The bacteriophage T4 dda protein is a 5'-3' DNA helicase that stimulates DNA replication and recombination reactions in vitro and seems to play a role in the initiation of T4 DNA replication in vivo. Oligonucleotide probes based on NH2-terminal amino acid sequence were used to precisely map the location of the dda gene on the T4 chromosome. Using polymerase chain reaction techniques, the dda gene was then cloned into an expression vector, and the overproduced protein was purified in two chromatography steps. Both the genomic and cloned dda genes were sequenced and found to be identical, encoding a protein of 439 amino acids. The dda protein contains amino acid sequences resembling those of other known helicases, and is most homologous to the Escherichia coli recD protein. Protein affinity chromatography was used to show a direct interaction between the dda protein and the T4 uvsX protein (a recA-type DNA recombinase).

Helicases have been isolated from a wide variety of eukaryotic and prokaryotic cells (reviewed by Matson and Kaiser-Rogers, 1990). Exact physiological roles have yet to be determined for many of these enzymes. However, they vary in a broad range of biochemical properties, including substrate unwound (RNA or DNA helices), direction of strand movement (5' to 3' or 3' to 5'), and nucleotide cofactor hydrolyzed, reflecting their variety of functions inside the cell.

Bacteriophage T4 has been found to encode all of its own replication proteins. These include two distinct DNA helicases, the gene 41 protein and the dda protein, which appear to be important in both DNA replication and recombination reactions (Krell et al., 1979; Alberts et al., 1980; Jongeneel et al., 1984a; Kodadek and Alberts, 1987). The gene 41 protein, which is essential for T4 DNA replication, is a highly processive DNA helicase that moves along a single-stranded DNA template in the 5' to 3' direction (i.e., along the lagging strand of a replication fork) (Liu and Alberts, 1981a; Venkatesan et al., 1982). The gene 61 protein (the DNA primase that makes the RNA primers for Okazaki fragment synthesis) and the gene 41 protein interact to form the T4 primosome (Liu and Alberts, 1980, 1981b; Nossal, 1980).

The dda protein was originally isolated as a DNA-dependent ATPase by Ebisuzaki and co-workers (Debreceni et al., 1970; Behme and Ebisuzaki, 1975). Mutant dda- phage show a substantial delay in DNA synthesis, but because near normal amounts of DNA are eventually produced phage burst size is reduced only slightly (Little, 1973). No UV sensitivity or defects in recombination have been detected in dda mutant infections (Behme and Ebisuzaki, 1975).

The dda protein and the gene 41 protein share some properties at a biochemical level. Both DNA helicases run in the 5' to 3' direction along single-stranded DNA. In the absence of the gene 41 protein, the dda protein stimulates the rate of DNA strand-displacement DNA synthesis at an in vitro replication fork (Jongeneel et al., 1984b). Since no increase in this rate is observed when the dda protein is added to reactions that have been stimulated by the gene 41 protein, the two DNA helicases do not appear to act synergistically at the fork (Jongeneel et al., 1984b).

The dda protein differs from the gene 41 protein in acting distributively (continuously dissociating and reassociating with the DNA molecule being unwound) rather than processively (Jongeneel et al., 1984a). In addition, the dda helicase does not form a primosome with the 61 protein. Unlike the 41 protein, the dda protein binds tightly to the T4 gene 32 protein (helix-destabilizing or single-stranded DNA-binding protein), and it is retained when T4 infected cell lysates are passed over a uvsX proteinagarose column (uvsX is a recA analog with a central role in T4 genetic recombination) (Jongeneel et al., 1984a; Formosa and Alberts, 1984). A role in recombination is further suggested by dda protein's 4-fold acceleration of the rate of uvsX protein-catalyzed DNA branch migration in in vitro reactions (Kodadek and Alberts, 1987).

In general, one suspects that the two T4 DNA helicases can partially substitute for each other for some of the helicase functions inside the T4 bacteriophage-infected cell. Evidence for this assertion comes from studies on the T4 gene 59 protein. A T4 gene 59 amber mutant alone on a non-suppressing strain shows normal DNA synthesis early in infection, followed by DNA synthesis arrest at late times of infection (Cunningham and Berger, 1977). However, if the phage is also dda-, almost no DNA is made. Thus, without the 59 gene product, the dda protein is essential for any phage growth. Recent biochemical characterization of the gene 59 protein has shown that it loads the T4 gene 41 helicase onto single-stranded DNA. Thus, the combined biochemical and genetic data suggest that the dda protein plays an important role in DNA metabolism in vivo but that its function can be partly
replaced with that of the gene 59-41 protein complex.

