The *uvxs* Protein of Bacteriophage T₄ Arranges Single-stranded and Double-stranded DNA into Similar Helical Nucleoprotein Filaments*

(Received for publication, November 5, 1984)

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The bacteriophage T₄ *uvxs* gene codes for a DNA-binding protein that is important for genetic recombination in T₄-infected cells. This protein is a DNA-dependent ATPase that resembles the *Escherichia coli* recA protein in many of its properties. We have examined the binding of purified *uvxs* protein to single-stranded DNA (ssDNA) and to double-stranded DNA (dsDNA) using electron microscopy to visualize the complexes that are formed and double label analysis to measure their protein content. We find that the *uvxs* protein binds cooperatively to dsDNA, forming filaments 14 nm in diameter with an apparently helical axial repeat of 12 nm. Each repeat contains about 42 base pairs and 9–12 *uvxs* protein monomers. In solutions containing Mg²⁺, the *uvxs* protein also binds cooperatively to ssDNA. The filaments that result are 14 nm in diameter, show a 12-nm axial repeat, and they are nearly identical in appearance to the filaments that contain dsDNA. In the filaments formed along ssDNA, each axial repeat contains about 49 DNA bases and 9–12 *uvxs* protein monomers. Both the filaments formed on the ssDNA and dsDNA show a strong tendency to align side-by-side. T₄ gene 32 protein also binds cooperatively to ssDNA and interacts both physically and functionally with *uvxs* protein. However, when gene 32 and *uvxs* proteins were added to ssDNA together, no interaction between the two proteins was detected.

The product of the bacteriophage T₄ *uvxs* gene is involved in the major pathway for genetic recombination in T₄-infected *Escherichia coli* cells. Lesions at the *uvxs* locus produce decreased recombination frequencies, a smaller burst size, an early arrest of DNA synthesis, and an increased sensitivity to ultraviolet radiation and other DNA-damaging agents (1, 2).

The *uvxs* protein has been purified by T. Yonesaki and T. Minagawa (Koyoto University, Koyoto, Japan), whose characterization has revealed several similarities between the *uvxs* protein and the *E. coli* recA protein. For example, both are DNA-dependent ATPases and both catalyze pairing of homologous DNA molecules.

The T₄ gene 32 protein is a single strand-binding or helixdestabilizing protein that is required for bacteriophage T₄ DNA replication, recombination, and repair (3). When agarose matrices containing covalently bound gene 32 protein were used to chromatograph lysates of T₄-infected cells, the *uvxs* protein was one of several T₄ proteins that was specifically retained (4). This association allowed us to partially purify the *uvxs* protein, which we have now further purified by standard chromatographic techniques to greater than 98% homogeneity as judged by electrophoresis on SDS–polyacrylamide gels.

Our characterization indicates that the *uvxs* protein has an apparent Mᵦ = 40,000 and is a ssDNA-dependent ATPase. Surprisingly, the ATPase produces a mixture of both ADP + Pᵦ and AMP + PPᵦ. Both the ADP- and AMP-producing activities exactly coelute from several chromatographic matrices, and identical ratios of ADP to AMP are produced using *uvxs* protein prepared by different purification schemes and from different types of infected cell. We therefore believe that both activities are intrinsic to the *uvxs* protein. As with the *E. coli* recA protein, dsDNA does not appear to serve as a cofactor for the *uvxs* ATPase activity.

Both we and Yonesaki and Minagawa have determined that homologous pairing and strand exchange of DNA molecules are catalyzed by the *uvxs* protein using assays similar to those developed for studying the *E. coli* recA protein (5–7).

When circular ssDNA is mixed with linear dsDNA, we find that the *uvxs* protein catalyzes extensive synopsis which is dependent on 1 homology between the DNA molecules, 2 ATP hydrolysis, and 3 the addition of the T₄ gene 32 protein. Gene 32 protein does not catalyze this reaction without *uvxs* protein. While we have not yet fully characterized the reaction, it appears that while synopsis is very efficiently promoted, a less extensive strand exchange occurs than has been reported with recA protein as the catalytic agent. This suggests that the *uvxs* protein catalyzes the directed branch migration that follows synopsis less efficiently than does the *E. coli* recA protein.

