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Association of φX174 DNA-dependent ATPase activity with an *Escherichia coli* protein, replication factor Y, required for *in vitro* synthesis of φX174 DNA

(DNA replication)

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ABSTRACT φX174 DNA-dependent DNA synthesis is catalyzed *in vitro* by the combination of at least 11 purified protein fractions: *dnaB, dnaC(D),* and *dnaG* gene products, DNA polymerase III, DNA elongation factors I and II, DNA binding protein, and replication factors W, X, Y, and Z. The reaction requires ATP, 4 dNTPs, and Mg^{+2} and is specific for φX174 (or φXahb) DNA. Purified replication factor Y contains φX174 (or φXahb) DNA-dependent ATPase (or dATPase) activity. The ATPase activity is poorly stimulated by other single-stranded DNA, by double-stranded DNA, or by RNA. The products of the φX174 DNA-dependent ATPase activity of factor Y are P_i and ADP (or dADP). The association of φX174 DNA-dependent ATPase activity with factor Y was shown in the following ways: (*a*) the two activities copurified with a constant ratio; (*b*) they comigrated on native polyacrylamide gel electrophoresis; (*c*) both activities were heat-inactivated at the same rate; and (*d*) both showed identical patterns of *N*-ethylmaleimide sensitivity.

Three pathways have been identified by which single-stranded circular phage DNAs are converted to duplex DNA in reactions catalyzed by purified proteins isolated from *Escherichia coli*. The least complex system uses fd phage DNA and requires all four NTPs, dNTPs, and Mg^{+2}; the proteins required include purified RNA polymerase, *E. coli* DNA binding protein, DNA elongation factors I and II, and DNA polymerase III (1–3). A second pathway for the conversion of single-stranded DNA to duplex DNA uses ST-1, φXtB, or G4‡ phage DNA; the proteins required for this reaction include *dnaG* gene product, *E. coli* DNA binding protein, DNA elongation factors I and II, and DNA polymerase III (7, 8). A third and more complex system used φX174 or φXahb§ phage DNA. DNA synthesis requires ATP, dNTPs, Mg^{+2}, and at least eleven protein preparations isolated from uninfected *E. coli* (10): *dnaB, dnaC(D), dnaE,* and *dnaG* gene products, DNA binding protein, DNA elongation factors I and II, and replication factors W, X, Y, and Z¶. It is possible that other factors will be found to be involved in these *in vitro* replicating systems. Each system is specific for the DNAs mentioned and does not catalyze DNA systhesis dependent on other DNAs using the reaction conditions described for each system (1, 2, 7, 8, 10).

Some of the proteins involved in these reactions have been characterized. Four of them, *dnaB, dnaC(D), dnaE,* and *dnaG* gene products, are required for the replication of the *E. coli* chromosome as well and are defined by mutants temperature-sensitive for DNA synthesis. The *dnaE* gene product is DNA polymerase III (11, 12). The *dnaB* gene product has an associated ribonucleoside triphosphatase activity which is stimulated by single-stranded DNA (13). No enzymatic activity is as yet associated with *dnaC(D)* gene product. We have found that *in vitro* the purified *dnaC(D)* gene product interacts physically and functionally with *dnaB* gene product (14). It has been suggested that *dnaG* gene product has RNA polymerase activity (7). Since the other seven proteins required for *in vitro* φX174 DNA-dependent DNA synthesis are as yet undefined by genetic loci, their role in *E. coli* chromosome replication, if any, is unknown. Three such proteins, DNA binding protein and DNA elongation factors I and II, have been studied in other *in vitro* reactions. DNA binding protein interacts physically with some proteins in the absence of DNA as well as affecting the activities of some enzymes by binding to single-stranded DNA (15–17). DNA elongation factors I and II are required for DNA synthesis catalyzed by DNA polymerase II or III in the presence of long single-stranded DNA primed with RNA or DNA (2). The other four protein fractions involved in the φX174 DNA synthesizing system are referred to as replication factors W, X, Y, and Z and are defined by their requirement in this system (10). To date, replication factors W, X, and Z have no known enzymatic activities. We have found replication factor Y contains φX174 DNA-dependent ATPase activity and will describe the activity in this communication.