In order to further characterize the dda helicase and its interaction with other proteins, we precisely mapped its location within the T4 chromosome, sequenced and cloned the dda gene, and overexpressed and purified the dda protein. In addition, the purified dda protein was chromatographed on a uvX protein affinity column to test for a direct uvX-dda protein interaction.

**MATERIALS AND METHODS**

**Reagents and Enzymes**—All restriction and DNA modifying enzymes (including the Taq DNA polymerase) were purchased from New England Biolabs unless otherwise noted. Polynucleotide kinase and dideoxynucleoside triphosphates were obtained from Pharmacia 1KB Biotechnology Inc., avian myeloblastosis virus reverse transcriptase from Life Sciences (St. Petersburg, FL), Sequenase was from United States Biochemical Corp., ampicillin from Roerig/Pfizer, lysozyme from Worthington, formamide from Fluka, and agarose from FMC Bioproducts. Dimethyl sulfate, pipercidine, and hydroxylamine were from Aldrich, formic acid from Fisher Scientific, and [γ-32P]ATP from Amerham Corp. The sequence of the 25 NDH-terminus amino acids of the dda protein that we purified from T4-infected cells by published procedures (Jongeneel et al., 1984a) was determined by Ken Williams (Yale University). G oligonucleotide primers were synthesized by the Biomolecular Resource Center at the University of Wisconsin, Madison. The uvX protein affinity column was prepared by Scott Morrical in this laboratory.

**Plasmids**—The ppl18xwd vector was obtained from Dr. T.-C. Lin (Yale University). This vector contains the large EcoRI-BamHI fragment from the pBR322 derivative, pUC19, ligated to the EcoRI-BamHI fragment from a pGEM derivative that contains the late promoter control region carrying the gene encoding the repressor cl
t17 (Lin et al., 1987). The λDNA has the rexA and rexB genes (map position 37,000-36,110) deleted, since they inhibit T4 infection when contained on a multicopy plasmid (Shinedling et al., 1987). The T4 X-DNA has the rexA and rexB genes (map position 7.3-10.78) was cut out of the gel, and the DNA was recovered from the gel pieces by melting the agarose at 65 °C followed by phenol extractions. The DNA was ethanol precipitated, resuspended in digest buffer, and cut with HindIII. The 3.6-kb DNA fragment (map position 10.608-10.295) for sequencing, which contains the X-DNA origin, was purchased from Stratagene.

**DNA Fragmentation**—Cells 1010 cells/ml in LB media were incubated at 30 °C. For complementation at 35 °C, samples were switched to a water bath at 35 °C 15 min prior to infection with a T4ts75(gene 32)sudl double mutant. Cells were infected, plated on T4 soft agar, and incubated at the appropriate temperature (30 or 35 °C).

**Plating of Phage**—E. coli Tab32-4 cells containing either plasmid pKHdda or pTL18xwd in LB media were incubated at 30 °C. For complementation at 35 °C, samples were switched to a water bath at 35 °C 15 min prior to infection with a T4ts75(gene 32)sudl double mutant. Cells were infected, plated on T4 soft agar, and incubated at the appropriate temperature (30 or 35 °C).

**DNA Sequencing**—The DNA was digested on the plasmid pKHdda was grown to 6 x 106 cells/ml in LB media containing 50 μg/ml ampicillin. The temperature was then quickly switched to 38 °C, and the incubation was continued for 3 h. After harvesting, the cells were stored at -20 °C.