These results suggest that the *uvxs* protein, by analogy to recA protein, may provide a protein scaffold upon which DNA synopsis and subsequent strand exchange reactions occur. An understanding of the mechanism by which these proteins, acting synergistically with their respective helixdestabilizing proteins, orchestrate genetic recombination events will require a detailed knowledge of the ultrastructure of the complexes they form with DNA. Such studies are being pursued in several laboratories with recA protein (8–12).

Here, we report our initial characterization of *uvxs* protein binding to DNA. We show that the *uvxs* protein has the properties one

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*This work was supported by Grant GM31819 from the National Institutes of Health and the National Cancer Institute Grant CA16086 to J. G. T. F was supported by National Institutes of Health Grant GM-24020. 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.

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1 The abbreviations used are: SDS, sodium dodecyl sulfate; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; ATPyS, adenosine 5'-0-(3'-thiotriphosphate); EM, electron microscopy; bp, base pairs.

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might expect of a recombinational scaffolding protein: 1) it forms regular protein helices along both ssDNA and dsDNA which show a strong tendency to align side-by-side; 2) the protein scaffold binds ssDNA to a length very close to that of dsDNA; and 3) the uvsX protein is required in proportionately large amounts (relative to the DNA template) to catalyze DNA strand exchange.

**EXPERIMENTAL PROCEDURES**

**Proteins and DNA**—Bacteriophage T₄, gene 32 protein was prepared as described (13). The uvsX protein was purified by a procedure to be described in detail elsewhere. Briefly, *E. coli* B cells infected with DNase 1 and cleared by centrifugation. The supernatant was prepared as described (13). The uvsX protein was purified by a procedure (80% Bio-Rad Bio-Gel HTP, 20% Whatman cellulose CF11) and of dsDNA; and 3) the uvsX protein is required in proportionately large amounts (relative to the DNA template) to catalyze DNA replication.

**Visualization of uvsX Protein-DNA Filaments**

Protein-DNA Filaments—To examine the binding of uvsX protein to dsDNA by EM, the uvsX protein was incubated for various times at 37 °C with duplex plasmid and viral DNAs at protein to DNA ratios from 1:1 to 100:1 (weight/weight) in the presence of different salts. Following incubation, the samples were prepared for EM and examined (“Experimental Procedures” and Fig. 1). The binding of uvsX protein to dsDNA produced thick, stiff nucleoprotein filaments with distinct axial repeats (Fig. 1). The binding was highly cooperative; most of the molecules were either fully protein-covered or were protein-free. In those complexes that were only partially covered, only one protein tract was seen, suggesting that nucleation is slow relative to the rapid addition of protein to the end of the growing filament. Networks in which the filaments were aligned side-by-side were a common observation, particularly when incubations were carried out at low Mg²⁺ concentration.

Filaments identical to those in Fig. 1 were observed when the incubations were carried out in TE (0.01 M Tris acetate, pH 7.5, 0.1 mM EDTA), TE with 1, 2, or 12 mM Mg²⁺, TE with 6 mM Mg²⁺ plus 2 mM ATP, or TE with 12 mM Mg²⁺ plus 5 mM ATP. Full coverage of the DNA was observed when the amount of the uvsX protein added exceeded 20 times the mass of the dsDNA. The rate of DNA coverage by the uvsX protein was temperature-dependent; aliquots from a reaction with M13mp7 dsDNA (see Fig. 2A) required 5 min of incubation at 37 °C to complete the coverage of the DNA, 10 min at 20 °C, and 20 min when incubations were carried out at 0 °C; in all cases, the appearance of the filaments remained the same, and single protein tracts were observed along the DNA. This suggests that the elongation of the growing uvsX protein tract was rate-limiting and temperature-dependent (as contrasted to nucleation on the dsDNA) and that about 12 bp were covered per s at 20 °C. Optimal conditions for DNA binding appeared to be incubation at 37 °C in TE with 12 mM Mg²⁺; however, when 0.1 M NaCl or 0.15 M potassium acetate was added to such a mixture, no binding was observed by EM.

The length of the uvsX-dsDNA filaments formed with linear or nicked circular M13mp7 dsDNA was measured following their preparation for EM by dehydration and shadowcasting. A value of 2.10 ± 0.08 pm (37 measurements) was obtained when the complexes were formed in TE with 2 mM Mg²⁺ plus 1 mM ATP and at a 50:1 protein to DNA (weight/weight) ratio. This value corresponds to 97 ± 4% the length of the protein-free M13mp7 dsDNA (2.17 nm) prepared for EM in the same manner. The same lengths were observed when the complexes were prepared in TE alone or TE with 12 mM Mg²⁺ plus 5 mM ATP.

