Proc. Nat. Acad. Sci. USA
Vol. 72, No. 6, pp. 2099–2103, June 1975

DNA Synthesis Involving a Complexed Form of DNA Polymerase I in Extracts of *Escherichia coli*

(modified DNA polymerase activity/enzyme complex/*recBC* enzyme)

RICHARD W. HENDLER, MICHAEL PEREIRA*, AND RAYMOND SCHARFF

Section on Membrane Enzymology, Laboratory of Cell Biology, National Heart & Lung Institute, National Institutes of Health, Bethesda, Maryland 20014

Communicated by Rollin D. Hotchkiss, February 28, 1975

ABSTRACT DNA polymerase I (EC 2.7.7.7; deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase) has been recovered as a complex of about 390,000 molecular weight. The complex displays an ATP-stimulated DNA-synthesizing activity that prefers native to heat-denatured DNA. Genetic evidence indicates that the *recBC* enzyme is associated with the polymerase in the complex. Preliminary evidence for complexes involving DNA polymerases II and III is also presented.

Three DNA polymerase activities have been found in *E. coli*. Only one of them (polymerase III) has been shown to be indispensable for DNA replication (1). However, all three may be capable of participating in replication, with backup systems available to partially substitute for the loss of polymerase I (EC 2.7.7.7; deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase) or II. The known enzymatic properties of all three polymerases are more suited to repair functions than replication (2). However, *in vivo*, the enzymes may function in complexes which alter their activities. The uncertainty as to the precise roles of the known polymerases of *E. coli* in replication also applies to their roles in repair and recombination (3, 4).

We describe here an ATP-stimulated, DNA-synthesizing system that depends primarily on the activities of DNA polymerase I and the *recBC* enzyme. The system has been characterized in a particulate fraction (P_3) shown to have a high specific activity for DNA synthesis (5) and then isolated as a soluble complex of molecular weight about 390,000.

MATERIALS AND METHODS

Cell Fractions. Penicillin spheroplasts of *E. coli* W-6 were prepared and broken by gentle homogenization as described (5, 6). P_3 is the pellet fraction obtained by centrifuging a 20,000 × *g* (15 min) supernate for 60 min at 105,000 × *g*. P_3 was stored in small amounts in liquid nitrogen until needed.

Assay for DNA Synthesis. Incubations (0.17–0.35 ml) were at 25° in a basic incubation medium (5) containing: 15 or 30 μM each dATP, dGTP, dCTP, and [^{3}H]dTTP (60–600 μCi/μmol); 60 μM Tris·HCl, pH 8.1; 1 μM 2-mercaptoethanol; 8 μM $MgCl_2$; and native or denatured (7) *E. coli* or calf thymus DNA. For P_3, 150 mM KCl was present; for isolated complex, 70 mM KCl, 10% (v/v) glycerol, and 100 μg/ml of bovine serum albumin. Aliquots (50–100 μl) were pipetted onto Whatman 3mm filter discs and washed (5). Nucleic acids were solubilized and radioactivity was measured: (*i*) by hydrolysis in perchloric acid (5) (counting efficiency = 16%) or (*ii*) by solubilization from wetted discs with 1 ml of NCS reagent and counting in 0.4% 2,5-diphenyloxazole in toluene (efficiency = 43%). Incorporation was linear for several hours after a lag of up to 20 min. Protein was determined by the method of Lowry *et al.* (8), with bovine serum albumin as a standard. Activity is expressed in terms of total nucleotide incorporated, calculated as four times the quantity of dTTP incorporated.

Other Procedures. Preparative acrylamide gel electrophoresis was performed in a Canalco apparatus. The multiphasic buffer system used was no. 2212 of Jovin *et al.* (9). The lower gel (12 ml) contained 2.5% acrylamide and 0.1% bis-acrylamide. The upper gel (6 ml) contained 2.5% acrylamide and 0.625% bis-acrylamide. The upper gel was overlayered with 0.3 ml of 0.2 M thioacetic acid/0.13 M imidazole, pH 4.6 (25°) in 40% (v/v) glycerol. The sample was then applied and electrophoresis carried out at 0°. The elution buffer was standard pH 7.2 buffer (described in section on isolation).

