Mutations in a Conserved Motif Inhibit Single-stranded DNA Binding and Recombination Mediator Activities of Bacteriophage T4 UvsY Protein*

Jill S. Bleuit, Yujie Ma, James Munro, and Scott W. Morrical‡

From the Departments of Biochemistry and Microbiology and Molecular Genetics, and Vermont Cancer Center, University of Vermont College of Medicine, Burlington, Vermont 05405

The UvsY recombination mediator protein is critical for homologous recombination in bacteriophage T4. UvsY uses both protein-protein and protein-DNA interactions to mediate the assembly of the T4 UvsX recombinase onto single-stranded (ss) DNA, forming presynaptic filaments that initiate DNA strand exchange. UvsY helps UvsX compete with Gp32, the T4 ssDNA-binding protein, for binding sites on ssDNA, in part by destabilizing Gp32-ssDNA interactions and in part by stabilizing UvsX-ssDNA interactions. The relative contributions of UvsY-ssDNA, UvsY-Gp32, UvsY-UvsX, and UvsY-UvsY interactions to these processes are only partially understood. The goal of this study was to isolate mutant forms of UvsY protein that are specifically defective in UvsY-ssDNA interactions, so that the contribution of this activity to recombination processes could be assessed independently of other factors. A conserved motif of UvsY found in other DNA-binding proteins was targeted for mutagenesis. Two missense mutants of UvsY were isolated in which ssDNA binding activity is compromised. These mutants retain self-association activity, and form stable associations with UvsX and Gp32 proteins in patterns similar to wild-type UvsY. Both mutants are partially, but not totally, defective in stimulating UvsX-catalyzed recombinase functions including ssDNA-dependent ATP hydrolysis and DNA strand exchange. The data are consistent with a model in which UvsY plays bipartite roles in presynaptic filament assembly. Its protein-ssDNA interactions are suggested to moderate the destabilization of Gp32-ssDNA, whereas its protein-protein contacts induce a conformational change of the UvsX protein, giving UvsX a higher affinity for the ssDNA and allowing it to compete more effectively with Gp32 for binding sites.

Recombination-dependent replication is a critical stage in the life cycle of the bacteriophage T4. During T4 infection of Escherichia coli, a switch from origin-dependent replication to an extremely productive recombination-dependent mode of DNA synthesis takes place. This recombination-dependent mode requires the phage-encoded proteins UvsX and UvsY (1). Null mutants at either locus have identical recombination-deficient and DNA-arrest phenotypes. They also exhibit small plaque morphology, and sensitivity to ultraviolet light (2–5).

These effects are in agreement with UvsX and UvsY being essential for initiating phage recombination-dependent replication and for mediating recombinational DNA repair.

The 43-kDa UvsX protein, functionally homologous to the E. coli RecA protein and to Rad51 in Saccharomyces cerevisiae, is an ATP-dependent DNA strand transferase (6, 7). Like RecA and Rad51, UvsX binds cooperatively to ssDNA, forming presynaptic filaments that catalyze DNA strand exchange. The formation of UvsX presynaptic filaments is assisted by the UvsY protein, the T4 recombination mediator protein (RMP), and by Gp32, the T4 ssDNA-binding protein. In the currently accepted model of T4 presynaptic filament assembly (8), Gp32 pre-coats the ssDNA, removing inhibitory secondary structure from the lattice. UvsX must then displace Gp32 from the ssDNA, but direct displacement is thermodynamically unfavorable and kinetically slow. Instead, UvsY protein mediates the formation of UvsX filaments on Gp32-saturated ssDNA, helping UvsX to displace Gp32 in the process. The mechanism of recombination mediation by UvsY is the focus of this study and of previous work by our laboratory and others (9–16).

UvsY is the prototype of a class of proteins referred to as recombination mediator proteins or RMPs (17). The common function of these proteins is to overcome thermodynamic and/or kinetic barriers imposed by ssDNA-binding proteins and to mediate the specific assembly of a recombinase-ssDNA complex. Other examples of RMPs include the human and S. cerevisiae Rad52 proteins, the S. cerevisiae Rad55/57 protein dimer, and the E. coli RecO/R protein complex. The 15.8-kDa UvsY protein lacks enzymatic activities of its own, but stimulates the enzymatic activities of UvsX. In vitro, the addition of relatively low concentrations of UvsY generally overcomes the inhibitory effects of high Gp32, high salt, and/or low UvsX concentrations, conditions regularly encountered in vivo, in UvsX-catalyzed ssDNA-dependent ATP hydrolysis, DNA strand exchange, and recombination-dependent replication reactions (8–13, 18). Biochemical properties of UvsY include non-cooperative and sequence nonspecific binding to ssDNA, as well as specific interactions with T4 recombination proteins UvsX, Gp32, Gp46, and Gp47 (8, 13, 15, 19–21).2 In addition, UvsY forms stable hexamers in solution and binds to ssDNA in this form (14). The ssDNA contacts multiple subunits of the

* This work was supported by National Institutes of Health Grant GM48847. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 802-656-8260; Fax: 802-862-8229; E-mail: smorrical@zoo.uvm.edu.

1 The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; RFI, supercoiled plasmid form of M13-derived dsDNA; RFII, linear form of M13-derived dsDNA; BME, 2-mercaptoethanol; RMP, recombination mediator protein; Gp32, bacteriophage T4 gene 32 protein; HAP, hydroxypapitate; dDNA, etheno-DNA; ATPγS, adenosine 5’-O-(thiotriphosphate).

