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Conversion of φX174 Viral DNA to Double-Stranded Form by Purified *Escherichia coli* Proteins

(*E. coli dna* gene products/*E. coli* DNA binding protein/DNA elongation factors I and II/ replication factor X and factor Y/DNA polymerase III)

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ABSTRACT **The *E. coli* proteins that catalyze the conversion of φX174 single-stranded DNA to duplex DNA have now been purified extensively. The reaction depends on *dnaB*, *dnaC(D)*, *dnaE*, and *dnaG* gene products, DNA elongation factors I and II, *E. coli* DNA binding protein, and two additional *E. coli* proteins, replication factors X and Y. DNA synthesis by these proteins requires φX174 viral DNA, dNTPs, Mg^{+2}, and ATP. The product synthesized is full-length linear φX174 DNA. The reaction has been resolved into two steps. The first step involves the interaction of ATP and φX174 DNA with *dnaB* and *dnaC(D)* gene products, *E. coli* DNA binding protein, and replication factors X and Y in the absence of dNTPs. Subsequent dNMP incorporation requires the addition of DNA polymerase III, DNA elongation factors I and II, *dnaG* gene product, and dNTPs.**

The formation of duplex DNA from φX174 single-stranded DNA is catalyzed by extracts of uninfected *Escherichia coli* (1). This reaction involves *dnaB*, *dnaC(D)*, *dnaE* [DNA polymerase III (2, 3)], and *dnaG* gene products (4, 5); it does not involve *dnaA* gene product (6) or RNA polymerase (4, 5). φX174 DNA-dependent DNA synthesis also requires ATP, dNTPs, and Mg^{2+}; none of the other NTPs are required and none will replace ATP (4). The product synthesized is full-length linear φX174 DNA.

Using complementation assays in this system, we have purified *dnaB*, *dnaC(D)*, *dnaE*, and *dnaG* gene products (6–8). Each isolated *dna* gene product specifically stimulated φX174 DNA synthesis in inactivated crude extracts prepared from cells temperature-sensitive for that gene product; each was thermolabile in complementing activity when prepared from temperature-sensitive cells and compared to the corresponding gene product prepared from wild-type cells.

In an attempt to reconstitute this system with purified proteins, we found that purified *dnaB*, *dnaC(D)*, and *dnaG* gene products did not catalyze φX174 DNA-dependent DNA synthesis in the presence of DNA polymerase III and DNA elongation factors I and II‡. However, the addition of crude fractions to the above six proteins resulted in φX174 DNA-dependent DNA synthesis. In this communication we describe the isolation of three additional *E. coli* proteins that are required in conjunction with the proteins mentioned above for DNA synthesis. One of these was shown to be *E. coli* DNA binding protein (12), and the other two are referred to as DNA replication factors X and Y.

‡ We previously showed that DNA elongation factors I and II were required for DNA synthesis catalyzed by DNA polymerase II or III in the presence of long single-stranded DNA primed with DNA or RNA (9, 10). Elongation factor I is probably identical to copolymerase III* (11), a protein required with DNA polymerase III* (defined as a complex form of DNA polymerase III) for DNA synthesis dependent on RNA or DNA primed single-stranded DNA. We found the requirement for DNA polymerase III* in this elongation factor I-dependent reaction could be satisfied by DNA polymerase II or III in conjunction with elongation factor II. For this reason, protein preparations used in these studies that have DNA polymerase III* activity will be referred to as DNA polymerase III fractions containing elongation factor II.

MATERIALS AND METHODS

Materials, Reagents, and Methods, unless otherwise indicated, were as previously described (4, 6–8).

Preparation of Proteins. DNA polymerase III, DNA elongation factor I, DNA elongation factor II, *E. coli* DNA binding protein, DNA polymerase III containing DNA elongation factor II, and *dnaB*, *dnaC(D)*, and *dnaG* gene products were purified by procedures modified from those in refs. 10, 10, 10, 12, 11, 13, 6, and 7, respectively. The assay and definition of units of DNA binding protein are given in ref. 9; the assays and definitions of units of other proteins are given in references above.