**Cells were lysed using a procedure** (Alberts and Frey, 1970) modified by J. Barry in this laboratory. Cells (28 g) were thawed and resuspended in 136 ml of buffer containing 20 mM Tris-HCl, pH 8.1, 1 mM Na2EDTA, 1 mM β-mercaptoethanol, 10 mM benzamidine-HCl, 1 mM phenylmethylsulfonyl fluoride. T4 lysozyme was added to a final concentration of 500 μg/ml, and the supernatant was incubated until viscous. The DNA was degraded by adding DNase I to 10 μg/ml and adjusting the extract to 10 mM MgCl2, 1 mM CaCl2. The suspension was incubated in ice-water, gently mixed until the viscosity decreased, and then sonicated with repeated 1-min bursts from the horn of a Branson sonifier operating at 40% duty until the DNA concentration dropped to 15% of its original value. Another 10 μg/ml of DNase I was added, and the suspension was incubated at 37 °C for 30 min. The DNA was then purified by phenol extraction, and the DNA was ethanol precipitated, resuspended in digest buffer, and cut with HindIII. The 3.6-kb DNA fragment (map position 10.608-10.295) for sequencing, which contains the X-DNA origin, was purchased from Stratagene.

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was added, and the extract was incubated at 15 °C for 20 min. The extract was centrifuged at 20,000 rpm (48,200 × g) in a Sorvall SS 34 rotor for 20 min to remove cell debris and then further clarified by centrifugation at 35,000 rpm (111,000 × g) in a Beckman VTi 50.2 rotor for 5 h. The supernatant was dialyzed against 4 two-liter changes of buffer A (20 mM Tris-HCl, pH 8.1, 5 mM Na,EDTA, 1 mM β-mercaptoethanol, 2 mM benzamidine-HCl, and 10% (v/v) glycerol) to remove the divalent cations necessary for DNase I activity.

A 55-ml single-stranded DNA-cellulose column was constructed (Alberts and Herrick, 1971), containing 1.5 mg of DNA/packed ml. The dialyzed extract (fraction 1, Table II) was pumped at 1 column volume (cv)/h through the column, washed with 1 cv of buffer A containing 0.10 M NaCl, followed by 1.5 cv of buffer A containing 0.25 M NaCl, and then eluted with a 2-cv linear gradient of 0.24–2 M NaCl in buffer A. Fractions of 4 ml were collected. ATPase activity was determined using a charcoal adsorption assay, as described previously (Liu and Alberts, 1981).

The fractions containing the dda protein were pooled and dialyzed against buffer B (20 mM Tris-HCl, pH 8.1, 20 mM Na,EDTA, 1 mM Na,EDTA, 2 mM β-mercaptoethanol, and 10% (v/v) glycerol). A 5-ml DEAE-cellulose column was constructed and equilibrated with buffer B. The pooled, dialyzed DNA-cellulose fractions (fraction II, see Table II) were loaded at 1 cv/h onto the column, eluted with 2 cv of buffer B containing 60 mM NaCl. Fractions of 1.2 ml were collected. The fractions containing dda protein were pooled and concentrated in a Centricon 30 ultrafiltration device (Amicon Corp., Danvers, MA), and adjusted to storage buffer (20 mM Tris-HCl, pH 7.7, 50 mM NaCl, 1 mM β-mercaptoethanol, 0.2 mM Na,EDTA, and 45% (v/v) glycerol). The dda preparation was free of detectable nuclease activities as evaluated by sensitive DNA gel electrophoresis assays (Morris et al., 1979).

**RESULTS**

*The dda Gene Is Located at the 10.5–9.0-kb Position on the T4 Chromosome—*Behme and Ebisuzaki (1975) mapped the dda gene by showing that its DNA-dependent ATPase activity was present in cells infected with the T4 del(39–56)1 mutant (deletion map position 6.15–10.34 kb), but was missing in cells infected with the T4 del(39–56)10 mutant (deletion map position 6.3–10.7 kb).

We used two 20-nucleotide-long probes derived from the NH$_2$-terminal amino acid sequence that we determined for the purified dda protein to further define the location of the dda gene in the T4 chromosome. Oligonucleotide 1 was derived from the first 6 amino acids of dda and was 128-fold degenerate. Oligonucleotide 2 was derived from amino acids 8–13 and was 192-fold degenerate.

Blot hybridization to restriction nuclease-digested T4cDNA was performed separately with oligonucleotides 1 and 2 according to standard methods (Maniatis et al., 1982). Each probe hybridized to multiple restriction fragments, but only restriction fragments that hybridized with both probes were judged to contain the dda gene. One of these fragments (fragment II in Table II) hybridized to a 2.0-kb HindIII fragment at map position 10.3–12.55 on the T4 linear chromosome and with a 1.7-kb EcoRI fragment at map position 8.94–10.59; thus the 5' end of the dda gene must lie between map position 10.3–10.6. Assuming that dda is transcribed in the counter-clockwise direction on the T4 circular map like other early and middle T4 genes (Kutter and Ruger, 1983), the dda gene should lie somewhere between map positions 10.6 and 8.8.

