Cloning of the Bacteriophage T4 uvsX Gene and Purification and Characterization of the T4 uvsX Recombination Protein*

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The bacteriophage T4 uvsX gene is a nonessential gene required for normal levels of DNA repair, recombination, and replication. We demonstrate that plasmids containing the T4 DNA approximately 300–2800 base pairs upstream of T4 gene 41 express a biologically active uvsX protein. This uvsX protein imparts increased survival to UV-irradiated T4 uvsX− phage and decreases the T4 uvsX− mutant suppression of a conditionally lethal T4 mutant in the gene 49 recombination nuclease.

The uvsX protein purified from cells with a uvsX+ plasmid catalyzes ATP hydrolysis to ADP and AMP and, in the presence of the T4 gene 32 helix-destabilizing protein, ATP-dependent strand exchange between homologous circular single-stranded and linear duplex DNA. These results agree with the recent characterization of uvsX protein from T4-infected cells by Yonesaki et al. (Yonesaki, T., Ryo, Y., Minagawa, T., and Takahashi, H. (1985) Eur. J. Biochem. 148, 127–134) and by Formosa and Alberts (Formosa, T., and Alberts, B. M. (1984) Cold Spring Harbor Symp. Quant. Biol. 49, 363–370). In addition, we find that under some reaction conditions strand exchange is catalyzed by uvsX protein in the absence of 32 protein.

The level of the uvsX protein expressed by the uvsX+ plasmids is high and independent of the orientation of the T4 DNA within the vector. This suggests that transcription promoter(s) lie upstream of the uvsX gene on the cloned T4 DNA. In vitro transcription of T4 DNA restriction fragments reveals two tandem promoters whose transcripts initiate approximately 500 and 600 nucleotides upstream of the uvsX gene and extend through the gene.

The genome of bacteriophage T4 is a linear duplex of 186 kilobase pairs which is circularly permuted and terminally redundant (1, 2). Genetic analyses of T4 mutants displaying altered DNA synthesis have implicated many phage genes in DNA replication and metabolism (3, 4). Generally, these mutants have been classified as belonging to one of the following three categories: DNA− (no DNA synthesis), DNA delay (delay in onset of DNA synthesis), and DNA arrest (early stop of DNA synthesis). In vitro studies (5–7) have demonstrated that the products of six DNA− genes (gene 43-DNA polymerase, 44-, 62-, 45-polymerase accessory proteins, 32-helix-destabilizing protein, and 41-primease component) plus one DNA delay gene (61-primease component) together catalyze efficient strand displacement synthesis of DNA on a nicked circular template and prime discontinuous synthesis on the displaced strand. These studies suggest that these seven T4 gene products are the proteins required in vivo for new DNA synthesis on the leading and lagging strands of a replication fork. As DNA synthesis proceeds in vivo, the main form of T4 DNA observed is a complex network (8) which sediments faster than T4 DNA extracted from the phage (9).

Mosig and co-workers have proposed (reviewed in Ref. 10) that this network arises from strand invasion by the single-stranded DNA present at the unreplicated terminus of one DNA molecule into the homologous duplex region of another T4 genome. It is proposed that the invading single strand is then extended by the complex of replication enzymes. Since the T4 genome is circularly permuted, such a recombination/replication process would result in the type of network of highly branched concatenated structures observed for replicating T4 DNA.

The T4 genes needed for the observation of the T4 concatenated structures constitute a recombination pathway (discussed in Refs. 11 and 12) which is thought to include (among other T4 genes) uvsX, uvsY, uswW, and gene 49. Proposals concerning the functions of these gene products are based in part on the following evidence. First, in vivo, conditionally lethal T4 gene 49 mutants, under nonpermissive conditions, accumulate more complex networks of T4 DNA (13) with even higher sedimentation values (14, 15). Extracts from T4 49+ infections, but not from T4 49−, convert this DNA to material which sediments more slowly (14). Furthermore, the purified gene 49 protein, a DNA endonuclease (16, 17), cleaves DNA substrates containing cruciforms or Holiday structures at the branch point to yield products which can be sealed with DNA ligase (18). Taken together, these studies support the proposal that the gene 49 protein is required to resolve the recombinational network into structures which can be processed for packaging (13, 14, 18). The nonessential T4 genes, uvsX, uvsY, and uswW, are needed for the wild type level of resistance to UV and ionizing radiation and chemical agents, implicating these gene products in DNA repair (for a review, see Ref. 19). In addition, these genes are also needed for normal T4 recombination. Mutants in each of these three genes display decreased recombination frequencies (11, 20, 21), and uvsX or uvsY mutants have a DNA arrest phenotype (22, 23) consistent with their inability to continue normal DNA synthesis which is thought to proceed through recombination intermediates. Mutations in uvsX or uvsY also suppress gene 49 mutations (22–24), and a uvsX− (or uvsY−) 49− double mutant infection does not produce the very fast sedimenting DNA observed after 49− infections (25). These results

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support the hypothesis that the uvsX and uvsY gene products contribute to the formation of the concatenated structures which require 49 nucleases for processing (12, 25). Presumably, a uvsX<sup>−49</sup> or uvsY<sup>−49</sup> mutant survives by packaging the few T4 progeny genomes produced by replication without the recombination intermediates.

A more precise role for the uvsX gene product in recombination has been elucidated by the biochemical studies of Yonesaki et al. (26) and by Formosa and Alberts (Ref. 27, cited in Refs. 28 and 29). These workers have purified the uvsX protein from T4-infected cells and report that this protein is a single-stranded DNA-dependent ATPase that promotes ATP-dependent homologous pairing and strand exchange of DNA. These DNA-pairing reactions are analogous to those carried out by the Escherichia coli recA protein. Griffith and Formosa (29) have recently shown that, again like the recA protein, uvsX protein binds cooperatively and tightly to double-stranded or single-stranded DNA to form filaments which can be observed by electron microscopy. Taken together, these in vitro observations suggest that the uvsX protein, through its promotion of homologous pairing, contributes directly to the formation of the concatenated structures of replicating T4 DNA observed in vivo.

