The Phage T4 uvsY Recombination Protein Stabilizes Presynaptic Filaments*

(Received for publication, April 21, 1989)

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The bacteriophage T4 uvsY protein is required for efficient recombination in T4-infected Escherichia coli cells. Previous in vitro work has shown that the purified uvsY protein is an accessory protein; it stimulates homologous pairing catalyzed by the phage uvsX protein (a RecA-like recombinase) under certain conditions. We show here that this effect can be traced, at least in part, to a UvsY-dependent stabilization of uvsX protein-single-stranded DNA complexes. These presynaptic filaments are one of the early obligatory intermediates in the strand exchange reaction between homologous single- and double-stranded DNAs. The mechanism of filament stabilization seems to involve a slower loss of UvsX subunits. A model that accounts for the data is presented in which both recombination proteins are incorporated into the presynaptic filament.

A central goal in the study of homologous recombination is to reconstitute in vitro an entire recombination reaction between two homologous, fully duplex DNA molecules using highly purified proteins. While no laboratory appears to be close to achieving this feat, many of the partial reactions that together make up the overall process have been reconstituted. Homologous strand exchange has been particularly heavily studied (1, 2). It is now thought that in Escherichia coli this reaction proceeds by initial formation of a presynaptic filament, a polymer of the RecA protein or analogous factors, along single-stranded (ss) DNA. This event may be preceded by complexation of single-stranded binding protein (SSB) if one is present. The presynaptic filament then binds a double-stranded (ds) molecule and begins a poorly understood “search for homology.” Once a complementary region of the duplex DNA is found the invading single strand pairs with its complement. This yields either a parancemic or plecometic joint depending on the topological constraints of the DNA substrates. For suitable partners (homologous single-stranded circles and duplex linear DNAs are often employed), branch migration can then occur, leading to partial or complete displacement of one of the original duplex strands.

We are interested in understanding the recombination machinery of the bacteriophage T4. T4 is highly recombinogenic, simple to work with, and appears to encode most of its own recombination functions, making it an attractive model system for the more complex recombination machines present in eukaryotic cells. Like the E. coli recA protein and related factors from other organisms (3-7), the T4 uvsX protein (8-10) is able to drive all phases of strand exchange in vitro. However, several lines of evidence suggest that this particular phase of the recombination process requires the coordinated action of several other factors in vivo (11). For example, pairing between M13 ss circular and linear dsDNA catalyzed by the bacteriophage T4 uvsX protein is stimulated tremendously by the T4 gene 32 product (8-10), a helix-stabilizing protein (12). Genetic studies of phage T4 have implicated other gene products as potential strand exchange factors as well. For example, T4 uvsY mutants exhibit phenotypes almost indistinguishable from uvsX+ phage (13).

Recent in vitro work employing purified uvsY protein has suggested an explanation for this observation. Under certain conditions, particularly in high salt buffers or at low levels of uvsX protein, the uvsY protein stimulates the ability of UvsX to mediate homologous pairing although the uvsY protein alone exhibits no pairing activity (14, 15). However, UvsY has no effect on recA protein-mediated reactions, suggesting that the direct UvsX-UvsY protein-protein interactions detected by protein affinity chromatography (16) play an important role in its ability to stimulate UvsX-mediated homologous pairing.

In order to appreciate the function of the recombination “machine” (17) in vivo, it is necessary to better understand the mechanism by which the uvsY protein operates. In this report, we examine the role of UvsY in the initial phase of homologous pairing: presynthesis. We find that the uvsY protein stabilizes the uvsX protein-ssDNA polymer greatly. The uvsY protein-dependent stabilization of the presynaptic filament at least partially explains the ability of UvsY to stimulate UvsX-catalyzed homologous pairing.

**EXPERIMENTAL PROCEDURES**

Purification of the uvsY Protein—The uvsY protein was purified from cells harboring pT251W, a plasmid carrying the uvsY gene under the control of the l promoter. This plasmid was constructed by Dr. T. C. Lin of Yale University, who kindly provided us with it. Upon induction of logarithmically growing cultures at 42°C for 4 h, the uvsY protein is expressed at a level of about 10% of the total protein mass, as estimated by Coomasie Blue-stained SDS-PAGE gels.

