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Dynamic DNA Helicase-DNA Polymerase Interactions Assure Processive Replication Fork Movement

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Published in:
Molecular Cell

DOI:
[10.1016/j.molcel.2007.06.020](https://doi.org/10.1016/j.molcel.2007.06.020)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2007

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Hamdan, S. M., Johnson, D. E., Tanner, N. A., Lee, J.-B., Qimron, U., Tabor, S., Oijen, A. M. V., & Richardson, C. C. (2007). Dynamic DNA Helicase-DNA Polymerase Interactions Assure Processive Replication Fork Movement. *Molecular Cell*, 27(4), 539-549. <https://doi.org/10.1016/j.molcel.2007.06.020>

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Supplemental Data

Dynamic DNA Helicase-DNA Polymerase

Interactions Assure Processive

Replication Fork Movement

Samir M. Hamdan, Donald E. Johnson, Nathan A. Tanner, Jong-Bong Lee, Udi Qimron, Stanley Tabor, Antoine M. van Oijen, and Charles C. Richardson

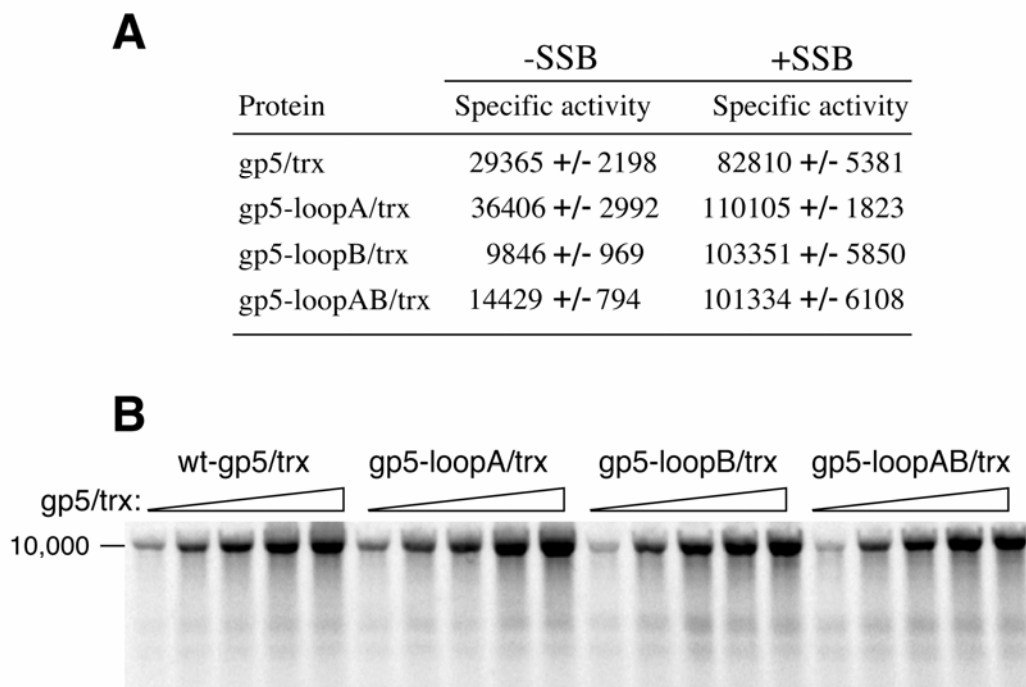


Figure S1. DNA Synthesis Catalyzed by gp5/trx Variants on Primed ssM13

A 24-mer primer

(5'-CGCCAGGGTTTTCCCAGTCACGAC-3') was annealed to M13 mGP1-2

ssDNA. The standard reaction mixture (13.5 μ l) contains 40 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 5 mM DTT, 50 mM NaCl, 0.2 mg/ml BSA, 500 μ M each of dATP, dCTP,

dGTP, dTTP, and [α - ^3H]dTTP (3 cpm/pmol), 16 nM primed-M13, 6 μM trx, and 4 nM gp5. SSB concentration when added to the reaction was 5 μM . Gp5 and trx were incubated in ice for 30 min and the rest of the components were added and the reaction was started by being transferred to 37 °C. The reactions are stopped after 4 min with EDTA to a final concentration of 150 mM. Aliquots were placed on DE81 filter paper, were washed with 0.3 M ammonium formate (pH 8.0), and then the amount of radioactively labeled synthesized DNA bound to the DE81 filter was measured with scintillation counter. (A) Specific activity of gp5/trx variants. Specific activity is measured as the amount of nmoles of [^3H]dTTP incorporated per minute per mg of enzyme. (B) Identity of DNA polymerization products by gp5/trx variants. The reaction is carried out as described in above except that DNA synthesis was monitored by the incorporation of [α - ^{33}P]dAMP and E. coli SSB is present at 5 μM . The concentrations of gp5 used are 1.6, 3.2, 6.3, 12.5, and 25 nM. The DNA products are denatured, and analyzed by electrophoresis in 0.8% alkaline agarose gel.