Preparation of the samples for shadowcasting necessitates a dehydration step which might alter the structure of the protein-DNA complexes. To examine this possibility, aliquots of uvsX protein-M13mp7 linear dsDNA complexes formed as above were embedded in a film of uranyl acetate immediately after fixation and examined directly. Linear filaments with a very distinct axial repeat were observed (Fig. 2A). The length of these filaments was the same as that measured after shadowcasting, within experimental error. The diameter of the
FIG. 1. Visualization of uvsX protein bound to dsDNA by shadowcasting. The uvsX protein (5 μg) was mixed with 0.1 μg of a mixture of 9.4-kilobase monomer and 18.8-kilobase dimer pB90 plasmid supertwisted circles (25) in 0.1 ml of TE buffer (0.01 M Tris acetate, pH 7.5, 0.1 mM EDTA) with 12 mM MgCl$_2$ and 5 mM ATP and then incubated for 8 min at 37 °C. The sample was fixed and prepared for EM, including shadowcasting with tungsten (see “Experimental Procedures”). A dimer circle is shown here in reverse contrast; bar equals 1 μm.

Filaments measured 14 ± 1.3 nm, and the axial repeat measured 12 ± 2 nm. When the same samples were examined by negative staining prior to fixation and filtration, no difference in either the appearance or the dimensions of the filaments was observed, which shows that the fixation step did not appreciably alter their structure. The 12-nm length of the axial repeat was confirmed by counting the total number of repeats on these linear filaments and dividing by the total filament length. From these values (170 repeats per filament and 7230 base pairs of DNA), we calculate that each axial repeat is 12 nm in length and contains 42 base pairs of DNA.

When the uvsX protein was incubated with supertwisted DNAs, circular nucleoprotein loops were observed that were twisted about themselves (Fig. 1), and there was no apparent difference in the rate of protein binding to either nicked or supertwisted DNA. The loops, however, appeared less twisted than the protein-free DNA. For example, when fd dsDNA, which was known to contain 35 ± 3 supertwists from direct band counting by agarose gel electrophoresis (18, 19), was covered with uvsX protein, the resulting complexes exhibited only 5 ± 2 twists (40 molecules counted). It is unlikely that the DNA had become nicked since the binding of uvsX protein to nicked circular DNAs yielded circular loops with no twists. Examination of the electron photomicrographs leads us to conclude that this reduction in twists could be due to the physical constraints of winding the thick filament about itself. If it were instead due to an unwinding of the DNA helix by the uvsX protein, the unwinding would amount to 1-2°/base pair.

The E. coli recA protein binds very tightly to dsDNA in the presence of the ATP analog, ATPγS, and in doing so extends the DNA 1.6-fold and unwinds the DNA helix by 12.5°/base pair (8, 11, 20). Similar incubation conditions were used for the uvsX protein and dsDNA in the presence of ATPγS or the βγ-imido-ATP analog. No evidence was found for a similar unwinding or linear extension of the DNA by the uvsX protein.

The uvsX Protein Binds to Single-stranded DNA—Visual-
Visualization of uvsX Protein-DNA Filaments

FIG. 2. Visualization of uvsX protein bound to ssDNA and dsDNA by negative staining. A, uvsX protein was complexed with linear M13mp7 dsDNA exactly as in Fig. 1 and then prepared for EM, including fixation and staining with 1% uranyl acetate (see “Experimental Procedures”). B, uvsX protein (5 μg) was complexed with 0.1 μg of M13mp7 ssDNA as described in Fig. 1 and prepared for EM as in A above. Insets show portions of each at higher magnification. Bar equals 0.2 μm (0.1 μm for insets).