Genetic mapping experiments have assigned the uvsX gene upstream of gene 41 (22, 30). We have located gene 41 on the physical map of T4 by showing that plasmids containing T4 map units 22.01 to 20.06 express 41 protein (31). In the process of constructing plasmids containing gene 41, we have found that the region upstream of gene 41 encodes a protein of about 46 kDa (this paper). We show that this protein is the T4 uvsX gene product and that the presence of a plasmid expressing uvsX protein in the cell complements T4 uvsX<sup>−</sup> phage. In addition, we have purified the protein expressed by a uvsX plasmid to homogeneity. Our preliminary characterization of this protein indicates that, like uvsX protein from T4-infected cells, it is a ssDNA-dependent ATPase and a dsDNA-binding protein which promotes strand exchange between linear duplex and homologous single-stranded DNA.

**Experimental Procedures**

**RESULTS**

The T4 DNA Upstream of Gene 41 Expresses a Protein with an Apparent Molecular Weight of 46,000—We have previously shown (31) that the plasmid pDH428 (T4 DNA from map units 24.3 to 20.06 (Fig. 1, lane B) contains gene 41 in the region between the BglII site at 22.01 and the HindIII site at 20.06. Although 41 protein is expressed by pDH428/DH1 cells at a level that can be easily detected in a complementation assay for DNA synthesis (31), the level of the protein is not high enough to observe a 46-kDa band co-migrating with 41 protein on a Coomassie Blue-stained polyacrylamide-SDS gel (Fig. 2, lanes 7 and 15). However, comparison of the proteins expressed by cells with the vector pDH428 with those expressed by cells containing the vector (lane 1) does reveal the presence of a band of another size (46 kDa) that is apparently dependent on the T4 DNA sequence. To determine whether this new protein is encoded by the T4 DNA, we constructed several plasmids (Fig. 1A) which include part of the 4500 bp of T4 DNA cloned into pDH428. We then asked whether cells containing these plasmids also express the new protein. As shown in Fig. 2 (lane 14), cells with the plasmid pDH428Δ1, in which 3980 bp of T4 and vector DNA downstream of the BglII site have been deleted, produce the 46-kDa band. This result suggests that only the T4 DNA region upstream of the BglII site is necessary for expression of this protein. Furthermore, production of the protein, at a level high enough to be observed in these crude extracts, must require at least part of the 360 bp of T4 DNA between the BglII site (map unit 22.37) and the BglIII site (map unit 22.01) since deletion of this region (pDH428Δ2) eliminates the protein band (lane 12).

Direct evidence that the protein is encoded by the T4 DNA upstream of gene 41 was obtained by observing in vitro translation products expressed by plasmids containing this region or expressed by T4 DNA restriction fragments (Fig. 3, A and B). Supercoiled plasmid DNA or linear T4 DNA restriction fragments were transcribed and translated in vitro in the presence of [<sup>35</sup>S]methionine using an E. coli S-30 extract. Transcription/translation of the vector plasmid (panel A, lane 2) gives one product of 34 kDa. We assign this protein as β-lactamase, the product of the amp<sup>+</sup> gene which is present in the pBR322 sequence and has been previously shown to be a major in vitro translation product of pBR322 (46). Insertion of the T4 DNA within the vector to yield the recombinant plasmids pDH428 (panel A, lanes 3 and 4) and pDH447 (panel A, lane 5) results in plasmids whose major translation product is a highly expressed species of 46 kDa. The 46-kDa band is again observed as the major species if just the T4 DNA from this region, a SalI restriction fragment (map units 24.8 to 2075) or an EcoRI fragment (map units 24.3 to 21.15) is transcribed and translated in vitro (Fig. 3B, lanes 2 and 3). These results support the previous in vivo results indicating that the gene for the 46-kDa protein is present on the T4 DNA.

We have inferred the direction of the 46-kDa gene based on the translation products expressed by the plasmid pDH428Δ5, a plasmid identical to pDH428 except that the 800 bp between the Clal sites at map units 22.85 and 22.00 have been deleted (Fig. 1A). The 46-kDa protein is not made by this plasmid in vivo (Fig. 2, lane 9) or in vitro (Fig. 3A, lane 6). However, in vitro, the major species is a highly expressed new protein of 30 kDa. We interpret the 30-kDa band as an abnormal protein generated by the fusion of the open reading frame of the 46-kDa protein to new DNA sequences. In addition, we assume that this fusion has occurred by joining the DNA encoding the N terminus of the normal 46-kDa protein to new sequences, rather than fusing the DNA of the C terminus to another start. This assumption is reasonable since the 30- and the 46-kDa proteins are similarly expressed at a very high level and represent the major translation products of pDH428Δ3 and pDH428 (or pDH447), respectively. Since the plasmid pDH428Δ1 produces normal levels of the 46-kDa protein in vivo (Fig. 2, lane 14), the gene for this protein cannot begin downstream of the BglII site at map unit 22.01. Thus, we conclude that the production of the abnormal 30-kDa protein by pDH428Δ5 indicates that the normal gene starts upstream of the Clal site at map unit 22.85 and proceeds toward gene 41. This direction is consistent with the previously determined general direction of early T4 transcription in vivo (2), the direction of the immediately down-
Cloning of T4 uvsX Gene