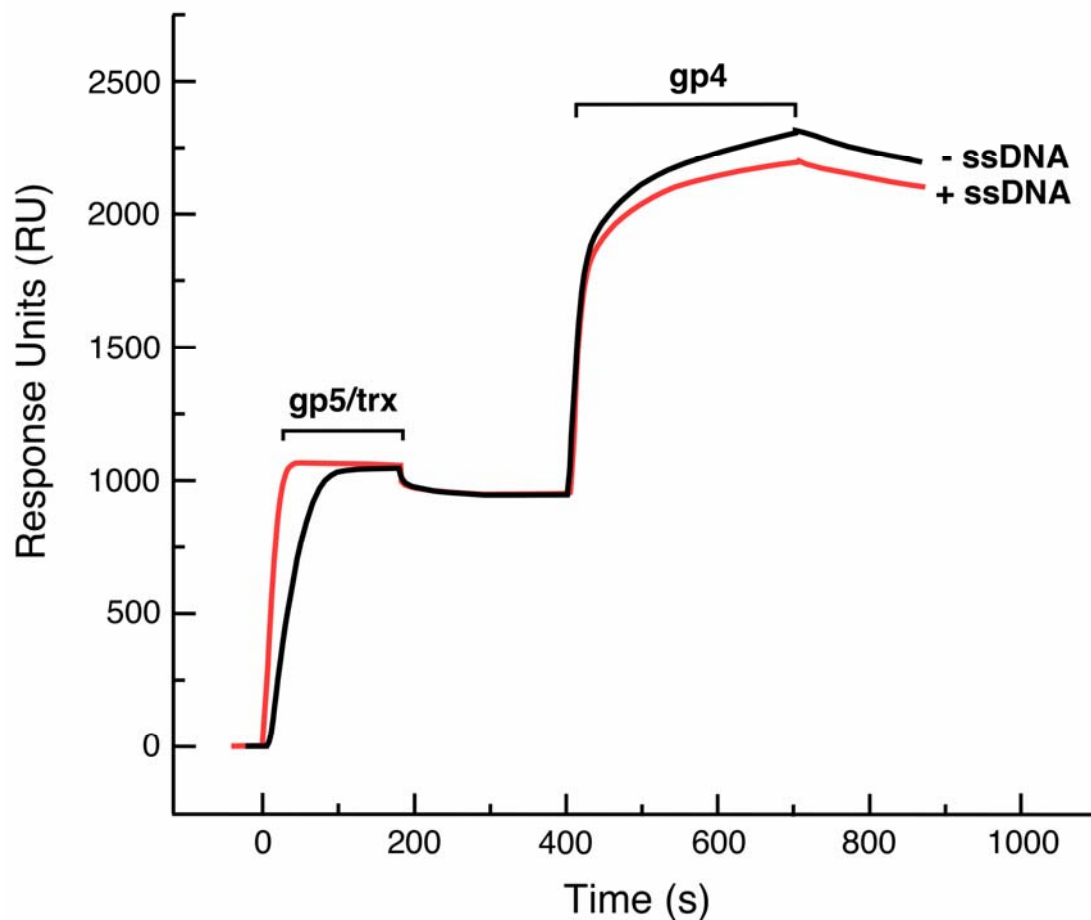


Figure S2. Gp4 Binding to gp5/trx Bound to a Primer-Template in the Presence and Absence of Excess ssDNA

The template (5'- Biotin TGA AAA AC TGG CAC TGG CCG TCG TGT TCA CG) was annealed to the primer (5'- CG TGA ACA CGA CGG CCA GTG CCA). The annealed primer-template differs from the one used in Fig 3 in the text in that the chain terminating nucleotide is ddGTP and the incoming nucleotide is dTTP (the nucleotide that is utilized by gp4). Coupling and binding studies were carried out as described in the Experimental Procedures. Nearly 215 RU of the biotinylated primer-template is coupled to the surface. The RU resulting from the coupling of the primer-template was subtracted from the baseline. Gp5/trx, is injected at a concentration of 0.3 μ M in a flow buffer containing 1.5

mM dTTP and 100 μ M ddGTP. In one experiment gp4 is injected at a concentration of 1 μ M (monomer) in flow buffer containing 1.5 mM dTTP. In another g4 at a concentration of 1 μ M (monomer) was pre-incubated with 61-mer ssDNA (0.3 μ M) in the presence of 1.5 mM dTTP and the mixtures was injected over the gp5/trx-DNA complex. The start and end of injections of gp4 and gp5/trx are indicated.

