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DNA Replication: Keep Moving and Don't Mind the Gap

Short Review

Lance D. Langston[1] and Mike O'Donnell[1,*]
[1] Howard Hughes Medical Institute
The Rockefeller University
1230 York Avenue
New York City, New York 10021

As the replication fork progresses, synthesis of the discontinuous lagging strand requires frequent priming and cycling of the lagging strand polymerase to the new primers. It appears that this mechanism also permits bypass of template lesions on both strands, leaving the damage behind in a single-strand gap and precluding fork stalling or collapse.

Introduction

The standard view of DNA replication is that it is semi-discontinuous, with the leading strand synthesized as a single uninterrupted chain and the lagging strand as a series of short (<2 kb) Okazaki fragments. Despite the prevalence of this view and the support it has gained from in vitro studies, it is not in agreement with cytological evidence in *E. coli*, which routinely shows discontinuities in both strands during DNA replication (reviewed in Wang [2005]). This does not necessarily imply that there is a regular cycle of reinitiation on the leading strand, but it suggests that priming can and does recur on the leading strand as the replication fork moves away from the origin, most likely in response to damage on the template DNA molecule. This review describes our present understanding of the process of discontinuous DNA synthesis on the lagging strand. We also discuss new emerging views of replication in which differences in the mechanisms of leading and lagging strand synthesis are blurred by the need to deal with ever-present lesions on both template strands.

Replisome Dynamics in *E. coli*

Numerous different proteins act together to advance a DNA replication fork. In aggregate, these diverse protein actors are referred to as a "replisome," and the structure and function of the *E. coli* replisome are illustrated in Figure 1. Parental duplex DNA is unwound by the homohexameric DnaB helicase as it translocates along the lagging strand template in the 5′ to 3′ direction just ahead of the leading strand polymerase (Figure 1A). The helicase activity of DnaB is greatly stimulated by interaction with the τ subunit of DNA polymerase III holoenzyme (Kim et al., 1996). DNA polymerase III holoenzyme is a multiprotein complex comprised of two heterotrimeric Pol III core polymerases ($\alpha\varepsilon\theta$ subunits), two homodimeric β sliding clamp processivity factors, and a single γ complex clamp loader ($\gamma_1\tau_2\delta\delta'\chi\psi$) (reviewed in Johnson and O'Donnell [2005]). The C termini of the τ subunits protrude from the γ complex clamp loader and bind two separate assemblies of Pol III core. These connections enable the clamp loader to serve as a structural bridge between the replicative helicase and the leading and lagging strand polymerases at the prow of the replication fork.

Cellular DNA polymerases cannot initiate synthesis in the absence of a nucleic acid primer, so the first step in DNA synthesis is the formation of a short RNA primer (~10 nt) by specialized RNA polymerases known as primases (Kornberg and Baker, 1992). In principle, leading strand synthesis requires only a single priming event, whereas frequent repriming is the hallmark of discontinuous lagging strand synthesis. The distribution of primers, ~1–2 kb apart on the lagging strand, is governed by dynamic interactions between DnaB and the DnaG primase (Tougu and Marians, 1996) and possibly, according to a recent report, by interactions between separate primase molecules bound to a single DnaB on the lagging strand (Corn et al., 2005).

Primase remains bound to the 3′ terminus of the RNA primer through contact with SSB, the single-strand DNA (ssDNA) binding protein, which binds and protects ssDNA ahead of the primer (Figure 1A). The Pol III core polymerase then replaces primase at the primer terminus in a three-part switch activated by the χ subunit of the γ complex clamp loader (Yuzhakov et al., 1999). χ displaces primase by competitive binding to SSB, whereas the clamp-loading subunits of γ complex (the AAA+ proteins γ, τ_2, δ, and δ') form a helical structure that completely encases the newly cleared primer-template junction (for a detailed review of clamp loaders and how their structures confer specificity for the primed site, see O'Donnell and Kuriyan [2006]). In the second part of the switch, the clamp loader binds ATP, opens the ring-shaped β clamp, and positions it around the RNA-DNA duplex (Figure 1B). ATP hydrolysis ejects the clamp loader from the primer-template junction and releases the clamp, allowing it to close around the duplex (Figure 1C) (Ason et al., 2003). Pol III core then binds the β clamp to complete the switch and begin synthesis of a new Okazaki fragment (Figure 1D). When bound to Pol III core, the β ring slides along the DNA with the polymerase, converting it into a highly processive enzyme capable of extending DNA chains over 50 kb without dissociating.

