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A DNA primase specified by I-like plasmids

(plasmid *drd* mutants/initiation of DNA replication/RNA priming/φX174, fd, and G4 DNA/*Escherichia coli dna* gene products)

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ABSTRACT **An enzyme has been isolated from *Escherichia coli* strains harboring the I-like plasmid R64*drd11*, which is capable of initiating DNA synthesis on the circular, single-stranded DNA of phages φX174, fd, and G4. In the conversion of these templates to duplex forms *in vitro*, the enzyme can substitute for the functions of *E. coli dna*B-*dna*C-*dna*G proteins, *E. coli* RNA polymerase, and *E. coli dnaG* protein, respectively. The enzyme requires all four ribonucleoside triphosphates for optimal activity, although a combination of ATP, CTP, and GTP can almost completely satisfy the rNTP requirement. The enzyme appears to cooperate specifically with DNA polymerase III because single-stranded DNA-dependent synthesis takes place in extracts deficient in DNA polymerases I and II but not in extracts from a *dnaZ* mutant. Highly purified enzyme preparations consist mostly of two major polypeptides, M_r 140,000 and 180,000, when analyzed by sodium dodecyl sulfate gel electrophoresis. These polypeptides cosediment with the enzyme activity through a glycerol gradient with a sedimentation coefficient of 3.6 S. DNA priming activity in extracts of *E. coli* strains harboring the mutant plasmids R64*drd11* or ColI*drd1*, which are derepressed in functions of conjugational DNA transfer, is severalfold higher than the activity from strains carrying the corresponding wild-type plasmid. This correlation suggests that the enzyme may play a role in conjugational DNA synthesis.**

The I-like plasmids (R64, R144, and ColI) of the incompatibility group Iα are large, circular DNA molecules with molecular weights ranging from 62–72 × 10^6 (1, 2). Transfer properties specified by I-like plasmids are generally repressed, but derepressed (*drd*) mutants have been isolated (3, 4) and used to study the transfer of conjugative plasmids from donor to recipient cells of *Escherichia coli* (5–7). Only one of the strands of the plasmid is transferred during conjugational DNA synthesis (5). Conjugational DNA synthesis is independent of the *E. coli dnaB* and *polA* functions but requires the *dnaE* gene product in the recipient cell (6, 7). In addition, *drd* mutants of plasmids R64, R144, and ColI, but not those of F-like plasmids, partially suppress the phenotype of *E. coli dnaG* mutants (8).

Many conjugative plasmids are able to suppress or enhance the temperature sensitivity of *E. coli dnaB* mutants (9), and five different R plasmids, including R64*drd11* and R144*drd3*, have been used to construct viable R-derivatives of the unsuppressed (*sup*$^+$) *E. coli dnaB*266 amber mutant (10). This has been interpreted as evidence for the presence of *dnaB* analog (*ban*) genes being associated with these plasmids (10). During our studies in collaboration with V. N. Iyer (Ottawa) designed to identify a *ban* protein of plasmid R64*drd11*, we have discovered an enzyme capable of initiating DNA synthesis on circular single-stranded (ss) DNA templates. A similar enzyme activity has subsequently been detected in extracts from strains harboring the plasmids R144 and ColI. The enzyme level in extracts from strains that carry the *drd* mutant plasmids of R64 and ColI is severalfold higher than the level observed in strains carrying the corresponding wild-type plasmid. The purification and initial characterization of the enzyme encoded by the plasmid R64*drd11* is described in this paper. The enzyme catalyzes the ssDNA-dependent incorporation of rNTPs into short oligoribonucleotide primers, and we therefore classify it as a novel DNA primase.

Table 1. DNA primase content of *E. coli* MS1 carrying conjugative plasmids

Plasmid donor strain	Plasmid type	Ref.	Trans-conjugants	Relative DNA primase content
PW2/R64*drd11*	I-like	10	R64-11/MS1	(100)*
WDII/R64	I-like	9	R64/MS1	0.4
BW66/R144*drd3*	I-like	8	R144-3/MS1	7.3
BW62/R144	I-like	8	R144/MS1	10.8
BW61/ColI*drd1*	I-like	8	ColI-1/MS1	39.9
BW162/ColI	I-like	8	ColI/MS1	0.8
BW162/R1*drd16*	F-like	8	R1-16/MS1	ND†
BW162/R100*drd1*	F-like	8	R100-1/MS1	ND
—	—	‡	MS1	ND

* (100) = 48.1 pmol of dTMP incorporated per μg of fraction I.
† ND, not detectable.
‡ Strain MS1 is a nalidixic acid-resistant derivative of *E. coli* Q1710*dnaB*266*supF* isolated in this laboratory. The plasmids were transferred from the indicated donor strains to recipient strain MS1 as described by Wilkins (8). Fraction I, prepared from the trans-conjugants, was assayed for DNA primase content in the presence of 30 μg of a DNA polymerase III fraction. Assay conditions were as described in *Materials and Methods* except that φX174 DNA was replaced by 0.15 μg of fd DNA.

