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Report:

Structural Analysis of *Bacillus subtilis* SPP1 Phage Helicase Loader Protein G*39*P
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Abstract

The *Bacillus subtilis* SPP1 phage-encoded protein G*39*P is a loader and inhibitor of the phage G*40*P replicative helicase involved in the initiation of DNA replication. We have carried out a full x-ray crystallographic and preliminary NMR analysis of G*39*P and functional studies of the protein, including assays for helicase binding by a number of truncated mutant forms, in an effort to improve our understanding of how it both interacts with the helicase and with the phage replisome organizer, G*38*P. Our structural analyses reveal that G*39*P has a completely unexpected bipartite structure comprising a folded N-terminal domain and an essentially unfolded C-terminal domain. Although G*39*P has been shown to bind its G*40*P target with a 6:6 stoichiometry, our crystal structure and other biophysical characterization data reveal that the protein probably exists predominantly as a monomer in solution. The G*39*P protein is proteolytically sensitive, and our binding assays show that the C-terminal domain is essential for helicase interaction and that removal of just the 14 C-terminal residues abolishes interaction with the helicase *in vitro*. We propose a number of possible scenarios in which the flexibility of the C-terminal domain of G*39*P and its proteolytic

sensitivity may have important roles for the function of G*39*P *in vivo* that are consistent with other data on SPP1 phage DNA replication.