The *E. coli* replication fork moves about 1 kb per second under normal circumstances. Okazaki fragments are 1–2 kb in *E. coli*, so new primers must be synthesized on the discontinuous lagging strand every few seconds. The clamp loader is capable of rapid and repeated loading of new β dimers onto primed sites as they are generated, so this is not a limiting step, but a longstanding conundrum in lagging strand synthesis has been to rationalize how the lagging strand polymerase lets go of DNA after it completes an Okazaki fragment (Figures 1B and 1C). The interaction between β and Pol III core on DNA is very tight, with a dissociation half-life of more than 5 min when bound to the primer-template junction. With only 10–20 molecules of polymerase in the cell, and new Okazaki fragments being produced every few seconds, the lagging polymerase must be used repeatedly. But how does the lagging strand Pol III core

*Correspondence: odonnel@mail.rockefeller.edu

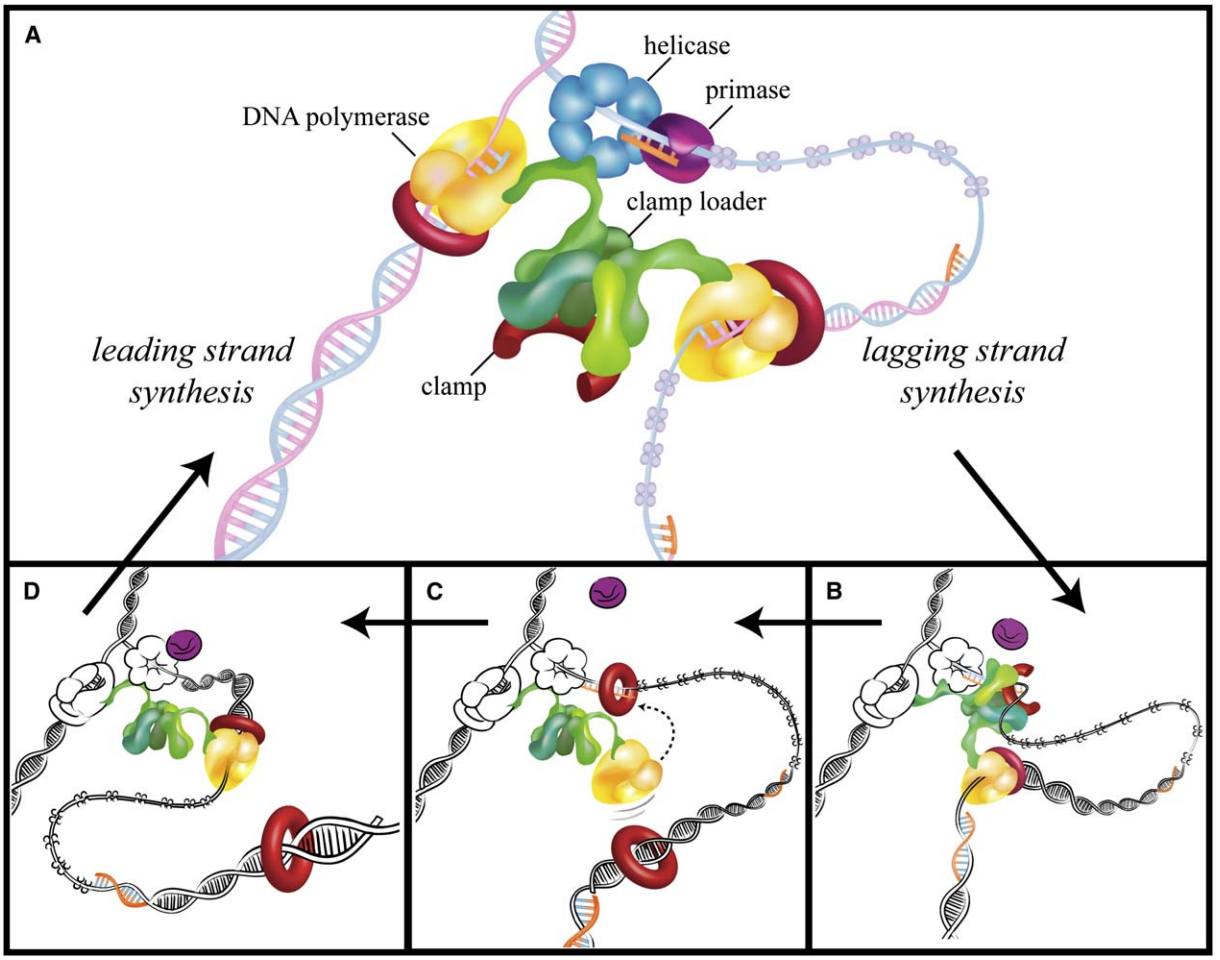

Figure 1. The Cycle of Lagging Strand Synthesis on an Undamaged Template

(A) As the lagging strand polymerase synthesizes an Okazaki fragment, the clamp loader opens a new clamp and the helicase recruits primase to the replication fork to initiate the next fragment.

(B) After synthesis of the RNA primer, the clamp loader displaces primase and loads the clamp onto the new primer/template junction.

(C) Completion of Okazaki fragment synthesis triggers recycling of the lagging strand polymerase to the newly loaded clamp, leaving the old clamp behind.

(D) The lagging strand polymerase synthesizes the new Okazaki fragment, completing a full cycle. Fork unwinding and leading strand synthesis continue throughout the cycle. The authors are grateful to Dr. Nina Yao for the artwork.

rapidly disconnect from the ring-shaped clamp when it finishes a fragment?