MATERIALS AND METHODS

Materials, Reagents, and Methods, unless otherwise indicated, were as previously described (10, 18).

Preparation of Proteins. *E. coli* DNA binding protein, *dnaB, dnaC(D),* and *dnaG* gene products, DNA elongation factors I and II, DNA polymerase III, and replication factor X were purified using procedures modified from those in refs. 15, 19, 20, 21, 2, 22, and 10, respectively. The assays and definitions of units of the proteins are given in references above. Replication factors W and Z were purified by their requirement for φX174 DNA-dependent DNA synthesis in the reaction described below. The procedures for the isolation of these factors will be published elsewhere. One unit of replication factor w or Z stimulated incorporation of 1 nmol of dTMP under the assay conditions described below with saturating amounts of the other proteins present.

‡ Phages ST-1, φXtB, and G4 are described in refs. 4, 5, and 6, respectively.
§ Phage φXahb is described in ref. 9.
¶ We have recently isolated another protein factor involved in this reaction and will refer to it as replication factor W.

Table 1. Copurification of replication factor Y activity and ϕX174 DNA-dependent ATPase activity

Enzyme fraction	Specific activity (U/mg)	Recovery (%)	Ratio of factor Y activity to ϕX174 DNA-dependent ATPase activity (×10)
1. Crude extract	—	—	—
2. Ammonium sulfate	0.5	100	—
3. DEAE-cellulose I	1.7	93	—
4. Phosphocellulose I	60	83	—
5. DEAE-cellulose II[a]	360	26	0.75
6. Phosphocellulose II	580	18	0.78
a. Glycerol gradient sedimentation[b]	1070	18	0.77
b. Polyacrylamide gel electrophoresis	—	—	0.79

Reaction mixtures were as described in *Materials and Methods*. Replication factor Y was purified as follows: *E. coli* [600 g of strain NY73 (11), grown and frozen in sucrose (18)] was lysed by incubation at 0° for 30 min and at 30° for 5 min with 0.2 mg/ml of lysozyme, 0.1% Brij 58, 0.05 M Tris·HCl (pH 7.5), 0.02 M EDTA, 1 mM dithiothreitol, 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 50% saturation with solid ammonium sulfate and centrifuged for 20 min at 10,000 × *g*. The pellet was suspended in 100 ml of 0.02 M Tris·HCl (pH 8.5), 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol (buffer A) and dialyzed 4 hr against 3 liters of the same buffer. The sample was diluted 4-fold (volume = 400 ml, 5800 mg of protein, 2900 U) and applied to a 5 × 30 cm DE-52-cellulose column equilibrated with buffer A. The column was developed with a 4-liter gradient from 0 to 0.8 M NaCl in buffer A. Fractions were collected; those eluting before 0.2 M NaCl were pooled (volume = 1.2 liters, 1600 mg of protein, 2700 U) and applied to a 3.5 × 35 cm phosphocellulose column equilibrated with buffer A. The column was washed with 300 ml of buffer A and eluted with a 2-liter gradient from 0 to 1.0 M NaCl in buffer A. Fractions (20 ml each) were collected and assayed for factor Y. Active fractions (eluting between 0.15 and 0.3 M NaCl) were pooled and adjusted to 50% saturation with solid ammonium sulfate. The precipitate was collected by centrifugation and dissolved in buffer A (volume = 5 ml, 40 mg of protein, 2400 U). This fraction was dialyzed against buffer A, diluted to 25 ml, and applied to a 1.8 × 20 cm column of DE-52-cellulose equilibrated with buffer A. The column was washed with 20 ml of buffer and eluted with a 400-ml gradient from 0 to 0.5 M NaCl in buffer A. Fractions (6 ml each) were collected and assayed for factor Y activity and for DNA-dependent ATPase activity with fd and ϕX174 DNA. Active fractions eluting between 0.03 and 0.08 M NaCl were pooled (see footnote[a]) and diluted with an equal volume (45 ml) of 0.02 M potassium phosphate (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, and 10% glycerol (buffer B) (volume = 90 ml, 2.1 mg of protein, 750 U). This material was applied to a 1.1 × 20 cm column of phosphocellulose equilibrated with buffer B. The column was eluted with a 100-ml gradient from 0.02 to 0.8 M potassium phosphate (pH 7.5) in buffer B. Fractions (0.8 ml each) were collected and assayed for factor Y and for ϕX174 DNA-dependent ATPase activity. Active fraction (eluting between 0.22 and 0.35 M potassium phosphate) were pooled and concentrated by vacuum dialysis against buffer A containing 0.2 M KCl (volume = 1.0 ml, 0.9 mg of protein, 525 U). A portion of the fraction was applied to a 5-ml 15–35% glycerol gradient containing 0.02 M Tris·HCl (pH 8.0), 1 mM EDTA, 1 mM dithiothreitol, and 0.5 M KCl. After centrifugation for 28 hr at 50,000 rpm in the SW 50.1 Spinco rotor, replication factor Y had sedimented through about one-half of the gradient. Nearly 100% of the activity applied was recovered. Active fractions were stored frozen and were stable for 6 months with repeated freezing and thawing.