2 J. S. Bleuit, Y. Ma, R. Schroock, K. Stemke-Hale, and S. W. Morrical, manuscript in preparation.
UvsY hexamer, suggesting that the lattice is wrapped. The UvsY protein contains an LKARLDY motif. As predicted, these mutants are defective in ssDNA binding, but are completely deficient in heteroprotein binding, ssDNA binding, or presynapsis. We have begun to study mutant forms of UvsY that are defective in heteroprotein binding, ssDNA binding, or self-association. Limited chemotryptpsinolysis digests the 137-amino acid UvsY protein into two putative domains: an amino-terminal fragment or domain containing the first 101 residues, and a carboxyl-terminal fragment or domain containing the remaining 36 residues (13). The NH2-terminal fragment retains weak ssDNA binding activity, but is completely deficient in self-association (hexamerization) and in UvsY-Gp32 and UvsY-UvsX interactions (13, 24). The decrease in ssDNA binding affinity appears to result from the loss of intrahexamer synergism or cooperativity in this monomeric form of UvsY, not from disruption of the ssDNA binding site itself (24). Despite the loss of protein-protein contacts, the NH2-terminal fragment of UvsY retains the ability to stimulate the ssDNA-dependent ATPase and DNA strand exchange activities of UvsX, but at dramatically lower levels than full-length UvsY (13). Thus both ATPase and DNA strand exchange activities of UvsX, but at dramatically lower levels than full-length UvsY (13). Therefore, both UvsY-Gp32 and UvsY-UvsX interactions contribute significantly to the stabilization of the UvsX-ssDNA polymer (10). To further explore the roles of protein-ssDNA and protein-protein interactions in presynapsis, we have begun to study mutant forms of UvsY that are defective in heteroprotein binding, ssDNA binding, or self-association. Limited chemotryptpsinolysis digests the 137-amino acid UvsY protein into two putative domains: an amino-terminal fragment or domain containing the first 101 residues, and a carboxyl-terminal fragment or domain containing the remaining 36 residues (13). The NH2-terminal fragment retains weak ssDNA binding activity, but is completely deficient in self-association (hexamerization) and in UvsY-Gp32 and UvsY-UvsX interactions (13, 24). The decrease in ssDNA binding affinity appears to result from the loss of intrahexamer synergism or cooperativity in this monomeric form of UvsY, not from disruption of the ssDNA binding site itself (24). Despite the loss of protein-protein contacts, the NH2-terminal fragment of UvsY retains the ability to stimulate the ssDNA-dependent ATPase and DNA strand exchange activities of UvsX, but at dramatically lower levels than full-length UvsY (13). Thus both protein-ssDNA and protein-protein interactions of UvsY appear to be important for assembling UvsX-ssDNA presynaptic filaments and for stimulating the activities of UvsX.

To isolate the specific contributions of UvsY-ssDNA interactions to UvsY function, it is necessary to construct a UvsY species containing point mutations that would impair its interaction with ssDNA without significantly altering its other assoicative properties. The UvsY protein contains an LKARLDY sequence motif at positions 57–63. This motif appears to be conserved in a number of DNA repair proteins including the S. cerevisiae Rad3 DNA helicase and the human ERCC2 protein, a putative DNA helicase involved in excision DNA repair and defective in one complementation group of Xeroderma pigmentosum (25). Its location in the N-domain plus alternating basic and hydrophilic residues suggested that it could be important for UvsY-ssDNA interactions. This paper describes the construction and characterization of two site-directed UvsY mutant proteins, UvsYkea and UvsYkea, which contain single and double missense mutations, respectively, within the LKARLDY motif. As predicted, these mutants are defective in ssDNA binding, however, they retain self- and heteroprotein-association activities similar to wild-type UvsY. We demonstrate that both mutants are partially but not totally defective in stimulating UvsX-catalyzed reactions, which has interesting implications for the mechanism by which UvsY mediates the assembly and function of T4 presynaptic filaments.

MATERIALS AND METHODS

Reagents, Enzymes, and Nucleic Acids—Chemicals, biochemicals, and commercial enzymes were purchased from New England Biolabs. Taq polymerase and PCR reagents were purchased from PerkinElmer Life Sciences. Oligonucleotides were purchased from Operon. Circular single-stranded DNA from the bacteriophage M13mp19 was isolated by extraction from purified phage particles (26). Supercoiled M13mp19 dsDNA (RFI) was isolated from phage-infected E. coli cells as described (26). RFI DNA was digested with the EcoRI restriction endonuclease to produce linear M13mp19 DNA (RFII), which was labeled with 32P at its 5’ ends as described (26). The concentrations of ssDNA and dsDNA were determined by the absorbance at 260 nm, using conversion factors of 36 and 50 μg/mL M13mp19, respectively. All DNA concentrations are expressed in units of micromoles of nucleotide residues per liter. Ethanolo-modified ssDNA (eDNA) was synthesized by treating either poly(dA) lattices with an average extinction coefficient of 69,760 M–1 cm–1 for UvsX, 19,180 M–1 cm–1 for UvsY, and 41,360 M–1 cm–1 for Gp32 (30).

Construction of UvsY Missense Mutants—UvsY missense mutants were constructed using a three-step PCR site-directed mutagenesis strategy (31). The overall scheme involves amplifying the gene in two portions, one of which contains a targeted mutation. Denaturing the two products and annealing them at their region of overlap, and then performing a polymerase fill-in reaction creates a template of the gene containing the mutation. This new template is subsequently amplified by PCR, gel purified, and cloned into a suitable expression vector.

The starting template used in this process was the wild-type UvsY-encoding plasmid pTL251W, which was a generous gift from Dr. T. C. Lin of Yale University. The 5’ end of the wild-type UvsY gene (nucleotide positions 1–169) was amplified using the following forward and reverse primers: forward (34-mer), primer 1, 5’-CAATTGAATAGAGATCTTTCATGCAGGACGTAATGAGATTAGATGGTATG-3’; and reverse (20-mer), primer 2, 5’-AGACGAATTCTTCTGTTGCT-3’.

The forward primer introduces an NdeI restriction site containing the start codon onto the 5’ end of the resultant PCR product. A second PCR amplifies the 3’ end of the UvsY gene (nucleotide positions 150–410) and introduces either one or two codon changes at sites described here. To construct the double mutant UvsYkea, a forward primer was used that contains an altered reverse sequence and that the codons for lysine 58 and arginine 60 are both changed to code for alanine. The forward primer is: forward (42-mer), primer 3a, 5’-GACACAGAAGAGGATGGTTCGTTCAAGAATGACT-3’; and reverse (20-mer), primer 2, 5’-AGACGAATTCTTCTGTTGCT-3’.

The same reverse primer was used to amplify the 3’ end of the UvsY gene containing both the single and double mutations. It introduces a BamHI site onto the 5’ end of the UvsY gene, immediately following the stop codon. It has the following sequence: reverse (31-mer), primer 3: 5’-GGCCGGCGGATCCCATGCGCAGTATGACGTTG-3’.