Assays for Proteins Involved in φX174 DNA Synthesis. Each assay (0.025 ml) contained 20 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 4 mM dithiothreitol, 1 mM ATP, 0.04 mM each of dATP, dCTP, dGTP, and [^{3}H]dTTP (500–1000 cpm/pmol), 10 μg/ml of rifampicin, 300 pmol of φX174 DNA, *dnaB* gene product (0.1 U, 0.12 μg), *dnaG* gene product (0.1 U, 0.07 μg), *dnaC(D)* gene product (0.1 U, 0.3 μg), DNA elongation factor I (0.2 U, 0.04 μg), *E. coli* DNA binding protein (0.2 U, 0.25 μg), replication factor X (0.05 U, 0.06 μg), replication factor Y (0.05 U, 0.35 μg), and DNA polymerase III (0.2 U containing 0.04 U of DNA elongation factor II, 0.2 μg) or DNA polymerase III (0.2 U, 0.05 μg) and DNA elongation factor II (0.1 U, 0.12 μg). After incubation at 30° for 30 min, acid-insoluble radioactivity was measured. One unit (U) of replication factor X or Y stimulated incorporation of 1 nmol of dTMP under the above conditions with saturating amounts of the other proteins present.

RESULTS

Characterization of Proteins Required for φX174 DNA-Dependent DNA Synthesis. The proteins required for φX174 DNA synthesis are shown in Table 1. The reaction depended on *dnaB*, *dnaC(D)*, and *dnaG* gene products, DNA polymerase III, DNA elongation factors I and II, replication factors X and Y, and *E. coli* DNA binding protein. DNA polymerase II in conjunction with DNA elongation factor II or DNA polymerase I in the absence of elongation factor II also satisfied the requirement for DNA polymerase III in catalyzing

TABLE 1. *Protein requirements for φX174 DNA-dependent DNA synthesis*

Additions	TMP incorporated (pmol/30 min) Exp. 1	Exp. 2
Complete	38.5	21.5
omit *dnaB* gene product	0.3	—
omit *dnaC(D)* gene product	0.3	—
omit *dnaG* gene product	<0.2	—
omit *E. coli* DNA binding protein	3.3	—
omit Replication factor X	1.1	—
omit Replication factor Y	0.4	—
omit DNA polymerase III (containing DNA elongation factor II)	0.5	—
omit DNA polymerase III	omit(—)	5.1
omit DNA elongation factor II	omit(—)	<0.2
omit DNA elongation factor I	2.0	0.5

