Proc. Natl. Acad. Sci. USA
Vol. 73, No. 10, pp. 3511–3515, October 1976
Biochemistry

Mechanism of DNA elongation catalyzed by *Escherichia coli* DNA polymerase III, *dnaZ* protein, and DNA elongation factors I and III

(DNA replication/ATP or dATP cofactor)

SUE WICKNER

Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

Communicated by Jerard Hurwitz, August 2, 1976

ABSTRACT Elongation of a primed single-stranded DNA template catalyzed by *E. coli* DNA polymerase III (DNA nucleotidyltransferase, deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) requires *dnaZ* protein and two other protein factors, DNA elongation factors I and III. The reaction occurs by the following mechanism: (*i*) *dnaZ* protein and DNA elongation factor III together catalyze the transfer of DNA elongation factor I to a primed DNA template. This transfer reaction requires ATP or dATP in addition to *dnaZ* protein, DNA elongation factors I and III, and primed template; it does not require DNA polymerase III. (*ii*) DNA polymerase III binds to the complex of DNA elongation factor I with primed template; it does not bind to primed template which is not complexed with DNA elongation factor I. This binding reaction proceeds in the absence of ATP or dATP as cofactor, *dnaZ* protein, and DNA elongation factor III and without additional DNA elongation factor I. (*iii*) The complex of DNA polymerase III, DNA elongation factor I, and primed template catalyzes DNA synthesis upon the addition of dNTPs.

S. Wickner and Hurwitz have shown that *in vitro* DNA elongation of primed single-stranded DNA templates requires three *Escherichia coli* proteins in addition to DNA polymerase III (DNA nucleotidyltransferase, deoxynucleosidetriphosphate: DNA deoxynucleotidyltransferase, EC 2.7.7.7): *dnaZ* protein, DNA EF I (elongation factor), and DNA EF III (1). The *dnaZ* protein is involved in *E. coli* chromosome elongation (2) and is required for growth of ϕX174 and fd bacteriophages (3, 4). Consistent with *in vivo* results, it is required for *in vitro* conversion of ϕX174, fd, and ST-1 single-stranded phage DNAs to duplex DNA (1) and for DNA synthesis dependent on ϕX174 RFI DNA (Sumida-Yasumoto and Hurwitz, personal communication). DNA polymerase III, the *dnaE* protein (5) is also essential for DNA replication *in vivo*. Since DNA EF I and DNA EF III are as yet genetically undefined, their importance in DNA replication can only be inferred from their involvement in *in vitro* replication systems*. Different combinations of six to twelve purified proteins catalyze ϕX174, fd, and ST-1 DNA-dependent DNA synthesis (6–8). The involvement of *dnaZ* protein, DNA EF I, DNA EF III, and DNA polymerase III in all three of these DNA synthesizing systems suggests that the three systems share a common mechanism of DNA elongation.

In this report, the mechanism of action of the DNA elongation components is examined.

MATERIALS AND METHODS

Materials, Reagents, and Methods. Unless otherwise indicated these were as previously described (1, 9).

Abbreviation: EF I, elongation factor.

* DNA EF II was defined as a protein required with DNA EF I and DNA polymerase II or III for DNA elongation (8). DNA EF II preparations were found to consist of *dnaZ* protein plus a protein referred to as DNA EF III (1).

Preparation of Proteins. DNA polymerase III and DNA binding protein were purified from *E. coli* strain NY73 (5) and *dnaZ* protein from strain NY73 or E4860 (5) by procedures modified from those in refs. 10, 11, and 1, respectively. Assay conditions and definitions of units for DNA polymerase III are in ref. 12; those for the other proteins are given in the above references. DNA EF I and DNA EF III were purified from strain NY73 by procedures to be published elsewhere using the DNA elongation assay described below. Based on the native molecular weights of DNA EF I (80,000), DNA EF III (63,000), and *dnaZ* protein (125,000) and calculating purity from native gel electrophoresis (13) 0.06 unit of DNA EF I (about 80% pure), 0.05 unit of DNA EF III (about 50% pure), and 0.03 unit of *dnaZ* protein (about 50% pure) equal 1 pmol of each, respectively.

