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Biochemical and Structural Characterization of Interactions between DNA Polymerase and Single-Stranded DNA Binding Protein in Bacteriophage RB69. Siyang Sun, Linda Geng, Yousif Shamoo, Dept. of Biochemistry and Cell Biology, Rice Univ., Houston TX 77005.

We are interested in studying the RB69 (a T4-like bacteriophage) replisome because it is very similar to those of eukaryotes and has been a prototype for studies on leading and lagging strand synthesis. RB69's replisomal proteins have been proven more amenable to x-ray diffraction studies than those of T4's. We have established solution conditions for the formation of a discrete and homogeneous complex of RB69 DNA polymerase (gp43), primer-template DNA, and RB69 single-stranded DNA binding protein (gp32) using equilibrium fluorescence and light scattering. We have characterized the interaction between DNA polymerase and single-stranded DNA binding protein and measured a 60-fold increase in the overall affinity of RB69 SSB for template-strand DNA in the presence of DNA polymerase that is the result of specific protein-protein interactions. We have also shown that a functional domain of RB69 single-stranded DNA-binding protein previously suggested to be the site of RB69 DNA polymerase-SSB interactions is dispensable. Since both gp43 and gp32 crystallize rather readily by themselves, previous attempts to crystallize the gp43•primer/template DNA•gp32 complex have not been successful. To prevent individual proteins from crystallizing and increase our chances for success, we fused gp32 to the N-terminus of gp43. gp32-gp43 fusion•primer/template DNA complex crystals have been obtained but diffracted poorly. The crystals have a high solvent content that has made freezing problematic. We are working to improve the quality of the crystals as well as freezing conditions. Meanwhile, we are also investigating on the processivity of the gp43•gp32 fusion. Our preliminary data shows that this fusion protein has higher processivity than wild-type gp43.