Supporting Information

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SI Text

SI Results ***Oligomerization of Gp4.*** Because truncation of gp4 can affect its ability to oligomerize, we assessed the oligomerization of 63-kDa-gp4 and the three gp4 variants by gel analysis. It was shown previously that gp4 oligomerizes to form dimers to hexamers as well as higher forms of oligomers (1). The functional form of gp4 that binds to DNA is a hexamer (2), and the binding of nucleotides to gp4 plays an essential role in the formation of stable hexamers (3). We have measured the ability of the 63-kDa gp4 and the three gp4 variants to oligomerize in the presence of various concentrations of β,γ-methylene dTTP ranging from 0.01 mM to 1 mM. Prior to electrophoresis of the proteins on a native gel we have incubated the proteins with glutaraldehyde in order to cross-link and stabilize the oligomeric forms. The reaction mixtures containing different gp4 were incubated for 20 min at 37 °C, and then the reactions were stopped by the addition of glutaraldehyde to 0.03% (*v*/*v*). The samples were analyzed on a nondenaturing 10% polyacrylamide gel in TBE (Fig. S1). The native gel analysis reveals that 63-kDa gp4, gp4ΔZBD, and gp4ΔC display similar oligomerization patterns. The oligomerization pattern of gp4Δprimase differs slightly from those observed with the other gp4. Gp4Δprimase contains a small content of lower order oligomers at concentrations of β,γ-methylene dTTP ranging from 0.01 mM to 0.5 mM.

DNA binding. We measured the ability of each protein to bind a 27-mer oligonucleotide in the presence of the non-hydrolysable dTTP analog β,γ-methylene dTTP using a nitrocellulose DNA binding assay (Fig. S2). The reaction mixtures containing different concentrations of gp4, 1 nM 5′-^{32}P-labeled 27-mer oligonucleotide, and 1 mM β,γ-methylene dTTP were incubated at 37 °C for 30 min and then filtered through a nitrocellulose and a ζ probe membrane. The quantities of protein-bound ssDNA and unbound ssDNA were measured in a phosphorimager by scanning the nitrocellulose and ζ probe membrane, respectively. The dissociation constants (K_d) were calculated by fitting the experimental data using an algorithm for the single-site saturation. Calculated K_d values are displayed in Fig. S2. The wild-type 63-kDa gp4 binds the oligonucleotide with a K_d of 143 ± 42 nM, similar to the binding of the gp4ΔZBD (K_d of 105 ± 44 nM) and gp4ΔC (K_d of 139 ± 50 nM). Gp4Δprimase binds the ssDNA approximately threefold less tightly (K_d of 370 ± 48 nM) and binds 30% of the oligonucleotide at the higher protein concentrations. In summary, 63-kDa gp4, gp4ΔZBD, and gp4ΔC display nearly identical binding affinities to a 27-mer ssDNA. The threefold poorer binding of gp4Δprimase to ssDNA is likely due to the fact that gp4Δprimase doesn't contain the RPD, which alone can bind to ssDNA (4).

SI Materials and Methods ***Protein expression and purification.*** Proteins were overproduced from plasmids overexpressing their genes and purified as described previously: 63-kDa gp4 and gp4ΔZBD (5), gp4ΔC (6), gp4Δprimase (7), gp4Δhelicase (8), gp5 lacking exonuclease activity, and trx (9).

Oligomerization assay. Gp4 preparations were examined for their ability to oligomerize in the presence of β,γ-methylene dTTP (8). The reaction mixtures (15 μL) containing 2 μM (monomer) gp4, 20 mM Tris-HCl, pH 7.5, 200 mM NaCl, 2 mM $MgCl_2$, 10 mM DTT and 0.01 to 1 mM dTTP were incubated for 20 min at 37 °C. The reactions were stopped by the addition of glutaraldehyde to 0.03% (*v*/*v*). The samples were then kept at 37 °C for another 5 min, and the reaction products were analyzed by electrophoresis through a nondenaturing 10% polyacrylamide gel in 0.25× TBE. The gels were stained with Coomassie Blue.

DNA binding assay. A nitrocellulose filter-binding assay was used to measure the binding of wild-type and altered gp4 to ssDNA. The reaction mixtures (20 μL) containing 1 nM 5′-^{32}P-labeled 27-mer oligonucleotide (5′-CAG TAG CGG GTC TAT TTC TCA GCG TCC-3′), 20 mM Tris-HCl, pH 7.5, 200 mM NaCl, 2 mM $MgCl_2$, 10 mM, 1 mM β,γ-methylene dTTP, and the indicated concentrations of wild-type or altered gp4 were incubated at 37 °C for 30 min. The reaction mixtures were then filtered through a nitrocellulose membrane laid above a ζ probe membrane in a dot-blot filtration apparatus. The quantities of protein-bound ssDNA and non-bound ssDNA were measured in a phosphorimager by scanning the nitrocellulose and ζ probe membrane, respectively. Dissociation constants were calculated using an algorithm for the single-site saturation using the software KaleidaGraph.

