The bacteriophage T4 gene 59 helicase assembly protein is required for recombination-dependent DNA replication, which is the predominant mode of DNA replication in the late stage of T4 infection. T4 gene 59 helicase assembly protein accelerates the loading of the T4 gene 41 helicase during DNA synthesis by the T4 replication system in vitro. T4 gene 59 helicase assembly protein binds to both T4 gene 41 helicase and T4 gene 32 single-stranded DNA binding protein, and to single and double-stranded DNA. We show here that T4 gene 59 helicase assembly protein binds most tightly to fork DNA substrates, with either single or almost entirely double-stranded arms. Our studies suggest that the helicase assembly protein is responsible for loading T4 gene 41 helicase specifically at replication forks, and that its binding sites for each arm must hold more than six, but not more than 12 nucleotides. The 1.45 Å resolution crystal structure of the full-length 217-residue monomeric T4 gene 59 helicase assembly protein reveals a novel alpha-helical bundle fold with two domains of similar size. Surface residues are predominantly basic (pI 9.37) with clusters of acidic residues but exposed hydrophobic residues suggest sites for potential contact with DNA and with other protein molecules. The N-terminal domain has structural similarity to the double-stranded DNA binding domain of rat HMG1A. We propose a speculative model of how the T4 gene 59 helicase assembly protein might bind to fork DNA based on the similarity to HMG1, the location of the basic and hydrophobic regions, and the site size of the fork arms needed for tight fork DNA binding. The fork-binding model suggests putative binding sites for the T4 gene 32 single-stranded DNA binding protein and for the hexameric T4 gene 41 helicase assembly.