Purification of the uvsY protein was done according to Dr. Lin, Dr. John Rush, and Dr. William Konigsberg (Yale University). The procedure summarized below is a minor modification of the procedure these workers kindly communicated to us prior to publication.

Buffers—Lysis buffer: 50 mM Tris-HCl (pH = 8.1), 2 mM EDTA, 1 mM β-mercaptoethanol. X buffer: 50 mM Tris-HCl (pH = 7.5), 2 mM EDTA, 10 mM β-mercaptoethanol, 0.5 M NaCl. X buf: same as X, except 0.2 NaCl. P buffer: 50 mM Tris-HCl (pH = 7.5), 0.1 mM EDTA, 5 mM β-mercaptoethanol, 10% (w/v) glycerol, 10 mM KCl. P buf: same as P, except 0.5 M KCl. DC buffer: 20 mM DC buffer: 20 mM

1 The abbreviations used are: ss, single-stranded; ds, double-stranded; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SSB, single-stranded binding protein.

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Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM β-mercaptoethanol, 10% (w/w) glycerol. DC13 buffer: same as DC8, except 100 mM NaCl. DC17 buffer: same as DC8, except 0.7 M NaCl. HP buffer: 10 mM potassium phosphate (pH = 7.4), 5 mM β-mercaptoethanol, 0.1 M NaCl, HP buffer: same as HP8, except 1 M NaCl. Storage buffer: 20 mM Tris-HCl (pH = 7.4), 0.2 mM EDTA, 1 mM β-mercaptoethanol, 100 mM NaCl, 50% (w/w) glycerol.

Growth of Cells—HB101 cells harboring pTL251W were grown in L broth containing 50 µg/ml ampicillin at 29 °C until the A600 was 0.5–0.6. The temperature was then raised to 42 °C for 4 h. The cells were harvested by low speed centrifugation and frozen in liquid nitrogen.

Preparation of the Cell Extract—11 g of cell paste was thawed and suspended in 100 ml of lysis buffer containing 2 mg of lysozyme, 0.05% deoxycholate, and 1 mM phenylmethylsulfonyl fluoride. After stirring for 30 min at room temperature, the suspension was sonicated until the A600 of a 200-fold diluted aliquot ceased to decrease. The temperature was not allowed to rise above 8 °C during this procedure. The lysate was cleared by centrifugation at 11,000 g for 10 min. The pellet was resuspended in 10 ml of X8 buffer with the aid of a Dounce homogenizer. Insoluble material was cleared by centrifugation at 11,000 × g for 20 min. 1.67 g of ammonium sulfate was then added to the supernatant with gentle stirring (30 min), followed by centrifugation at 30,000 × g for 20 min. Most of the uvsY protein is found in the supernatant. To this solution was added a further 1.52 g of ammonium sulfate. After centrifugation at the same speed for 30 min, the pellet, containing the uvsY protein, was redissolved in 5 ml of X8 buffer and dialyzed against HP8 buffer (two changes of 1 liter each). This cleared lysate is referred to as Fraction I.

Phosphocellulose Chromatography—Fraction I was loaded onto a 10-ml P1 column (Whatman) that had been extensively washed with P8 buffer. After washing with 30 ml of P8 buffer, the uvsY protein was eluted by passing 20 ml of P8 buffer through the column. The uvsY protein-containing fractions were pooled and diluted with the appropriate volume of DC8 buffer to bring the NaCl concentration to 100 mM. This is designated Fraction II.

DNA-cellulose Chromatography—Fraction II was loaded onto a 50-ml DNA-cellulose column (1 mg of DNA/packed ml). The column was washed with 150 ml of DC17 buffer followed by 100 ml of DC8 buffer. The uvsY protein elutes in the latter step. The pooled fractions (40 ml) are dialyzed against two 1-liter changes of HP8 buffer. This is designated Fraction III.

Hydroxyapatite Chromatography—Fraction III was loaded onto a 5-ml hydroxyapatite column. The column was then washed with 10 ml of HP8 buffer and developed with a linear gradient of 50 ml of HP8 to 50 ml of HP9. The uvsY protein elutes between 0.5 M and 0.7 M NaCl. The uvsY protein-containing fractions (fraction IV) were pooled (10 ml) and dialyzed into storage buffer. 9 mg of ≥95% pure uvsY protein (as judged by Coomasie Blue-stained SDS-PAGE gels) was loaded onto a 50-ml HP9 column. The UVSY protein elutes in the latter step. The pooled fractions (40 ml) are dialyzed against two 1-liter changes of HP8 buffer. This is designated Fraction V.