Fig. 1. The bacteriophage T4 genome in the region of genes 41, 61, and uvsX. Panel A shows the T4 DNA from a SalI site at map unit 24.8 through gene 61. (Map units refer to those in Ref. 58 and represent approximately 1000 bp.) The positions of genes 41 and 61 have been determined previously (31, 32, 59, 60). The identification and approximate position of the uvsX gene is given in this paper. The position of the uvsX gene is denoted by dashes to emphasize remaining uncertainties in the exact position of the 5' and 3' termini of the gene (see "Results"). The position of two E. coli RNA polymerase promoters active in vitro are designated as and have been located upstream of uvsX (see Fig. 4). Beneath the restriction map, the regions of T4 DNA inserted in various plasmids are shown by open bars. Solid bars denote deleted regions. Details of the plasmid constructions are given under "Experimental Procedures" and in Ref. 31. (pDH428A1 represents a deletion of DNA in pDH428 between the BglII site at 22.01 and the NdeI site within the vector shown in panel B. Thus, the vector DNA present in this plasmid differs from the other plasmids shown.) Restriction sites referred to in the text are shown and have been determined using T4 dC-DNA and/or the plasmids containing cloned T4 DNA from this region. We have observed differences from the reported restriction sites (58) in the region between the EcoRI site at map unit 24.3 and the XbaI site at 22.37. We do not observe a PstI site reported to lie at map unit 22.40 after restriction of the purified T4 SalI fragment (map units 24.8 to 20.75) or restriction of the plasmids. In addition, our calculation of distances between the EcoRI site at 24.3 and the XbaI site at 22.37, again using the purified SalI fragment and the plasmids, differs from reported values (58). We have indicated our values of these distances which were used to determine the start of the in vitro transcripts (see text). Panel B shows the plasmid pDH428 (31) which contains T4 DNA from the EcoRI site at map unit 24.3 to the HindIII site at map unit 20.06. The T4 DNA has been ligated to a fragment of the gal expression vector pKG1810 (37) which contains E. coli sequence (---) with the IS2 transcription terminator (T) and the galK gene followed by pBR322 sequence (--------) from 2068 to 4363 (61). amp' designates the ampicillin resistance gene expressing β-lactamase.

stream genes 41 and 61 (31, 32), and the position of the major promoters in this region as determined in vitro (see below). Based on the size of the abnormal pDH428Δ3 product (30 kDa), we estimate that the farthest upstream position for the 5' end of the 46-kDa gene (assuming no introns) is 820 bp upstream of the ClaI site at map unit 22.85 (enough sequence to encode a protein of 30,000 daltons). Since the 46-kDa protein is observed in vivo in cells containing pDH428Δ1 (Fig. 2, lane 14) but is not observed in those containing pDH428Δ2 (Fig. 2, lane 12), we conclude that the 3' end of the gene
were from DH1 cells containing the following plasmids: pKG1810, pDH428, pDH447, pDH428A2, and pDH4212. See Fig. 1A for DNA contained in each plasmid.

probably lies between the XbaI site at map unit 22.37 and the BglII site at 22.01. Thus, we position the gene for the 46-kDa protein upstream of gene 41 on the T4 genome as indicated in Fig. 1A.

**In Vitro Transcription Demonstrates That the T4 DNA Upstream of the 46-kDa Gene Contains Two Promoters for E. coli RNA Polymerase**—In the previous translation experiments, we observed a high level of the 46-kDa protein both in vivo and in vitro. In addition, the expression of the protein is independent of the orientation of the T4 DNA within the plasmid (Fig. 2: compare pDH4426 with pDH447 [lanes 4 and 5] and pDH428 with pDH421 [lanes 7 and 8]). These results suggest either that the T4 DNA contains its own transcription promoter(s) for the expression of the 46-kDa gene or (less likely) that the gene is translated at a very high level from upstream of the NdeI site. Initiation at these promoters generates transcripts that extend in vitro through the region we have assigned to the gene for the 46-kDa protein.

The 46-kDa Protein Is a T4 dsDNA-binding Protein, Absent After Infection of a T4 uswX Phage—The region we have assigned to the 46-kDa gene lies in a part of the T4 genome which is transcribed early after infection in vivo and which contains several genes for proteins that interact with DNA (2). To test whether the 46-kDa protein might also have an affinity for DNA, we examined the dsDNA cellulose-binding proteins expressed by DH1 cells containing either the vector pKG1810R3, the plasmid pDH447, or infected with T4 uswX phage (Fig. 5, left panel). The 46-kDa protein expressed by pDH447 binds to the dsDNA cellulose in the presence of 0.1 M NaCl, is eluted by stepping the salt concentration to 0.5 M NaCl (lane E), and co-migrates with a similarly bound protein of mol wt 68,000 (bovine serum albumin), 43,000 (ovalbumin), and 30,000 (carbonic anhydrase) as well as those of 14C-labeled standards (molecular weights of 68,000 (bovine serum albumin), 43,000 (ovalbumin), and 30,000 (carbonic anhydrase)). As of the major transcript(s), we cleaved the Sall fragment with the restriction endonucleases BglII, XbaI, CiaI, PstI, and NdeI. (The XbaI digest was only 50% complete.) We then transcribed these substrates in the presence of [35S]methionine as described under "Experimental Procedures."
FIG. 4. In vitro transcription of T4 DNA restriction fragments demonstrates two transcripts initiating upstream of the gene for the 46-kDa protein. T4 restriction fragments were transcribed in vitro as described under "Experimental Procedures" and in the text. Panel A, RNA products were obtained after transcription of (lane 1) the 4330-bp SalI fragment (map units 24.8 to 20.75) or (lane 2) the 3400-bp EcoRI fragment (map units 24.3 to 21.15) in the presence of [α-32P]UTP and displayed on a 1% nondenaturing agarose gel. The positions of heat-denatured 32P-labeled HindIII fragments of λ DNA are indicated. Note that the sizes of the RNAs cannot be determined from this gel because the gel is nondenaturing. Panel B, denaturing 4% polyacrylamide, 7 M urea gel showing RNA products (labeled by [α-32P]CTP) obtained after transcription of the above SalI fragment (lane 1) or the fragment digested with BglII (lane 2), XbaI (lane 3), Clal (lane 4), PstI (lane 5), or Bdel (lane 6). (The XbaI digestion was 50% complete). The positions of size markers obtained by digesting λ DNA with HindIII and EcoRI are shown. The doublet of transcripts observed after digestion of the SalI fragment with the various restriction enzymes (see text) is indicated by dots. We calculate the sites of these transcripts as follows: 1800 and 1690 bases (lane 3); 1320 and 1200 bases (lane 4); 880 and 780 bases (lane 5); and 600 and 500 bases (lane 6).