Assay for Replication Factor Y. Each assay mixture (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, 200 pmol of ϕX174 DNA, 0.5 mg/ml of bovine serum albumin, *dnaB* gene product (0.05 U, 0.06 μg), *dnaG* gene product (0.08 U, 0.06 μg), *dnaC(D)* gene product (0.08 U, 0.1 μg), DNA elongation factor I (0.1 U, 0.05 μg), *E. coli* DNA binding protein (0.4 μg), DNA replication factor X (0.05 U, 0.06 μg), replication factor Z (0.08 U, 0.1 μg), replication factor W (0.05 U, 0.03 μg), DNA polymerase III (0.1 U, 0.02 μg), DNA elongation factor II (0.15 U, 0.03 μg), and protein fractions containing replication factor Y activity. After incubation at 30° for 30 min, acid-insoluble radioactivity was measured. In the absence of factor Y, 0.5 to 1.5 pmol of dTMP were incorporated using the above conditions; in the presence of saturating amounts of factor Y, 20 to 30 pmol of dTMP were incorporated. One unit (U) of replication factor Y stimulated incorporation of 1 nmol of dTMP under the above conditions with saturating amounts of the other proteins present.

Assay for ATPase Activity. Each assay mixture (0.04 ml) contained 50 mM Tris·HCl (pH 8.0), 1 mM dithiothreitol, 20 mM KCl, 0.1 mg/ml of bovine serum albumin, 10 μg/ml of rifampicin, 0.7 mM $MgCl_2$, 0.7 mM [γ-^{32}P]ATP (10–50 cpm/pmol), 200 pmol of ϕX174 DNA, and protein fractions as indicated. After 30 min at 30°, $^{32}P_i$ was determined by the method of Conway and Lipmann (23). One unit of ATPase catalyzed the production of 1 μmol of $^{32}P_i$ using the above conditions.

RESULTS

Evidence for identity of replication factor Y and ϕX174 DNA-dependent ATPase activity

Replication factor Y activity and ϕX174 DNA-dependent ATPase activity copurified over the last two steps of a 2000-fold purification procedure (Table 1). These two activities coeluted on phosphocellulose column chromatography with a constant ratio of factor Y to ATPase activity across the peak (Fig. 1A). When this fraction was sedimented through a glycerol gradient, the two activities cosedimented with a constant ratio and a molecular weight of about 55,000 (Fig. 1B). Native polyacrylamide gel electrophoresis (Fig. 2) and DEAE-Sephadex column chromatography (data not shown) of the phosphocellulose-II fraction also showed that the two activities were associated and in a constant ratio of factor Y to ϕX174 DNA-dependent ATPase activity.

In addition to their copurification, replication factor Y and ϕX174 DNA-dependent ATPase activity showed identical rates of heat inactivation (Fig. 3). Under the conditions used, both activities were 50% inactivated by heating 4 min at 49°, suggesting one protein species contains both activities. Also, both activities were inactivated equally by *N*-ethylmaleimide. Fractions (phosphocellulose-II) were incubated

[a] Prior to step 5, factor Y preparations contained DNA-independent ATPase activity and nonspecific DNA stimulated ATPase activity. At this step, factor Y fractions that had a high ratio of ϕX174 DNA to fd DNA-dependent ATPase activity were pooled for further purification.