The Clal–HindIII DNA fragment, encompassing map positions 10.7–10.4, was isolated from a digest of T4 cytosine-containing DNA (T4cDNA). This fragment was sequenced using the Maxam-Gilbert technique to determine the precise location of the dda gene's 5' end and confirm the expected orientation of the gene on the T4 chromosome. The results showed that the 5' end of the dda gene lies at the 10.5 position on the chromosome with the predicted orientation; therefore, the 3' end must lie at position 9.0 (assuming that the gene encodes a 50-kDa protein and does not contain an intron).

**Sequence Analysis of the dda Gene—**Repeated attempts to clone restriction fragments encompassing the entire dda gene by conventional methods failed, suggesting that the dda gene product is deleterious to *E. coli* when under the control of its own promoter. Fortunately, both T4cDNA and T4 mRNA are easily obtained from T4-infected cells, and by employing these nucleic acids as single-stranded templates we were able to use theideoxy method to determine the sequence of the dda gene. The strategy used for sequencing is presented in Fig. 1. The dda gene sequence is shown in Fig. 2.

Sequence analysis predicts a dda protein sequence of 439 amino acids. The calculated mass of 49,947 daltons agrees with the estimated mass from SDS-polyacrylamide gel electrophoresis of 56,000 (Krell et al., 1979), 50,000 (Burkey and Ebisuzaki, 1977), or 48,000 daltons (Jongeneel et al., 1984a). The dda sequence contains a consensus nucleoside triphosphate-binding site and six regions of homology with other helicases (see "Discussion").

**Amplification, Modification, and Cloning of the dda Gene for Overproduction of the Gene Product—**We used the PCR technique to amplify the dda gene while simultaneously changing its Shine–Dalgarno sequence in preparation for cloning in a tightly regulated expression vector. The sequences of the two primers used for PCR are shown in Fig. 2. T4cDNA was used as the template, and the PCR reaction was carried out as described by Kogan et al. (1987), with the following modifications: the time and temperature used for polymerase incubation was increased to 4 min at 65 °C, primers were allowed to anneal at 42 °C for 24 s, and no additional Taq DNA polymerase was added during the cycles. On a 1% agarose gel, more than 90% of the DNA product migrated at 1.3 kb, the length of the dda gene; 30 rounds of synthesis yielded a 13,000-fold amplification (1.3 μg of DNA).

The 1.3-kb DNA fragment containing the dda gene was placed in an expression vector, pTL19xwd, downstream of the λ late promoter. This promoter is controlled by the c$_{67}$ repressor produced by the vector. The plasmid contains the 39,178–34,500 region of bacteriophage λ with the rexA and B genes deleted; the rex gene products inhibit T4 growth when contained on a plasmid (Shinedding et al., 1987), which would complicate genetic studies.

DNA strands from the cloned dda gene, complementary to those sequenced from T4 genomic nucleic acids (see Fig. 1A), were sequenced to confirm the sequence obtained from the T4 genomic nucleic acids and to determine if any mutations were introduced during the cloning of the dda gene. The approach used to sequence the cloned dda gene, pKHdda, is shown in Fig. 1B. The sequence of both the genomic and the cloned dda gene were found to be identical, and the sequence of the first 25 amino acids encoded by the gene is the same as that determined for the dda protein isolated from phage T4-infected cells.

The production of functional dda protein was tested genetically by assaying the ability of the cloned dda gene to com-
plement a bacteriophage T4 gene 32 temperature-sensitive, 
*su* deletion double mutant (T4ts75*su*). The *su* and *dda* genes are believed to be the same (Jongeneel et al., 1984; Doherty et al., 1982). Whereas either the T4 ts75 (gene 32) or 
*su*1 mutant alone will grow at both 30 and 35 °C, the 
T4ts75(gene 32)*su*1 double mutant phage will not grow at 
either temperature on a Tab32-4 *E. coli* strain (Doherty 
et al., 1982); this strain restricts the growth of many gene 32 
temperature-sensitive mutants at normally permissive tem-
peratures, without affecting wild-type T4 phage (Nelson 
and Gold, 1982). As shown in Table I, the T4ts75(gene 32)*su*1 
mutant phage grows on Tab32-4 containing only the vector (pTL19xwd), at both 
30 and 35 °C. This result shows that the *dda* gene product 
encoded by the plasmid is active *in vivo*, and it further 
supports the previous evidence that *su* and *dda* are the same 
gene.