MATERIALS AND METHODS

Materials, Reagents, and Methods. Unless otherwise indicated, these were as described (11–13). The following *E. coli* strains were used for preparation of receptor extracts: BT1071 *dnaB* (14), BC1304 *dnaB dnaC* (15), PC22 *dnaC* (16), BT1011 *dnaG* (14), AX727 *dnaZ* (17), and HMS83 *dna*$^+$, *polA*, *polB* (18). *E. coli dnaB groP*A15 (19) was used as a source for DNA polymerase III. *E. coli* strains used as donor to introduce by conjugation the plasmids into *E. coli* MS1 are described in Table 1. Phage G4 DNA was prepared as described by Zechel *et al.* (20). Phage fd DNA was a gift of H. Schaller (Heidelberg). Agarose SeaKem HGT(P) was from Marine Colloids, Rockland, ME. DNA-agarose was prepared as described (21).

The composition of buffers A–F is described in the legend to Table 2. All buffers contained, in addition, 1 mM dithiothreitol and 12% (wt/vol) glycerol. Purified enzyme preparations were diluted into buffers containing 100 μg of bovine serum albumin per ml.

Preparation of Ammonium Sulfate Fraction. Growth and lysis of cells, streptomycin sulfate treatment, and precipitation of proteins with 43% saturated ammonium sulfate (fraction I) were as described (12). Fraction I of *dna*$^+$ wild-type cells is able

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Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; ss, single-stranded.

Table 2. Purification of DNA primase

Fraction	Total activity, nmol dTMP/ incorporated	Specific activity, nmol dTMP/ mg protein
I. Ammonium sulfate	88,310	35.8
II. DEAE-Sephacel I	65,190	111
III. ssDNA-agarose	48,710	2,080
IV. Sepharose 6B	27,740	3,060
V. DEAE-Sephacel II	17,100	8,070
VI. Hydroxyapatite	13,390	16,530
VII. Phosphocellulose	7,640	50,910

DNA primase was purified from 732 g of *E. coli* R64-11/MS1. Fraction I (3.22 g of protein) in 640 ml of buffer A (20 mM Tris·HCl, pH 7.6/50 mM NaCl/1 mM $MgCl_2$/0.1 mM EDTA) was applied to a DEAE-Sephacel column (19.8 cm^2 × 16.2 cm) equilibrated in buffer A. The column was washed with 1 column vol of buffer A, and DNA primase was eluted with a 4.8-liter linear gradient of 50–600 mM NaCl in buffer A. Active fractions eluting at 220 mM NaCl (see Fig. 3) were pooled and the protein was precipitated by ammonium sulfate (369 mg/ml). The precipitate was dissolved in buffer B (20 mM Tris·HCl, pH 7.6/50 mM NaCl/1 mM EDTA) and, after dialysis (fraction II, 585 mg of protein), applied to a ssDNA-agarose column (19.8 cm^2 × 15.2 cm). The column was washed with 500 ml of buffer B followed by 500 ml of buffer B containing 0.3 M NaCl. The 0.3 M NaCl eluate containing the primase activity was concentrated by ammonium sulfate precipitation (fraction III, 23.4 mg of protein) and filtered through a Sepharose 6B-column (5.3 cm^2 × 94 cm) equilibrated with buffer C (20 mM Tris·HCl, pH 7.6/1 M NaCl/0.1 mM EDTA). Active fractions were pooled and concentrated by dialysis against buffer D [20 mM Tris·HCl, pH 7.6/0.1 mM EDTA/20% (wt/vol) polyethylene glycol 20,000] followed by dialysis against buffer B (fraction IV, 9.1 mg of protein). Fraction IV was applied to a second DEAE-Sephacel column (0.64 cm^2 × 7 cm), and DNA primase was eluted with a 100-ml gradient of 50–500 mM NaCl in buffer B. Active fractions were pooled (fraction V, 2.1 mg of protein) and applied to a hydroxyapatite column (2 cm^2 × 2 cm) equilibrated with buffer E (20 mM potassium phosphate, pH 6.8/50 mM KCl/0.1 mM EDTA). The column was washed and DNA primase was eluted with a 100-ml gradient of 20–500 mM phosphate. Active fractions eluting at 70 mM phosphate were pooled (fraction VI, 0.81 mg of protein), dialyzed against buffer F (5 mM Tris·H_3PO_4, pH 7.0/1 mM EDTA), and loaded on a phosphocellulose column (0.64 cm^2 × 3.1 cm). The column was washed and DNA primase was eluted with a 50-ml gradient of 0–600 mM NaCl in buffer F. Active fractions eluting at 200 mM NaCl were pooled and concentrated by dialysis against buffer D followed by dialysis against buffer B containing 50% (wt/vol) glycerol (fraction VII, 0.15 mg of protein). DNA primase was assayed by its ability to restore φX174 DNA synthesis in fraction I of *E. coli* BC1304 *dnaB dnaC* (65 μg of protein) as described in *Materials and Methods.*