Visualization of uvsX protein bound to circular M13mp7 ssDNA revealed the formation of circular nucleoprotein filaments nearly identical in appearance to those formed with duplex DNA and at a rate severalfold faster than the assembly onto dsDNA. These filaments, like those containing dsDNA, readily formed intertangled networks with the individual filaments aligned side-by-side. Since the uvsX protein hydrolyzes ATP in the presence of ssDNA, the appearance of filaments formed in the presence of uvsX protein, ssDNA, and ATP might be expected to be time-dependent. When uvsX protein was incubated with M13mp7 ssDNA in TE with 12 mM Mg2+ plus 5 mM ATP, filament loops with distinct axial repeats were observed within 15 s (Fig. 2B). The greatest number of complexes were observed after incubating from 1 to 10 min. Fewer complexes were seen after 2-h incubations when all of the ATP was hydrolyzed, but those observed were similar in appearance to the ones seen at 15 s. The lack of partially coated molecules suggests that the dissolution occurs in a cooperative fashion, much as assembly does. Full coverage of the ssDNA was achieved when the amount of uvsX protein added exceeded 35 times the mass of the ssDNA, a significantly greater ratio than that required to coat dsDNA.

When incubations were carried out in TE plus 2-12 mM Mg2+ without ATP, apparently identical complexes were observed, except that they were stable to prolonged incubation. When ATP was added after uvsX protein had been assembled onto ssDNA in the presence of Mg2+, the protein was eventually released. These results suggest that the same complex was formed in the presence of Mg2+ whether or not ATP was added and that the dissolution was dependent on the products of ATP hydrolysis. The binding of uvsX protein was inhibited by 0.1 M NaCl, but unlike results obtained with dsDNA, binding to ssDNA was observed in TE plus 12 mM Mg2+ and 0.15 M potassium acetate.

The length of the circular filaments formed with M13mp7 ssDNA in the presence of Mg2+ plus or minus ATP measured 1.85 ± 0.1 μm (25 measured) which corresponds to 85 ± 5% the length of the duplex, protein-free form of the DNA (2.17 μm). The diameter of the filaments, measured in negatively stained images (Fig. 2B), was 14 ± 1 nm (71 measurements), and the axial repeat measured 12.5 ± 1.4 nm (92 measurements). The axial repeat was independently estimated to be 12.5 nm by counting the total number of repeats in 10 circles (average of 150) and dividing into the filament length (1.85 μm). Thus, we calculate that there are 49 bases in each 12.5-nm axial repeat of the uvsX protein-ssDNA filaments formed in the presence of Mg2+.

When the uvsX protein was incubated with M13mp7 ssDNA in TE buffer alone (without Mg2+), some individual nucleoprotein loops were observed (Fig. 3A); however, an even greater fraction of the DNA was present in large networks with the filaments aligned side-by-side (Fig. 3B). The contour
length of the ssDNA-uvsX protein filaments formed in TE, however, was less than that observed with Mg²⁺ present: 1.2-1.3 μm (38 molecules measured) which corresponds to 60% the length of the M13mp7 dsDNA (2.17 μm). The axial repeat length (12 nm) and filament diameter (14 nm) measured the same as in filaments formed in the presence of Mg²⁺. One explanation is that Mg²⁺ is required for full coverage of the DNA and that small protein-free gaps were present but remained undetected by these EM methods.

The uvsX and Gene 32 Proteins Binding to Single-stranded DNA Independently—The T₄ uvsX protein binds to agarose matrices containing covalently bound gene 32 protein (4), and gene 32 protein binds to a uvsX protein-agarose column (21). Since gene 32 protein is required for the uvsX protein-cata-lyzed homologous pairing between ssDNA circles and dsDNA linears, it seemed possible that a complex of the two proteins might form that arranges ssDNA into a structure different from that produced by either protein alone. To investigate this possibility, the gene 32 protein and uvsX protein were incubated together in TE with 12 mM Mg²⁺ and 5 mM ATP at 37 °C for 5 min and then added to M13mp7 ssDNA in the same buffer. Samples were taken for examination by EM after incubating at 37 °C for periods from 15 s to 2 h. In the experiment illustrated in Fig. 4, a uvsX protein/ssDNA ratio of 100:1 (weight/weight) and a gene 32 protein/ssDNA ratio of 20:1 (weight/weight) were employed. All of the ssDNA was extended into open nucleoprotein loops after as little as a 15-s incubation. Many of the ssDNA circles appeared to be covered exclusively by either uvsX protein or gene 32 protein. In those ssDNA circles that showed both proteins bound, there were long tracts typical of each type of complex. Upon 1-2 h incubation at 37 °C, a greater fraction of the ssDNA appeared to be complexed by gene 32 protein, probably due to the inhibition of uvsX protein binding by the products of ATP hydrolysis. At short times with this input ratio (5:1 ratio of uvsX protein to gene 32 protein), roughly half of the total ssDNA was covered by uvsX protein and half by gene 32 protein. Increasing or decreasing the uvsX protein/gene 32 protein ratio led to the expected increase or decrease in coverage by one or the other protein.