Materials Were Obtained from the Following: dNTPs (Calbiochem or Sigma); ATP, 2,5-diphenyloxazole, and sodium-*p*-chloromercuribenzoate (Calbiochem); Whatman 3mm filter paper discs (Arthur Thomas and Co.); calf thymus DNA, *E. coli* DNA, and DNase I (deoxyribonuclease I, bovine pancreas, 3.1.4.5, Worthington DPFF, RNase-free) (Worthington); NCS reagent (Amersham/Searle); *E. coli* DNA polymerase I (grade 1) (Boehringer Mannheim); Blue Dextran 2000 (Pharmacia); bovine albumin powder (Armour Laboratories); [*methyl*-^{3}H] TTP (18 Ci/mmol) and [*4,5*-^{3}H]-leucine (10 Ci/mmol) (Schwartz/Mann); antibody to DNA polymerase I (Dr. I. R. Lehman, Stanford University); 1β-D-arabinofuranosylcytosinetriphosphate (ara-CTP) (Dr. Robert A. Sanchez, Salk Institute).

Strains of E. coli: W6 *pro-*(ATCC no. 25377); *rec*$^+$(N2257) *recB21* AB2470, and *recB21C22*(N2258) (obtained from Dr. M Gellert); BT 1026 (*endo*$^-$*polA*$_1^-$*thy*$^-$*str*$^-$*dnaE*$_{ts}$*1026*) (obtained from Dr. James A. Wechsler).

RESULTS

Using assay conditions suitable for DNA polymerase I (10), ATP-stimulated DNA synthesis was observed in a particulate fraction (P_3) obtained from *E. coli:* 137 ± 4 pmol/min per mg of protein without ATP, 179 ± 4 with 1 mM ATP($P <$

Abbreviations: ara-CTP, 1β-D-arabinofuranosylcytosinetriphosphate; complex, the material isolated from a Bio-Gel A-1.5m column in the elution volume corresponding to a molecular weight of 390,000 and possessing ATP-stimulated DNA polymerase activity.

* Present address: New York Blood Center, 310 East 67th Street, New York, N.Y. 10021.

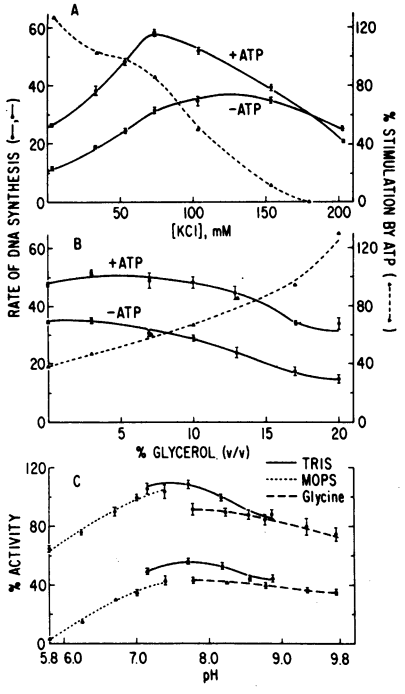

FIG. 1. (A) Effect of KCl on P_3 activity. The rate of DNA synthesis is expressed as pmol of nucleotides incorporated per min/mg of protein. Each point represents the mean ±SEM of four determinations. Standard incubation conditions were used with 200 μg/ml of *E. coli* DNA, all four dNTPs at 30 μM each, and 10% (v/v) glycerol. P_3 was present at a protein concentration of 106 μg/ml and counting method *ii* was used. ATP, when present, was 1 mM. (B) Effect of glycerol on P_3 activity. Assay conditions were as described in panel A with 70 mM KCl. P_3 was present at a protein concentration of 68 μg/ml. (C) Effect of pH on P_3 activity. The rate of DNA synthesis is expressed as a percentage of the activity in Tris buffer at pH 8.2 in the presence of 1 mM ATP. Each point is the mean ±SEM for three determinations in glycine buffer and for seven or more determinations in Tris or MOPS (morpholinopropanesulfonic acid) buffers. Assay conditions were as described in panel A with 70 mM KCl. P_3 was present at a protein concentration of 108 μg/ml. The pH of each buffer was measured at room temperature on a 0.05 M solution.