to support φX174 complementary (−)strand synthesis. However, fraction I of the *E. coli dna* temperature-sensitive mutants listed above is inactive, with the exception of strain BT1071 *dnaB*. The residual *dnaB* activity is destroyed by heating fraction I (37 mg of protein per ml) for 1.5 min at 37°C if used as *dnaB* receptor extract.

Preparation of DNA Polymerase III Fraction. Fraction I of *E. coli groP*A15 (1.3 g of protein) was chromatographed on a DEAE-Sephacel column as described in the legend to Table 2. DNA polymerase III, eluting at 150 mM NaCl, was assayed by adding purified DNA primase (fraction IV) to aliquots of each fraction. DNA synthesis was measured as described below. The resulting fraction (560 mg of protein) is called DNA polymerase III fraction for convenience, although its identity was not proven unequivocally.

Assay for DNA Primase. Assay mixtures (50 μl) contained 20 mM Tris·HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM dithiothreitol, 2.5 mM spermidine·3HCl, 25 μg of rifampicin per ml, 2 mM ATP, 125 μM each of CTP, GTP, and UTP, 12.5 μM each of dATP, dCTP, dGTP, and [*methyl*-^{3}H]dTTP (1200 cpm/pmol), 0.48 nmol of φX174 DNA, and protein fractions as indicated in the legends to figures and tables. [^{3}H]dTMP incorporation into acid-insoluble material was measured after incubation for 30 min at 30°C.

RESULTS

Rifampicin-Resistant DNA Priming Activity Specified by I-Like Plasmids. Fraction I protein of *E. coli*, insoluble in 43% saturated ammonium sulfate, contains a sufficient level of replication proteins to replicate φX174 (11) and fd DNA. Conversion of φX174 ss to duplex DNA is rifampicin resistant and requires, in addition to other proteins, the *dnaB* gene product (22, 23). Consequently φX174 DNA synthesis catalyzed by fraction I of *E. coli* MS1 is strongly inhibited by antibody directed against *dnaB* protein (Fig. 1). When φX174 DNA synthesis was measured with fraction I from strain R64-11/MS1, we noticed, however, that antibody to the *dnaB* protein had only a slight inhibitory effect. On the other hand, the response of fraction I from strain R64/MS1 to *dnaB* antibody was comparable to that of fraction I from strain MS1 (Fig. 1).

The differential response of these extracts to *dnaB* antibody was also reflected in their different potential to support fd DNA synthesis. Priming of fd (−)strand synthesis requires *E. coli* RNA polymerase (24) and is therefore sensitive to rifampicin. This is true for fraction I of strains MS1 and R64/MS1, although in the latter case a low level of rifampicin-resistant synthesis is observed. However, fd DNA synthesis by fraction I of strain R64-11/MS1 is rifampicin resistant (Fig. 2). These results indicated that plasmid R64 might code for a new DNA priming protein whose synthesis is repressed in strains carrying the wild-type plasmid but which is expressed as a consequence of the *drd* mutation.