The UvsYkea single and UvsYkea double mutants were cloned using identical methods. After the 5’ end of the UvsY gene had been PCR-amplified by standard procedures (26) with primers 1 and 2, the 5’ end had been amplified with either primer 3a or primer 3b for the downstream end, and primer 4 for the downstream primer. The resulting fragment was gel-purified. The two pools of fragments were mixed and added to standard PCR buffer containing free nucleotides, and then subjected to 94 °C for 30 s, followed by 50 °C for 30 s. These conditions cause the respective fragments to denature and then re-anneal. Because of the overlapping regions in the two separate fragments, some of

2 H. T. H. Beernink, M. A. Sweezy, and S. W. Morrical, manuscript in preparation.
Site-directed Mutagenesis of UvsY Recombination Mediator Protein

...the unmutated species are composites, with one strand from the UvsY5′ end pool and one strand from the UvsY3′ end pool. After the annealing step, Taq polymerase was added to the reaction to allow nucleotide fill-in of the un-annealed overhangs. A unique fragment is generated that contains a complete sequence of the UvsY gene, with point mutations mapping to codons 58 and/or 60, and with 5′-NdeI and 3′-BamHI sites. By adding primers 1 and 4 to the reaction, this mutated UvsY gene fragment can be PCR-amplified by standard methods. The final PCR products containing mutated UvsY genes were ligated into the pET3a expression vector to form the constructs designated pUvsYK58A,R60A or pUvsYK58A, respectively. Their sequences were verified by DNA sequencing, using the dyeode method (26) and an ABI automated sequencer.

Expression and Purification of UvsY Mutants—The pUvsYK58A,R60A and pUvsYK58A plasmids were transformed into competent BL21(DE3) E. coli cells. Single colonies of pUvsYK58A,R60A or pUvsYK58A, harboring transformants grown on LB/ampicillin plates were selected and grown at 37 °C in liquid LB media, containing 100 μg/ml ampicillin, to a cell density of A600 = 0.6, then induced by adding 1 μM isopropyl-1-thio-β-D-galactopyranoside and continuing growth for 3 h at 37 °C. For the UvsYK58A,R60A preparation, 19 g of induced cells were harvested from a 6-liter culture and resuspended in 100 ml of lysis buffer (20 mM Tris-HCl, pH 7.4, 5 mM MgCl2, 5 mM 2-mercaptoethanol (BME), 1 mM NaCl, 10 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride). DNase I was added to a final concentration of 20 μg/ml. The cells were sonicated with 1-mm diameter tungsten heads at 4 °C. All subsequent steps were performed at 4 °C. The cell lysate was centrifuged for 30 min at 10,000 × g in a Sorvall SS-34 rotor, and then centrifuged for 2 h at 30,000 × g in a Beckman Ti-45 rotor. The supernatant was recovered and dialyzed exhaustively against 2 × 4 liters of PC-100 buffer (20 mM Tris-HCl, pH 7.4, 2.2 mM EDTA, 1 mM BME, 100 mM NaCl, and 10% (w/v) glycerol). The dialysate was then loaded onto a Whatman P-11 phosphocellulose column (bed volume = 180 ml) that had been pre-equilibrated with PC-100 buffer. A 1,200-ml linear gradient from PC-100 to PC-100 (1000 to 1000 mM NaCl) was run. UvSYK58A,R60A eluted with a peak at ~325 mM NaCl. Fractions were analyzed by Coomassie Blue staining of SDS-polyacrylamide gels, and UvSYK58A,R60A-containing fractions were pooled. The pooled fraction was dialyzed against 2 × 2 liters of 20 mM Tris-HCl, pH 8.1, 5 mM EDTA, 1 mM BME, 100 mM NaCl, and 10% (w/v) glycerol), and then loaded onto a 50-ml ssDNA-cellulose (DC) column that had been prepared as described (32), and pre-equilibrated in the same buffer. The DC column was washed with DC-100 buffer, and then eluted with a step gradient of DC-200 (same as DC-100, except the NaCl concentration is 200 mM), followed by DC-600 (NaCl = 600 mM). UvSYK58A,R60A did not adhere to the DC column, but instead came off in the wash flow-through. Two minor fractions were pooled and dialyzed into DEAE-50 buffer (20 mM Tris-HCl, pH 8.1, 5 mM EDTA, 1 mM BME, 50 mM NaCl, and 10% (w/v) glycerol) and loaded onto a 40-ml DEAE-cellulose column that had been pre-equilibrated in the same buffer. The DEAE column was washed with DEAE-50 buffer, and then eluted with a 400-ml gradient from HAP-100 to HAP-900 (potassium phosphate concentration = 900 mM). UvSYK58A,R60A eluted with a peak at ~460 mM potassium phosphate. Fractions were analyzed by Coomassie Blue staining of SDS-polyacrylamide gels and the peak fractions were pooled and dialyzed against 2 × 2 liters of UvSY prestorage buffer (20 mM Tris-HCl, pH 7.4, 0.2 mM EDTA, 1 mM BME, 100 mM NaCl, and 20% (w/v) glycerol), and then against 2 × 2 liters of UvSY storage buffer (20 mM Tris-HCl, pH 7.4, 0.2 mM EDTA, 1 mM BME, 100 mM NaCl, and 65% (w/v) glycerol). The final yield was ~66 mg of UvSYK58A,R60A. The protein was >99% homogeneous and both nuclease- and ATPase-free, as determined by methods described for native UvSY (15). Concentration was performed by the absorbance at 280 nm, using an extinction coefficient of 19,180 M−1 cm−1 for UvSYK58A,R60A. This extinction coefficient was calculated using the method of Gill and von Hippel (30).

The UvSYK58A single mutant protein was expressed as described for the double mutant protein, but was subjected to additional purification steps. The UvSYK58A was dialyzed against 20 mM Tris-HCl, pH 8.1, 5 mM EDTA, 1 mM BME, 50 mM NaCl, and 10% (w/v) glycerol) and loaded onto a 40-ml DEAE-cellulose column that had been pre-equilibrated in the same buffer. The DEAE column was washed with DEAE-50 buffer, and then eluted with a 400-ml gradient from HAP-100 to HAP-900 (potassium phosphate concentration = 900 mM). UvSYK58A did not adhere to the DEAE-cellulose column, but instead came off in the wash flow-through. Two minor fractions were pooled and dialyzed against 20 mM Tris-acetate (pH 7.4), 90 mM potassium acetate (KOAc), 10 mM magnesium acetate, and NaCl ranging from 0.18 to 1.0 M. UvSYK58A was analyzed using the van Holde and Weischet method (33).

Protein Affinity Chromatography Experiments—Uvx-, Gp32-, and bovine serum albumin-agarose columns were prepared by covalently coupling the proteins to Bio-Rad Affi-Gel 10 beads as described (34). In each case, total immobilized protein was determined to be 1.5 to 2.0 mg of protein/ml of bed volume. 2 ml columns were poured and equilibrated with running buffer RB-50 (20 mM Tris-HCl, pH 5.1, 1 mM EDTA, 5 mM MgCl2, 50 mM NaCl, and 10% (w/v) glycerol). All chromatography steps were conducted at 4 °C. 100-μg quantities of UvSY wild-type, UvSYK58A,R60A, or UvSYK58A were dialyzed into RB-50 and loaded onto the columns by gravity flow. The flow-through was collected and protein was quantified. The columns were washed with 4 ml of buffer RB-500 and then loaded with UvSYK58A,R60A or UvSYK58A. Protein concentrations were determined by the Bradford assay and SDS-PAGE. Exact NaCl concentrations at which proteins eluted were determined by solution conductivity comparisons against NaCl standards.