—, not done. Reaction mixtures were as described in *Materials and Methods* with 0.05 U each of replication factors X and Y. DNA polymerase III (0.2 U) containing 0.04 U of DNA elongation factor II was used in Exp. 1; DNA polymerase III (0.2 U) and DNA elongation factor II (0.1 U) were used in Exp. 2. Replication factor X was isolated as follows: *E. coli* [600 g of strain NY73 (2), grown and frozen in sucrose (4)] was lysed by incubation at 0° for 45 min and at 30° for 5 min with 0.2 mg/ml of lysozyme, 0.1% Brij 58, 0.01 M Tris·HCl (pH 7.5), 20 mM EDTA, 1 mM dithiothreitol, and 0.15 M KCl and centrifuged for 40 min at 50,000 × *g*. The supernatant was adjusted to a final concentration of 4% with a solution of 20% streptomycin sulfate and centrifuged for 20 min at 10,000 × *g*. The supernatant was adjusted to 45% saturation with solid ammonium sulfate and centrifuged for 20 min at 10,000 × *g*. The pellet was suspended in 20 mM Tris·HCl (pH 7.5), 10% glycerol, 1 mM EDTA, and 10 mM 2-mercaptoethanol (buffer A) and dialyzed for 4 hr against 3 liters of the same buffer. The sample was diluted 3-fold (volume = 160 ml, 1800 mg of protein, 2900 U) and applied to a 3 × 30-cm DE-52 cellulose column equilibrated with buffer A. The column was developed with a 1-liter gradient from 0 to 0.6 M KCl in buffer A. Active fractions (eluting with 0.2 M KCl) were pooled, adjusted to 50% saturation with solid ammonium sulfate, and centrifuged. The pellet was dissolved in 20 mM potassium phosphate (pH 7.5), 10% glycerol, 10 mM 2-mercaptoethanol, and 1 mM EDTA (buffer B), dialyzed for 2 hr against this buffer, and diluted 3-fold (volume = 60 ml, 343 mg of protein, 2600 U). The fraction was applied to a 3 × 30-cm column of phosphocellulose (P-11) equilibrated with buffer B, and the activity was eluted after the main peak of protein by washing the column with 300 ml of buffer B. The activity was concentrated by ammonium sulfate precipitation (50%), resuspended in and dialyzed for 2 hr against 20 mM potassium phosphate (pH 6.8), 10% glycerol, 1 mM EDTA, and 10 mM 2-mercaptoethanol, applied to a 2 × 15-cm DE-52 cellulose column, and eluted with a 400-ml gradient from 0 to 0.5 M KCl in the same buffer. Active fractions were concentrated by ammonium sulfate (50%); the pellet was resuspended in 50 mM Tris·HCl (pH 8.2), 50 mM KCl, 1 mM EDTA, 10% glycerol, and 10 mM 2-mercaptoethanol, dialyzed for 2 hr against this buffer (volume 10 ml, 17 mg of protein, 860 U), and applied to a 2 × 15-cm DEAE-Sephadex column. The column was developed with a 350-ml gradient from 0.05 to 0.6 M KCl in the same buffer. Active fractions (volume = 36 ml, 9 mg of protein, 720 U) were adjusted to 40% saturation with solid ammonium sulfate. After centrifugation, the pellet was washed successively with 30% and 20% saturated ammonium sulfate in buffer A. The fraction that remained insoluble after extraction contained the majority of the activity (volume = 0.75 ml, 0.9 mg of protein, 700 U). This fraction was subjected to sedimentation through a 5-ml, 15–35% glycerol gradient containing 50 mM Tris·HCl (pH 7.5), 0.5 M KCl, 1 mM EDTA, and 1 mM dithiothreitol. After centrifugation for 30 hr at 50,000 rpm in the SW 50.1 Spinco rotor, replication factor X had sedimented through half of the gradient. Active fractions were stored frozen and were stable to repeated freezing and thawing. Replication factor Y was isolated as follows: preparation of crude extract [from 350 g of *E. coli*, strain HMS-83 (14)], treatment with streptomycin sulfate, and ammonium sulfate precipitation were as described above. The ammonium sulfate precipitate was dissolved in and dialyzed against buffer A. The dialyzed sample (volume = 50 ml, 480 mg of protein, 875 U) was diluted 3-fold and applied to a 3 × 25-cm column of DE-52 cellulose equilibrated with buffer A. The column was developed with a 700-ml gradient from 0 to 0.8 M KCl in buffer A. Fractions eluting between 0.05 and 0.15 M KCl were pooled, adjusted to 50% saturation with solid ammonium sulfate, and centrifuged. The pellet was dissolved in buffer A (volume = 44 ml, 440 mg of protein, 1300 U), dialyzed against this buffer, and applied to a 3 × 30-cm column of phosphocellulose equilibrated with buffer A. The column was eluted with a 750-ml gradient from 0 to 1 M KCl in buffer A. Active fractions (eluting with 0.25 M KCl) were concentrated by ammonium sulfate precipitation (50%) (volume = 13 ml, 31 mg of protein, 920 U). The sample was dialyzed against buffer B and applied to a 2 × 18-cm column of phosphocellulose equilibrated with buffer B. The column was eluted with a 300-ml gradient from 0.02–0.9 M potassium phosphate (pH 7.5) in buffer B. Activity eluted with 0.2 M potassium phosphate (volume = 40 ml, 4 mg of protein, 550 U). This fraction was concentrated by ammonium sulfate precipitation (50%) and stored frozen. It was further purified by glycerol gradient sedimentation, under conditions described for sedimentation of replication factor X.

dTMP incorporation dependent on φX174 DNA and *E. coli dna* gene products.

On fractionation of extracts stimulating φX174 DNA synthesis in the presence of *dnaB*, *C(D)*, and *G* gene products, DNA polymerase III, and DNA elongation factors I and II, one of the components isolated [purified to 50% of homogeneity as determined by migration of protein and activity on native polyacrylamide gel electrophoresis (15)] had the properties of *E. coli* DNA binding protein. These properties included: (*a*) protection of single-stranded DNA from degradation by nuclease S1 and *Neurospora* nuclease; (*b*) stability at 70° and 100° for 10 and 2 min, respectively; (*c*) insensitivity to *N*-ethylmaleimide; (*d*) native molecular weight of about 80,000 (12); (*e*) stimulation of dNMP incorporation catalyzed by DNA polymerase II with heat-denatured salmon sperm DNA as template (12); and (*f*) inhibition of RNA synthesis catalyzed by RNA polymerase in reactions dependent on single-stranded DNA templates and not on double-stranded DNA templates§. Furthermore, purified *E. coli* DNA binding protein satisfied the requirement for this factor in the φX174 DNA-dependent reaction.