The uvsX Protein-DNA Filaments Contain 10 uvsX Protein Monomers in Each Axial Repeat—To measure the number of uvsX protein monomers in each axial repeat of these filaments, ³H-labeled uvsX protein was complexed with ¹²⁵I-labeled dsDNA (see “Experimental Procedures”) under conditions where the DNA was completely covered by protein. The samples were fixed (as for EM) and then chromatographed on Sepharose 4B columns as shown in Fig. 5A. Full coverage of the dsDNA was verified by EM. From the ratio of counts in the excluded complex peak (Fig. 5A), we calculate that the protein/dsDNA mass ratio is 14:1. Assuming a molecular weight for uvsX protein of 40,000, this corresponds to 1 monomer of uvsX protein for every 4.2 base pairs, or 10 monomers of protein/each axial repeat which contains 42 bp of DNA.

In experiments with ssDNA, complexes formed in TE with 12 mM Mg²⁺ and 5 mM ATP were incubated for 60 s (Fig. 5B) or for 2 h (Fig. 5C) at 37 °C. A third sample was incubated for 30 min in the same buffer but without ATP (Fig. 5D). Mass ratios of 150:1 uvsX protein/ssDNA were used to ensure complete coverage of the DNA. From the specific activities, we calculate that the uvsX protein/ssDNA mass ratio in the peak fractions was 22:1 (weight/weight) for the 60-s sample and 25:1 for the sample incubated for 30 min without ATP. In the sample incubated for 2 h, the ratio was 18:1, indicating that some protein had been released. A value of 22-25:1 corresponds to about 1 monomer of uvsX protein/5 nucleotides, or 10 monomers/49 bases: the number of bases in each of the repeating units in the fiber.

**DISCUSSION**

In this study we have examined the binding of the uvsX protein to both ssDNA and dsDNA by electron microscopy. We observe the same structures whether the samples are fixed or not and whether they are dehydrated for shadowcasting or embedded in a film of uranyl acetate. This argues against the possibility that artifactual changes are induced by the EM preparative procedures. Unpublished EM observations of T.
Visualization of uvsX Protein-DNA Filaments

**FIG. 4.** Visualization of shadowcasting of uvsX and gene 32 proteins bound to ssDNA. uvsX protein (0.5 pg) and gene 32 protein (1 pg) were incubated in 0.1 ml of TE buffer with 12 mM Mg²⁺ and 5 mM ATP for 10 min at 37 °C and then added to 0.1 µg of M13 mp7ssDNA in the same buffer. Following incubation for 5 more, the sample was fixed and prepared for EM (see "Experimental Procedures"). The micrographs are shown in reverse contrast. Bar equals 0.2 µm (A) and 0.4 µm (B and C).

Minigawa on the binding of uvsX protein to DNA cited in Footnote 1 add further support to our results.

We have found that each axial repeat on filaments formed with either ssDNA (in solutions containing Mg²⁺) or dsDNA contains the same number of uvsX protein monomers. Also, each type of complex appears indistinguishable by EM. These observations suggest that the filament structure is shaped more by protein-protein interactions than by protein-DNA interactions. The value of 10 uvsX protein monomers/helical repeat is based on assumptions that there is full coverage of the DNA by the uvsX protein, and that the fixation process does not cause a net release or gain of protein from the DNA. Direct EM visualization of the purified double-labeled complexes from which these values were obtained support these assumptions. (When the complexes were separated from free uvsX protein by gel filtration without fixation, a variable amount of protein release was observed by EM.) From a consideration of all possible errors, the extreme values are likely to be between 9 and 12 monomers/repeat, and our estimate of 10, if in error, is more likely to be low than high.