Phosphocellulose Chromatography—Fraction V was loaded onto a 10-ml P1 column (Whatman) that had been extensively washed with P8 buffer. After washing with 30 ml of P8 buffer, the uvsY protein was eluted by passing 20 ml of P8 buffer through the column. The uvsY protein-containing fractions were pooled and diluted with the appropriate volume of DC8 buffer to bring the NaCl concentration to 100 mM. This is designated Fraction II.

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Other Proteins—The uvsX and gene32 proteins were purified as described previously (11, 18). Pyruvate kinase and lactate dehydrogenase were purchased from Sigma.

DNA—The DNA employed is a 57-mer oligonucleotide complementary to nucleotides 6371–28 of wild type M13 DNA. The oligonucleotide was synthesized on an Applied Biosystems DNA synthesizer interfaced to a computer. These manipulations required 10 s.

ATP Hydrolysis Experiments—Each reaction contained 10 mM ATP, and DNA in a buffer composed of 10 mM Tris acetate (pH = 7.4), 10 mM magnesium acetate, 1 mM dithiothreitol, 0.5 mM EDTA, and 90 mM potassium acetate (unless specified otherwise). For the spectrophotometric assays (19), an ATP regeneration system was included composed of 2 mM phosphoenolpyruvate, 0.24 mM NADH, 5 units of 1 ml of lactic dehydrogenase, and 5 units of 1 ml of pyruvate kinase. All reactions were conducted at 37 °C in a total volume of 1 ml. All of the components except ATP were mixed and preincubated in an Eppendorf tube at 37 °C for 5 min. The reactions were initiated by the addition of ATP (unless stated otherwise), mixed thoroughly, and transferred to a quartz cuvette in the temperature-regulated cavity of a Beckman DU-64 UV-Vis spectrometer interfaced to a computer. These manipulations required 10 s. The data were collected and plotted. Rates were obtained by linear regression analysis using the Beckman Data Leader software package.

ATPase assays employing polyethyleneimine plates were performed as described (9). The specific activity of [α-32P]ATP was 40,500 cpm/nmol.

In all cases, the concentrations of the DNA, uvsX, and uvsY proteins employed are given in the figure legends.

Nitrocellulose Filter Binding Assays—5'-32P-labeled 57-mer (30.7 nM) (3671 cpm/pmol) was incubated in the presence of the indicated amount of uvsY protein for 15 min at 37 °C. Twenty ml of the solution was then spotted onto nitrocellulose filter disks (Whatman). The disks were washed thoroughly with cold buffer, dried, and counted in a Beckman LS 1701 liquid scintillation counter. The standard ATP hydrolysis assay buffer was employed, except for the experiments in Fig. 8. In these runs, the potassium acetate concentration was varied as indicated.

RESULTS

Purification of the uvsY Protein—The uvsY protein was purified to greater than 95% homogeneity from E. coli cells harboring pTL251W, a plasmid containing the uvsY gene under the control of the λ PL promoter. The final fraction did not contain any detectable nuclease or ATPase activities (data not shown). The plasmid was constructed by Dr. T. C. Lin of Yale University. The purification scheme is a minor modification of a procedure provided to us by T. C. Lin, John Rush, and William Konigsberg. We gratefully acknowledge these workers for providing us with pTL251W and the uvsY protein purification procedure prior to publication. Details are provided under “Experimental Procedures.”

The uvsY Protein Stabilizes the Presynaptic Filament at High Salt—The uvsX protein is a DNA-dependent ATPase (9). Therefore, its binding to single-stranded DNA can be followed by a spectrophotometric assay in which the hydrolysis of ATP is coupled enzymatically to the oxidation of NADH, an event conveniently monitored at 340 nm (19). We employed a 57-mer oligonucleotide as the DNA template in order to minimize any complications due to secondary structure.

Fig. 2a shows that the rate of uvsX protein-catalyzed ATP
suggested that the uvsY protein acts stoichiometrically.