As expected, no such protein is expressed by cells containing the vector.

Previously, the genes for two T4 proteins which interact with DNA have been genetically mapped upstream of gene 41. One of these genes encodes β-glucosyltransferase, a protein of 46,000 daltons (47) which adds monoglucosyl moieties to DNA (reviewed in Ref. 48). We find no β-glucosyltransferase activity (see “Experimental Procedures”) in crude extracts of cells with plasmids expressing the 46-kDa protein or in the dsDNA cellulose-binding fraction of pDH447/DH1 cells (data not shown). The other T4 protein which interacts with DNA and whose gene has been mapped upstream of gene 41 is uvsX. As indicated in the Introduction, this protein has been shown to be analogous to the E. coli recA protein in promoting ATP-dependent homologous pairing in vitro (26–29). We have identified the 46-kDa dsDNA-binding protein present in cells with pDH447 or after infection with wild type T4D as the uvsX gene product, since this protein is not found after infection with the T4 uvsX- mutant, fdsA (Fig. 5, right panel, lane E) or with T4 uvsX am11 (not shown). Previous workers (26, 28, 49) have estimated the size of the uvsX protein as approximately 40 kDa, in contrast to its apparent size of 46 kDa on our gels. This discrepancy is due to the conditions of gel electrophoresis since the uvsX protein made by pDH447 and that purified from T4-infected cells (the generous gift of T. Formosa, University of California, San Francisco) migrate as 46 kDa under our conditions (data not shown) and as 40 kDa under conditions (50) similar to those used by Formosa and Alberts.3,4 The reason for the anomalous electrophoretic behavior of the uvsX protein is not clear. Although for convenience we have referred to the uvsX protein as the 46-kDa band on protein gels, its actual size will have to be determined by other techniques.

The uvsX Protein Expressed by the Recombinant Plasmids Complements T4 uvsX- Phage—The phenotype of T4 uvsX- mutants is pleiotropic including increased sensitivity to UV light, ionizing radiation, and chemical agents, as well as arrest of DNA synthesis and decreased burst size (see Introduction). In collaboration with J. W. Drake (National Institute of Environmental Health Sciences), we tested the biological activity of the uvsX protein expressed by our clones by asking whether UV-irradiated T4 uvsX- phage have increased survival in cells containing the 46-kDa protein. T4x, (uvsX-) phage were irradiated and then plated on DH1 cells without plasmid, with the vector, or with pDH447. As controls, wild type T4D or T4 deficient in two other genes associated with recombination and repair, uvsY and denV, were also irradiated and plated. Each control phage has a distinctive survival curve which is not altered by the host (Fig. 6, solid symbols). However, after 83 s of irradiation, the irradiated uvsX- phage survive approximately 8-fold better (to a level nearly like that of T4D) when plated on the pDH447/DH1 cells (open triangles) than on DH1 or DH1 with the vector (open circles and squares). This experiment indicates that the uvsX protein expressed by the clone can substitute for the T4 uvsX gene product in vivo in responding to damage by UV light.

As discussed in the Introduction, T4 uvsX- mutants have been shown to suppress conditionally lethal mutations in the T4 gene 49 (22–24). Thus, at the nonpermissive temperature, T4 uvsX- ts gene 49 phage will grow but T4 ts gene 49 phage will not. We asked whether providing uvsX protein from the uvsX+ plasmid pDH428 could reduce this suppression. As shown in Table I, use of the pDH428/DH1 host lowers the efficiency of plating of the T4 fdsA (uvsX-)—tsC9 (ts gene 49) phage at the nonpermissive temperature by greater than 20-fold. The use of cells containing the vector or the plasmid pDH428A3 (with the 800-bp deletion which eliminates the 46-kDa product) does not affect the efficiency of plating. In addition, the ts gene 49 mutation by itself displays a tighter phenotype on pDH428/DH1 cells than on cells that do not express uvsX protein. Taken together, the results of these complementation experiments indicate that the uvsX protein expressed by our clones behaves like a biologically active uvsX gene product.

Purification of the uvsX Protein from pDH447/DH1 Cells—The purification of the uvsX protein (see “Experimental

3 T. Formosa, personal communication.
4 D. M. Hinton and N. G. Nossal, unpublished results.
Procedures”) is summarized in Table II and Fig. 7. Most of the purification is achieved by chromatography of the crude extract over dsDNA cellulose, which yields a fraction containing the uvsX protein as the major protein (Fig. 7, lane C). The remaining DEAE and phosphocellulose steps are modifications of procedures developed for the purification of uvsX protein from T4-infected cells by Formosa (27-29). During the last chromatographic step (phosphocellulose column) uvsX protein, apparently homogeneous by gel electrophoresis, co-chromatographs with a ssDNA-dependent ATPase activity (Fig. 7) which gives both ADP and AMP as products (see Footnote e to Table II). The purified protein is free of nucleases acting on single-stranded, double-stranded, or supercoiled DNA under the conditions used for the strand exchange reactions below (Figs. 8 and 9).