0.01). The P_3 fraction was not stimulated by ATP in the absence of added calf thymus DNA (64 ± 4 compared with 64 ± 2). Heat-denatured DNA was inferior to native DNA both for stimulating DNA synthesis (95 ± 7 compared with 137 ± 4, $P < 0.01$) and for supporting an ATP stimulation (115 ± 7 compared with 179 ± 4, $P < 0.01$). The synthesis was sensitive to DNase (>95% inhibition by 10 μg/ml) and depended on the presence of added unlabeled nucleotides (omission of dATP or of dATP, dCTP, and dGTP inhibited 80%). The same behavior was seen with *E. coli* DNA. When *E. coli* DNA was used, the synthesized DNA had the same density as *E. coli* DNA labeled *in vivo*, as determined by isopycnic banding in neutral and alkaline CsCl density gradients (not shown). The observed ATP stimulation is not large, but it was reproducible and served as a convenient marker for further studies with the system.

DNA synthesis by P_3 was markedly sensitive to antibody to polymerase I (>95% inhibition in the presence of DNA and ATP). It was not inhibited by 10^{-4} M ara-CTP, a concentration which inhibits polymerase II by 70% and polymercurimerase III by 30% (11). *p*-Chloromercuribenzoate at 0.3 mM [which completely inhibits polymerase II (12)] inhibited P_3 DNA synthesis by only 32%, and 10 mM *p*-chloromercuribenzoate was needed to obtain >90% inhibition. The major part of the DNA synthesizing activity is, therefore, due to polymerase I.

TABLE 1. *Comparison of P_3 preparations from E. coli W6 and recB21*

Source of P_3	Heated *E. coli* DNA		Native *E. coli* DNA	
	−ATP	+ATP	−ATP	+ATP
W_6	306(8)	287(6)	404(13)	496(13)
recB21	315(6)	260(6)	394(10)	365(10)

Each value is pmol of nucleotides incorporated per min per mg of protein, for the number of determinations in parentheses, using assay conditions as described in *Materials and Methods*, with 150 mM KCl, all four dNTPs (15 μM each), *E. coli* DNA (200 μg/ml), and 1 mM ATP where indicated. Proteins were 158 μg/ml for W6, 130 μg/ml for *recB21*. The ATP stimulation of W6 was statistically significant ($P < 0.01$, *t* test of significance).

Because polymerase I is not an ATPase, the participation of another enzyme in the ATP-stimulated synthesizing system is indicated. The ATP-stimulated DNase coded for by the *recB* and *recC* gene locations (13–18) was tested for its possible involvement by use of a *recB* mutant. DNA synthesis by P_3 made from both the wild type (W6) and the mutant (recB21) responds to added DNA (data not shown). The preference of native over heated DNA is shown by both (Table 1), although, under these conditions, the ATP stimulation in the presence of added native DNA is seen only in W6. These data suggest that the *recBC* enzyme is involved in the ATP-stimulated DNA synthesis observed in this system. Conditions under which *recBC* mutant systems can be stimulated by ATP are discussed below.

Conditions for Maximal ATP Dependence of DNA Synthesis. Based on the data of Fig. 1, a modified medium containing 70 mM KCl and 10% (v/v) glycerol was used to maximize the ATP-dependent DNA synthesis. Optimal concentration was 8–12 mM for Mg^{2+} and 1–7 mM for ATP in this medium (data not shown). The soluble fraction (S_3, the supernate from P_3) was not stimulated by ATP in the high salt (150 mM KCl) medium but was fully stimulated by ATP (+100%) in the low salt plus glycerol medium. Mixing experiments at the higher salt concentrations (data not shown) reveal the presence in S_3 of soluble components which raise the non-ATP-stimulated levels of synthesis, thereby decreasing or abolishing the ATP stimulation. Therefore, under the modified conditions, the polymerase activities of both particulate and soluble fractions are ATP-responsive.