[b] Glycerol gradient sedimentation was carried out on a portion of phosphocellulose II fraction. The results are calculated for the entire preparation.

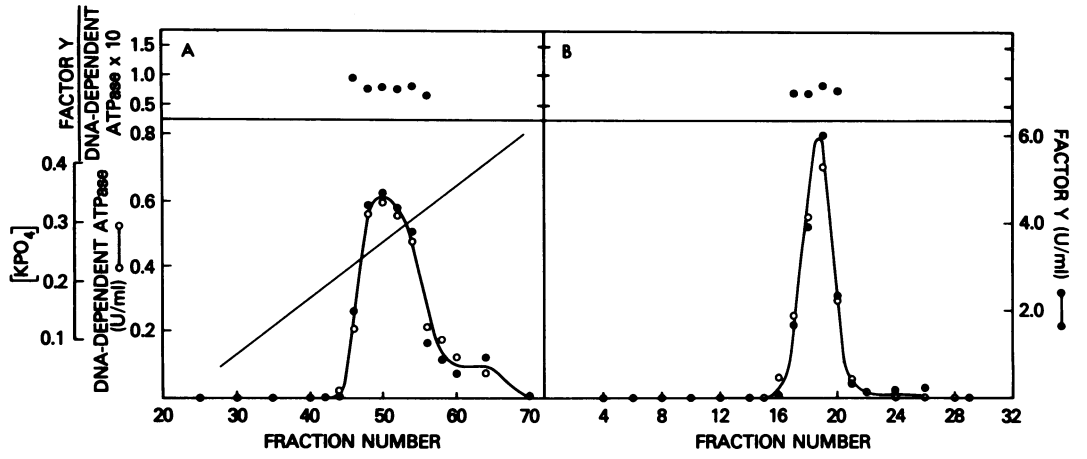

FIG. 1. Association of replication factor Y and ϕX174 DNA-dependent ATPase activity. (A) Phosphocellulose column chromatography. Factor Y was isolated as described in Table 1 through fraction 6. Fractions eluting from the phosphocellulose II column were assayed for ϕX174 DNA-dependent ATPase activity (O) and replication factor Y activity (●). (B) Glycerol gradient sedimentation. Fractions 48–52 were pooled and concentrated by vacuum dialysis. A portion was subjected to glycerol gradient centrifugation as described in Table 1. Fractions were assayed for ϕX174 DNA-dependent ATPase activity (O) and factor Y activity (●).

with 20 mM *N*-ethylmaleimide for 20 min at 25°, adjusted to 50 mM dithiothreitol, and assayed for both activities. Under these conditions, both replication factor Y activity and DNA-dependent ATPase activity were inactivated 61%. These results suggest that ϕX174 DNA-dependent ATPase activity is associated with replication factor Y.

Characterization of ϕX174 DNA-dependent ATPase activity of replication factor Y

The requirements for ϕX174 DNA-dependent ATP hydrolysis are shown in Table 2. In the absence of ϕX174 DNA, insignificant amounts of ATP were hydrolyzed (1–3% of that in the presence of ϕX174 DNA). Using the conditions described in *Materials and Methods*, maximal activity required 200–300 pmol of ϕX174 DNA; half-maximal activity was seen with 40 pmol of ϕX174 DNA per assay. Maximal activity was seen with 1 mM ATP in the presence of 1.25 mM $MgCl_2$; the K_m for ATP was 0.25 mM in the presence of 0.25 mM $MgCl_2$. Mg^{+2} was required for activity; Mn^{+2} but not Zn^{+2} satisfied this requirement; the K_m for Mg^{+2} was 0.125 mM in the presence of 1 mM ATP. dATP was hydrolyzed at the same rate as ATP; CTP, GTP, UTP, dCTP, dGTP, and dTTP were hydrolyzed at 1–5% the rate of ATP or dATP. The products of the reaction were P_i and ADP or dADP, which were formed in stoichiometric amounts. ϕX174 DNA-dependent ATPase activity occurred maximally at pH 9.0 (glycine buffer); the activity at pH 9.5 (glycine buffer), pH 7.5 (Tris buffer), pH 7.5 (potassium phosphate buffer), and pH 6.5 (potassium phosphate buffer) was 85%, 60%, 50%, and 30% of that at pH 9.0, respectively. The reaction was sensitive to salt; the addition of 0.05 M NaCl or NH_4Cl inhibited the activity 50%. The reaction was linear with time for more than 40 min at 30° or until 75% of the