Assays for DNA Elongation Factors I and III. Reaction mixtures (30 μl) contained 20 mM Tris·HCl (pH 7.5), 7 mM $MgCl_2$, 2 mM dithiothreitol, 50 μg/ml of bovine serum albumin, 0.1 mM ATP, 50 μM each of dATP, dCTP, dGTP, and [^{3}H]dTTP (500–1000 cpm/pmol), 2 nmol of ϕX174 DNA primed with RNA [prepared using *E. coli* RNA polymerase (8)], DNA polymerase III (0.3 unit, 0.7 μg), and *dnaZ* protein (0.3 unit, 30 ng). The assay for DNA EF I contained DNA EF III (0.3 unit, 80 ng); that for DNA EF III contained DNA EF I (0.3 unit, 0.3 μg). After 20 min at 30°, acid insoluble radioactivity was measured. One unit of DNA EF I or DNA EF III activity stimulated incorporation of 1 nmol of dTMP under the above conditions. The reactions were linear with increasing amounts of protein over a 20- to 50-fold range with 0.2–1 pmol of dTMP incorporated in the absence of added DNA EF I or DNA EF III.

Assay for ATP Hydrolysis. Reaction mixtures (30 μl) contained 20 mM Tris·HCl (pH 7.5), 1 mM $MgCl_2$, 2 mM dithiothreitol, 50 μg/ml of bovine serum albumin, 0.1 mM [γ-^{32}P]ATP, 2 nmol of ϕX174 DNA primed with RNA, and proteins as indicated. After 20 min at 30°, $^{32}P_i$ was measured (14).

RESULTS

Requirements for DNA elongation of primed single-stranded DNA

Elongation of primed single-stranded DNA catalyzed by DNA polymerase III requires *dnaZ* protein, DNA EF I, and DNA EF III; *E. coli* DNA binding protein and spermidine are not required (Table 1, ref. 1). ATP as well as dTTP is required when the template is poly(dA)·oligo(dT). Since the ATP requirement is satisfied by dATP, the cofactor requirement cannot be detected with templates that require the incorporation of dAMP. The K_m for ATP and dATP is about 10 μM.

Complex formation between DNA elongation factor I and primed DNA template

The *dnaZ* protein and DNA EF III catalyze the transfer of DNA EF I to primed DNA templates in an ATP- or dATP-dependent reaction. This was shown by the following experiment: DNA EF I, DNA EF III, *dnaZ* protein, ATP, and poly(dA)·oligo(dT) were incubated and the products of the reaction subjected to gel filtration (Fig. 1). The excluded volume contained DNA and associated proteins, the partially included volume contained free proteins, and the fully included volume contained ATP. The isolated protein–DNA complex carried out DNA synthesis upon the addition of dTTP and DNA polymerase III; ATP was removed from the protein–DNA complex by filtration and was not required for dTMP incorporation (Fig. 1A).

Column fractions were assayed for various proteins: (*i*) DNA EF I activity (assayed as in *Materials and Methods*) was associated with the primed template (Fig. 1B); it was in excess of DNA synthetic activity by the isolated protein–DNA complex supplemented with DNA polymerase III and dTTP. The possibility existed that DNA EF I was not in the complex, but that the primed template had been altered such that DNA synthesis no longer depended on DNA EF I. To eliminate this possibility, the protein–DNA complex was dissociated with salt, the salt removed, and the material assayed. After this treatment, DNA EF I activity could still be detected even though the material could no longer carry out DNA synthesis upon the addition of DNA polymerase III and dTTP (Fig. 1, legend). In addition to being dissociated by salt, the protein–DNA complex was dissociated partially by the gel filtration procedure; DNA EF I was found in fractions 15 to 30 as well as in fractions containing DNA. (*ii*) In contrast to DNA EF I, *dnaZ* protein and DNA EF III were not detected in the protein–DNA complex when the