Extracts of strain MS1 carrying various conjugative plasmids were screened for priming activity (Table 1). Activity was detected in extracts of cells harboring the plasmids R64, R144, and ColI but not in extracts of cells that carry the F-like plasmids R1 or R100. For R64 and ColI, at least 50-fold higher levels of primase were induced by the derepressed mutant plasmid than by the corresponding wild-type plasmid. Such a difference in primase content, however, was not observed between R144-3/MS1 and R144/MS1.

Purification of DNA Primase Encoded by Plasmid R64*drd11*. In pilot experiments it was found that the DNA priming activity of strain R64-11/MS1 complemented not only an *E. coli dnaB* extract, but also an extract of the *E. coli dnaB dnaC* double mutant BC1304. We therefore used the stimulation of φX174 DNA synthesis in extracts of strain BC1304 as an assay for purification of the DNA priming activity. The

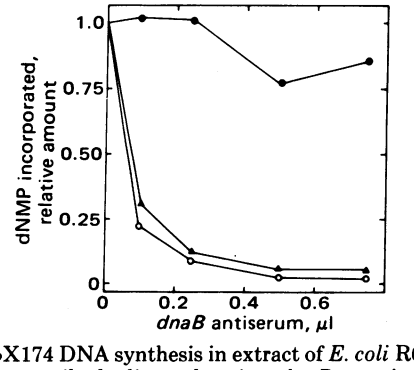

FIG. 1. φX174 DNA synthesis in extract of *E. coli* R64-11/MS1 is insensitive to antibody directed against *dnaB* protein. DNA synthesis was measured with fraction I of MS1 (120 μg of protein) (○), R64/MS1 (60 μg) (▲), and R64-11/MS1 (50 μg) (●). Fraction I was incubated with increasing amounts of *dnaB* antiserum for 10 min at 0°C before being added to the 50-μl assay mixture. Incorporation values without *dnaB* antiserum (1.0) were 27–30 pmol of dNMP.

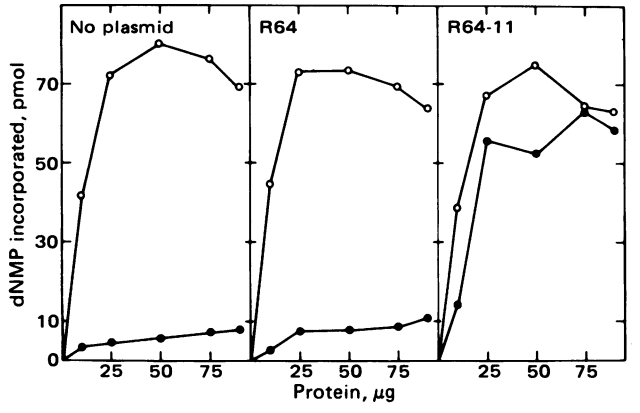

FIG. 2. fd DNA synthesis in extract of *E. coli* R64-11/MS1 is resistant to rifampicin. DNA synthesis with (●) and without (○) rifampicin was measured with fraction I of MS1, R64/MS1, and R64-11/MS1. ϕX174 DNA was replaced by 66 ng of fd DNA.

enzyme was purified approximately 8500-fold with a 9% yield (Table 2). The activity was eluted from DEAE-Sephacel far ahead of the *dnaB* complementing activity (Fig. 3). Its elution profile overlaps with that of the *dnaZ* complementing activity (Fig. 3, *Inset*) and also with the DNA polymerase III activity. Overlapping of the different activities is probably the reason for the ability of the fractions to replicate ϕX174 DNA in the absence of BC1304 receptor extract (Fig. 3, *Inset*). The primase binds to ssDNA-agarose and can be eluted from the column with 0.3 M NaCl. When the enzyme was filtered through a Sepharose 6B column, it eluted slightly ahead of aldolase (M_r 149,000). Purification of the corresponding enzyme of strain PW2/R64*drd*11 gave results similar to that shown in Table 2 for R64-11/MS1.

Physical Properties of DNA Primase. Fraction VII of DNA primase from PW2/R64*drd11* was analyzed by zonal sedimentation through a glycerol gradient (Fig. 4). A single peak of activity containing 70% of the applied activity was recovered from the gradient. The enzyme sedimented with the same velocity as ovalbumin (M_r 43,000, $s_{20,w}$ = 3.6 S). Analysis of the gradient fractions by sodium dodecyl sulfate (NaDodSO$_4$)/polyacrylamide gel electrophoresis revealed that two major polypeptides cosedimented with the enzyme activity. The electrophoretic mobilities indicated molecular weights of 140,000 and 180,000 as compared to myosin (M_r 200,000), *E. coli* RNA polymerase subunits β and β' (M_r 155,000 and 165,000), and β-galactosidase (M_r 130,000) (data not shown). No Coomassie blue-stainable material was detected on the denaturing gel at the position expected for a protein of M_r 40,000–60,000. The enzyme is therefore possibly a highly anisometric molecule.