DNA Binding Studies—Fluorescence experiments were carried out in an SLM8000 fluorimeter as described (15, 27, 28). Data in each titration were corrected for the effects of sample dilution, intrinsically protein fluorescence, inner filter effects, and/or baseline fluorescence of DNA depending on the type of experiment (15, 27, 35). Titrations of UvSY species into blank solutions containing no DNA were used to identify fluorescence changes because of protein addition. This signal change was a linear function of protein concentration in all experiments. Data were not corrected for photobleaching of the DNA because photobleaching was found to be negligible over the time period of all experiments. As a general precaution against photobleaching, samples were only exposed to the light source during the 10–15 s data acquisition interval following each addition and equilibration of titrant.

ATPase Assays—Rates of UvSY-catalyzed ssDNA-dependent ATP hydrolysis were determined by a coupled spectrophotometric assay, using a slight modification of the procedure of Morrill et al. (37). ATPase time courses were recorded on a Hitachi U-2000 spectrophotometer equipped with a water-jacketed cuvette holder to maintain a constant temperature of 37 °C. Final reaction volumes of 700 μl were contained in quartz cuvettes of 1-cm path length. Assays contained 20 mM Tris acetate (pH 7.4), 90 mM potassium phosphate (KOAcl), 10 mM magnesium acetate, 2 mM ATP, 6 units/ml pyruvate kinase, 6 units/ml lactate dehydrogenase, 2.3 mM phosphoenolpyruvate, and 0.23 mM NADH. In all reactions contained 4.5 μM UvSY and 5 μM M13mp19 ssDNA, plus UvSY (10 μg/ml), Gp32 (50 μg/ml), and UvSY wild-type (0.5–5 μg/ml), or UvSYK58A (0.5–5 μg/ml) as indicated. Identical control buffer conditions were maintained between experiments by adding protein storage buffers to reagents in appropriate amounts. All reaction components except UvSY protein were preincubated for 5 min at 37 °C, and then reactions were initiated by adding UvSY. Reaction velocities were calculated from the...
change in absorbance at 380 nm as described (37) using the linear portions of time courses.

DNA Strand Exchange Assays—We used an assay based on the protocol of Formosa and Alberts (7). Reaction mixtures contained 20 mM Tris acetate (pH 7.4), 90 mM potassium acetate, 10 mM magnesium acetate, 100 μM bovine serum albumin, 1 mM dithiothreitol, 2 mM ATP, 10 mM creatine phosphate, 10 μg/ml creatine phosphokinase, 4.5 μM 5'-32P-labeled M13mp19 ssDNA, 4.5 μM 5'-32P-labeled M13mp19-RFII DNA, 35 μg/ml Gp32, variable amounts of UvX (0–50 μg/ml) and variable amounts of UvS (0–5 μg/ml), UvS undergoing ATPase activity (20). The data demonstrate that UvS undergoes ATPase activity (20).

RESULTS

Expression and Purification of UvS K58A and UvS K58A,R60A Mutant Proteins—UvS K58A and UvS K58A,R60A mutant proteins were generated, overexpressed, and purified as described under “Materials and Methods.” Results of overexpression and purification are shown in Fig. 1. The mutant proteins were purified to > 95% and > 99% homogeneity, respectively, and were free of any detectable nuclease or ATPase contamination. The final purified fractions of UvS K58A and UvS K58A,R60A shown in Fig. 1 were used for all of the solution studies reported here. Note that both UvS K58A and UvS K58A,R60A were passed over ssDNA-cellulose affinity columns in the course of purification; neither mutant protein bound to ssDNA-cellulose in buffer containing 100 mM NaCl, whereas wild-type UvS binds quantitatively to ssDNA-cellulose at the same salt concentration, and requires 600 mM NaCl for elution (15) (see Table I). The ssDNA-cellulose results indicate major ssDNA-binding defects in UvS K58A and UvS K58A,R60A, which we explore in greater detail in a later section.

Self-Association Properties of UvS K58A and UvS K58A,R60A Mutant Proteins—Wild-type UvS protein exists predominantly as a 6.0 S hexamer in analytical ultracentrifugation buffers containing NaCl concentrations greater than 0.2 M, and forms progressively larger oligomers as salt concentration decreases below the 0.2 M threshold (14). Conversely, a truncated form of UvS protein lacking the COOH-terminal 36 amino acid residues sediments as a 2.1 S monomer at all salt concentrations examined (24). The self-association properties of missense mutants UvS K58A and UvS K58A,R60A were examined by sedimentation velocity. Fig. 2 shows the integral distribution of g 20,w for both mutant proteins as a function of NaCl concentration. The data demonstrate that UvS K58A and UvS K58A,R60A both resemble wild-type UvS in their capacity to oligomerize. Both mutants sediment at ~6 S at higher salt concentrations, indicative of the stable hexameric form observed with wild-type UvS (Table I and Ref. 14). Neither mutant shows any tendency to dissociate into quaternary structures smaller than hexamers under the wide range of solution conditions tested here (0.18–1.0 M NaCl in analytical ultracentrifugation buffer; Fig. 2). Therefore it is reasonable to conclude that the K58A and K58A,R60A missense mutations, unlike COOH-terminal truncation (24), do not destabilize the fundamental hexameric quaternary structure of the UvS protein. There are, however, differences observed in the sedimentation properties of UvS K58A and UvS K58A,R60A compared with wild-type UvS (Table I). Both mutants appear to form higher order associations more readily than wild-type, as evidenced by the protein concentration dependence of g 20,w observed for UvS K58A and UvS K58A,R60A in salt concentrations ranging from 0.3 to 1.0 M NaCl (Fig. 2). This trend, which may indicate some degree of polydispersity on the part of UvS K58A and UvS K58A,R60A, stands in contrast to wild-type UvS, which is highly monodisperse under equivalent conditions (Table I and Ref. 14). Therefore the K58A and K58A,R60A missense mutations, while preserving the hexamerization properties of UvS, may also render the protein more susceptible to aggregation.
Both UvsY<sub>K58A</sub> and UvsY<sub>K58A,R60A</sub> Retain Heteroprotein Interactions with Gp32 and UvsX—Protein affinity chromatography experiments were performed to examine interactions between the UvsY<sub>K58A</sub> and UvsY<sub>K58A,R60A</sub> mutants, respectively, and either UvsX or Gp32, depending on the experiment. Results are summarized in Table II. When either UvsY<sub>K58A</sub> or UvsY<sub>K58A,R60A</sub> was passed over a column containing Gp32 immobilized on agarose beads, it bound quantitatively, requiring
Salt-directed Mutagenesis of UvsY Recombination Mediator Protein