Table 1. Requirements for DNA elongation of oligo(dT)·poly(dA)

Additions	dTMP incorporated
	(pmol/20 min)
Complete	37.6
–DNA elongation factor I	<0.2
–*dnaZ* protein	0.3
–DNA elongation factor III	0.3
–DNA polymerase III	<0.2
–oligo(dT)	<0.2
–ATP	<0.2
–ATP + dATP (0.1 mM)	28.0
–ATP + GTP, CTP, UTP, dGTP or dCTP (0.1 mM)	<0.2
–ATP + α,β-methylene ATP or β,γ-methylene ATP (0.1 mM)	<0.2
+DNA binding protein (4 μg)	24.8

Reactions (30 μl) contained 20 mM Tris·HCl (pH 7.5), 7 mM $MgCl_2$, 2 mM dithiothreitol, 50 μg/ml of bovine serum albumin, 0.1 mM ATP, 50 μM [^{3}H]dTTP (500 cpm/pmol), 1 nmol each of poly(dA) and oligo(dT), DNA EF I (0.1 unit), DNA EF III (0.1 unit), *dnaZ* protein (0.3 unit), and DNA polymerase III (0.3 unit). After 20 min at 25°, acid-insoluble radioactivity was measured. dATP was treated with sodium periodate prior to use.