**Strand Exchange Catalyzed by the uvsX Protein in Vitro**—We have verified that the uvsX protein from the clone catalyzes D loop formation (data not shown) and strand exchange between circular ssDNA and homologous dsDNA (Figs. 8 and 9), as originally reported for the uvsX protein from T4-infected cells (26-29). At the lower concentra-tion of ssDNA, strand exchange is catalyzed by the uvsX protein alone (Fig. 8, lane 5) but is strongly stimulated by the T4 gene 32 helix-destabilizing protein (lane 4). At the higher ssDNA concentration, both the uvsX and 32 proteins are apparently required (Fig. 8, lane 1), since there are no visible products with only the uvsX protein (lane 2) or 32 protein (not shown). A portion of the products in lanes 1, 4, and 5 co-migrate with marker nicked φX RF II, the expected product of complete strand exchange. Since we have not further characterized these products, it is possible that products other than simple nicked circles co-migrate with the nicked circle marker. There are also products migrating behind the nicked circle marker, which may be intermediates in which the linear duplex is only partially unwound. Products running behind the nicked circle are also seen in similar reactions catalyzed by recA protein (51). In Fig. 8, there are, in addition, large products which do not enter the gel and which are more prominent at higher ratios of dsDNA:ssDNA (lanes 4 and 5 versus lane 1). These may be complex structures resulting from incomplete strand exchange involving several molecules. Fig. 9 demonstrates that the strand exchange catalyzed by the uvsX protein alone is also stimulated by increasing the concentration of dsDNA. As described under “Experimental Procedures,” we labeled the 5224-bp XhoI/PstI fragment of φX174 RF DNA at the 3′ terminus of the (−)-strand by adding nucleotides to the 3′ OH of the XhoI end using the large fragment of E. coli polymerase I to give blunt ends. At the lower dsDNA concentration, the uvsX protein alone does not yield visible products (lane 4). However, raising the dsDNA concentration 2-fold allows a reaction by the uvsX protein alone (compare lanes 7 and 4). The addition of 32 protein (Fig. 9, lanes 3 and 6) increases the extent of the reaction mediated by uvsX protein at both concentrations of dsDNA. Note that products co-migrating with nicked circular DNA and presumably representing extensive branch migra-

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**Fig. 5.** The 46-kDa protein is a dsDNA-binding protein, copurifying with a protein present after infection by T4 phage but absent after infection by T4 with the fdsA mutation in the uvsX gene. Cell lysates were partially purified by chromatography on dsDNA cellulose columns as described under “Experimental Procedures” and the proteins displayed on 10% (left) or 12.5% (right) polyacrylamide-SDS gels. For each extract, lane A represents 5 μl (left gel) or 3 μl (right gel) of the dialyzed 130,000 × g supernatant; lane B, 5 μl (left gel) or 3 μl (right gel) of the proteins flowing through the column; and lanes C–F, 15 μl (left gel) or 20 μl (right gel) of the proteins eluting with DC buffer plus 0.05, 0.1, 0.5, and 1.0 M NaCl, respectively. The positions of the 46-kDa protein as well as protein standards having molecular weights of 68,000 (bovine serum albumin) and 43,000 (ovalbumin) are indicated. The left gel shows extracts from DH1 cells containing the vector (pKC1810R3), the plasmid pDH447, or infected with T4amN81 (gene 41). The right gel shows extracts from DH1 or pDH447/DH1 cells infected with T4D or T4 fdsA (uvsX)
Cloning of the Bacteriophage T4 Gene uvsX and Preliminary Characterization of the uvsX Protein Expressed by the Clone—

The bacteriophage T4 uvsX gene is a nonessential gene whose product, along with other proteins, is required for wild type resistance to radiation and chemical treatment, normal recombination frequencies, and the formation of a network of T4 DNA thought to arise through homologous recombination among replicating T4 genomes (see Introduction). We have found that plasmids containing the T4 DNA approximately 300–2900 bp upstream of gene 41 express a 46-kDa protein whose presence both imparts increased survival to UV-irradiated T4 uvsX- phage (Fig. 6) and decreases the suppression of a conditionally lethal T4 ts gene 49 mutant by a T4 uvsX- mutant (Table 1). These in vivo results are consistent with our having cloned the gene for a biologically active uvsX protein, and our position for the uvsX gene agrees with genetic mapping of the uvsX gene between T4 genes 41 and 42 (25, 30).

DISCUSSION

Cloning of the Bacteriophage T4 Gene uvsX and Preliminary Characterization of the uvsX Protein Expressed by the Clone—

The bacteriophage T4 uvsX gene is a nonessential gene whose product, along with other proteins, is required for wild type resistance to radiation and chemical treatment, normal recombination frequencies, and the formation of a network of T4 DNA thought to arise through homologous recombination

Fig. 6. The presence of pDH447 (uvsX+ plasmid) specifically increases the survival of UV-irradiated T4 uvsX- phage. T4D, T4 fdb (uvsY-), T4C (den V-), and T4x (uvsX-) were irradiated for the indicated times at a UV dose rate of 0.3 J m-2 s-1 and plated on DH1 without plasmid (●, ○), pKG1810R3 (vector, ■, □), or pDH447/DH1 (uvsX+) plasmid, ▲, △. The uvsX- phage is indicated by open symbols; all other phage are shown with closed symbols. The surviving fraction represents the titer obtained after irradiation relative to that prior to UV treatment.