Response of DNA Polymerase I to ATP in the Presence of P_3. In order to see if free DNA polymerase I, in the presence of P_3, showed an ATP stimulation, DNA polymerase I (2.2 units/ml), having a specific activity of 4840 units/mg (19) [corresponding to step VII in the isolation of Jovin *et al.* (20)], was added to P_3 (0.195 mg of protein per ml) both at 70 mM and 150 mM KCl. The increment of polymerizing activity found, in both cases, corresponded to the calculated activity for the amount of polymerase added and represented about double the level of endogenous polymerizing activity (data not shown). At 70 mM KCl, the additional activity

TABLE 2. *Purification of the complex*

	Protein, mg	Total activity, nmol/min		Specific activity, nmol/(min × mg)		Yield, %		Degree of purification	% ATP stimulation
		−ATP	+ATP[e]	−ATP	+ATP	−ATP	+ATP	+ATP	
1. Lysed spheroplasts[a]	18,000	—[b]	2,360	—[b]	0.131	—[b]	(100)	(1)	—[b]
2. 20,000 × *g* Supernatant fraction	7,920	1,720	1,980	0.218	0.251	(100)	84	1.92	+15
3. Bio-Gel A-50m[c]	880	1,530	1,910	1.74	2.26	89	81	17.3	+25
4. Bio-Gel A-1.5m[c,f]	(<0.2)[d]	672	1,240	(>3,360)	(<6,200)	39	53	(>47,400)	+85

DNA polymerase activity was assayed as described in *Materials and Methods* with 70 mM KCl, 100 μg/ml of bovine serum albumin, 10% (v/v) glycerol, *E. coli* DNA present at 100 μg/ml, and all four dNTPs at 32 μM each.

[a] The starting material was 170 g (wet weight) of *E. coli* W6 spheroplasts.

[b] The enzymatic activity of the lysed spheroplasts in the absence of ATP is not dependable since incorporation ceases within 60 min.

[c] In each of these cases, data were obtained on pooled fractions.

[d] Although the fractions containing active material were concentrated from about 100 to 6.5 ml, the protein concentration was too low to be measured. When the limit of detection is estimated to be about 25 μg/ml, less than 6.5 × 0.025 mg = 0.16 mg was present.

[e] ATP, when present, was at 1 mM.

[f] The void fraction obtained from Bio-Gel A-50m was treated with 150 μg/ml of DNase I for 1.5 hr at 30°, pH 5.7, before being applied to Bio-Gel A1.5m.

was the same in the presence and absence of 1 mM ATP. At 150 mM KCl, there was no apparent stimulation by 1 mM ATP, but instead, an inhibition. If it is assumed that no contaminating factors in the added enzyme fundamentally altered the properties of P_3, it appears that the ATP stimulation seen in P_3 alone is not due to free DNA polymerase I.

Isolation of an Enzyme Complex Containing DNA Polymerase I. The following procedure is based on the fact that in the crude extract the DNA synthesizing activity is strongly associated with DNA. All steps were performed at 4° and the standard buffer contained 15 mM KCl, 0.02% NaN_3, 10^{-4} M dithiothreitol, 10% (v/v) glycerol, and 10 mM Tris·HCl at a final pH 7.2, unless otherwise noted. A summary of a typical preparation is given in Table 2 and described below.

Lysate: An unwashed centrifugal pellet of *E. coli* W6 spheroplasts was passed through two layers of cheese cloth and resuspended to 10× volume (1700 ml) in standard buffer containing 10 mM $MgCl_2$. The suspension was vigorously stirred with a magnetic stirrer for 15 min with the vortex formed reaching the bottom of the beaker. The suspension was then centrifuged for 15 min at 20,000 × *g* (average).