**Overproduction and Purification of the Cloned *dda* Gene Product**—The *dda* protein was overproduced from plasmid 
pKHdda in *E. coli* SG934 cells, which contain a mutation in 
the *htpR* gene. The *htpR* gene is essential for the transcription of 
heat shock genes, and proteases normally induced upon 
heat shock are not expressed (for review, see Neidhardt et al., 
1984). The expression of the *dda* protein was induced at 38 °C 
for 3 h. After induction, the *dda* protein represented 1% of 
the total soluble protein, which is 10-fold more than obtained 
from T4-infected cells. Further overexpression of the *dda* 
protein could be obtained at higher temperatures, but this 
resulted in the formation of insoluble *dda* protein aggregates 
and a reduced final yield.

**Purification of the Overexpressed *dda* Protein**—The over-
expression of the *dda* protein allowed us to simplify our 
previous procedure for *dda* purification from T4-infected cells, 
which involved five columns and resulted in a 5% yield of 
pure *dda* protein (Jongeneel et al., 1984a). From our induced 
cells, the *dda* protein can be purified free of nuclease after 
only two columns with a yield of 60%, as detailed in Table II.

In the new procedure, the crude lysate is passed through a 
single-stranded DNA-cellulose column, from which the *dda* 
protein is eluted with a steep NaCl gradient. The fractions 
that contain the highly purified *dda* protein are pooled and 
chromatographed over a DEAE-cellulose column under con-
ditions in which the *dda* protein flows through. The results of 
an SDS-polyacrylamide gel analysis at each stage of the 
purification are shown in Fig. 3.

**The Overexpressed *dda* Protein Has DNA Helicase Activity**—An assay was carried out to determine whether the 
overexpressed *dda* protein purified from T4-uninfected cells 
has DNA helicase activity, since it is conceivable that the *dda* 
protein requires post-translational modification or some other 
component picked up during T4 infection to become an active 
helicase.

To assay the *dda* protein for helicase activity, we con-
structed a DNA substrate that contains a fully complementary 
5′ end-labeled 393-nucleotide DNA fragment annealed to 
single-stranded genomic M13 DNA. The unwinding of this 
substrate by a helicase changes the mobility of the labeled 
DNA fragment on a non-denaturing agarose gel and is readily 
detected by autoradiography (Jongeneel et al., 1984a).

The unwinding of the DNA substrate by the overexpressed
purified dda protein is presented in Fig. 4. The dda protein does not require post-translation modification by phage T4 proteins for helicase activity. The percentage of DNA unwound greatly increases when the dda concentration is raised from 4 to 8 μg/ml, which increases the ratio of dda protein molecules to DNA nucleotides from 1:2 to 1:1. Three dda protein molecules/nucleotide are required for the unwinding of all of the DNA molecules. One dda protein molecule from phage T4-infected cells/three DNA nucleotides was needed to unwind 84% of the somewhat different DNA molecules used in our earlier study (Jongeneel et al., 1984a).

The dda Gene Product Binds Directly to the T4 uvsX Protein—Overexpression of the dda gene product made it possible to obtain large enough quantities of the protein to produce a dda protein affinity column. Affinity chromatography with other T4 proteins involved in DNA metabolism attached to an agarose matrix showed a tight interaction of the dda protein with the gene 32 protein (Formosa et al., 1983). To to an agarose matrix, Affi-Gel 10, as described by Formosa et al. (1983). Although a column containing 2 mg of dda protein/
packed ml was prepared, we did not detect any binding of the purified gene 32 protein to this column (data not shown). This result suggests that the dda protein is inactivated during its attachment to the column. Interestingly, of the eight other T4 proteins that have previously been attached to this agarose matrix, the only one that was similarly inactivated was the other DNA helicase, the T4 gene 41 protein.