The uvsY Protein Stabilizes the Presynaptic Filament at High Dilution—We measured the rate of uvSX protein-dependent ATP hydrolysis at 90 mM potassium acetate as a function of the protein concentration at a constant, rather low (0.5 μg/ml, 1.54 μM) DNA concentration. The sigmoidal nature of the curve (Fig. 3A) provides further evidence for the fact that the active ATPase is the uvSX protein-DNA filament, and not noncooperatively bound protein monomers.

**FIG. 2.** The uvSX protein stabilizes UvSX-containing presynaptic filaments at high ionic strength. a, the rate of ATP hydrolysis was monitored by the spectrophotometric assay in reactions containing 2.5 μg/ml (7.7 μM) DNA, 10 μg/ml uvSX protein (0.25 μM), and either 0 μM (open squares) or 0.25 μM uvSY protein (black diamonds). The concentration of potassium acetate was varied as indicated. b, titration with the uvSY protein suggests the presence of a protein-protein complex of defined stoichiometry. The ATP hydrolysis rates of reactions containing 7.7 μM DNA, 0.25 μM uvSX protein, 300 mM potassium acetate, and the indicated amount of the uvSY protein were measured. The rate of hydrolysis in the absence of uvSY is almost nil; addition of the accessory factor stimulates the rate, which plateaus at 0.25 μM uvSY protein (equimolar with UvSX). Addition of excess UvSY does not further increase the rate.

The concentration of potassium acetate was varied (Fig. uvsX protein was held constant while that of the uvsY protein was varied (Fig. 2b). The rate increased with the UvSY concentration until the molar ratio of the two proteins was 1. Further increases did not result in a greater rate of hydrolysis,

**FIG. 3.** The uvSY protein stabilizes presynaptic filaments at high dilution. A, a plot of the rates of ATP hydrolysis in reactions containing 0.5 μg/ml (1.54 μM) DNA and the indicated amount of uvSX protein. One plot (open squares) shows the rates of reactions lacking the uvSY protein, the other (black diamonds), rates of reactions containing UvSY (equimolar with UvSX). In the presence of the accessory protein, the rate of ATP hydrolysis saturates at a level of uvSX protein (0.39 μM) sufficient to cover 100% of the DNA present, assuming a site size of 4 nucleotides/uvSX monomer. The reaction lacking UvSY exhibited a very different titration curve. Much more uvSX protein was required to achieve maximal levels of ATP hydrolysis. B, absorbance versus time data for reactions containing UvSX, but not the uvSY protein. The amount of uvSX protein (μM) added is indicated adjacent to each line. These data were employed to calculate the rates for the "−UvSY" reactions in A. C, absorbance versus time data for reactions containing equimolar levels of UvSX and UvSY. The concentration of uvSX protein (μM) in each run is indicated next to the experimental curve. The rates for the "+UvSY" reactions shown in A were calculated using these data, from the decrease in absorption at late times (the maximum rates observed).
This is in agreement with previous electron microscopic studies by Griffith and co-workers (20, 21) who visualized very long filaments by electron microscopy and Formosa and Alberts (9), who showed that oligonucleotides smaller than about 25 residues do not support UvsX-catalyzed ATP hydrolysis. One of the former studies also demonstrated that the binding site size of the uvsX protein is 3–4 nucleotides (20).

Interestingly, the reaction shown in Fig. 3A (open squares) saturated only at a level of uvsX protein sufficient to cover 300% of the DNA template (1.15 μm), assuming a size of four nucleotides per protein monomer. We do not believe that this is due to a protein preparation of low specific activity since more than 95% of our uvsX protein binds to a DNA-agarose resin (data not shown). Rather, it shows that the uvsX protein-DNA filament is dynamic and that under these conditions all of the protein monomers are not incorporated into filaments in the steady state. When the experiment was repeated with a 1:1 ratio of the uvsX and uvsY proteins a different result was obtained (Fig. 3A, closed diamonds); the rate cleanly saturated at a uvsX protein concentration (0.39 μM) sufficient to cover 100% of the DNA. Indeed, addition of excess protein inhibited the rate somewhat. These experiments are consistent with the idea that the uvsY protein somehow stabilizes the normally dynamic UvsX-ssDNA filament at low uvsX protein and DNA concentration as well as at high salt.