Bio-Gel A-50m: The 20,000 × *g* supernatant fraction was concentrated by ultrafiltration (Amicon HIDX50 hollow fiber) to approximately the 1X volume (170 ml). The concentrate was layered onto a Bio-Gel A-50m column (50–100 mesh, 5 × 74 cm, and reservoir height = 50 cm) equilibrated with standard buffer. The column was eluted at 60 ml/hr with standard buffer. The 290 ml eluted after the first sign of turbidity was collected and concentrated by ultrafiltration (Amicon HIDX 50 hollow fiber) to 60 ml. This "void volume" fraction contained 97% of the applied activity [Table 2, Yield (+ATP)].

DNase treatment: To the concentrate was added dithiothreitol to a final concentration of 10^{-4} M, $MgSO_4$ (7.5 mM), sodium acetate (0.02 M, pH 5.7), and DNase I (150 μg/ml). The reaction mixture was incubated 1.5 hr at 30°, cooled over ice, and centrifuged at 109,000 × *g* (average) for 1.5 hr.

Bio-Gel A-1.5m: The supernatant fraction from above was concentrated by ultrafiltration (Amicon XM50) to 10 ml. After the addition of Blue Dextran-2000 and [*4,5*-^{3}H]leucine (5 μCi), the concentrate was layered onto a Bio-Gel A-1.5m column (100–200 mesh, 5 × 85.5 cm, and reservoir height = 60 cm) equilibrated with standard buffer. The column was eluted at 45 ml/hr with standard buffer, and 9 ml fractions were collected. A single peak of DNA-synthesizing activity was eluted (Fig. 2A). This peak contained 65% of the applied activity [Table 2 rows 3 and 4 (Yield, +ATP)] and 100% of the eluted activity (Fig. 2A). Fig. 2B shows that the polymerase activity isolated from the bacterial extract (peak at elution volume 965 ml) is easily separable from that of added DNA polymerase I (peak at elution volume 1240 ml). Fractions containing 80% of the eluted activity (elution volume 800–1000 ml) were pooled and concentrated by ultrafiltration (Amicon XM50) to 6.5 ml. Fig. 2C is placed here for comparison to 2A and 2B. It will be discussed below.

An aliquot (3.5 ml) of the concentrate from the Bio-Gel A-1.5m column (after the addition of 50 μl of 0.1% bromophenol blue) was subjected to preparative polyacrylamide gel electrophoresis. DNA polymerase activity was eluted as a single peak containing 80% of the applied activity (not shown). The average stimulation by 1 mM ATP of the DNA-synthesizing activity was 107%.

Properties of the Isolated Complex. The response of the isolated complex to KCl, Mg^{2+}, glycerol, and ATP was essentially the same as that seen in the crude system (data not shown). The complex required the addition of DNA, Mg^{2+}, and all four deoxynucleoside-5′-triphosphates (Table 3). In the absence of added ATP, native DNA was more effective for stimulating DNA synthesis than was heat-denatured DNA. Stimulation of synthesis by ATP was 17% in the presence of heated DNA and 83% in the presence of native DNA (Table 3).

Molecular Weight of the Complex. An aliquot of the concentrated eluate from the Bio-Gel A-1.5 m column was mixed with a solution of protein standards containing Blue Dextran 2000 as a void volume marker and [*4,5*-^{3}H]leucine as a salt volume marker. The mixture was then reapplied to the Bio-Gel A-1.5m column. The DNA synthesizing activity of the complex was eluted in a peak corresponding to a molecular