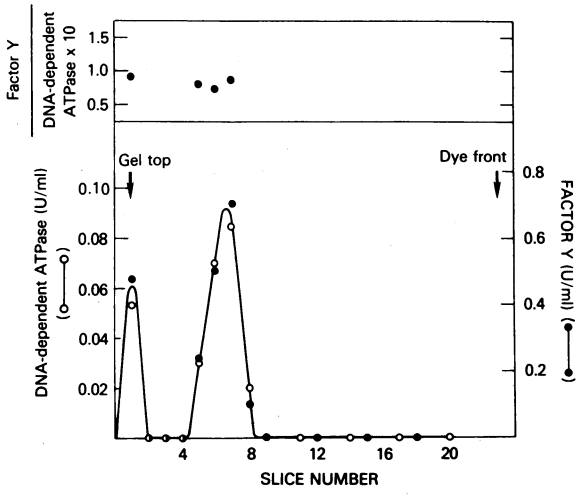

FIG. 2. Association of replication factor Y and ϕX174 DNA-dependent ATPase activity on native polyacrylamide gel electrophoresis. Replication factor Y (1.0 U of phosphocellulose II fraction) was subjected to polyacrylamide gel electrophoresis as described by Jovin *et al.* (24). The gel was sliced into 24 fractions; each was suspended in 0.1 ml of 0.02 M potassium phosphate (pH 7.0), 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, and 0.1 mg/ml of bovine serum albumin. Fractions were assayed for replication factor Y activity (●) and for ϕX174 DNA-dependent ATPase activity (O). Approximately 20% of the activity applied to the gel was recovered. The activity in slice 1 probably reflected aggregated protein that did not enter the gel. No protein was detected on an identical gel that was stained with Coomassie blue.

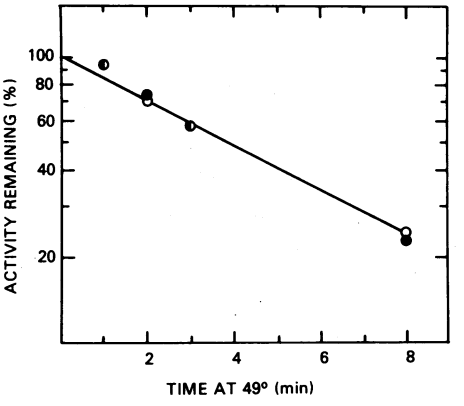

FIG. 3. Heat-inactivation of replication factor Y activity and ϕX174 DNA-dependent ATPase activity. Replication factor Y (phosphocellulose II fraction) was heated at 49°. At the times indicated, portions were removed and assayed for replication factor Y activity (●) and for ϕX174 DNA-dependent ATPase activity (O) as described in *Materials and Methods*. The initial replication factor Y activity was 7.5 U/ml; 1, 2, and 5 μl of each sample were assayed.

Table 2. Requirements for ϕX174 DNA-dependent ATPase associated with factor Y

Additions	ATP or dATP hydrolysis	
	P_i	ADP or dADP
	(nmol/30 min)	
Complete	10.0	10.6
—ϕX174 DNA	<0.1	<0.2
—ATP + dATP	—	9.4
—Mg^{++}	<0.1	—

Assays were as described in *Materials and Methods* with [α- or γ-^{32}P]ATP or [α-^{32}P]dATP and 0.07 U of replication factor Y (glycerol gradient fraction). ADP and dADP were measured by PEI-cellulose thin-layer chromatography by the method of Randerath (25).