**Table I**

| Sedimentation coefficient (s20,w) ranges of UvsY wild-type and mutant proteins as a function of NaCl concentration |
|-------------------------------------------------|
| Salt concentration in analytical ultracentrifugation buffer | UvsY6 | UvsYK58A | UvsYK58A,R60A |
|-------------------------------------------------|
| 150 mM NaCl | 6–9 | 7–11 | 8–11 |
| 300 mM NaCl | 6 | 6–8 | 6–8 |
| 500 mM NaCl | 6 | 6 | 6–8 |
| 1 M NaCl | 6 | 6–7 | 6–7 |

a Data from Beernink and Morrical (14).

b Data derived from Fig. 2A, this study.

c Data derived from Fig. 2B, this study.

**Table II**

| Salt elution properties of UvsY wild-type and mutant proteins from affinity columns |
|-------------------------------------------------|
| Protein loaded | ssDNA-cellulose | UvsX-agarose | Gp32-agarose | Bovine serum albumin-agarose |
|-------------------------------------------------|
| UvsY | 600 mM | 600 mM | 400 mM | FT* |
| UvsYK58A | NaCl | NaCl | NaCl | NaCl |
| UvsYK58A,R60A | FT* | 200 mM | 200 mM | FT* |
| UvsYK58A,R60A | NaCl | NaCl | NaCl | NaCl |

* For bovine serum albumin-agarose control column, FT denotes protein eluted in the 50 mM NaCl flow-through fraction (RB-50 buffer).

**Figure 3**

a salt concentration of 200 mM NaCl to elute. When either mutant protein was passed over a UvsX-agarose column, it also bound quantitatively and required 200 mM NaCl for elution. However, when either UvsYK58A or UvsYK58A,R60A was passed over a bovine serum albumin-agarose control column, neither was retained, and each respective protein was recovered quantitatively in the flow-through fraction. In separate experiments, wild-type UvsY protein also bound to the same Gp32- and UvsX-agarose columns quantitatively, and could be eluted off the UvsX column with 600 mM NaCl, and the Gp32 column with 400 mM NaCl. This agrees with the previously published values of 450 and 360 mM NaCl for elution of UvsY from UvsX- and Gp32-agarose columns, respectively (7). The data indicate that both UvsYK58A and UvsYK58A,R60A mutants retain the ability to bind to UvsX and Gp32 proteins specifically, albeit with somewhat reduced stabilities compared with wild-type UvsY (Table II).

**Etheno-DNA Binding Properties of UvsYK58A and UvsYK58A,R60A Mutants**—The ssDNA-binding defects of UvsYK58A and UvsYK58A,R60A, first revealed by ssDNA-cellulose affinity chromatography (see above), were examined in greater detail using etheno-DNA (eDNA) fluorescence enhancement assays. Previous studies established that both wild-type and COOH-terminal truncated forms of UvsY enhance the fluorescence of eDNA (15, 24), allowing binding to be detected and quantified by this method. Here, we compared the abilities of UvsYK58A,R60A, UvsYK58A, and wild-type UvsY to enhance eDNA fluorescence. Protein-eDNA interactions were studied as a function of NaCl concentration, using salt-back titration profiles as indicators of relative binding affinity. The experiments depicted in Fig. 3 used a poly(dA) lattice with an average chain length of 310 nucleotide residues. Similar results were obtained with random-sequence eDNA derived from M13mp19 ssDNA circles (data not shown). The salt-back assay consisted of preincubating eDNA with a slight excess of native UvsY, UvsYK58A, or UvsYK58A,R60A (1.2-fold saturating, assuming a binding site size of n = 4 nucleotide residues per monomer, Ref. 15) in a low-ionic strength buffer containing 5 mM NaCl, then titrating each starting mixture with increasing concentrations of NaCl. Results are shown in Fig. 3.

The double mutant UvsYK58A,R60A exhibited no detectable increase in eDNA fluorescence, relative to eDNA-only control, under any salt conditions examined (5–75 mM NaCl in 20 mM Tris-HCl (pH 7.4), 1 mM MgCl2) (Fig. 3). Thus UvsYK58A,R60A appears to avoid being of ssDNA binding activity by two different criteria: eDNA and ssDNA-cellulose methods (see Table II). UvsYK58A enhanced the fluorescence of eDNA at NaCl concentrations ~25–30 mM (Fig. 3), indicating very weak, residual ssDNA binding activity for this single-mutant form. The apparent salt midpoint for dissociation of UvsYK58A-eDNA complexes was ~15 mM NaCl, and the complexes appeared to be completely dissociated by [NaCl] = 30 mM, thus explaining why no binding of this mutant to ssDNA-cellulose was observed in buffer containing 100 mM NaCl (Table II). UvsYK58A binding to eDNA appears to be even weaker than that of COOH-terminal truncated forms such as UvsY6*, which exhibits eDNA salt-dissociation midpoints of 65 mM NaCl and 145 mM KOAc, respectively, and affinity for ssDNA generally 104-fold lower than wild-type UvsY (24). Even allowing for some anion-specific stabilization of UvsYK58A-eDNA interactions (i.e. improved binding in acetate versus chloride as seen with UvsY and UvsY6*), it is questionable whether UvsYK58A could form any productive complexes with ssDNA at all under buffer conditions used in ATPase and strand exchange assays described in subsequent sections.

Control experiments with wild-type UvsY validate the results of our eDNA binding studies. Wild-type UvsY induces a large enhancement of eDNA fluorescence at low NaCl concentrations (Fig. 3), consistent with previous results (15). Titration of wild-type UvsY-eDNA complexes with NaCl causes the magnitude of fluorescence enhancement to decrease (Fig. 3); eventually a plateau is reached in which an ~3-fold level of fluorescence enhancement is maintained up to a NaCl concentration of ~300 mM (data not shown). Further titration leads to UvsY-eDNA complex dissociation at a salt midpoint of ~450 mM NaCl (data not shown). The behavior of wild-type UvsY-eDNA complexes in this study parallels that observed by Sweezy and Morrical (15, 16). Therefore the poor to non-existent binding of UvsYK58A and UvsYK58A,R60A, respectively, to eDNA reflects true ssDNA-binding defects in these mutant proteins, and not a failure of our fluorescence assays to detect protein-eDNA complex formation.