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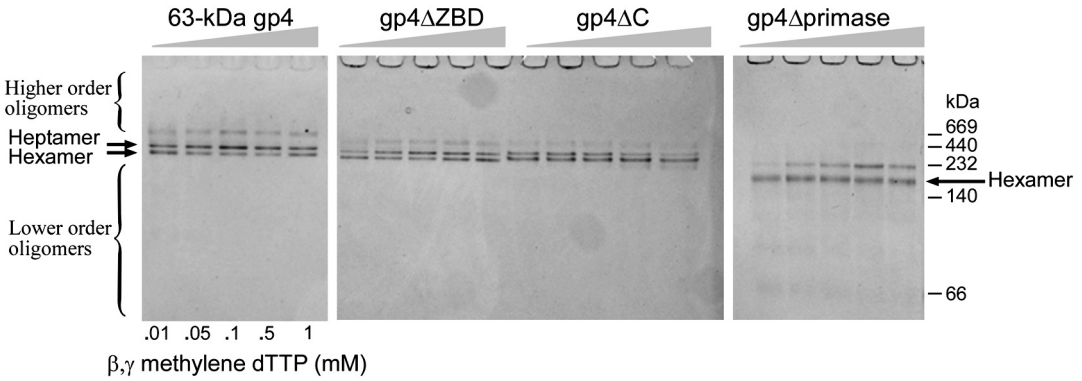

Fig. S1. Oligomerization of gp4. Oligomerization reactions contained 2 μM 63-kDa gp4, gp4ΔZBD, gp4ΔC or gp4Δprimase, and 0.01–1 mM β,γ-methylene dTTP. After incubation at 37 °C for 20 min, glutaraldehyde was added to 0.03% (*v*/*v*) to stabilize the oligomeric forms. The proteins were analyzed by electrophoresis on a 10% nondenaturing polyacrylamide gel. The state of the oligomerization of the proteins was determined after staining the gel with Coomassie Blue. The formation of hexamers, heptamers, higher order oligomers, and lower order oligomers are shown in the gel picture.

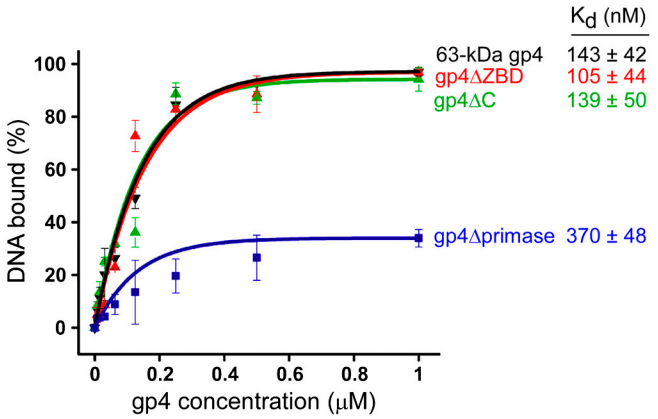

Fig. S2. Binding of gp4 to a primer/template. The dissociation constants for wild-type and the variant gp4 were determined in a nitrocellulose DNA-binding assay. The reactions were carried out in a 20 μL volume containing a range of concentrations of gp4, 1 nM 5′-^{32}P-labeled 27-mer oligonucleotide, and 1 mM β,γ–methylene dTTP. After incubation for 30 min at 37 °C the reaction mixtures were filtered through a nitrocellulose membrane laid above a ζ probe membrane in a dot-blot filtration apparatus. The quantity of protein bound ssDNA and free ssDNA was measured in a phosphorimager by scanning the nitrocellulose and ζ probe membrane, respectively. The binding by the gp4 variants to a 27-mer ssDNA was compared with the DNA-binding activity of a wild-type protein. The graph shows results of the experiment in which increasing concentrations of different gp4 helicases [63-kDa gp4 (black triangles), gp4ΔZBD (red triangles), gp4ΔC (green triangles), gp4Δprimase (blue squares)] were titrated into reaction mixtures containing 1 nM 5′-^{32}P-labeled 27-mer ssDNA. The error bars represent standard deviations of data points obtained in three independent experiments. The dissociation constants for binding of gp4 helicases to a 27-mer ssDNA were determined by fitting the experimental data with an algorithm for the single-site saturation in the software KaleidaGraph.