Fractionation of extracts that stimulated φX174 DNA synthesis in the presence of *dnaB*, *C(D)*, and *G* gene products, *E. coli* DNA binding protein, DNA polymerase III, and DNA elongation factors I and II resulted in the isolation of two

§ The activity that stimulated the φX174 system in the absence of DNA binding protein cosedimented through glycerol gradients and comigrated on native polyacrylamide gel electrophoresis with the DNA polymerase II stimulating activity and the RNA polymerase inhibitory activity.

TABLE 2. *Requirements for φX174 DNA-dependent DNA synthesis by purified proteins*

Additions	dTMP incorporated (pmol/30 min)
Complete	19.0
− φX174 DNA	<0.2
− dATP, dCTP, dGTP	<0.2
− ATP	0.2
− ATP; + 1 mM UTP, GTP, or CTP	0.2–0.3
− ATP; + 1 mM dATP	3.1
− rifampicin	18.7
− rifampicin; + 0.2 mM UTP, GTP, and CTP	22.6
Complete; + 0.2 mM UTP, GTP, and CTP	22.8

Reactions were as described in *Materials and Methods*, with NTPs purified by Dowex chromatography and dNTPs treated with periodate. Where dTMP incorporation was observed, it was dependent upon *E. coli dna* gene products.

additional proteins, DNA replication factors X and Y (Table 1). Replication factor X (purified 500-fold from the first ammonium sulfate fraction to the final ammonium sulfate fractionation) has a molecular weight of about 45,000 based on glycerol gradient sedimentation with malate dehydrogenase as marker. Replication factor X is insensitive to *N*-ethylmaleimide, while factor Y is sensitive. Factors X and Y were 50% inactivated by heating 1 and 10 min, respectively, at 55°. No RNA polymerase activity was detected with the factor preparations using calf thymus DNA or φX174 DNA as template (<0.2 pmol of GMP incorporated per 20 min at 30° with 0.1 U of factor X or Y); a small amount of DNA polymerase activity was detected with DNase-treated salmon sperm DNA (1.2 and < 0.2 pmol of dTMP incorporated per 30 min at 30° with 0.1 U of factor X and Y, respectively). They did not affect RNA or DNA synthesis on these templates catalyzed by *E. coli* RNA

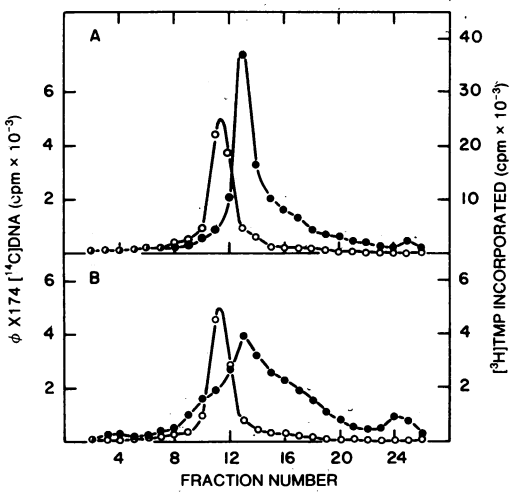

FIG. 1. Alkaline sucrose gradient sedimentation of reaction product. Reactions were incubated for 30 min as described in *Materials and Methods* and sedimented through 10–25% sucrose gradients containing 0.8 M NaCl, 0.2 M NaOH, and 1 mM EDTA. φX174 [^{14}C]DNA was included as marker, and acid-insoluble radioactivity was measured. (A) [^{3}H]dTMP product formed in the presence of all required components. (B) [^{3}H]dTMP product formed in the absence of *E. coli* DNA binding protein.

