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Essential protein interactions within the replisome regulate DNA replication

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Replisomes are protein assemblies that function as molecular motors to mediate template-directed polymerization of nucleotides, unwinding of DNA, synthesis of RNA primers and the assembly of proteins at the replication fork. A major role of the replisome is to coordinate the reactions mediated by its protein constituents, so that leading- and lagging-strand synthesis proceed at the same rate. The replisome is a dynamic structure with the release and recruitment of proteins as the replisome proceeds.

Bacteriophage T7 has evolved an efficient replisome consisting of four proteins; gene 5 DNA polymerase (gp5), *Escherichia coli* thioredoxin (trx)-processivity factor, gene 4 helicase-primase (gp4) and gene 2.5 ssDNA binding protein (gp2.5)[1] (**Fig. 1**). Gp5 forms a high affinity complex with trx (gp5/trx), increasing the processivity from a few to 800 nucleotides. The helicase domain of gp4 assembles on DNA as a hexamer to unwind the DNA. Gp5/trx forms a high affinity complex with the helicase during DNA synthesis to increase the processivity to 5 kb. The primase domain of gp4 catalyzes the template-directed synthesis of oligoribonucleotides to initiate the synthesis of Okazaki fragments on the lagging-strand. Gp2.5 coats the ssDNA to remove its secondary structures and to assist in the coordination of DNA synthesis.

In a second interaction, the helicase can also capture dissociating gp5/trx and return it to the leading strand, increasing processivity to > 17 kb. This process involves an electrostatic interaction between the acidic C-terminal tail of the helicase and two basic loops (exchange patch) in the trx-binding domain of gp5 (**Fig. 1B**).[2] This interaction also provides for a reservoir of gp5/trx recruited from solution to enable the exchange of gp5/trx without affecting processivity. A similar exchange occurs in the bacteriophage T4 system.[3]

More recently, we identified a third interaction of gp5/trx with helicase, an interaction essential for assembling the gp5/trx-helicase complex for leading-strand synthesis. This interaction involves another basic patch (K587, K589, R590 and R591, loading patch) on the polymerase that also serves as a docking site for the C-terminal tail of the helicase[4] (**Fig. 1A**). Neutralization of this loading patch abolishes its ability to mediate leading-strand DNA synthesis. Nonetheless, the altered polymerase can exchange with the replicating gp5/trx and continue ongoing leading-strand DNA synthesis.

These three interactions between gp5/trx and the helicase involve distinct steps, depicted in **Figure 1**.[4] Gp5/trx is loaded onto the DNA by an interaction of its loading patch with the C-terminal tail of the helicase (**Fig. 1A**). Once polymerase is engaged in nucleotide polymerization, there is a switch to a stable interaction that does not involve the C-terminal tail or either basic patch on gp5. This latter interaction is essential for the processivity of 5 kb. Gp5/trx that dissociates from the DNA can be captured and re-loaded by an interaction of its exchange patch with the tail of the helicase, increasing processivity to 17 kb[2] (**Fig. 1B**). Gp2.5 and the helicase have similar acidic C-terminal tails (**Fig. 1**) that interact with the basic patches on gp5, providing for three-way switches.

The T4 replisome consists of eight proteins and the *E. coli,* 14.[1] However, mechanisms in the T7 system are also found in these more complex systems. In *E. coli*, a clamp loader loads a β sliding-clamp onto DNA and then dissociates, with the free clamp for loading DNA polymerase;[5] similar events occur in the T4 system.[6] In T7, trx and the trx-binding domain of gp5 encircle the DNA to form a clamp. Thus the loading patch of gp5 resembles a clamp loader. In *E. coli*, the clamp loader stabilizes DNA polymerase and helicase at the replisome.[7] In T7, the stable interaction between helicase and polymerase retain the two polymerases at the replication fork. In *E. coli*, the β clamp recruits DNA polymerases during ongoing replication.[8] In T7, the interaction of the tail of the helicase and the exchange patch on gp5 recruits dissociating polymerase to the replication fork.

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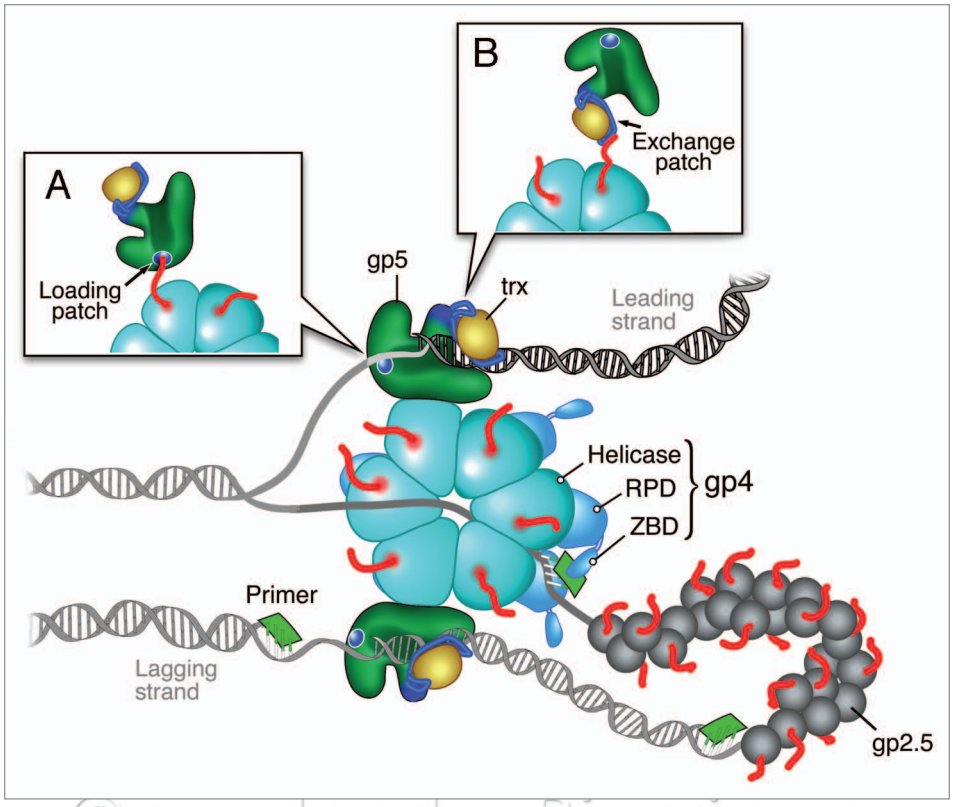

Figure 1. Model of the bacteriophage T7 replisome. The T7 replisome consists of DNA polymerase (gp5), the processivity factor thioredoxin (trx), the gene 4 helicase-primase (gp4) and the ssDNA-binding protein (gp2.5). The C-terminal tail of the helicase interacts with the loading patch (K587, K589, R590 and R591) (A) and the exchange patch on the trx-binding domain of gp5 (B).