Table I

Suppression of a T4 49- mutation by a uvsX- mutation is eliminated in cells with a uvsX+ plasmid

| Host       | uvsX gene product? | Phage T4D | Phage fdbA (uvsX-) | Phage tc9 (gene 49) | Phage fdbA-tc9 |
|------------|---------------------|-----------|--------------------|---------------------|----------------|
| pKG1810R3/DH1 | No                  | 0.95      | 1.11               | 0.23b               | 0.78           |
| pDH428/DH1    | Yes                 | 0.99      | 0.90               | <0.003              | 0.04           |
| pDH428Δ3/DH1  | No                  | 0.81      | 0.93               | 0.22b               | 0.98           |

a The T4 phage were plated as described under "Experimental Procedures." The efficiency of plating 44 °C/29 °C represents the titer of the phage at the nonpermissive temperature (44 °C) relative to that at the permissive temperature (29 °C).

b Calculated as if total extract purified through each step.

c This is uvsX protein in peak fractions used for phosphocellulose chromatography. Total uvsX protein recovered from DEAE column (corrected as in Footnote c) was 17 mg.

d DNA-dependent ATPase cannot be measured reliably in earlier fractions. In both fractions III and IV, the ratio of AMP to ADP was 0.28, and there was no ATP hydrolysis in the absence of ssDNA.

Fig. 7. Polyacrylamide-SDS gel electrophoresis and ssDNA-dependent ATPase activity of phosphocellulose fractions of the uvsX protein. Lane A, bovine serum albumin (M, = 68,000) and ovalbumin (M, = 43,000) marker proteins; lane B, dialyzed crude extract (2 μl); lane C, dsDNA cellulose-pooled enzyme (5 μl); lane D, DEAE-cellulose-pooled enzyme (5 μl); and lanes 2–26, 5 μl of the corresponding even-numbered phosphocellulose fractions. The 10% polyacrylamide gel was stained with Coomassie Blue. Single-stranded øX174 DNA-dependent ATPase was measured with 0.5 μl of the indicated fractions. (Dashed line is NaCl concentration.)

Among replicating T4 genomes (see Introduction). We have found that plasmids containing the T4 DNA approximately 300–2900 bp upstream of gene 41 express a 46-kDa protein whose presence both imparts increased survival to UV-irradiated T4 uvsX- phage (Fig. 6) and decreases the suppression of a conditionally lethal T4 ts gene 49 mutant by a T4 uvsX- mutant (Table 1). These in vivo results are consistent with our having cloned the gene for a biologically active uvsX protein, and our position for the uvsX gene agrees with genetic mapping of the uvsX gene between T4 genes 41 and 42 (25, 30). The T4 uvsX+ plasmids described here, together with the T4 uvsY+ and uvsW+ plasmids previously described (19, 52),
The uvsX protein that we have purified from plasmid-containing cells is a ssDNA-dependent ATPase which promotes homologous strand exchange in vitro, as has been previously shown for uvsX protein from T4-infected cells (26-29). Our finding that the plasmid-encoded uvsX protein hydrolyzes ATP to both ADP and AMP (Table II) is in agreement with the more extensive analysis of the products formed by the uvsX protein from infected cells (27-29) and eliminates the possibility that one of these products was produced by a contaminating phage protein. uvsX protein from cells with the plasmid mediates ATP-dependent strand exchange between the (-)-strand of a linear duplex and its single-stranded circular (+)-strand complement in a reaction which is stimulated by, but not absolutely dependent upon, the T4 gene 32 helix-stabilizing protein (Figs. 8 and 9). Strand exchange in the absence of 32 protein is highly dependent on the concentration of both ssDNA and dsDNA (Figs. 8 and 9), which may explain previous reports of a 32 protein requirement for the strand exchange reaction (cited in Ref. 29). However, we cannot rule out the possibility of a fundamental difference between the uvsX protein from the clone and that from T4-infected cells. Our results, indicating a stimulation but not a requirement for 32 protein in uvsX protein-mediated strand exchange, resemble those previously observed for the E. coli ssDNA binding (SSB) protein and the E. coli recombination protein recA (51); for a recent review, see Ref. 53). More detailed investigations of the T4 recombination proteins will be required in order to understand precisely how homologous recombination is accomplished by purified T4 proteins in vitro as well as during the life cycle of T4.

Expression of the uvsX Protein—Our plasmids which contain the T4 uvsX gene cloned in a multicopy plasmid vector express a high level of the protein both in vivo (Fig. 2) and in vitro (Fig. 3). We have presented evidence that at least part of this expression is due to transcription promoter(s) on the T4 DNA upstream of the uvsX gene. First, expression of the protein is independent of the orientation of the T4 DNA within the plasmid (Fig. 2), suggesting that in the plasmid the uvsX gene is not expressed simply by read-through transcription from the vector DNA. Second, our in vitro transcription of T4 DNA fragments from this region identifies 2 tandem promoters lying upstream of the uvsX gene which produce transcripts that extend through the uvsX gene (Fig. 4). We have observed transcripts from these promoters under conditions (see "Experimental Procedures" and "Results") which should favor specific initiation from stronger promoters over nonspecific starts or initiation at weaker promoters (54). A previous study by Gram et al. (55) to identify strong T4 promoters used in vitro by E. coli RNA polymerase failed to reveal the ones we have observed upstream of the uvsX gene. This discrepancy probably reflects the difference in salt concentrations used in their study (200 mM KCl) and our study) (75 mM) since we find that the intensity of the RNA bands observed after transcription of the T4 Saff fragment (map units 23.8 to 20.75) is diminished severalfold using the higher salt concentration. However, even at the higher salt concentration, the initiation of transcription is very specific, yielding product which comigrates with the major transcription band observed in Fig. 4A, lane 1.