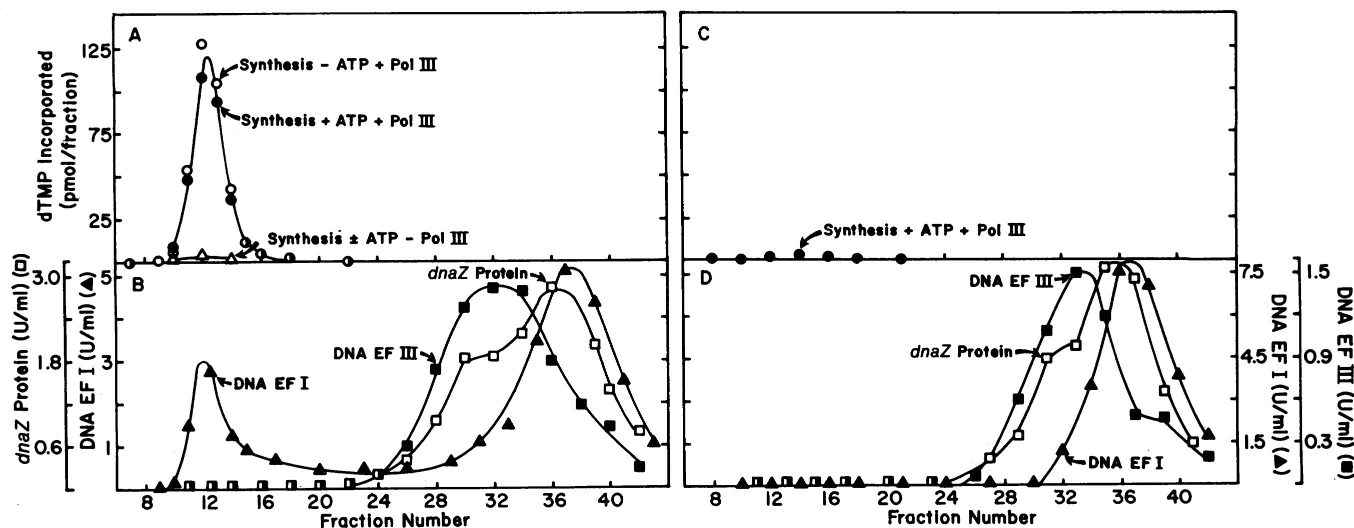

FIG. 1. Isolation of complex of DNA elongation factor I with primed DNA by gel filtration. (A) The first reaction mixture (80 μl) contained 20 mM Tris·HCl (pH 7.5), 50 μg/ml of bovine serum albumin, 7 mM $MgCl_2$, 2 mM dithiothreitol, 0.1 mM ATP, 25 nmol each of poly(dA) and oligo(dT), DNA EF I (2 units), DNA EF III (0.4 unit), and *dnaZ* protein (2.4 units). After 20 min at 25°, the sample was applied at room temperature to a 25 × 0.6 cm column of Bio-Gel A5m equilibrated with 30 mM Tris·HCl (pH 7.5), 2 mM dithiothreitol, 50 μg/ml of bovine serum albumin, 1 mM $MgCl_2$, 50 mM KCl, and 5% glycerol. The column was developed with the same buffer and 0.1 ml fractions were collected. Portions of excluded volume fractions (40 μl) were incubated with 50 μM [^{3}H]dTTP (2000–4000 cpm/pmol) and 0.2 unit of DNA polymerase III (Pol III) in the presence (●) or absence (○) of 0.1 mM ATP, and in the absence of DNA polymerase III ± ATP (△). Incorporation of dTMP was measured after 20 min at 25°. (B) Portions of fractions from the column described in Fig. 1A were assayed for *dnaZ* protein (□), DNA EF I (▲), and DNA EF III (■) as described in *Materials and Methods*. The amount of DNA EF I, DNA EF III, and *dnaZ* protein in the protein–DNA complex region was determined in two ways: First, DNA synthesis by the complex plus DNA polymerase III was subtracted from the amount of DNA synthesis obtained using assays for individual proteins. Second, the complex was dissociated and then assayed for activities: protein–DNA complex (isolated as in Fig. 1A) which supported 350 pmol of dTMP incorporation following addition of DNA polymerase III, was incubated on ice 1 hr with 1 M NaCl and applied to a 0.6 × 20 cm column of Sephadex G-25. The void volume contained 0.96 unit of DNA EF I, less than 0.03 unit of *dnaZ* protein, and less than 0.03 unit of DNA EF III activity; it supported less than 2 pmol of dTMP incorporation upon addition of DNA polymerase III. (C) The first reaction mixture was as described in Fig. 1A but minus ATP. The sample was subjected to column chromatography and DNA synthesis after addition of DNA polymerase III, 0.1 mM ATP, and dTTP was measured as described in Fig. 1A. (D) Portions of fractions from the column described in Fig. 1C were assayed for proteins. U refers to units.

Table 2. Requirements for DNA elongation factor I–DNA complex formation

First reaction components				Isolated protein-DNA complex			
Template and ATP	Proteins: DNA EF I	: DNA EF III	: *dnaZ* protein	DNA synthesis with DNA polymerase III	DNA EF I	DNA EF III	*dnaZ* Protein
				(pmol dTMP)	(U)	(U)	(U)
Complete	(1	1	1)	125	0.22	<0.01	<0.01
-ATP	1	1	1	<5	<0.01	<0.01	<0.01
-primer	1	1	1	<5	<0.01	<0.01	<0.01
Complete	0.1	1	1	32	0.06	<0.01	<0.01
Complete	—	1	1	<5	<0.01	<0.01	<0.01
Complete	1	0.2	1	22	0.02	<0.01	<0.01
Complete	1	—	1	<5	<0.01	<0.01	<0.01
Complete	1	1	0.1	<5	<0.01	<0.01	<0.01
Complete	1	1	—	<5	<0.01	<0.01	<0.01

First reactions were performed as in Fig. 1A but contained 20 nmol of ϕX174 DNA·RNA hybrid in place of poly(dA)·oligo(dT). Reactions contained 2 units of DNA EF I, 0.4 unit of DNA EF III, and 2.4 units of *dnaZ* protein; this ratio of these components was defined arbitrarily as 1:1:1. Protein–DNA complexes were isolated as in Fig. 1A. Incorporation of dTMP by 40 μl of DNA-protein complex was measured after the addition of DNA polymerase III (0.2 unit) and dNTPs plus any component omitted from the first reaction (0.1 mM ATP, 0.2 unit of DNA EF I, 0.2 unit of DNA EF III, or 0.1 unit of *dnaZ* protein). Incorporation of dTMP has been calculated for the entire volume of each isolated complex. DNA EF I, DNA EF III, and *dnaZ* protein in the complex were measured as described in *Materials and Methods*. The values shown are the total units of each protein in each isolated complex.