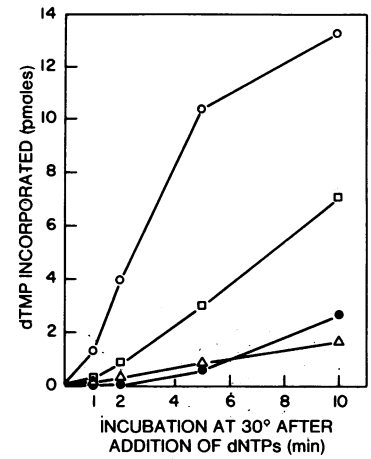

FIG. 2. Effect of incubation in the absence of dNTPs on DNA synthesis after addition of dNTPs. Reactions were as described in *Materials and Methods*, including all required proteins, φX174 DNA, ATP, Mg^{+2}, and buffer but omitting the four dNTPs. After incubation for 30 min at 30° (○), 30 min at 0° (△), 10 min at 30° (□), or 0 min (●), dNTPs were added and the rate of dTMP incorporation was measured.

polymerase or DNA polymerase III. No exonuclease activity was detected with [^{3}H]poly(dA-T) (<0.2 pmol rendered acid-soluble after 20 min at 30°).

Characterization of φX174 DNA-Synthesizing Reaction. The requirements for dTMP incorporation are shown in Table 2. The reaction depended on φX174 DNA; with 0.1, 0.3, 1.7, and 3.4 nmol of template, 10.7, 17.2, 15.5, and 8.2 pmol of dTMP were incorporated, respectively, under the conditions described in *Materials and Methods;* in the absence of *dnaB* gene product <0.2, 0.2, 1.4, and 2.0 pmol were incorporated, respectively. Thus, with increasing amounts of DNA, the extent of conversion of template to product and the specificity of the reaction decreased. ATP was essential for DNA synthesis; the optimum concentration was 0.75 mM. This requirement was not satisfied by equal concentrations of dATP, UTP, CTP, GTP, αβ methylene ATP, βγ methylene ATP, ADP, or AMP. As observed with crude extracts (4), there was no stimulation of dTMP incorporation by the combined addition of 0.2 mM UTP, CTP, and GTP in the presence of 0.2 or 1.0 mM ATP. The omission of rifampicin had no effect on the reaction with purified fractions, but it was added to prevent any possible involvement of *E. coli* RNA polymerase¶. The reaction was sensitive to salt; the addition of 50 mM KCl inhibited the activity 50%. Spermidine (0.5–5 mM) had no effect on the activity. When various other templates were substituted for φX174 viral DNA, including fd viral DNA, native and heat-denatured *E. coli* and T7 DNAs, colicin E_1 DNA, poly(dA), poly(dA-T) and poly(dT), the amount of DNA synthesis decreased 10-fold.

The reaction was linear with time for more than 60 min of incubation at 30° after a short lag of about 5 min. The re-

¶ φX174 DNA is a template for dTMP incorporation catalyzed by RNA polymerase, DNA polymerase III, and DNA elongation factors I and II (9–11). Thus, the formation of RNA-primed φX174 DNA obviates the requirement for *dnaB*, *dnaC*(*D*), and *dnaG* gene products, *E. coli* DNA binding protein, and replication factors X and Y for DNA synthesis.

TABLE 3. *Requirements of the separated steps of the φX174 DNA-dependent reaction*

Additions for first incubation in absence of dNTPs (40 min, 30°)	Additions for second incubation in presence of dNTPs	dTMP incorporated during second incubation at 15°, pmol/20 min	dTMP incorporated during second incubation at 6°, pmol/30 min
Complete	—	13.4	9.1
Complete; + dATP, dCTP, dGTP	—	19.0	8.8
− ATP	ATP	0.9	<0.2
− φX174 DNA	φX174 DNA	3.3	0.3
− *dnaB* gene product	*dnaB* gene product	2.1	<0.2
− *dnaC* gene product	*dnaC* gene product	1.8	0.7
− DNA binding protein	DNA binding protein	2.1	0.2
− Replication factor X	Replication factor X	2.0	0.9
− Replication factor Y	Replication factor Y	2.3	0.8
− *dnaG* gene product	*dnaG* gene product	13.7	7.3
− *dnaG* and *dnaB* gene products	*dnaG* gene product	<0.2	—
− DNA polymerase III	DNA polymerase III	16.4	7.8
− DNA elongation factor I	DNA elongation factor I	12.2	7.4
− *dnaG* gene product, DNA polymerase III, and DNA elongation factor I	*dnaG* gene product, DNA polymerase III, and DNA elongation factor I	8.2	—