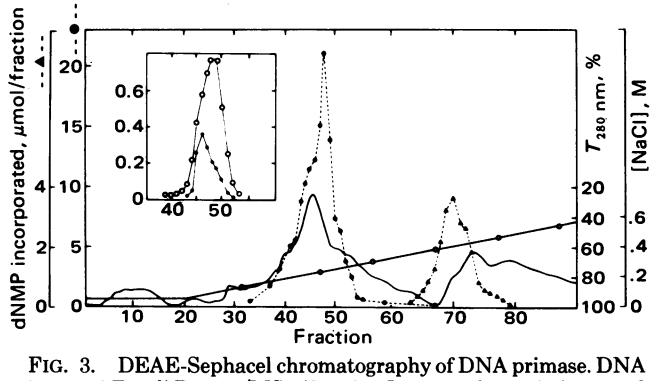

FIG. 3. DEAE-Sephacel chromatography of DNA primase. DNA primase of *E. coli* R64-11/MS1 (fraction I, 1.37 g of protein in 275 ml of buffer A) was applied to a DEAE-Sephacel column (7.5 cm^2 × 18.7 cm). The column was washed with 350 ml of buffer A and the protein was eluted with a 2.1-liter gradient of 50–600 mM NaCl in buffer A. (—) Transmittance; (○ — ○) NaCl concentration. Fractions (30 ml) were collected and aliquots were used to complement the following mutant extracts (fraction I) for DNA synthesis: ●, *E. coli* BC1304 *dnaB dnaC* (65 μg of protein per assay); ▲, *E. coli* BT1071 *dnaB* (110 μg). (*Inset*) ○, *E. coli* AX727 *dnaZ* (107 μg); ●, without receptor.

Agarose gel electrophoresis of DNA primase under native conditions revealed a single, relatively broad protein band (Fig. 5). The peak of DNA primase activity from an identical unstained native gel coincided with the stained protein band. Analysis of the Coomassie-positive material from the agarose gel by NaDodSO$_4$ gel electrophoresis under denaturing conditions again revealed two major polypeptides with M_r of 140,000 and 180,000. This analysis further shows that the mobility of the two polypeptides on the native gel was slightly different. Only the smaller polypeptide appears to run coincidently with the enzyme activity, as indicated by a nearly constant ratio of the amount of protein to activity (Fig. 5, *Inset*).

Enzymatic Characterization of DNA Primase. Purified DNA primase from strain PW2/R64*drd11* was tested for its ability to substitute for *E. coli* initiation functions in the conversion of ϕX174, G4, and fd DNA to duplex forms. The enzyme can fully restore ϕX174 DNA synthesis in extracts deficient in *dnaB*, *dnaC*, or *dnaG* proteins (Table 3). Similarly, the enzyme can substitute for the function of *E. coli* primase (*dnaG*