complex was assayed before or after dissociation (Fig. 1B). Using conditions in which 12 pmol of DNA EF I were associated with the protein–DNA complex, less than 0.1 pmol of *dnaZ* protein and less than 0.5 pmol of DNA EF III were associated with the complex (calculated as in *Materials and Methods* from the number of units recovered). The possibility remains that *dnaZ* protein and DNA EF III were in the protein–DNA complex in forms which could not be detected.

The requirements for the transfer of DNA EF I to primed DNA have been examined. In the absence of ATP in the first reaction mixture, there was no formation of a protein–DNA complex capable of carrying out dTMP incorporation on addition of DNA polymerase III, dTTP and ATP (Fig. 1C). Furthermore, neither DNA EF I, *dnaZ* protein, nor DNA EF III were associated with the primed template (Fig. 1D). Other requirements for protein–DNA complex formation included primed template, *dnaZ* protein, DNA EF I, and DNA EF III (Table 2). The primer requirement for DNA EF I-DNA complex formation suggests the possibility that DNA EF I is bound to 3′-OH ends of gaps in double-stranded nucleic acid. The amount of complex formed was nearly proportional to the amount of each protein added. With conditions where complex formation was limited by DNA EF I, about 80% of the input DNA EF I was recovered as a protein–DNA complex. With conditions where DNA EF III or *dnaZ* protein was limiting, all of the DNA EF III and *dnaZ* protein recovered after gel filtration was found in the partially included volume; this represented 50–100% of the *dnaZ* protein and DNA EF III applied to the column.

DNA EF I-DNA complex formation increased with time for 20 min at 25° and did not occur at 0°. DNA synthesis by isolated complex was proportional to the volume of the complex added; with 5, 10, 20, and 30 μl of complex prepared as in Fig. 1A, 4.8, 11.6, 20.8, and 30 pmol of dTMP, respectively, were incorporated after 20 min at 30° in the presence of dTTP and DNA polymerase III (in 40 μl of reactions). DNA synthesis by the isolated complex was rapid; after 2, 5, 10, and 20 min of incubation at 30°, 9.3, 24.4, 26.8, and 30 pmol of dTMP were incorporated, respectively, by 30 μl of complex supplemented with dTTP and DNA polymerase III. The isolated complex decayed on ice with a half-life of about 6 hr.

Involvement of ATP or dATP in DNA elongation factor I-DNA complex formation

The ATP or dATP-dependent reaction involves the transfer of DNA EF I to a primed template; ATP is not required for DNA synthesis by DNA polymerase III and the DNA EF I-DNA complex (Fig. 1). The ATP-dependent reaction has not been further resolved. An experiment performed in an effort to do this was as follows: *dnaZ* protein, DNA EF I, DNA EF III, oligo(dT), and ATP were incubated [conditions as described in Fig. 1A but minus poly(dA)] and the reaction was filtered through Sephadex G-25 to remove ATP (as described in Fig. 1A for Bio-Gel A5m). Although all three proteins and oligo(dT) were present in the excluded volume, DNA synthesis by that fraction required further addition of ATP as well as DNA polymerase III, dTTP, and poly(dA).

Specific ATP binding to the protein–DNA complex produced by DNA EF I, DNA EF III, and *dnaZ* protein has not been detected. A first reaction mixture as described in Fig. 1A, but with 0.1 mM [γ-^{32}P]ATP (10^4 cpm/pmol) and with 20 nmol of ϕX174 DNA·RNA hybrid in place of poly(dA)·oligo(dT), was subjected to gel filtration as in Fig. 1A. The isolated complex contained 3 pmol of DNA EF I (calculated as in *Materials and Methods* from the number of units recovered) and less than 0.15 pmol of ^{32}P; it catalyzed the incorporation of 120 pmol of dTMP upon the addition of DNA polymerase III and dNTPs (using conditions described in Fig. 1A). Similar results were obtained when [α-^{32}P]ATP was used in place of [γ-^{32}P]ATP.