Reaction mixtures for the first incubation were as described in legend of Fig. 2, but were carried out *in the absence of dNTPs* (except where described) and the indicated components. After 40 min at 30°, the omitted components and all four dNTPs were added. Mixtures were then incubated for either 20 min at 15° or 30 min at 6° and dTMP incorporation was measured. In each case, when the component omitted from the first step was not added in the second step, between <0.2 and 0.3 pmol of dTMP was incorporated. The DNA polymerase III used in these experiments contained elongation factor II.

action plateaued when 75% of the template DNA was converted to duplex DNA. The DNA products synthesized were full-length linear molecules, as determined by alkaline sucrose sedimentation (Fig. 1A). The amount of product synthesized in the absence of *E. coli* DNA binding protein was 10% of that synthesized by the complete system and was heterogeneous in size (Fig. 1B). None of the protein fractions used contained detectable DNA ligase activity [measured by their ability to protect [^{3}H]poly(dA-T) from degradation by exonuclease III]; the product size was not decreased by the addition of 10 mM nicotinamide mononucleotide; and at the earliest time examined (5 min), the product synthesized was full-length φX174 DNA. Thus, this DNA synthesis appears to be continuous.

Using the assay conditions described in *Materials and Methods* with [^{3}H]ATP and [α-^{32}P]dTTP there was <0.02 pmol of acid-precipitable AMP detected, while 86 pmol of dTMP were incorporated. Since there was less than 1 mol of AMP incorporated per mol of φX174 DNA synthesized, either poly(A) is not priming DNA synthesis or it is removed under the assay conditions used.

Separation of Reaction into Two Steps. Incubating the required proteins, ATP, and φX174 DNA in the absence of dNTPs (step 1) increased the initial rate of DNA synthesis after addition of dNTPs (step 2). As shown in Fig. 2, there was a 5-fold stimulation when step 1 was carried out for 10 min at 30° and a 15-fold stimulation after 30 min at 30°; there was no stimulation when step 1 was done at 0° for 30 min. Only the initial rate of DNA synthesis in step 2 was increased by the step 1 incubation; after 20 min of incubation with dNTPs, the rate of dNMP incorporation was the same whether or not reactions were subjected to step 1. As shown in Table 3, the rapid initial DNA synthesis in the second step depended on the presence of ATP, φX174 DNA, *dnaB*, and *dnaC(D)* gene products, replication factors X and Y, and DNA binding protein during the first step; the stimulation of incorporation was not seen when any one of these components was added during step 2 only. The ATP requirement during step 1 was not satisfied by dATP, UTP, CTP, or GTP. In contrast to these components, DNA polymerase III (containing elongation factor II), elongation factor I, and *dnaG* gene product were not required during the first step; the initial rate of dNMP incorporation observed was the same whether they were present during step 1 and step 2 or during step 2 only. Under these incubation conditions they were required for dTMP incorporation; the omission of any one from step 2 as well as step 1 resulted in the incorporation of <0.2 pmol of dTMP. It is not known yet if components required in the first step are further required in the second reaction step. Thus, the φX174 DNA-synthesizing system has been divided into two steps, with the first step involving reactions prior to dNMP incorporation.

DISCUSSION

We have purified the *E. coli* proteins that catalyze the conversion of φX174 single-stranded circular DNA to duplex DNA. In every respect this reaction is identical to that catalyzed by crude extracts (1, 4, 5): both are specific for φX174 DNA and poorly utilize fd single-stranded circular DNA; require *dnaB*, *C(D)*, *E*, and *G* gene products, dNTPs, Mg^{+2}, and only ATP of the NTPs; and produce full-length linear φX174 DNA molecules. The purified system involves two *E. coli* proteins, replication factors X and Y, in addition to seven previously characterized proteins: *dnaB*, *dnaC(D)*, *dnaE*, and *dnaG* gene products, DNA elongation factors I and II, and *E. coli* DNA binding protein. The *dnaB*, *C(D)*, *E*, and *G* gene products are defined by mutants temperature-sensitive for DNA synthesis and, thus, are involved in *E. coli* DNA replication. Until mutants are isolated, the physiological function of the other proteins involved in the φX174 DNA-synthesizing system and

their possible involvement in *E. coli* replication will remain unknown.