UvsYK58A and UvsYK58A,R60A Are Partially Defective in Stimulating UvsX-catalyzed ssDNA-dependent ATPase Activity—Gp32 protein inhibits UvsX-catalyzed ssDNA-dependent ATP hydrolysis by competing with UvsX for binding sites on ssDNA (7, 9, 38). Wild-type UvsY overcomes this inhibition by nucleating UvsX filament formation onto Gp32-ssDNA complexes, and presumably by helping UvsX to displace Gp32 from the ssDNA (9, 12, 39). We tested the abilities of UvsYK58A and UvsYK58A,R60A mutants to stimulate UvsX-catalyzed ATPase activity in the presence of Gp32-saturated ssDNA. Results are summarized in Table III. Reactions were performed under conditions in which the ssDNA-dependent ATPase activity of UvsX is absolutely dependent on functional UvsY protein, i.e. sub-saturating UvsX (0.2-fold) and saturating Gp32 (2.3-fold) with respect to binding sites on ssDNA (assuming n = 4 and 7 nucleotide residues for UvsX and Gp32, respectively (36, 40–42)). Under these conditions, reactions lacking UvsY had velocities indistinguishable from background, whereas the addition of wild-type UvsY (1.2-fold molar excess with respect to

---

**Note:** The document contains scientific data and analysis, including tables and figures, that require a background in molecular biology and biochemistry for full comprehension. The data and analysis presented are crucial for understanding the function and properties of UvsY and its mutants in the context of recombinant DNA and protein interactions. The text provides insights into the mutagenesis of UvsY and its effects on ssDNA binding and ATPase activity, highlighting the importance of specific amino acid modifications on protein function and stability. The experimental methods and results are foundational for further research in the field of recombinant DNA technology and protein engineering.
Controls lacked UvsX protein, but were otherwise identical to reactions dependent on UvsX and UvsY. Fig. 4 (tions were established in which DNA strand exchange is co-de-
UvsX-catalyzed DNA strand exchange reactions. First, condi-
UvsX concentration of 50

Velocities of UvsX-catalyzed ssDNA-dependent ATP hydrolysis in the presence/absence of UvsY wild-type and mutant proteins

Spectrophotometric ATPase assays were performed as described under “Materials and Methods.” Complete reactions contained 2 mM ATP, 4.5 μM (nucleotides) M13mp19 ssDNA, 10 μg/ml (0.23 μM) UvsX, and 50 μg/ml (1.5 μM) Gp32. UvsY wild-type or mutant proteins, when present, were at a concentration of 5 μg/ml (0.31 μM). All other reaction components and conditions were as described under “Materials and Methods.” Controls lacked UvsX protein, but were otherwise identical to “complete reactions” in the same row of the table.

![Fig. 3. Interactions of UvsY sp. with eDNA as a function of NaCl concentration. 7.5 μM UvsY (■), UvsYK58A (□), or UvsYK58A,R60A (○) was preincubated with 2.25 μM eDNA in low-salt mixtures as described under “Materials and Methods,” and any protein-eDNA complex formation was monitored by the increase in eDNA fluorescence. Each mixture was then titrated with NaCl and the disruption of complexes was monitored by the resulting decrease in eDNA fluorescence. The fluorescence contribution of the UvsY protein species alone was subtracted from each data point.](image)

Table III

| Proteins | ATP hydrolysis rate |
|----------|---------------------|
|          | Complete reaction  | Control (minus UvsX) |
|          | nmol/μg/min         |                     |
| UvsX alone | 0                  | 0                   |
| UvsX + UvsY | 7.7               | 0                   |
| UvsX + UvsYK58A | 2.3             | 0                   |
| UvsX + UvsYK58A,R60A | 2.9          | 0                   |

UvsX produced a high level of UvsX-catalyzed ATPase activity (Table III). With an identical concentration of either UvsYK58A or UvsYK58A,R60A replacing wild-type UvsY in the reaction, the ATPase velocity was approximately one-third of that obtained with wild-type UvsY (Table III). Thus, the two UvsY mutants are only partially defective in facilitating UvsX-ssDNA complex assembly on Gp32-saturated ssDNA. UvsYK58A and UvsYK58A,R60A retain significant and approximately equal abilities to promote UvsX-catalyzed ssDNA-dependent ATP hydrolysis under these conditions. Note that control reactions lacking UvsX enzyme had velocities indistinguishable from background, demonstrating that all three UvsY species were free of contaminating ATPase activity (Table III).

Effects of UvsYK58A and UvsYK58A,R60A Mutants on UvsX-catalyzed DNA Strand Exchange—We compared the abilities of wild-type UvsY, UvsYK58A, and UvsYK58A,R60A to stimulate UvsX-catalyzed DNA strand exchange reactions. First, conditions were established in which DNA strand exchange is co-de-

rating with respect to binding sites on ssDNA (24), DNA strand exchange occurs readily in the absence of UvsY protein. Reactions are characterized by the formation of DNA networks that do not enter the gel, a reaction outcome typical of UvsX-catalyzed strand exchange (7, 10, 18). Each reaction sample was treated with Proteinase K prior to gel loading to ensure that the aggregates that arose were results of the strand exchange and not from nonspecific aggregation of DNA by binding proteins (13, 43). Networks appear only 5 min after reaction initiation, the earliest time point examined (Fig. 4, lane 2). In contrast to these results, no strand exchange products are observed over a 40-min time course when the UvsX concentration is lowered to 10 μg/ml (0.23 μM), which is only 20% saturating with respect to binding sites on ssDNA (Fig. 4, lanes 5–9). Under these restrictive conditions, the addition of wild-type UvsY protein (5 μg/ml or 0.31 μM, a slight molar excess with respect to UvsX) activates the DNA strand exchange reaction, and the majority of 32P-labeled RIII substrate DNA is rapidly converted to well bound networks (Fig. 4, lanes 10–14). Smaller D-loop intermediates, which do enter the gel but run slower than the 32P-linear dsDNA substrate, are also evident early in the reaction but are rapidly converted into networks (Fig. 4, lanes 10–14). Substitution of an equal concentration of UvsYK58A,R60A double mutant for wild-type UvsY under otherwise identical conditions causes a severe defect in DNA strand exchange (Fig. 4, lanes 20–24). The reaction is not abolished, but formation of the network products is largely suppressed over the 40-min reaction time course, whereas D-
loop intermediates accumulate at a greatly reduced rate. Other-
wise identical experiments performed with UvsYK58A alone reveal that this single mutant is also partially defective in activating DNA strand exchange (Fig. 4, lanes 15–19). The defect is not as severe as that observed with UvsYK58A,R60A, but nevertheless, results in a lag time of about 10 min before network products begin to form, relative to reactions with wild-type UvsY. Thus the UvsYK58A and UvsYK58A,R60A mutants affect strand exchange differentially under restrictive conditions in which the reaction is co-dependent on UvsX and UvsY.
indicating a partial defect in mediated presynaptic filament assembly. Likewise, both UvsY mutants show greatly reduced abilities to mediate UvsX-catalyzed DNA strand exchange reactions (Fig. 4). In this case the strand exchange defect is more severe with the double than with the single mutant form of UvsY (Fig. 4), suggesting that the residual, extremely weak ssDNA binding ability observed with UvsYK58A (Fig. 4) might facilitate some function in promoting DNA strand exchange that is not available in UvsYK58A,R60A. Surprisingly, neither mutant is totally defective in facilitating UvsX-catalyzed ssDNA-dependent ATPase and DNA strand exchange reactions.