ATP was hydrolyzed. The reaction was proportional to the amount of replication factor Y added (between 0.01 and 0.1 U).

Replication factor Y (glycerol gradient fraction) did not catalyze ϕX174 DNA-dependent exchange between $^{32}P_i$ and ATP [<0.03 pmol/20 min at 30° with 0.07 U of replication factor Y using reaction mixtures as described in *Materials and Methods* with 2.5 nmol of $^{32}P_i$ (640 cpm/pmol)] or between [α-^{32}P] ADP and ATP [<0.02 pmol/30 min at 30° with 0.07 U of replication factor Y using reaction conditions as described in *Materials and Methods* with 2 nmol of [α-^{32}P] ADP added (320 cpm/pmol)]. PP_i from [γ-^{32}P]ATP (<0.01 nmol with 0.14 U of replication factor Y) and AMP from [γ-^{32}P]ATP (<0.01 nmol with 0.07 U) were not produced during the reaction. The purified protein preparation (glycerol gradient fraction) was free of exonuclease activity as measured by acid solubilization of [^{3}H]poly(dA-T) (<0.05 pmol was solubilized by 0.14 U of replication factor Y after 30 min at 30°). It was also free of endonuclease activity as determined by electron microscopy of reactions as described in *Materials and Methods* containing 0.3 U of replication factor Y (glycerol gradient fraction) and 300 pmol of ϕX174 DNA. Factor Y (phosphocellulose-II fraction) was free of the other protein components involved in the ϕX174 DNA-synthesizing system including *dnaB*, *dnaC(D)*, and *dnaG* gene products, DNA polymerase III, DNA elongation factors I and II, DNA binding protein, and replication factors W, X, and Z (assayed as described in *Materials and Methods* using 0.2 U of factor Y).

ϕX174 DNA specificity of DNA-dependent ATPase activity of factor Y.

The DNA specificity of the factor Y ATPase activity is shown in Table 3. Maximal activity was observed with either ϕX174 or ϕXahb DNA. We have found that these are the only two single-stranded circular DNAs tested to date which are replicated by the combination of the purified components of the ϕX174 DNA synthesizing system (8, 10). Other single-stranded circular DNAs including ST-1, ϕXtB, or fd which are replicated *in vitro* by other DNA-synthesizing systems not involving replication factor Y, stimulated replication factor Y ATPase activity only slightly. Factor Y ATPase activity did not require intact circular ϕX174 DNA, but circular DNA was more effective than fragmented DNA. In these experients ϕX174 DNA was treated with pancreatic DNase using conditions which produced nearly full-length molecules and left no circular molecules (as determined by electron microscopy). With 15, 45,. and 150 pmol of either DNase-treated or untreated DNA and 0.05 U of factor Y per assay, 41, 51, and 80% of factor Y ATPase activity was seen with DNase-treated DNA as compared to untreated DNA, respectively.

Table 3. Polynucleotide specificity of ATPase of replication factor Y

Additions	ATP hydrolyzed (nmol P_i/20 min)
A. None	0.2
B. Single-stranded DNA	
ϕX174	8.0
ϕXahb	8.3
ϕXtB	0.6
ST-1	0.8
fd	1.3
Heat-denatured *E. coli*	1.3
Heat-denatured T7	1.5
Heat-denatured λ	0.7
Poly(dT), poly(dC), poly(dG), poly(dA)	0.1–0.5
C. Double-stranded DNA	
ϕX174 RFI	0.1
Colicin E1	0.2
E. coli	0.3
D. RNA	
poly(A)	0.1
tRNA	0.2
Qβ	0.2

Reactions were as described in *Materials and Methods* with 300 to 500 pmol of various polynucleotides as indicated and 0.07 U of replication factor Y (glycerol gradient fraction).

As shown in Table 3 all other natural and synthetic single-stranded polydeoxynucleotides tested stimulated factor Y ATPase activity poorly. However, the specificity of factor Y ATPase activity depended on the assay conditions; lower pH, higher Mg^{+2} concentration, and lower salt concentration decreased the ratio of ϕX174 to fd DNA-dependent ATPase activity from about 6 to 8 to about 3 to 5. Table 3 also shows that duplex DNA and RNA were ineffective in stimulating ATPase activity. Thus with the assay conditions used there is a direct relation between DNAs that stimulate efficiently replication factor Y ATPase activity and those whose *in vitro* replication requires replication factor Y.

