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Minireview

Polymerase Switching in DNA Replication

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Our view of DNA replication has been of two coupled DNA polymerases anchored to the replication fork helicase in a "replisome" complex, synthesizing leading and lagging strands simultaneously. New evidence suggests that three DNA polymerases can be accommodated into the replisome and that polymerases and repair factors are dynamically recruited and engaged without dismantling of the replisome.

Current View of the Replication Machine: Right and Wrong

Bruce Alberts first suggested that DNA replication occurred within a large replisome complex, with coupled polymerases catalyzing leading- and lagging-strand synthesis at the same rate, anchored to the replication fork helicase (Alberts et al., 1975). Elements of this model, known as the "trombone model," (Figure 1) have been confirmed in a number of systems: leading- and lagging-strand polymerases are coupled but have asymmetric properties, DNA loops predicted to be formed on the lagging strand can be observed by electron microscopy, and the lagging-strand polymerase recycles to continue synthesis without dissociation from the fork.

Nonetheless, the simple (and prevalent) view that single DNA polymerases are bound continuously to their substrates appears to be incorrect. Rather, recent experiments in bacterial and bacteriophage reconstituted replication systems demonstrate a highly dynamic exchange of polymerases. This should probably not surprise us, since replication of a bacterial genome would otherwise demand extraordinary performance from a single molecule, more than 10^6 synthetic reactions over the course of close to an hour, without falter. Polymerase exchange most likely plays a critical role to correct lesions, overcome barriers in the DNA template, or replace a damaged polymerase without dismantling of the replisome structure.

Bacteriophage T7: A Simple Dynamic System

Bacteriophage T7 replication can be reconstituted with only four proteins and reveals a valuable, streamlined system to examine interactions that solve the problems inherent in DNA replication (background references may be found in Benkovic et al. [2001]). The T7 DNA polymerase consists of the T7 gene 5 product (gp5) in a tight complex with the *E. coli* host protein, thioredoxin. Unlike bacterial DNA and T4 bacteriophage polymerase, the T7 DNA polymerase does not employ a separate protein "clamp" to ensure processive synthesis; rather, thioredoxin binding promotes the C-terminal region of gp5 (the thioredoxin binding domain, TBD) to assume a claw-like structure, allowing it to encircle and tether the polymerase to the DNA substrate. DNA helicase and primase activities reside in the multifunctional gp4 protein. T7 gp2.5 protein, the single-strand DNA binding protein (SSB), makes interactions with both the DNA polymerase and the helicase.

The T7 replication system illustrates a mode of "dynamic processivity" revealed recently by two types of experiments (Hamdan et al., 2007; Johnson et al., 2007) similar to the reconstituted T4 replication system as described previously in a landmark paper (Yang et al., 2004). In the first experiment, replication proteins bind the labeled replication substrates and are later diluted into unlabeled DNA mixtures that will trap any dissociated protein. Under such conditions, the amount of DNA synthesized provides a measure of processivity of the enzyme, which is many thousands of nucleotides. In the second type of reaction, synthesis of the leading strand or coupled leading and lagging strands is initiated with a mutant DNA polymerase insensitive to inhibition by dideoxynucleotides and then challenged by the addition of wild-type polymerase, fully sensitive to inhibition. Under such conditions, synthesis ceases almost immediately: rapid polymerase exchange is indeed observed.

How can the observed high polymerase processivity be accommodated with free polymerase exchange? The idea is that the polymerase experiences transient dissociations from its substrate while remaining bound to the fork via interactions with the gp4 helicase. The helicase could bind "spare" polymerases, allowing them to be swapped onto the template during one of these excursions of the initial polymerase (Figure 2).

This dynamic behavior appears to involve multiple modes of interaction with the helicase protein: one while the polymerase is engaged with its substrate and another in reserve mode. When T7 DNA polymerase is bound to a primer-template DNA structure, the interaction with gp4 helicase is very tight and occurs at an unknown site within both proteins (Hamdan et al., 2007). When not bound to a primer-template DNA molecule, the polymerase interacts more loosely via basic loops in the C-terminal region of gp5 and the acidic C-terminal tail of gp4 (Hamdan et al., 2005; Lee et al., 2006). This latter electrostatic interaction is required for very high processivity and for