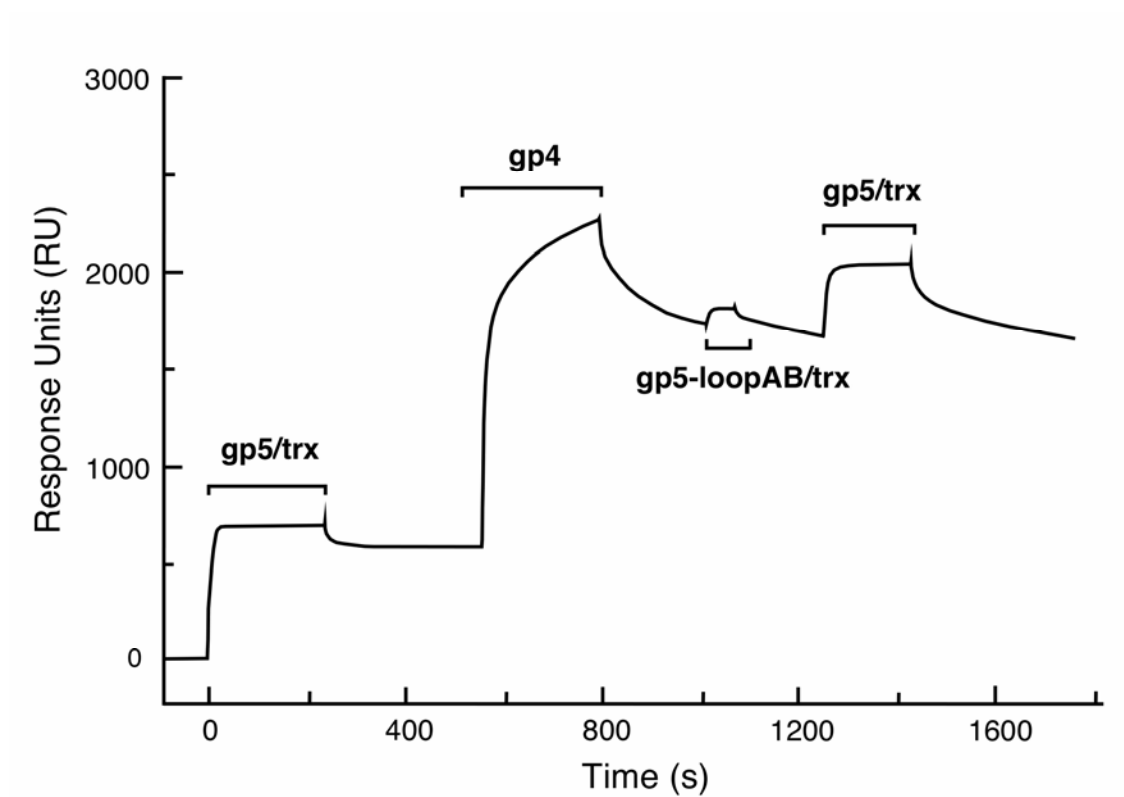


Figure S3. Binding of Excess Polymerase to gp4 that Is Bound to gp5/trx-DNA Complex

The experimental procedure is similar to that in Fig 3 in the text. Coupling and binding studies were performed as described in the Experimental Procedures. Nearly 120 RU of the 5'-bitinylated primer-template strand were coupled to the surface. The RU resulting from the coupling of the primer-template was subtracted from the baseline. Wild-type

gp5/trx was injected at a concentration of 0.2 μ M in buffer containing 1 mM dGTP and 10 μ M ddATP. Gp4 was then injected at concentration 1.5 μ M (monomer) in buffer containing 0.1 mM ATP and 2 mM dGTP. When the signal from the gp4 stable complex with the polymerase is reached, gp5-loopAB/trx was injected over at a concentration of 0.2 μ M in buffer containing 0.1 mM ATP and 2 mM dGTP. Following washing with buffer, wild-type gp5/trx was injected over at a concentration of 0.2 μ M in buffer containing 0.1 mM ATP and 2 mM dGTP. The start and end of injections of gp4, gp5/trx, and gp5-loopAB/trx are indicated.