The interaction of the dda protein with the T4 uvsX protein (a recA protein analogue) was previously suggested when the dda protein in an extract of T4-infected cells was retained on a uvsX protein-agarose column. Since the dda protein coeluted with the gene 32 protein from this column, the interaction of the dda protein with the uvsX protein could have been indirect (Formosa and Alberts, 1984). To determine if the uvsX and dda proteins bind directly to one another, we chromatographed a mixture of the pure dda protein with albumin on a uvsX protein affinity column. As shown in Fig. 5, the albumin is not retained by the column, whereas the dda protein binds to the column and is eluted by 50 mM NaCl. The dda protein and the albumin behaved identically on an agarose control column, and the dda protein was not retained on an albumin-agarose control column (data not shown). These results demonstrate that there is a direct, albeit weak, interaction between the uvsX and dda proteins. Similar weak interactions have been observed between the protein subunits of the T4 DNA polymerase holoenzyme. On protein affinity columns both the interactions between T4 DNA polymerase accessory proteins (the gene 45 protein and the 44/62 protein complex) and the interaction of the T4 DNA polymerase with the gene 45 protein are disrupted by washing with 50 mM NaCl (Formosa and Alberts, 1984; Formosa, 1985).

**DISCUSSION**

Direct sequencing of nucleic acids (DNA and RNA) produced from bacteriophage T4-infected cells has allowed us to use the PCR technique to engineer an appropriate vector to produce the dda protein in *E. coli*. The dda gene product was thereby overexpressed to approximately 1% of the total cellular protein in *E. coli* htpR mutant cells. The cloning and overexpression of the dda protein greatly simplified its purification, since its association with nucleases in the infected cell and with the tightly binding T4 gene 32 protein was avoided. Thus, the dda protein purified from overexpressing cells is more than 99% pure and free of nucleases after only two chromatography steps. The yield is greatly increased such that 7 mg of dda protein is obtained from 28 g of cells. The overexpressed dda protein has DNA helicase activity, and a direct interaction of the this protein with the T4 uvsX protein (a recA analogue essential for T4 genetic recombination) was detected on a uvsX protein-agarose column.

Both the genomic and cloned dda genes were sequenced using the dyeoxy method. The dda amino acid sequence shares six conserved motifs with a superfamily of ATPases identified by Gorbalenya et al. (1988) and independently by Hodgman (1988). The consensus sequences for the superfamily and the alignment of dda with the family of *E. coli* helicases within the six regions is shown in Fig. 6. Motif I contains the sequence common to many GTP- and ATP-binding domains, originally described by Walker et al. (1982). This motif forms a loop that binds the ATP phosphate (La Cour et al., 1985; Jurnack, 1985; Fry et al., 1986). Motif II most likely binds the ATP phosphate indirectly via a magnesium ion (Jurnack, 1985). Motif III is also conserved among many DNA and RNA polymerases (Hodgman, 1986). Motif VI is believed to be involved in DNA binding because of its occurrence in putative DNA-binding proteins (Hodgman, 1988). Neither the structure nor function of motifs IV and V is known.

Among this superfamily of more than 20 proteins, the dda protein has the greatest homology with the *E. coli* recD protein. The recD protein is the most diverged member of the *E. coli* helicase family, which is composed additionally of the recB, rep, and uvrD proteins (Hodgman, 1988). recD is a subunit of the recBCD (exonuclease V) complex which plays a central role in homologous genetic recombination (for review, see Telander-Muskavitch et al., 1981; Taylor, 1988). Exonuclease V moves along the DNA creating looped structures that are periodically cut by the enzyme (Taylor, 1988). The dda and recD protein share 38% amino acid identity within the six conserved sequence motifs and a Monte Carlo score of 4.5 when the amino acids NH$_2$-terminal to the first motif and COOH-terminal to the last motif are deleted from the analysis. For comparison, recD shares a 28, 31.5, and 30% amino acid identity within the conserved motifs with recB, rep, and uvrD, respectively, and Monte Carlo scores throughout the region encompassing all of the conserved motifs of 6.8, 4.4, 2.9, respectively.