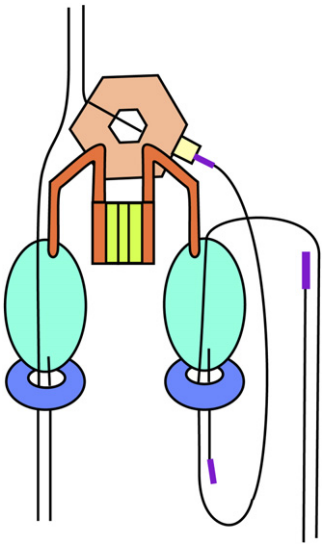

Figure 1. The Trombone Model for Coupled Leading- and Lagging-Strand Polymerization, as Modeled for the *E. coli* Replisome

Two DNA polymerases (turquoise) bound to processivity clamps (blue rings) are coupled to each other and to the fork helicase (tan hexagon) via interactions with τ (orange) of the clamp loader complex (other subunits in green). RNA primers (purple) to initiate Okazaki fragments are synthesized by primase (yellow) associated with the helicase.

polymerase exchange—that is, the "reserve" mode (Hamdan et al., 2007). Richardson and coworkers propose a switch between the two binding modes, responsive to primer-template DNA (Hamdan et al., 2007). When the polymerase disengages from its template, the electrostatic mode is enlisted to ensure association of polymerase with the replisome. Upon rebinding to the primer-template DNA, the polymerase is locked down tightly via other interactions with the helicase.

The helicase may therefore travel with two engaged polymerases and a spare, switching to the spare occasionally, which achieves high processivity of the complex concomitant with polymerase exchange. With three potential polymerases in the replisome, it is possible that the lagging strand engages a DNA polymerase on a new primer prior to completion of the previous Okazaki fragments, with two engaged lagging-strand polymerases (see Figure 3D, below). Recent electron microscopic analysis of T4 replication forks with biotin-labeled DNA polymerases suggests that almost one-third of forks carry three polymerases, and some of these exhibit multiple DNA loops, consistent with the engagement of two polymerases on the lagging strand at the same time (Nossal et al., 2007).

The *E. coli* Replisome: A Scaffold for Three Polymerases

The *E. coli* replisome exhibits dynamic properties, important for replication fork processivity and repair (background references and details can be found in Johnson and O'Donnell [2005]). *E. coli* DNA polymerase III is the replicative enzyme: the core DNA Pol III complex consists of a polymerase subunit, α, encoded by *dnaE*; a 3′ to 5′ editing subunit, ε, encoded by *dnaA*/*mutD*; and an additional protein, θ, *holE*. The processivity clamp, β, the *dnaN* gene product, encircles the DNA and recruits DNA pol III core complexes. Clamps are loaded and unloaded via a pentamer of three subunits of DnaX products (γ or τ) and one subunit each of δ (*holA*) and δ′ (*holB*). Accessory to the γ or τ complex are ψ (*holD*) and χ (*holC*), which interact with SSB, facilitate switching between DNA polymerase and primase at RNA primers, and stabilize the clamp loader complex. The fork helicase is a hexamer of DnaB subunits, which encircles the lagging template strand and unwinds DNA at the replication fork. DnaB recruits the primase, DnaG, which distributively synthesizes RNA primers on the lagging strand. τ of the clamp loader complex makes interactions that couple the DNA polymerase cores with the loader and DnaB (Figures 1 and 3).

The composition of the clamp loader complex has been subject to some revision over the years. The complexity

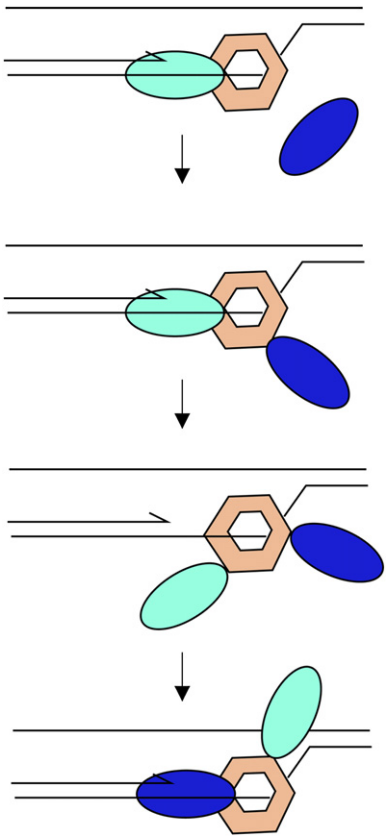

Figure 2. Polymerase Exchange, as in the T7 Bacteriophage Replication System

Only leading-strand synthesis is shown for simplicity. DNA polymerase (turquoise oval) is bound to the primer terminus and template and tightly associated with the fork helicase (tan hexagon). Other polymerases (blue oval) can bind more loosely to other sites on the helicase. The engaged polymerase releases the terminus transiently, allowing polymerase exchange.