to mimic the intermediate formed if synthesis terminates on the leading strand before the lagging strand (Figure 1). A similar fork was shown to be the best substrate for RecG in vitro (McGlynn and Lloyd, 2001), but it should be noted that it is atypical of stalled or collapsed replication forks in general, which may contain a wide variety of local DNA structures. The structure of RecG protein reveals a monomer consisting of three domains. The large N-terminal domain 1 is involved in binding the three-way junction in a square planar arrangement. This is reminiscent of the DNA structure in the RuvA-Holliday junction complex, but with one arm missing, hinting that the product of RecG catalysis could indeed be a four-way junction. Domain 1 contains a "wedge" structure, where the splitting of the duplexes at the junction is overseen by aromatic stacking contacts with orphan bases. One base is even caught in the process of flipping between the template and lagging arms.

Ahead of the DNA fork, the template arm extends toward domains 2 and 3. These contain the SF2 helicase motifs and form a tandem "RecA-like" domain structure characteristic of many helicases. Although the substrate does not reach the expected site of interaction with these motor domains, the authors draw on lessons learned from structural and biochemical studies of other SF2 helicases to fill in the gaps. Superposition of the helicase domains of the HCV NS3 helicase-ssDNA complex onto the equivalent regions of RecG reveals the probable pathway of the template arm. The authors' proposal that the template arm will contact the helicase domains as intact duplex suggests a simple model for fork reversal (Figure 1). Pulling the template duplex over the surface of these domains would force the leading and lagging strand duplexes to separate at the wedge domain, resealing the parental duplex. The complementary nascent single strands released in this process would then be suitably positioned to associate and form the fourth duplex arm of a Holliday junction. Interestingly, this elegant model for fork reversal would be equally applicable to R and D loop displacement and branch migration reactions, explaining the substrate promiscuity observed in studies of RecG in vitro.

Aside from the functional insights into RecG, the authors' model has an intriguing mechanistic implication for the large group of proteins containing helicase motifs. Nearly a decade ago, sequence analyses resulted in the classification of helicases into five families and the identification of seven motifs characteristic of strand separation activity (Gorbalenya and Koonin, 1993). With the recent explosion of genomic sequence information, many more proteins have been classified as helicases, but it is becoming clear that some do not possess the ability to separate nucleic acid strands. On the basis of structural and biochemical studies of PcrA, an SF1 DNA helicase, Wigley and colleagues proposed that the so-called "helicase" motifs form domains that are in fact responsible for an autonomous ssDNA translocation activity (Velankar et al., 1999). This hypothesis is now supported and extended by the RecG structure. The authors' model envisages the helicase domains simply as a double-stranded DNA translocation motor. Although strand separation does occur between the template and nascent strands behind the reversing fork, this is a consequence of the manner in which the leading and lagging duplexes are guided apart by the wedge domain. The *direct* function of the helicase domains is only to act as a motor, moving the protein along the template strands and resealing the parental duplex. Indeed, this activity might be thought of as quite the opposite of a classical strand separation reaction. Their model implies that the canonical helicase reaction is only performed by a subset of those enzymes containing the signature motifs. In reality, the motifs may define a modular structure that is found in a diverse group of ss- or dsDNA translocases. The motor activity could be harnessed in a variety of ways by accessory domains to accomplish a range of DNA processing events. Such a model would explain the presence of these motifs in proteins that do not appear to catalyze strand separation and/or for which

there is no need to invoke strand separation to explain biological function.

The new structural data are a leap forward in our understanding of RecG function at the molecular level. However, recombination-dependent replication restart is thought to involve upward of two dozen proteins operating in multiple, poorly defined pathways. This probably reflects the need for different strategies to reset collapsed or stalled replication forks that contain a variety of local DNA structures. Clearly much genetic, structural, and biochemical work remains before we fully understand the complex interplay and regulation of these essential processes.

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