In order to test this postulate further, rapid dilution experiments were performed. Two reactions containing high concentrations of the uvsX protein (100 μg/ml) and DNA (4 μg/ml) were incubated in the presence of ATP at 90 mM salt for 2 min, then diluted 10-fold into a buffer containing [γ-32P]ATP, but no uvsX protein or DNA. The rate of ATP hydrolysis in the diluted solutions was then monitored by the polyethyleneimine plate assay. In one case, a molar equivalent of the uvsY protein was included in the original concentrated reaction, while in the other it was omitted. The simple prediction is that if the presynaptic filaments formed during the 3-min incubation at high concentrations are stable, dilution should have no effect on the rate of ATP hydrolysis, when expressed in terms of turnovers/protein monomer/min. We find that the reaction lacking the uvsY protein (Fig. 4a, open squares) is very slow (only 7% of the undiluted reaction rate), demonstrating that the nucleoprotein filament is indeed dynamic and must lose subunits to the solution. However, in the presence of the uvsY protein (Fig. 4a, black diamonds), a respectable rate of hydrolysis is observed initially (65% of the undiluted rate), showing that the accessory factor stabilizes the uvsX protein-DNA filament. The velocity of the reaction slowly diminishes with time, suggesting that loss of UvsX subunits from the uvsY protein-stabilized filament does occur, but at a much reduced rate.

A Lag Period Is Observed in Dilute Reactions Containing the uvsY Protein—A curious feature of the diluted reactions containing the uvsY protein is that they exhibit a lag period prior to attaining the steady state (Fig. 3c). The rate of ATP hydrolysis at early times is very similar to that observed in the corresponding reactions lacking the accessory factor. This suggests that the formation of some important uvsY protein-containing structure is initially rate-limiting under these conditions. The lag phase is drastically reduced at high DNA and protein concentrations (data not shown). This rules out any artifact having to do with the ATP regeneration system or similar trivial explanations. It also argues against a rate-limiting break-up of protein aggregates since higher concentrations should exacerbate this problem.

Our standard procedure is to preincubate all of the components in the absence of ATP and then add the nucleotide triphosphate to initiate the reaction. We therefore experimented with other orders of addition. A similar lag was observed when ATP was included in the preincubation mix and the reaction was initiated by the addition of the uvsX protein. However, when UvsX, UvsY, and ATP were preincubated in the absence of DNA, and the reaction was begun by addition of the 57-mer, the lag period was reduced drastically (Fig. 5). Fig. 6 shows a run in which the reaction was initiated in the absence of UvsY; the accessory protein was then added after a 2.5-min incubation. As expected, the rate of ATP hydrolysis was accelerated considerably, but only after a lag phase. Together, these experiments demonstrate that the rate-limiting step in assembling a tightly bound presynaptic filament under dilute conditions is formation of a complex consisting of UvsX, UvsY, and ATP.
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**Fig. 5.** Formation of a complex containing UvsX, UvsY, and ATP, but not DNA, is rate-limiting at high dilution. UvsX protein-catalyzed ATP hydrolysis in the presence of the uvsY protein (equimolar with UvsX) was monitored by the spectrophotometric assay under high dilution conditions. The three runs shown are identical except for the order of addition of the reaction components: ——, DNA added last; — — — ATP added last; — — — uvsX protein added last. All reactions contained 9 pg/ml (0.22 μM) uvsX protein and 0.5 pg/ml (1.54 μM) DNA.

**Fig. 6.** The uvsY protein accelerates UvsX-catalyzed ATP hydrolysis when added to a reaction "in progress," but a lag period is observed. Reactions containing 11 μg/ml (0.275 μM) uvsX protein and 0.5 μg/ml (1.54 μM) DNA were initiated by addition of ATP to 1 mM and allowed to proceed for 2.5 min. At this time, the uvsY protein (0.275 μM) (solid line) or protein storage buffer (broken line) was added, and the solution was mixed thoroughly. A pronounced acceleration of the hydrolysis reaction was observed when UvsY was added but only after a noticeable lag period. The reaction lacking the accessory protein was unaffected. The large break in the absorbance curves is due to opening the spectrometer cavity.