Replication factor Y bound to single-stranded DNA, including ϕX174 and fd (measured by association of factor Y with either DNA on A-5m agarose gel filtration) and heat-denatured calf thymus (measured by binding to denatured calf thymus DNA agarose columns). The lack of DNA specificity for factor Y DNA binding but not for ATP hydrolysis suggests that DNA binding and ATP hydrolysis are independent properties of factor Y. The following experiment is consistent with this. Factor Y and fd DNA were incubated for 10 min, during which time little ATP was hydrolyzed. Then ϕX174 DNA was added and the rate of ATP hydrolysis was measured. Not until 10 min after the addition of ϕX174 DNA did the rate of hydrolysis reach the rate seen from time zero when factor Y was incubated with ϕX174 DNA alone. These results suggest that factor Y reversibly binds to fd DNA and dissociates slowly.

DISCUSSION

We have presented here a partial characterization of ϕX174 DNA-dependent ATPase activity associated with replication

factor Y, one of the components required for *in vitro* ϕX174 DNA-dependent DNA synthesis. The *dnaB* gene product, another component of the reconstituted ϕX174 DNA synthesizing system, and several other *E. coli* proteins have been shown to have DNA-dependent ATPase activity (13, 26, 27). Although it is not clear why two components of the ϕX174 DNA system have ATPase activities, the activities differ in several ways: (*a*) replication factor Y hydrolyzes ATP or dATP and no other NTPs or dNTPs while *dnaB* gene product hydrolyzes all four NTPs and no dNTPs; (*b*) replication factor Y does not hydrolyze ATP in the absence of single-stranded DNA while *dnaB* gene product does; and (*c*) factor Y ATPase activity is stimulated about 10 times more effectively by ϕX174 or ϕXahb DNA than by any other single-stranded DNA tested whereas *dnaB* gene product ATPase activity is stimulated by all single-stranded DNA.

It is not known yet how the triphosphatase activities of replication factor Y and *dnaB* gene product are related to their roles in DNA synthesis. In the presence of ϕX174 DNA in the absence of the other components required for *in vitro* ϕX174 DNA synthesis, both replication factor Y and *dnaB* gene product (13) hydrolyze large quantities of ATP. We have shown that they hydrolyze ATP at 100 times the rate that they stimulate dNMP incorporation in the reconstituted ϕX174 DNA synthesizing system. Thus, if these two ATPase activities were unregulated, then DNA synthesis would be an energetically costly process. We have recently found that the DNA-independent ATPase of *dnaB* gene product is inhibited by *dnaC(D)* gene product and that these two proteins form a physical complex which specifically requires ATP (14). If the interaction of *dnaB* and *dnaC(D)* and the accompanying inhibition of ATPase are physiological reactions, then the hydrolysis of ATP by *dnaB* in the absence of *dnaC(D)* gene product may be an uncoupled reaction. Similarly, the ϕX174 DNA-dependent ATP hydrolysis by replication factor Y may be an uncoupled reaction; other proteins may be required to couple this ATP hydrolysis to other reactions involved in DNA synthesis.

So far several ATP-dependent reactions involving components of the reconstituted ϕX174 DNA synthesizing system have been described which may contribute to the ATP requirement for DNA synthesis by this system as well as other replicating systems: (*a*) *dnaB* gene product contains ATPase (NTPase) activity which is stimulated by single-stranded DNA (13); (*b*) complex formation by *dnaB* and *dnaC(D)* gene products specifically requires ATP (14); (*c*) replication factor Y contains ϕX174 DNA-dependent ATPase (dATPase) activity; and (*d*) elongation of primed single-stranded DNA by DNA polymerase III and DNA elongation factors I and II requires ATP (dATP) (2). These activities are sufficient to account for the specific ATP requirement of the system; however, there may be still other ATP-dependent partial reactions.

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