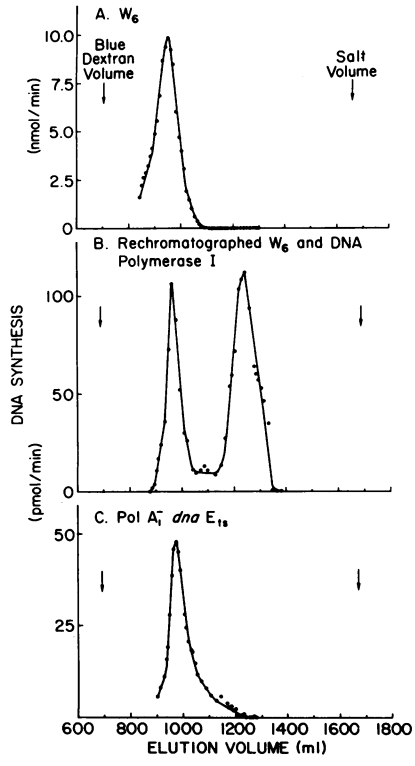

FIG. 2. Bio-Gel A-1.5m chromatography of DNA-synthesizing complex from wild type (A and B) and mutant (C). A portion of the material in the peak eluted from *E. coli* W6 (part A) was mixed with commercially purified DNA polymerase I and rechromatographed (part B). The void volume was determined with Blue Dextran 2000 and the salt volume with L-[^{3}H]leucine. Polymerase activity is expressed per ml and was assayed as in Table 2 in the absence of ATP. The activity shown in Fig. 1A was derived from 170 g wet weight of spheroplasts and that in Fig. 1C from 19.5 g. Note difference in ordinate units from A as opposed to B and C.

weight of 390,000. The enzymatic activity of the complex exhibited an 81–104% ATP stimulation throughout the peak (data not shown).

Properties of Complexes Isolated from a recBC Mutant and Its Parent Strain. Both the single mutant *recB21* and the double mutant *recB21C22* are missing the exo- and endonuclease activities of the *recBC* enzyme (13–16). Complex was isolated from *recB21C22* and from its *rec*$^+$ parent. Using ATP stimulation of DNA synthesis as a measure of complex

TABLE 3. *DNA synthesis with the isolated complex*

	No ATP (%)	+ATP (%)
Heated DNA	100	117
Native DNA	163	298
Native DNA − dGTP	15	24
Native DNA − (dATP, dGTP, and dCTP)	4	24
Native DNA − Mg^{+2}	—	2
−DNA	0.7	(−)0.5

DNA synthesis, assayed in triplicate as in Table 2, is expressed as a percent of the activity with heated DNA minus ATP (205 ± 5 pmol/min per ml). The complex derived from 70 mg of spheroplasts was used per ml of incubation medium.

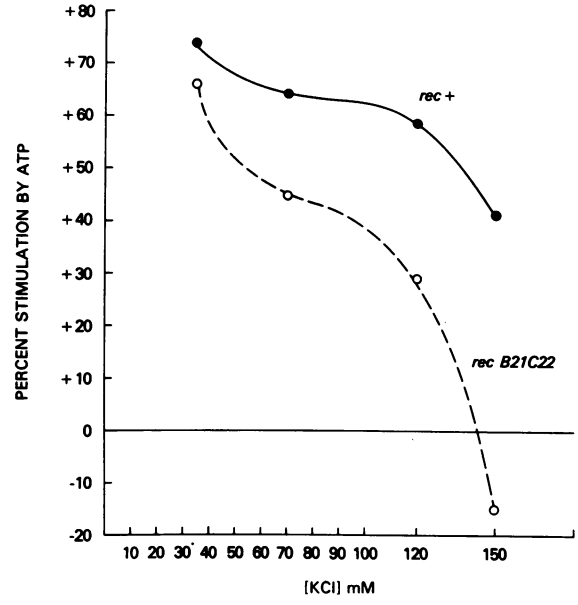

FIG. 3. Complexes isolated from *rec*$^+$ and *recB21C22* cells were assayed for nucleotide incorporation plus or minus 1 mM ATP, using the conditions described for Fig. 1A. Each point represents six incubations, three with and three without ATP. The complexes present in the incubation volume (0.2 ml) were derived from 5.8 mg wet weight of *rec*$^+$ spheroplasts and 8.9 mg wet weight of *recB21C22* spheroplasts. At 70 M KCl, the rate of nucleotide incorporation in the "—ATP" incubation was 121 pmol/min per ml for *rec*$^+$ and 67.2 pmol/min per ml for *recB21C22*.

integrity, we found that the mutant complex was much more sensitive to KCl concentration than was the parent complex (Fig. 3). At 35 mM KCl, the ATP-stimulation of the mutant complex approached that of the wild type. When isolated complex from the *rec*$^+$ strain W6 was exposed to 0.28 M KCl, it was found that the loss of ATP stimulation was accompanied by breakdown of the complex and release of DNA polymerase I (data not shown). Using P_3 preparations from *recB21*, *recB21C22*, and the *rec*$^+$ parent, we found that both of the mutant preparations had a low salt tolerance compared to that of the *rec*$^+$ preparation (data not shown).