The uvsY Protein Binds to Single-stranded DNA in the Absence of UvsX—The uvsY protein is retained by single-stranded DNA-cellulose columns. While this DNA-binding activity can easily be imagined to play an important role in the uvsY protein's function, it has not been examined in detail. A common method for studying nonspecific ssDNA-protein interactions is to monitor quenching of the intrinsic fluorescence of the protein upon DNA complexation. However, we have not as yet been successful in applying this methodology to the study of uvsY protein-DNA interactions. While the protein does possess a healthy fluorescence spectrum, addition of DNA does not lead to clean quenching under the conditions explored thus far, and irreproducible results are often obtained. This is unfortunate, since the aspects of the DNA-binding activity we are most interested in, namely the degree of cooperativity and the monomer site size, are most easily assessed by fluorescence titrations. Although we are continuing to work on these experiments, we turned to nitrocellulose filter binding assays using 5'-32P-labeled 57-mer as the DNA substrate.

Fig. 7 displays the results of adding increasing amounts of the uvsY protein to solutions containing 10 μg/ml (30.7 μM as nucleotides) of the oligonucleotide. DNA binding is clearly saturable, the maximum being observed at about 10 μg uvsY protein, a 3:1 molar ratio of nucleotides to UvsY monomers.

An important point in considering the relevance of UvsY-ssDNA interactions to the protein's role in presynapsis is its salt sensitivity, since UvsY rescues the ATPase activity of the uvsX protein at high salt. Fig. 8 presents the results of filter binding experiments conducted at increasing ionic strengths. While the DNA binding is somewhat salt-sensitive, significant amounts of protein-DNA complex are observed even at 300–400 mM potassium acetate, the highest ionic strength tested. The DNA binding is saturable, the maximum being observed at a ratio of approximately 3 eq of nucleotide per UvsY monomer. However, this apparent "site size" of three must be interpreted with caution (see text). The open squares represent control reactions in which the uvsY protein was heat-denatured prior to incubation.

**Fig. 7.** The uvsY protein has single-stranded DNA binding activity. Solutions containing 5'-32P-labeled 57-mer (30.7 μM, 3670 cpm/pmol) and the indicated amount of uvsY protein (black squares) were assayed by the nitrocellulose filter binding method. UvsY-DNA binding is saturable, the maximum being observed at a ratio of approximately 3 eq of nucleotide per UvsY monomer. However, this apparent "site size" of three must be interpreted with caution (see text). The open squares represent control reactions in which the uvsY protein was heat-denatured prior to incubation.

**Fig. 8.** Salt dependence of the uvsY protein-ssDNA interaction. Solutions containing 30.7 μM 5' labeled 57-mer, 10 μM uvsY protein, and the indicated amount of potassium acetate were analyzed as described in Fig. 6. Although uvsY protein-DNA complexation is somewhat salt-sensitive, the accessory protein retains considerable DNA-binding activity at ionic strengths sufficient to knock out the uvsX protein-catalyzed ATP hydrolysis reaction. This suggests that uvsY protein-DNA interaction could play a role in the observed UvsY-dependent rescue of this reaction at high salt.
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The experiments reported here bear on two important issues in T4 recombination: 1) the dynamics of uvsX protein-ssDNA (presynaptic) filaments and 2) the role of the uvsY protein in modulating the properties of these filaments.

Previous studies (22) of the uvsX and gene 32 protein-mediated strand exchange reaction led to the suggestion that the uvsX protein-DNA filaments that drive this reaction are dynamic in nature, constantly assembling and disassembling in a process coupled to ATP hydrolysis. This report is completely consistent with that notion. At high dilution, titration experiments (Fig. 3A) reveal that much greater than stoichiometric amounts of the uvsX protein are required to saturate the DNA. In addition, presynaptic filaments formed at high uvsX protein and ssDNA concentrations are quite sensitive to dilution. Therefore, a stable presynaptic filament that does not exchange protein subunits can be ruled out. A study of competitive reactions between the uvsx protein and the gene 32 and E. coli SSB proteins, to be reported elsewhere, is also consistent with a dynamic filament.