So far, two mechanisms have been described for disconnecting Pol III core from β on the lagging strand. One of these is an intramolecular signaling process within the Pol III holoenzyme that is triggered when Pol III core finishes an Okazaki fragment and encounters the 5′ terminus of a previous fragment. Often referred to as the "collision mechanism," this intramolecular signal is mediated by the τ subunit of γ complex, which attenuates the strength of the β-Pol III core interaction and reduces the dissociation half-life from 5 min to far less than 1 s (Leu et al., 2003; Stukenberg et al., 1994). This frees the lagging strand DNA polymerase from β and allows it to cycle to the new primer-template junction to begin synthesis of the next Okazaki fragment (Figure 1C). A similar mechanism has been shown to function during replication of bacteriophage T4 (Hacker and Alberts, 1994). When the lagging strand Pol III core cycles to a new primer, the β clamp is left behind on the completed DNA molecule. β binds to many other proteins, including DNA polymerase I and DNA ligase, and may coordinate their actions in removing RNA primers and covalent joining of the completed Okazaki fragments into a continuous DNA molecule (Johnson and O'Donnell, 2005).

An alternative mechanism involves premature release of the polymerase, which disengages from its clamp before Okazaki fragment synthesis is complete (Li and Marians, 2000). A recent study suggests that the premature release mechanism may be common during bacteriophage T4 replication (Yang et al., 2006). The authors propose that the signal for premature release is the assembly of a new clamp on an upstream primer; however, this is difficult to prove conclusively, as a clamp must be loaded on the primer in order to obtain an extension signal in the first place. The relative contribution of the collision and premature release mechanisms to ordinary cycling of the lagging strand polymerase remains to be determined, but it seems likely that the ability of