The Monte Carlo scores give a statistical evaluation of the homology of the motifs and the regions between them, encompassing a larger part of the gene than just the conserved motifs. It is calculated by aligning the sequences using the SS2 algorithm to produce a similarity score, subtracting from the original similarity score the mean scores from compari-

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**TABLE I**

| Plasmid       | Temperature | Plating efficiency |
|---------------|-------------|--------------------|
| pKHddα        | 35°C        | (1.00)             |
| pKHddα        | 30°C        | 0.28               |
| "Vector only" control | 35°C        | <0.007             |
| "Vector only" control | 30°C        | <0.007             |

**TABLE II**

**Summary of dda protein purification**

| Fraction | Step              | Volume (ml) | Protein (mg) | Units ATPase/ml | Total units | Yield (%) | Units/mg protein | Enzyme purification |
|----------|------------------|-------------|--------------|----------------|-------------|-----------|------------------|-------------------|
| I        | Cleared lysate   | 135         | 1039         | ND*            | ND          | (100)     | (1.0)           |fold              |
| II       | DNA-cellulose    | 44          | 11.9         | 43.2           | 1901        | 88        | 160              |80                |
| III      | DEAE-cellulose   | 35          | 7.0          | 37.2           | 1302        | 60        | 186              |100               |

*One unit of ATPase activity is defined as the amount of enzyme required to hydrolyze 1 µmole of ATP in 1 min at 37 °C.
*Not possible to determine due to high ATPase activity by proteins other than dda; ND, not determined.
*Calculated by determining the amount of dda protein in fractions I and II by SDS-polyacrylamide gel analysis.
sons with the randomized sequence, and then dividing by the standard deviation. A Monte Carlo score of between 3 and 6 indicates possible homology, whereas scores greater than 6 indicate a probable homology (Barker and Dayhoff, 1972; Argos and Vingron, 1990).

The overall homology between the recD and dda proteins is not enough to suggest a strong structural or functional homology. It is nevertheless worth nothing that their genetic phenotypes display some similarities. The dda- mutant phage show a DNA delay phenotype, but they eventually attain a phage burst size that is close to normal (Little, 1973; P. Gauss, personal communication). Only in a T4 gene 59- background is the dda gene essential for DNA synthesis. The complex of recBC, missing recD, lacks exonuclease V activity in vitro. But recD mutant cells show a hyper-recombination phenotype and are viable (Chaudhury and Smith, 1984). Thus, like the dda gene, recD is a nonessential gene. However, in a recF mutant (recF pathway gene) background, the recD gene is required for chromosome recombination and UV resistance; this suggests that the recBCD and recF pathways are somewhat redundant (Lovett et al., 1988). Similarly, the dda DNA helicase seems to be partially replaceable by the gene 41 DNA helicase, providing that an accessory protein for 41 protein function, the gene 59 protein is present.

What are the physiological roles of the dda protein? The DNA delay phenotype of dda mutant phage, which is extended to a severe block in early DNA synthesis when the 59 protein is absent, suggests an important function for the dda protein in the initiation of T4 DNA replication. Determination of its exact role in initiation is likely to require the reconstitution of the initiation process in a purified in vitro system containing a T4 replication origin (Kreuzer and Alberts, 1985; Menkens and Kreuzer, 1988). In addition, the direct interaction of the dda and uvsX proteins reported here suggests that the dda protein serves as a specific accessory factor in T4 uvsX protein-catalyzed genetic recombination. A role in DNA recombination is also suggested by the dda protein's 4-fold stimulation of uvsX-catalyzed DNA branch migration rates (Kodadek and Alberts, 1987). However, the observed binding of the dda protein to both 32 protein and uvsX protein could have the alternative function of promoting access of the dda protein to single-stranded DNA, which is believed to be completely covered by one or the other of these proteins in a T4-infected cell.

In summary, we have cloned and sequenced the dda gene and used the sequence to overproduce the dda protein. The overproduced dda protein has allowed us to demonstrate its direct interaction with the uvsX protein. This result supports a role of the dda protein in DNA recombination, but many questions remain. The availability of the clone and large
amounts of the dda protein should facilitate the further characterization of this DNA helicase's roles during T4 bacteriophage infection.

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Fig. 6. Alignment of the dda protein with motifs conserved among a family of E. coli helicases. These motifs were independently identified by Gorbalenya et al. (1988) and by Hodgman (1988). The dda protein was aligned using the SS2 algorithm of Altschul and Erickson (1986). Residues placed in boxes are absolutely conserved among six protein families of putative ATPases (Gorbalenya et al., 1988; Hodgman, 1988). Small letters are used in the consensus sequence to denote the range of observed amino acids at those positions where four or less alternatives exist among all members of the families. The numbers indicate the length of the gap between conserved motifs.

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