In vitro transcription/translation of the plasmid with the largest T4 DNA insert, pDH426, produces several minor species (Fig. 5) in addition to the uvsX protein. Longer

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**FIG. 8.** T4 uvsX protein catalyzes strand exchange between single-stranded φX174 circular DNA and linear duplex φX174 RF DNA in a reaction stimulated by T4 32 protein. Reaction mixtures contained no proteins (lanes 3 and 6), uvsX protein (412 µg/ml) alone (lanes 2 and 5), or uvsX protein plus T4 32 protein (37 µg/ml in lanes 1 and 4). φX174 RF III DNA (cut by PstI nuclease) was 17 µM (nucleotide), and ssDNA concentration was either 5.8 µM (nucleotide) in lanes 1-3 or 2.9 µM in lanes 4-6. The positions of φX174 single-stranded circular DNA (ssc), double-stranded linear DNA (dsl), and nicked circle RF II DNA (nc) markers are indicated.

**TABLE II**

| ssDNA | 5.8 µM | 2.9 µM |
|-------|-------|-------|
| uvsX  | ++    | +     |
| 32    | 1 2 3 | 4 5 6 |

**FIG. 9.** Increasing the linear dsDNA concentration facilitates strand exchange with circular ssDNA by the uvsX protein in the absence of 32 protein. Lanes 1-5 and lanes 6-8 contained the blunt end 5224-bp [32P]Xho-Pst fragment of φX174 RF DNA (see "Experimental Procedures") at final concentrations of approximately 1.4 and 2.8 µM (nucleotide), respectively. All reactions contained 2.3 µM (nucleotide) single-stranded φX174 viral DNA and, where indicated, 412 µg/ml uvsX protein and 110 µg/ml 32 protein. Lanes 5 and 8, proteins omitted; lanes 4 and 7, uvsX protein; lanes 3 and 6, uvsX and 32 proteins; lane 2, 32 protein; and lane 1, uvsX and 32 proteins without ATP. The positions of φX174 nicked circle RF II DNA (nc) and double-stranded linear DNA (dsl) markers are indicated.

**TABLE III**

| dsDNA | 1.4 µM | 2.8 µM |
|-------|-------|-------|
| ATP   | ++    | +     |
| uvsX  | +     | +     |
| 32    | +     | +     |

| nc    | dsl   |
|-------|-------|

5 D. M. Hinton, unpublished experiments.
exposures of this autoradiogram reveal that two of the fainter of these bands co-migrate with either β-lactamase, the amp′ gene product observed with the vector alone, or with T4 gene 41 protein, which we have previously shown is expressed by pDH428 in vivo (31). Most of the remainder of the bands migrate faster than the 46-kDa uvsX product and are dependent on the T4 DNA for expression. These minor bands could arise from premature transcription or translation stops of the highly expressed uvsX gene or could be unidentified T4 proteins. In fact, T4 gene 40, whose product, a 14-kDa protein, migrate faster than the 46-kDa UVSX product and are depend-
arise from premature transcription or translation stops of the is required for proper phage head assembly has been mapped, T4 genes cloned in pDH428. If the promoters we have detected minor protein bands we have observed after translation of the pDH428 protein and personal communications. We also thank David Rogerson to Alberts (University of California, San Francisco) for showing us their laboratory for the UV sensitivity experiment and to T. Formosa Sciences, Research Triangle Park, NC) for phage and the use of his on the T4 genome.

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Supplementary Material 20

Cloning of T4 uvsX Gene

FOR PROTECTION AND CHARACTERIZATION OF THE T4 UVB RECOMBINANT PROTEIN

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Experimental Procedures

Unless otherwise indicated, all materials and methods were described previously (31, 32).

Plasmids, Strains, and Preparations—E. coli DH5α, a K12- derived strain, was described (33). T4 λ phage strain (32, 33), T4 λ phage λ36 (33)

T4 λ phage λ41 (33, 34), T4 λ phage λ42 (33, 34), T4 λ phage λ64 (33, 34), T4 λ phage λ84 (33, 34), T4 λ phage λ63 (33, 34), and T4 λ phage λR1 (34) were obtained from J. W. Kingston (National Institute of Environmental Health Sciences).

The resulting DNA plasmids were isolated from the plasmid pVH627 (described above). The products were transformed with each of the restriction enzymes indicated. After digestion, the DNA solution in TBE buffer plus 50 mM NaCl was treated with T4 DNA polymerase in the presence of 0.2 mM dNTPs (31). The resulting DNA products were isolated from the plasmid pVH627 (described above). The products were transformed with T4 DNA polymerase to produce the recombinant plasmid pVH625 (pVH627a) and ligated again. pVH627a and pVH628, two products of this procedure, represent isolations of the 1400 bp EcoRI fragment in opposite orientations.

To construct the control plasmid pVH61093, the T4 λ λ phage vector pVH61093 was digested with restriction enzymes and ligated in the presence of the XhoI oligonucleotide and XhoI linker pGEM4X-Z (35). In Vitro Transcription/Translation Reactions—Plasmid DNA isolated DNA was digested with HindIII and EcoRI to allow equilibration with high-speed-centrifuged chloroplast DNA (31). After removal of the ethidium bromide with extraction with CCl4-saturated isopropanol, the DNA solution was dialyzed against TE buffer (10 mM Tris-Cl, pH 7.9, 1 mM EDTA) and the DNA was precipitated with 50% ethanol. The DNA pellet was washed with 70% ethanol, dried, and resuspended in TE buffer to give a final concentration of 0.2 to 1.5 mg/ml. The DNA was stored at -20°C.