The possibility that ATP hydrolysis accompanies DNA EF I-DNA complex formation has not been eliminated. One unit of DNA EF I, DNA EF III, and *dnaZ* protein hydrolyzed 144, 304, and 34 pmol of ATP in the absence of primed template and 140, 418, and 104 pmol in the presence of primed template, respectively (assayed as in *Materials and Methods*). ATP hydrolysis by mixtures of these proteins in the presence or absence of primed template was the sum of hydrolysis by the individual

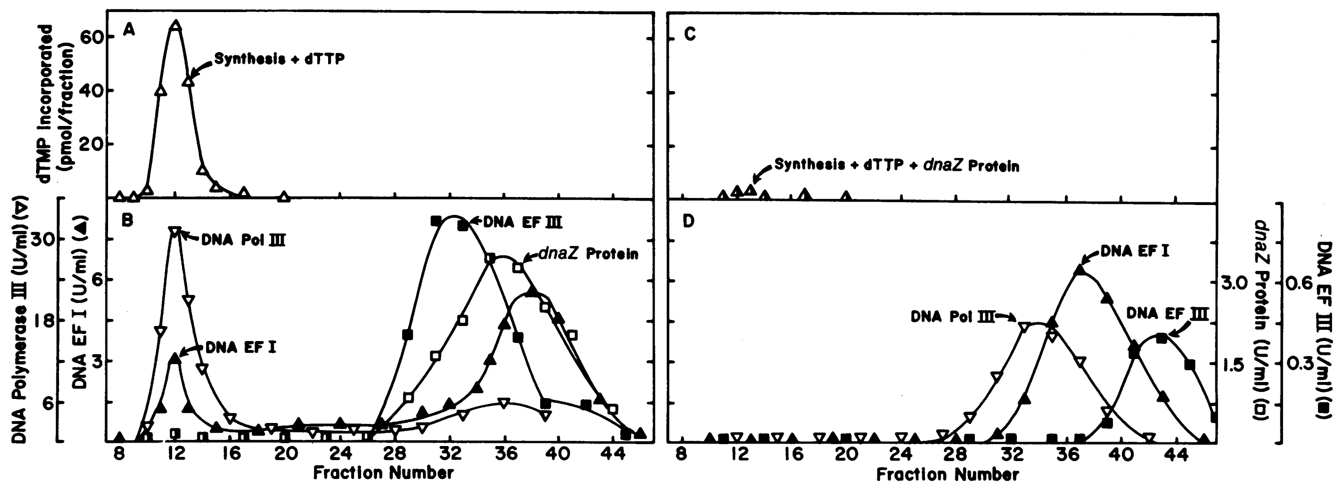

FIG. 2. Binding of DNA polymerase III (Pol III) to complex of DNA elongation factor I with primed DNA template. (A) The first reaction mixture was as in Fig. 1A but also contained 7 units of DNA polymerase III. The sample was subjected to column chromatography as in Fig. 1A. DNA synthesis by the isolated protein-DNA complex was measured by incubating 40 μl of each fraction with 50 μM [^{3}H]dTTP (△) for 20 min at 25°. (B) Portions of fractions described in Fig. 2A were assayed for *dnaZ* protein (□), DNA EF I (▲), DNA EF III (■), and DNA polymerase III (▽) as described in *Materials and Methods.* (C) The first reaction was as in Fig. 2A but *dnaZ* protein was omitted. The sample was chromatographed as in Fig. 1A. DNA synthesis was measured by incubating 40 μl fractions with 50 μM [^{3}H]dTTP and 0.2 unit of *dnaZ* protein (▲) for 20 min at 25°. (D) Fractions from the column described in Fig. 2C were assayed for proteins.

components. In summary, ATP functions in the transfer of DNA EF I to primed DNA but its precise role remains unclear.