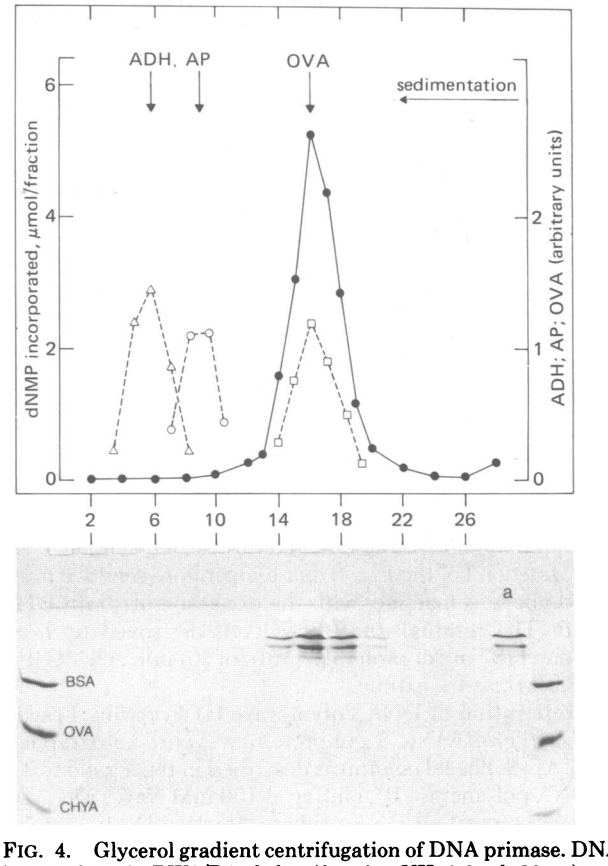

FIG. 4. Glycerol gradient centrifugation of DNA primase. DNA primase of strain PW2/R64*drd11* (fraction VII, 0.2 ml, 86 μg) was layered on a 4.8-ml, 18–43% (wt/vol) glycerol gradient in 20 mM Tris·HCl, pH 7.6/200 mM NaCl/0.1 mM EDTA/1 mM dithiothreitol. Centrifugation was at 60,000 rpm for 25 hr at 2°C (SW65 rotor). Fractions were tested for DNA primase activity (●) as described in the legend to Table 2. Aliquots were subjected to NaDodSO$_4$ gel electrophoresis according to Laemmli (25). Yeast alcohol dehydrogenase (ADH, M_r 141,000), *E. coli* alkaline phosphatase (AP, M_r 80,000), and ovalbumin (OVA, M_r 43,000) were run in parallel as markers. (*Lower*) a, Material applied to the gradient (8.6 μg of protein). BSA, bovine serum albumin; CHYA, chymotrypsinogen A.

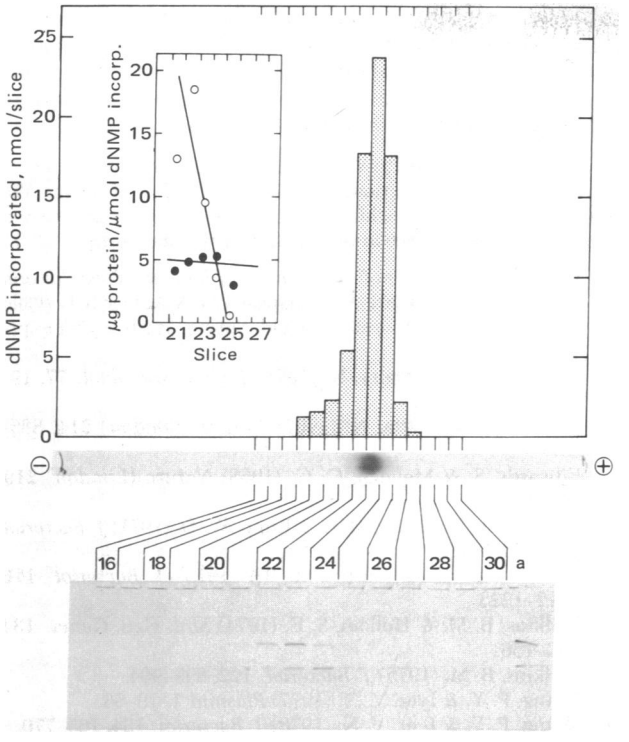

FIG. 5. Electrophoretic analysis of DNA primase under native and denaturing conditions. DNA primase of strain PW2/R64*drd11* (fraction VII, 4.3 μg) was subjected to electrophoresis on a cylindrical 1% agarose gel (0.5 × 11.5 cm) in 50 mM H_3PO_4/25 mM glycine/50 mM NaCl/1 mM EDTA/1 mM dithiothreitol/12% (wt/vol) glycerol, adjusted to pH 8.0 with Tris base. Electrophoresis was for 1 hr at 2.2 V/cm, followed by a 13-hr run at 4.5 V/cm (3.3 mA per tube) at 4°C. The gel was cut into 2.6-mm slices, which were incubated for 15 hr at 4°C in 50 μl of buffer B to elute the protein. Aliquots were assayed for DNA primase activity as described in the legend to Table 2. Recovery of activity from the gel was 30%. (*Middle*) An identical gel that was stained with Coomassie blue. (*Bottom*) Stainable material of the sliced agarose gel and an aliquot of fraction VII (1.7 μg, a) were electrophoresed on a 15% NaDodSO$_4$/polyacrylamide gel. The amount of protein in the two major bands was quantitated by microdensitometer tracing and correlated with the primase activity across the enzyme peak. (*Inset*) ○, Upper band; ●, lower band.

protein; refs. 20, 26, and 27) in G4 (−)strand synthesis and for *E. coli* RNA polymerase in the initiation of fd DNA synthesis (24). The enzyme was, however, unable to complement *dnaG* mutant extracts for ColE1 DNA synthesis (W. L. Staudenbauer, personal communication).