In vitro transcription/translation reactions (10 ml) contained DNA (amount given in legend to Fig. 3), 6 ml of 50 mM Tris, pH 7.5, 1 mM MgCl2, 20 mM KCl, 0.5 mM each 100 ml ATP, GTP, CTP, UTP, 10% glycerol, 1 ml of 10% rabbit reticulocyte lysate, 10% each rabbit reticulocyte lysate, and 1 μM of 100 μl in 0.1 ml loading solution (39, 40). Reactions were started by adding 100 ml loading solution (39, 40) and were incubated for 10 minutes. After incubation was complete, the reaction mixture was centrifuged for 10 minutes at 4°C. The supernatant was dialyzed against 300 ml of 50 mM NaCl, 1 mM EDTA, 10 mM Tris-Cl, pH 8.0, 150 μg/ml RNAse, 0.5 mg/ml glycerol, 10 μg/ml leupeptin, 0.6 mg/ml cycloheximide, and 10 μg/ml actinomycin D. After incubation at 37°C for 40 minutes, the mixture was centrifuged at 4°C for 10 minutes at 100,000 × g. After centrifugation, the supernatant was used for analysis of protein synthesis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The resulting reactions were incubated at 15°C for 45 minutes with 25 μg pancreatin (Nutritional Diets, Noblesville, IN) and then centrifuged for 30 minutes at 10,000 × g in a Beckman 50 Ti rotor. The supernatant was used for analysis of protein synthesis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

In Vitro Transcription/Translation Reactions—Plasmid DNA isolated (30 μg) was digested with HindIII and EcoRI to allow equilibration with high-speed-centrifuged chloroplast DNA (31). After removal of the ethidium bromide with extraction with CCl4-saturated isopropanol, the DNA solution was dialyzed against TE buffer (10 mM Tris-Cl, pH 7.9, 1 mM EDTA) and the DNA was precipitated with 50% ethanol. The DNA pellet was washed with 70% ethanol, dried, and resuspended in TE buffer to give a final concentration of 0.2 to 1.5 mg/ml. The DNA was stored at -20°C.

In vitro transcription/translation reactions (10 ml) contained DNA (amount given in legend to Fig. 3), 6 ml of 50 mM Tris, pH 7.5, 1 mM MgCl2, 20 mM KCl, 0.5 mM each 100 ml ATP, GTP, CTP, UTP, 10% glycerol, 1 ml of 10% rabbit reticulocyte lysate, 10% each rabbit reticulocyte lysate, and 1 μM of 100 μl in 0.1 ml loading solution (39, 40). Reactions were started by adding 100 ml loading solution (39, 40) and were incubated for 10 minutes. After incubation was complete, the reaction mixture was centrifuged for 10 minutes at 4°C. The supernatant was dialyzed against 300 ml of 50 mM NaCl, 1 mM EDTA, 10 mM Tris-Cl, pH 8.0, 150 μg/ml RNAse, 0.5 mg/ml glycerol, 10 μg/ml leupeptin, 0.6 mg/ml cycloheximide, and 10 μg/ml actinomycin D. After incubation at 37°C for 40 minutes, the mixture was centrifuged at 4°C for 10 minutes at 100,000 × g. After centrifugation, the supernatant was used for analysis of protein synthesis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Cloning of T4 uvsX Gene

Cloning of the T4 phage uvsX gene was achieved by the use of an E. coli λ λ phage strain and lambda RSF1010 (31, 32). The resulting DNA plasmids were isolated from the plasmid pVH627 (described above). The products were transformed with T4 DNA polymerase to produce the recombinant plasmid pVH625 (pVH627a) and ligated again. pVH627a and pVH628, two products of this procedure, represent isolations of the 1400 bp EcoRI fragment in opposite orientations.

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Cloning of T4 uvsX Gene

Phosphocellulose Chromatography — Fifty percent of the free peak fractions (2.5 ml total) from the DEAE column was diluted with an equal volume of buffer A and loaded onto a 2-ml column of Whatman DE-11 phosphocellulose which had been equilibrated with buffer A and washed with 0.1 M NaCl. The column was then washed with 4 ml of buffer A with 0.1 M NaCl and then with a linear gradient of 0.1 to 0.8 M NaCl in buffer A (40 ml total). Greater than 99% of essentially homogeneous uvsX protein was eluted in a single 1-ml fraction at 0.15 M NaCl.

uvsX ATPase Assay — Reaction mixtures (3 ml) containing 50 mM Tris-Cl, pH 7.5, 5 mM MgCl₂, 100 µg/ml bovine serum albumin, 58 mM (dithiothreitol), 250 µM radioactive 32P-ATP (3 cpm/µmol), and enzyme were incubated for 30 minutes at 37°C. The nucleotide products were separated by chromatography on PEI thin layers (Brinkman) in 1.2% 90% linear gradient of 0.1 to 1.0 M LiCl, and 50 mM potassium phosphate, pH 7.4. Cleared lysates were then treated after centrifugation at 10,000 g. Aliquots of these lysates or of the 0.5 M NaCl eluates from dsDNA cellulose column (see above) were assayed for β-glucosyltransferase activity essentially as described (44) in a reaction mixture (4 ml) containing 100 mM potassium phosphate, pH 7.0, 60 µM bacteriophage T2 DNA (nucleotide equivalents), 25 mM MgCl₂, and 50 µM uridine diphosphate glucose (glucose-14C) (0.026 Ci/mmol). After reaction mixtures were incubated at 30°C for 15 minutes, transfer of glucose to DNA was determined by acid-insoluble radioactivity (45).