The UvsY protein has a dramatic effect on the properties of the presynaptic filament. In general, it can be said that the uvsY protein is a stabilizing factor. For example, it renders the uvsX protein-ssDNA complex far less susceptible to dilution and increases in ionic strength. These observations therefore suggest that the previously reported UvsY-dependent rescue of uvsX protein-mediated homologous pairing at high salt or low UvsX concentrations can be attributed at least in part to the stabilization of the presynaptic filament. It is also clear that direct protein-protein interactions between UvsY and UvsX are important, as evidenced by the inability of UvsY to stimulate RecA-catalyzed ATP hydrolysis under a variety of conditions and the kinetic observation of an intermediate composed of UvsX, UvsY, and ATP (Fig. 5).

What is the stoichiometry of the UvsX-UvsY complex? First of all, it is clear that one cannot think about this complex in terms of, for example, a simple heterodimer. Both the uvsX and uvsY proteins form large, heterogeneous aggregates of unknown structure. For example, when present at the concentrations and in the buffers employed in this report, either protein can be quantitatively pelleted by a 20-s spin in a table top microcentrifuge. This is also true of reactions containing DNA, which is not surprising given that the products of UvsX-mediated homologous pairing are huge aggregated DNA networks (8, 22, 23). Unfortunately, these colligative properties have frustrated our attempts to characterize discreet uvsX-uvsY protein complexes by native gel electrophoresis, gradient sedimentation, or other techniques (data not shown). Titration of the uvsX protein-catalyzed ATPase reactions at high salt (Fig. 2) with increasing amounts of uvsY protein clearly shows that the stimulatory effect is saturable, demonstrating that there is indeed a defined UvsX-UvsY stoichiometry. Since saturation is observed at equimolar concentrations of the uvsX and uvsY proteins, our data suggest that this functional stoichiometry is 1:1, or more properly, n:n, given that these proteins act in the context of filaments polymerized along DNA. We have observed the same apparent stoichiometry in homologous pairing assays as well as competitive DNA-binding reactions containing the SSB or gene 32 proteins. Of course, the accuracy of this value depends upon a knowledge of the percentage of active protein in our preps. More than 95% of the protein in our final uvsX and uvsY protein preparations is retained on a single-stranded DNA-agarose column even after months of storage (data not shown), suggesting that the specific activity of both proteins is very high. Nonetheless, we cannot rigorously rule out that either protein does lose some activity relevant to the catalysis of ATP hydrolysis but is still able to bind DNA, so the 1:1 stoichiometry must remain tentative until other independent measures of activity are examined. It should be noted that Harris and Griffith obtained evidence for a 3:1 UvsX to UvsY stoichiometry using similar methods (14).

How is stabilization of the uvsX protein-DNA complex achieved? Formation and maintenance of the presynaptic filament includes at least three phases: nucleation, initial growth, and, finally, a steady state flux of subunits through the filament coupled to ATP hydrolysis (22). In principle, the uvsY protein could affect any or all of these stages. A model that is consistent with our data is shown in Fig. 9. It implies that the uvsY protein is incorporated throughout the length of the presynaptic filament. Given the ssDNA-binding activity of the uvsY protein, we postulate that both UvsX and UvsY contact the nucleic acid. This feature of the model...
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would explain why the uvsY protein-aided ATPase activity of the uvsX protein is less salt-sensitive than the DNA-binding activity of UvsY (Fig. 8); the UvsX-UvsY-DNA complex may be more stable than either protein-DNA complex alone. Of course, it has not been shown unambiguously that UvsY-DNA binding is crucial to filament stabilization, so this feature remains speculative.

Filament stabilization could be achieved in two general ways. The most obvious is that the uvsY protein acts to slow loss of the uvsX protein from the filament. Alternatively, it is possible that the rate of filament decomposition is unaffected by UvsY, but that the rate of nucleation and/or filament growth is vastly stimulated. At the steady state, both schemes would yield similar results since the amount of uvsX protein complexed to DNA is determined by the ratio of the on and off rates. The kinetics of the diluted reaction (Fig. 4a) and studies of reactions in which the gene 32 or SSB proteins are present as competitors lead us to favor the slow decomposition version of the model. However, it will be necessary to directly measure association and dissociation rates of UvsX-DNA complexes in the presence and absence of the uvsY protein to distinguish rigorously between these schemes. Finally, it should be added that the two models are not mutually exclusive, it may be that both $k_{on}$ and $k_{off}$ (Fig. 9) are affected by UvsY.

REFERENCES