The enzyme appears to cooperate specifically with DNA polymerase III holoenzyme (28), as indicated by its ability to stimulate DNA synthesis in extracts deficient in DNA polymerases I and II and by its inability to restore DNA synthesis in a *dnaZ* mutant extract (Table 3). The product of the *dnaZ*

Table 3. φX174 DNA synthesis in *E. coli dna* mutant extracts in the presence of plasmid-specified DNA primase

Extract	dNMP incorporated, pmol		
	Complete	−DNA primase	−rNTPs
dnaB	70	2.4	3.1
dnaC	59	2.6	2.2
dnaB dnaC	50	1.9	1.8
dnaG	69	8.5	2.7
dnaZ	1	1.5	1.1
polA polB	54	5.2	3.5

The complete system contained fraction I of *E. coli dna* temperature-sensitive mutants (95–130 μg of protein) or of *E. coli* HMS83 (70 μg) and purified DNA primase from strain PW2/R64*drd11* (0.67 ng) per 50-μl assay mixture.

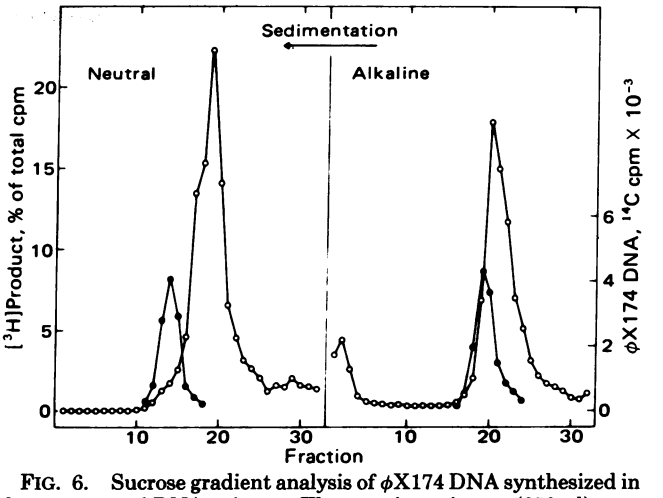

FIG. 6. Sucrose gradient analysis of φX174 DNA synthesized in the presence of DNA primase. The reaction mixture (250 μl) containing all four rNTPs was composed as described in the legend to Table 4. After incubation for 40 min at 30°C, 85-μl aliquots were withdrawn and the reaction was terminated by addition of (*i*) 85 μl of 3% Sarkosyl/1 M NaCl/10 mM EDTA (neutral sucrose gradient) and (*ii*) 85 μl of 3% Sarkosyl/0.8 M NaCl/10 mM EDTA/0.2 M NaOH (alkaline sucrose gradient). φX174 [^{14}C]DNA (4.5 μg, 5100 cpm/μg) was added to each sample as a sedimentation marker. Sucrose gradient centrifugation was performed as described (11). ●, φX174 [^{14}C]DNA; ○, product [^{3}H]DNA (265 pmol of dNMP per gradient).

gene is an integral part of DNA polymerase III holoenzyme (28, 29). A DEAE-Sephacel fraction containing DNA polymerase III, *dnaZ* protein, and DNA binding protein but no *dnaB*, *dnaC*, and *dnaG* protein was found to be sufficient to achieve φX174 (−)strand synthesis in conjunction with purified DNA primase. Neither DNA polymerase I nor T7 DNA polymerase could substitute for the DNA polymerase III fraction (data not shown).

The product synthesized in a reaction containing DNA primase and the DNA polymerase III fraction with φX174 DNA as template was analyzed by sucrose gradient centrifugation. In alkaline sucrose the majority of the product sedimented as full-length linear molecules (16S), whereas under neutral conditions the material sedimented to the expected position of φX174 replicative form II DNA (Fig. 6).

Priming of φX174 (−)strand synthesis was absolutely dependent on the presence of rNTPs (Tables 3 and 4). Maximum DNA synthesis was observed in the presence of all four rNTPs. The requirement for ATP and CTP appears to be crucial because any combination lacking ATP or CTP was ineffective.