Evidence for Complexes of DNA Polymerase II and III. When the DNA polymerase I-deficient and DNA polymerase III thermosensitive (*polA*$_1$-,*dnaE*$_{ts}$) mutant was used as a potential source of complex, all of the DNA synthesizing activity was eluted from Bio-Gel A-1.5m in the same elution volume as the wild-type complex (compare Fig. 2C and A). The amount of activity (at 25°) was 5% or less of that isolated from an equal weight of W6 spheroplasts (not shown). This suggests that complexes involving DNA polymerases II and III are present. ATP stimulation was seen at 25° at three KCl concentrations (i.e., 55% at 200 mM, 22% at 50 mM, and 7% at 2.5 mM). No ATP stimulation was seen at 45°. These results indicate that a complex involving polymerase III is able to be stimulated by ATP.

DISCUSSION

We describe an *in vitro* system for DNA synthesis that utilizes a complexed form of DNA polymerase I. The system is exceedingly stable, prefers native to heat-denatured DNA templates, and is stimulated by ATP. These properties, as

well as its preference for KCl concentrations around 70 mM and glycerol concentrations above 10% (v/v), distinguish the activity from that of free DNA polymerase I. The system has been isolated as a complex of about 390,000 daltons. From the observations that a *recBC* mutant lost the ability to respond to ATP (at 150 mM KCl), that complex isolated from a *recBC* mutant is more labile to KCl than the wild-type complex, and that the weight of the complex is almost equal to the combined weights of DNA polymerase I [about 109,000 (21)] and the *recBC* enzyme [about 270,000 (19)], we feel that the complex probably contains both enzymes. DNA polymerase I, alone and in the presence of P_3, is not affected by 1 mM ATP at 70 mM KCl and is markedly inhibited by 1 mM ATP at 150 mM KCl. From the known nucleolytic properties of the *recBC* enzyme it would not be expected that it could activate a DNA duplex for polymerase activity in the manner of pancreatic DNase. In the first place, *recBC* DNase appears incapable of producing internal nicks or gaps in duplex DNA (22). Secondly, since it can simultaneously degrade, from a given end, both strands of a DNA molecule and because the *recBC* DNase does not appear to dissociate from the DNA strand until digestion is completed (22, 23), attachment of DNA polymerase I to a potential primer strand would be prevented.

The *recBC* enzyme has four known functions. It is an ATP-dependent double- and single-strand exonuclease, an ATP-stimulated single-strand endonuclease, and a DNA-dependent ATPase (13–18, 22, 23). The *recB21* and *recB21C22* mutants are missing the endonuclease and exonuclease functions, but no information on the ATPase function has been published. Mutants in the *recBC* ATPase function have not been described. Our data suggest that it is the ATPase function, and not the nuclease activities, that is important for the functioning of the complex. This is based on the finding that an ATP-stimulatable, 390,000-dalton complex can be isolated from *recB21C22* cells, but its stability to KCl concentrations >70 mM is much less than that of the wild type. Linn and coworkers have suggested that the DNA-stimulated ATPase function is involved in unwinding the duplex DNA and allowing the enzyme to track along the DNA strands (23).

The high background of polymerase activity seen in the absence of ATP may be due to two factors. We have found that dATP (but not dGTP, dCTP, or dTTP) can effectively substitute for rATP so that, even in the absence of added rATP, the system is not without an effective ATP. Secondly, the crude system contains nucleases (other than *recBC*) which, in the absence of ATP, can prepare DNA for polymerase activity. Our preparation of complex may still contain some other nuclease activity.

Preliminary evidence for the existence of complexes containing polymerase II and polymerase III was obtained with a $polA_1^-$,$dnaE_{ts}$ mutant. Although the activity isolated from this mutant was less than 5% of that obtained from polA$^+$ strains, all of the activity was found in a fraction corresponding to a molecular weight of about 390,000. The ATP stimulation of activity was heat-sensitive, indicating that the complex involving polymerase III is stimulated by ATP while that involving polymerase II is not.