Based on previous biochemical studies of UvsY properties (13–16, 24), we proposed a mechanistic model for UvsY-mediated assembly of the T4 presynaptic filament, which is shown in Fig. 5A. This model includes a critical role for UvsY-ssDNA interactions in destabilizing Gp32-ssDNA interactions, which is necessary for nucleating UvsX-ssDNA filament assembly. The destabilization of Gp32-ssDNA occurs independently of UvsY-Gp32 protein-protein interactions (16). The proposed mechanism of destabilization involves wrapping of ssDNA around hexameric UvsY, which would disrupt cooperative interactions between neighboring Gp32 molecules. Based on this model, one might reasonably predict that mutations abrogating ssDNA binding interactions would completely inhibit UvsY mediator function, but results of our current study show that this is not the case. Instead, the data indicate that UvsY heteroprotein interactions alone are sufficient to promote an attenuated level of presynaptic filament assembly and to provide partial activation of UvsX-catalyzed reactions. Because UvsY-Gp32 interactions are not critical for the Gp32-ssDNA destabilization step of presynapsis (Ref. 16, see Fig. 5A), we propose that the partial mediator activities observed with UvsYK58A and UvsYK58A,R60A mutants arise predominantly through UvsY- UvsX interactions, as shown schematically in Fig. 5B. Here, binding of UvsYK58A or UvsYK58A,R60A to UvsX induces a high affinity ssDNA binding conformation of the recombinase. The high affinity UvsY mutant-UvsX complex competes more effectively with Gp32 for binding sites on the ssDNA than does UvsX alone, allowing the formation of a limited number of nucleation sites for presynaptic filaments (Fig. 5B).

The models in Fig. 5, A and B, predict that the wild-type UvsY protein uses at least two different mechanistic effects to mediate presynaptic filament assembly. The first effect is destabilization of Gp32-ssDNA interactions, an effect dependent on UvsY-ssDNA interactions (16). This idea is consistent with the observation that the isolated ssDNA-binding domain of UvsY can still weakly stimulate UvsX-catalyzed reactions in...
the presence of ssDNA-saturating concentrations of Gp32 (13). The second effect is induction of high affinity UvsX-ssDNA interactions, an effect dependent on UvsY-UvsX interactions. The latter idea is supported by the observation that the salt stability of T4 presynaptic filaments is increased in the presence of UvsY (10), and by fluorescence anisotropy data suggesting that UvsY and the nucleotide analog ATPγS (which stabilizes UvsX-ssDNA complexes; Ref. 40) induce similar conformational changes in UvsX protein. Other effects are possible and are not necessarily mutually exclusive with the models shown in Fig. 5, A and B. The role of UvsY-Gp32 interactions, although poorly understood, could be important for maintaining appropriate spatial organization of the four macromolecular components, UvsY, Gp32, UvsX, and ssDNA, during the early stages of presynaptic filament assembly. This idea is presented schematically in Fig. 5C. Such an effect could help to explain the partial activities of UvsY mutants described in this study, i.e. Gp32 could recruit and orient the UvsY mutant into a suboptimal but nucleation-competent complex, even though the ssDNA binding activity of the mutant is compromised. This could, in turn, explain why the UvsY<sub>K58A</sub> single mutant performs better than the UvsY<sub>K58A,R60A</sub> double mutant in strand exchange reactions (Fig. 4); the residual ssDNA binding activity of UvsY<sub>K58A</sub> might make it that much easier to form a reasonably ordered nucleation complex in response to interactions with Gp32 and/or UvsX. The scheme in Fig. 5C might also explain the observation that UvsY cannot assemble UvsX onto ssDNA covered with Gp32-A, a truncated form of Gp32 lacking the domain for protein-protein interactions with UvsY and UvsX (21). Although UvsY destabilizes Gp32-A-ssDNA complexes (16), presumably Gp32-A fails to properly orient UvsY molecules for subsequent downstream steps in presynapsis and strand exchange.

The involvement of the LKARLDY motif of the UvsY protein in ssDNA binding suggests that this sequence element might be important for polynucleotide binding in other proteins as well. In addition to its conservation in the human ERCC2 nucleotide excision repair protein and the yeast Rad3 helicase (25), close matches appear in other DNA repair proteins including the UvrB excision repair protein of Thermus thermophilus (44), the RecB protein (exonuclease V subunit) of Pseudomonas syringae (Protein Data Bank accession number AAL79572), and the ERCC2/TFIIH orthologs of various eukaryotes (22, 23, 25, 46–49). Each of these proteins is known to bind to DNA. Because mutagenesis of the LKARLDY motif in T4 UvsY protein affects recombination functions, then similar mutations in other proteins conceivably could alter DNA recombination/repair outcomes and thereby impact genomic stability.

The close functional conservation of RMPs from bacteriophage to humans clearly demonstrates their importance in homologous recombination and recombinational DNA repair (17). Studies of the role of the T4 RMP, UvsY protein, have illuminated some of the biochemical trickery used to remodel protein-ssDNA complexes and to assemble enzymatically active

\[ \text{UvsX-ssDNA complex} \]

\[ \text{UvsY-ssDNA complex} \]

\[ \text{Gp32-ssDNA complex} \]

\[ \text{UvsX-Gp32 complex} \]

\[ \text{UvsY-Gp32 complex} \]

\[ \text{UvsY-UvsX complex} \]

\[ \text{UvsY-Gp32-UvsX complex} \]

\[ \text{UvsY-Gp32-UvsX-ssDNA complex} \]

The high affinity form of UvsX competes more effectively with Gp32 for binding sites on ssDNA, allowing an attenuated level of filament nucleation to occur. C. UvsY LKARLDY-motif mutants lacking ssDNA binding activity could also act as adapters between UvsX and Gp32-ssDNA, using protein-protein interactions to tether UvsX to the complex. The tethered UvsX could displace Gp32 from the ssDNA locally by adopting a high affinity conformation as in B) and/or via high local concentration effects.