Table 4. Ribonucleoside triphosphate requirement of DNA primase

rNTPs	DNA synthesis
A,C,G,U	(100)*
A,C,G,–	75
A,C,–,U	43
–,C,G,U	8
A,–,G,U	5
A,C,–,–	34
–,C,G,–	10
A,–,G,–	3
A,–,–,U	2
–,–,G,U	0.4
–,C,–,U	0.2

Each assay mixture (50 μl) contained the DNA polymerase III fraction (30 μg of protein) and DNA primase of strain PW2/R64*drd11* (fraction VII, 0.67 ng). When present, the concentration of rNTPs was 125 μM each.

* (100) = 151 pmol of dNMP incorporated.

With ATP and CTP alone, 34% of the maximum amount of DNA synthesis was obtained.

The dependence of the replication reaction on rNTPs reflects their need for RNA primer synthesis. The purified primase catalyzes, in the presence of natural ssDNA, the synthesis of very short oligoribonucleotides composed of A, C, and G residues. The enzyme does not catalyze the polymerization of dNTPs either in the presence or absence of rNTPs and it is free of DNA-dependent ATPase activity (unpublished results).

DISCUSSION

The development of *in vitro* systems for the replication of circular phage ssDNAs has proved invaluable for unravelling the biochemistry of DNA chain initiation (22, 23). The protein requirements for priming turned out to depend on the particular template used. For DNA synthesis to start on M13 and fd DNA, rifampicin-sensitive *E. coli* RNA polymerase is needed (24). Initiation of ϕX174 and G4 DNA synthesis instead requires *E. coli dnaG* protein as a primer-synthesizing enzyme (27, 28). By using the small phage DNAs as probes, we have discovered an additional priming activity in extracts of bacteria carrying the conjugative plasmids R64, R144, or ColI (Table 1). The R64 plasmid primase is, like *dnaG* protein, a rifampicin-resistant primer-generating polymerase but has a larger polypeptide molecular weight. Unlike the *dnaG* primase, the plasmid primase does not discriminate among ϕX174, fd, and G4 DNA templates. In this respect the enzyme resembles the phage T7 gene-4 primase, which shows a similar lack of template specificity (30). In addition, whereas the *dnaG* primase action on ϕX174 DNA is strictly dependent on accessory replication proteins such as *dnaB* and *dnaC* (22, 23, 31), plasmid primase has no such dependency (Table 3). The oligonucleotide-synthesizing activity of the two priming enzymes are also different; the plasmid primase, unlike the *dnaG* primase, does not incorporate deoxyribonucleotides into mixed ribo-deoxyribopolymers (26, 27).

The short oligoribonucleotides produced by plasmid primase serve as primers for subsequent DNA synthesis by a DNA polymerase III fraction. Chain elongation requires at least *E. coli dnaZ* function in addition to the polymerizing activity (Table 3). The apparent cooperation of plasmid primase with DNA polymerase III suggests that the enzyme may play an important role in DNA replication. What might this function be?

An attractive possibility is the involvement of plasmid primase in the conjugational DNA transfer synthesis. The fact that high levels of enzyme are found whenever transfer functions are derepressed is in support of this assumption (Table 1). During conjugation, only one DNA strand is transferred with its 5′ terminus as the leading extremity from the donor to the recipient cell (5, 6). Synthesis of the complementary strand requires DNA polymerase III but is rifampicin resistant (7). Moreover, it occurs in *E. coli dnaB* (7) and *dnaG* (8) recipient cells at the restrictive temperature. This means that none of the known DNA priming mechanisms is required. The plasmid primase may therefore be a candidate for the initiation of complementary strand synthesis during conjugational DNA transfer. It is proposed that the enzyme conveys the DNA from the donor to the recipient cell, where it synthesizes the primer. Alternatively, it could generate the primer in the donor, and the transferred single strand would, in this case, arrive in the recipient cell complete with primers attached.

Wilkins (8) has reported that derepressed mutant plasmids of the I type can partially restore the capacity of *E. coli dnaG* mutants to synthesize DNA and to form colonies at the restrictive temperature. In contrast, F-like plasmids fail to suppress the *dnaG* mutant phenotype. From our detection of priming activity in I-like but not in F-like plasmid-carrying cells, it would appear that the enzyme described in this paper is a potential candidate for *dnaG* suppression.

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