Wickner *et al.* (25, 26) have recently described complex forms of polymerase III: Pol III*, a dimer of two molecules of polymerase III (26), and holoenzyme polymerase III, a tetramer of 330,000 daltons consisting of two subunits of polymerase III and two subunits of copolymerase III. The relationship of Pol III* and the holoenzyme, if any, to the complex we have found is unknown.

In recent work, we have been successful in reversibly dissociating the complex from wild-type cells. The formation of complexes from purified polymerases and the *recBC* enzyme will be studied. The construction of a complex from its highly purified constituents will provide a rigorous proof of its composition.

M.P. was a fellow of the Damon Runyan Cancer Society during the course of the investigation described here.

1. Gefter, M. L., Hirota, Y., Kornberg, T., Wechsler, J. A. & Barnoux, C. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3150–3153.
2. Moses, R. E., Campbell, J. L., Fleischman, R. A., Frankel, G. D., Mulcahy, H. L., Shizuya, H. & Richardson, C. C. (1972) *Fed. Proc.* **5**, 1415–1421.
3. Howard-Flanders, P. (1973) *Brit. Med. Bull.* **29**, 226–236.
4. Tait, R. C., Harris, A. L. & Smith, D. W. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 675–679.
5. Scharff, R., Hendler, R. W., Nanninga, N. & Burgess, A. H. (1972) *J. Cell Biol.* **53**, 1–23.
6. Hendler, R. W., Burgess, A. H. & Scharff, R. (1969) *J. Cell Biol.* **42**, 715–732.
7. Momparler, R. L. (1969) *Biochem. Biophys. Res. Commun.* **34**, 465–471.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
9. Jovin, T. M., Dante, M. L. & Chrambach, A. (1970) in *Multiphasic Buffer Systems Output* (National Technical Information Service, Springfield, Va. 22151), PB196085–196091.
10. Klenow, H. & Henningsen, I. (1969) *Eur. J. Biochem.* **9**, 133–141.
11. Rama Reddy, G. V., Goulian, M. & Hendler, S. S. (1971) *Nature New Biol.* **234**, 286–288.
12. Kornberg, T. & Gefter, M. L. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1348–1355.
13. Oishi, M. (1969) *Proc. Nat. Acad. Sci. USA* **64**, 1292–1299.
14. Goldmark, P. J. & Linn, S. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 434–441.
15. Wright, M., Buttin, G. & Hurwitz, J. (1971) *J. Biol. Chem.* **246**, 6543–6555.
16. Barbour, S. D. & Clark, A. J. (1970) *Proc. Nat. Acad. Sci. USA* **65**, 955–961.
17. Nobrega, F. G., Rola, F. H., Pasetto-Nobrega, M. & Oishi, M. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 15–19.
18. Goldmark, P. J. & Linn, S. (1972) *J. Biol. Chem.* **247**, 1849–1860.
19. Richardson, C. C., Schildkraut, C. L., Aposhian, H. V. & Kornberg, A. (1964) *J. Biol. Chem.* **239**, 222–232.
20. Jovin, T. M., Englund, P. T. & Bertsch, L. L. (1969) *J. Biol. Chem.* **244**, 2996–3008.
21. Kornberg, A. (1969) *Science* **163**, 1410–1418.
22. Karu, A. E., MacKay, V., Goldmark, P. J. & Linn, S. (1973) *J. Biol. Chem.* **248**, 4874–4884.
23. MacKay, V. & Linn, S. (1974) *J. Biol. Chem.* **249**, 4286–4294.
24. Kornberg, A. (1974) in *DNA Synthesis* (W. H. Freeman and Co., San Francisco), p. 129.
25. Wickner, W., Schekman, R., Geider, K. & Kornberg, A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1764–1767.
26. Wickner, W. & Kornberg, A. (1974) *J. Biol. Chem.* **249**, 6244–6249.