Using purified proteins and ϕX174 viral DNA, it is now possible to study detailed mechanisms involved in initiation of DNA synthesis and elongation catalyzed by this system. The reaction has been resolved into two steps. In the first step, *E. coli* DNA binding protein, *dnaB* and *dnaC(D)* gene products, replication factors X and Y, ATP, and ϕX174 DNA participate in reactions prior to dNMP incorporation. DNA polymerase III, DNA elongation factors I and II, and *dnaG* gene product are not required during step 1 but are required for dNMP incorporation during the second step after the addition of dNTPs. DNA polymerase III and factors I and II probably function in DNA elongation in the second step as they do in elongation of RNA- or DNA-primed single-stranded DNA (9–11). The role of *dnaG* gene product in the second step is unclear. To date, *dnaG* gene product has had no effect on dNMP incorporation catalyzed by DNA polymerase III and elongation factors I and II with DNase-treated DNA or with DNA- or RNA-primed single-stranded DNA as template. Possibly *dnaG* gene product functions in generating primer ends from protein–DNA complexes formed during step 1; DNA polymerase III plus elongation factors might then elongate the primer. Since ATP is the only nucleotide added during step 1, the primer may consist of a protein–AMP–DNA complex. Some functions of ATP in the ϕX174 DNA replicating system have already been suggested. We have found that the *dnaB* gene product is associated with ribonucleoside triphosphatase activity, which is stimulated 10-fold by single-stranded DNA (13). The physiological role of this activity is unknown. Also, elongation of RNA or DNA primers catalyzed by DNA polymerase III and elongation factors I and II requires ATP (10, 16) or dATP (10). Since these two activities cannot account for the specific ATP requirement of the system, it is likely that other functions of ATP will be found.

Although the purified ϕX174 DNA-synthesizing system is specific for ϕX174 DNA under the assay conditions described, many of the *E. coli* proteins involved are also involved in other DNA replicating systems. For example, the conversion of fd single-stranded circular DNA to duplex DNA requires four of the proteins required for ϕX174 DNA synthesis: DNA polymerase III, elongation factors I and II, and *E. coli* DNA binding protein (9, 10, 17). In contrast to the ϕX174 system, the fd system requires RNA polymerase (1, 4, 5) and does not require *dnaB* (5), *dnaC(D)*, and *dnaG* gene products (4), or replication factors X and Y (unpublished observations). Thus, while some of the reactions catalyzed by each of these systems may be novel to that system, others may be universal to DNA replication.

Note Added in Proof. Upon further purification, DNA replication factor Y appears to consist of at least two separable proteins.

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1. Wickner, W. T., Brutlag, D., Schekman, R. & Kornberg, A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 965–969.
2. Gefter, M. L., Hirota, Y., Kornberg, T., Wechsler, J. & Barnoux, C. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3150–3153.
3. Nüsslein, V., Otto, B., Bonhoeffer, F. & Schaller, H. (1971) *Nature New Biol.* **234**, 285–286.
4. Wickner, R. B., Wright, M., Wickner, S. & Hurwitz, J. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3233–3237.
5. Schekman, R., Wickner, W. T., Westergaard, O., Brutlag, D., Geider, K., Bertch, L. L. & Kornberg, A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2691–2695.
6. Wickner, S., Berkower, I., Wright, M. & Hurwitz, J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2369–2373.
7. Wickner, S., Wright, M. & Hurwitz, J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1613–1618.
8. Wright, M., Wickner, S. & Hurwitz, J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3120–3124.
9. Hurwitz, J., Wickner, S. & Wright, M. (1973) *Biochem. Biophys. Res. Commun.* **51**, 257–267.
10. Hurwitz, J. & Wickner, S. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 6–10.
11. Wickner, W., Schekman, R., Geider, K. & Kornberg, A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1764–1767.
12. Sigal, N., Delius, H., Kornberg, T. Gefter, M. L. & Alberts, B. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3537–3541.
13. Wickner, S., Wright, M. & Hurwitz, J. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 783–787.
14. Campbell, J. L., Soll, L. & Richardson, C. C. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2090–2094.
15. Jovin, T. M., Chrambach, A. & Naughton, M. A. (1964) *Anal. Biochem.* **9**, 351–369.
16. Wickner, W. & Kornberg, A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3679–3683.
17. Geider, K. & Kornberg, A. (1974) *J. Biol. Chem.* **249**, 3999–4005.