The length of the uvsX-dsDNA filaments corresponds to 3.0 A/bp, and each helical repeat contains 42 bp. The rise of natural protein-free dsDNA in solution is 3.0 A/bp (22), and there are 10.5 bp/turn (23). A repeat of 42 bp contains exactly four repeats of such a helix. These correlations suggest that each helical repeat contains four turns of the DNA helix lying along the core of the filament with 9–12 monomers of uvsX protein arranged in a helical path about the DNA. This model agrees with our finding that the pitch of the DNA helix is not appreciably altered by uvsX protein binding.

Greater differences were observed between the binding of the uvsX and recA protein to dsDNA. In the presence of the nonhydrolyzable ATP analog ATP-γ-S, recA protein binds tightly to dsDNA and in doing so extends the DNA by 1.6-fold and unwinds the helix by 12.5°/base pair (8, 9, 11). We observed no evidence for a parallel effect of ATP analogs on uvsX binding to dsDNA. Optimal rates of strand exchange catalyzed by recA protein are observed in TE buffer with 12 mM Mg²⁺ plus 3 mM ATP (24). Under these conditions, recA protein does not bind to dsDNA as visualized by EM, whereas uvsX protein binds dsDNA avidly. The catalysis of strand exchange by uvsX protein, however, appears to require higher

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\(^4\) J. Griffith and T. Formosa, unpublished observations.
Measurement of the protein stoichiometry of the uvsX protein-DNA complexes. A, uvsX protein labeled with $^{125}$I (18 µg, 150 cpm/µg) was mixed with $^3$H-labeled M13mp7 dsDNA (0.4 µg, 16,300 cpm/µg), and the mixture was incubated for 10 min at 37°C. The sample was then fixed as for EM and filtered on a 1 x 30 cm Sepharose 4B column. B-D, complexes of $^{125}$I-labeled uvsX protein salt (0.15 M potassium acetate). At this salt concentration uvsX protein was observed to bind to ssDNA but not to dsDNA. Thus, when compared under the conditions where each protein optimally catalyzes strand exchange, the dsDNA binding properties of the recA and uvsX proteins are rather similar.

Both the uvsX and recA proteins catalyze DNA strand exchange, they form regular protein helices along both ssDNA and dsDNA that show a strong tendency to align side-by-side, and they extend ssDNA to a length close to that of dsDNA. The relatively large amount of recA or uvsX protein required in these reactions (relative to the DNA templates) suggests that formation of nucleoprotein filaments like those seen by EM is an essential step in the reactions. Proteins that exhibit these properties could be termed recombinational scaffolding proteins. We do not yet understand in detail how proteins of this class participate in the catalysis of DNA synopsis and strand exchange. However, current models, shaped by our knowledge of the DNA binding properties of the recA protein, envision that during pairing, the two DNA partners are held in close association over an extensive region by their envelopment into helical recA protein-DNA filaments or scaffolds (11). A cyclic unwinding of the dsDNA may occur within these filaments as the point of DNA strand transfer moves forward (8). In a pairing event between ssDNA and dsDNA, the arrangement of ssDNA into a conformation similar to that of dsDNA would naturally seem to facilitate pairing and exchange.

These studies represent an initial investigation of the DNA-binding properties of the bacteriophage T4 uvsX protein. Many questions remain unanswered. In particular, no function for the gene 32 protein in these reactions has been suggested by our study despite its dramatic effect on the uvsX protein activity. Also, the relatively slow rate of catalyzed branch migration observed in vitro may mean that either the reaction conditions are not optimal or that other protein factors are involved. Further studies will be required to approach these questions and to ultimately describe the molecular mechanism by which the uvsX protein, the gene 32 protein, and other bacteriophage T4 phage-induced proteins orchestrate genetic recombination events in the infected cell.

Acknowledgment—We thank Dr. Alberts for help and critical reading of the manuscript.

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(18 µg, 150 cpm/µg) and $^3$H-labeled M13mp7 ssDNA (0.27 µg; 44,400 cpm/µg) were prepared in TE buffer with 12 mM Mg$^2+$, 5 mM ATP (B and C), or in TE buffer with 12 mM Mg$^2+$ only (D). Incubations were at 37°C for 60 (B), 2 h (C), and 30 min (D). Following incubation, the samples were fixed and filtered on Sepharose 4B as above. Protein/DNA ratios determined from the ratio of counts after correction for spillover (see "Experimental Procedures") are described in the text.
Visualization of uvsX Protein-DNA Filaments

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