Fig. 5. Models for the roles of UvsY wild-type and mutants in presynaptic filament assembly. See text for details. A, model for nucleation of the T4 presynaptic filament by wild-type UvsY protein, adapted from Beernink and Morrical (17) and Bleuit et al. (8). UvsY destabilizes Gp32-ssDNA interactions, an effect mediated predominantly by UvsY-ssDNA interactions. UvsY then recruits UvsX recombinase via protein-protein interactions, nucleating presynaptic filament assembly with concomitant expulsion of Gp32 from the complex. B, UvsY LKARLDY-motif mutants lacking ssDNA binding activity may still promote presynaptic filament assembly by inducing a high affinity ssDNA-binding conformation of UvsX via protein-protein interactions. The high affinity form of UvsX competes more effectively with Gp32 for binding sites on ssDNA, allowing an attenuated level of filament nucleation to occur. C, UvsY LKARLDY-motif mutants lacking ssDNA binding activity could also act as adapters between UvsX and Gp32-ssDNA, using protein-protein interactions to tether UvsX to the complex. The tethered UvsX could displace Gp32 from the ssDNA locally by adopting a high affinity conformation (as in B) and/or via high local concentration effects.

4 J. Farb and S. Morrical, unpublished data.
presynaptic filaments from inactive precursors. Through continued physical and biochemical studies of UvsY and its mutants, we hope to further refine a rigorous model of UvsY structure and function, one that will hopefully shed light on recombination mechanisms used by many organisms.

Acknowledgments—We thank Drs. Hans Beerink, Mark Sweezy, and Richard Ando for help with the mutagenesis design.

REFERENCES

1. Kreuzer, K. N., and Morrical, S. W. (1994) in Molecular Biology of Bacteriophage T4 (Karam, J. D., ed.) pp. 28–42, American Society for Microbiology, Washington, D.C.
2. Cunningham, R. P., and Berger, H. (1977) Virology 60, 67–82.
3. Melamede, R. J., and Wallace, S. S. (1978) FEBS Lett. 78, 3–12.
4. Melamede, R. J., and Wallace, S. S. (1982) Mol. Gen. Genet. 177, 510–519.
5. Hinot, D. M., and Nossal, N. G. (1986) J. Biol. Chem. 261, 5663–5671.
6. Formosa, T., and Alberts, B. M. (1986) J. Biol. Chem. 261, 6107–6118.
7. Bleut, J. S., Xu, H., Ma, Y., Wang, T., Liu, J., and Morrical, S. W. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8288–8293.
8. Yonesaki, T. and Minagawa, T. (1989) J. Mol. Biol. 209, 389–418.
9. Jiang, H., Giedroc, D., and Kodate, T. (1993) J. Biol. Chem. 268, 7904–7911.
10. Weber, C. A., Kirchner, J. M., Salazar, E. P., and Takayama, K. (1994) Mutat. Res. 324, 147–152.
11. Straubell, R. G., Feingold, E. A., Grouse, H. L., Derge, J. G., Kraus, R. D., Collins, F. S., Wagner, L. Shennan, C. M., Shuler, G. D., Altschul, S. F., Zeeberg, B., Buetow, K. H., Schafer, C. F., Bhat, N. K., Hopkins, R. P., Jordan, H., Moore, T. Max, G. I., Wang, J. Hasei, F., Diatchenko, L., Marusina, K., Farmer, A. A., Rubin, G. M., Hong, L., Stapleton, M., Soares, M. B., Balsano, M. F., Casavant, T. L., Hecht, T. E., Brownstein, M. J., Uden, T. B., Toshijuki, S., Carcini, P., Prange, C., Raha, S. S., Lequillano, N. A., Peters, G. J., Abramson, R. D., Mullah, S. J., Bosak, S. A., McEwan, P. J., McKernan, K. J., Male, J. A., Gunaratne, P. H., Richards, S., Moritzot, D. C., and Morizot, D. C. (1983) J. Biol. Chem. 258, 5663–5671.
12. Formosa, T., and Alberts, B. M. (1990) J. Biol. Chem. 265, 275–285.
13. Sweezy, M. A., and Morrical, S. W. (1996) Trends Biochem. Sci. 21, 605–600.
14. Ando, R. A., and Morrical, S. W. (1998) J. Biol. Chem. 273, 6954–6959.
15. Harris, L. D., and Griffith, J. D. (1986) Biochemistry 25, 2258–2360.
16. Sweezy, M. A., and Morrical, S. W. (1997) Biochemistry 36, 936–944.
17. Sweezy, M. A., and Morrical, S. W. (1999) Biochemistry 38, 956–944.
18. Sweezy, M. A., and Morrical, S. W. (1999) Trends Biochem. Sci. 24, 385–389.
19. Formosa, T., and Alberts, B. M. (1991) J. Biol. Chem. 266, 14031–14038.
20. Formosa, T., and Alberts, B. M. (1984) Cold Spring Harbor Symp. Quant. Biol. 49, 363–370.
21. Jiang, H., Giedroc, D., and Kodate, T. (1993) J. Biol. Chem. 268, 7904–7911.
22. Yonesaki, T. and Minagawa, T. (1989) J. Biol. Chem. 264, 7814–7820.
23. Kodate, T., Gan, D.-C., and Stemke-Hale, K. (1989) J. Biol. Chem. 264, 16453–16457.
24. Harris, L. D., and Griffith, J. D. (1989) J. Mol. Biol. 206, 19–27.
25. Melamede, R. J., and Wallace, S. S. (1982) Biochimie (Paris) 79, 275–285.
26. Sweezy, M. A., and Morrical, S. W. (1997) J. Mol. Biol. 266, 927–938.
27. Morrical, S. W., Hempstead, K., and Morrical, M. D. (1994) in Molecular Biology of Bacteriophage T4 (Karam, J. D., ed.) pp. 28–42, American Society for Microbiology, Washington, D.C.
28. Formosa, T., and Alberts, B. M. (1990) J. Biol. Chem. 265, 275–285.
29. Morrical, S. W., Hempstead, K., and Morrical, M. D. (1994) in Molecular Biology of Bacteriophage T4 (Karam, J. D., ed.) pp. 28–42, American Society for Microbiology, Washington, D.C.