Binding of DNA polymerase III to complex of DNA elongation factor I and primed DNA template

DNA polymerase III binds to the complex of DNA EF I and primed template. To show this, DNA polymerase III, DNA EF I, DNA EF III, *dnaZ* protein, ATP, and poly(dA)·oligo(dT) were incubated and subjected to gel filtration. The isolated protein-DNA complex carried out dTMP incorporation following addition of dTTP (Fig. 2A). As in the experiments presented in Fig. 1, ATP had no effect on dTMP incorporation by the complex. Fig. 2B shows that DNA polymerase III as well as DNA EF I was associated with the poly(dA)·oligo(dT); it again shows that *dnaZ* protein and DNA EF III were not associated with the DNA. When *dnaZ* protein was omitted from the first reaction, a protein-DNA complex capable of carrying out dTMP incorporation after addition of dTTP and *dnaZ* protein was not formed (Fig. 2C). Neither DNA polymerase III nor DNA EF I was associated with the DNA (Fig. 2D). These results suggest that, with the conditions described, DNA polymerase III forms a stable complex with a primed DNA template only when the template is complexed with DNA EF I. Furthermore, the binding of DNA polymerase III to the DNA EF I-DNA complex does not displace DNA EF I from the complex. Whether or not DNA EF I is released as DNA polymerase III catalyzes the incorporation of dTMP is not known.

The requirements for DNA polymerase III binding were identical to those for DNA EF I binding: ATP, primed template, DNA EF I, DNA EF III, and *dnaZ* protein. The complex decayed on ice with a half-life of about 6 hr. DNA synthesis by the complex was proportional to the amount of the fraction added; it was complete within 5-10 min of incubation with dTTP.

Fig. 2B and 2D suggest that *dnaZ* protein and DNA EF III form a physical complex. DNA EF III [which has a native molecular weight of about 63,000 (1)] eluted with an apparent molecular weight of 200,000 in the presence of *dnaZ* protein [which has a native molecular weight of about 125,000 (1)]. In the absence of *dnaZ* protein, DNA EF III eluted as expected for a protein of 63,000 (Fig. 2D). Unpublished results have shown: (*i*) protein complex formation requires *dnaZ* protein and DNA EF III; (*ii*) ATP, primed template, DNA polymerase III, and DNA EF I are not required; and (*iii*) the interaction depends on the ratio of *dnaZ* protein and DNA EF III. The existence of the *dnaZ*-DNA EF III protein complex in reaction mixtures which catalyze DNA EF I-DNA complex formation,

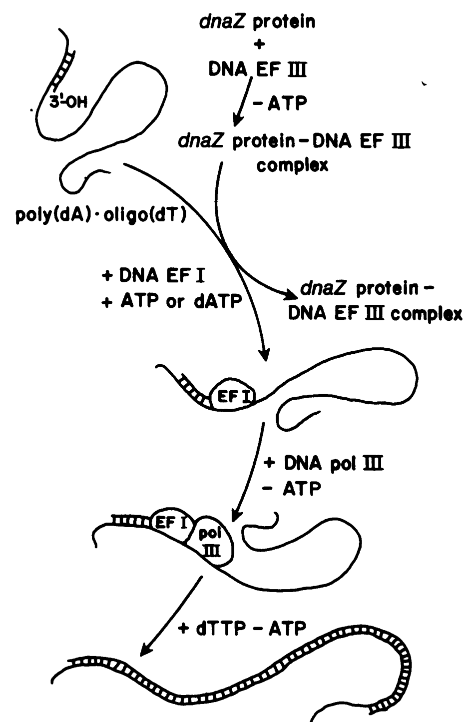

FIG. 3. Mechanism of DNA elongation of a primed DNA template. Pol III refers to DNA polymerase III.

suggests it may have a functional role in the transfer reaction.

DISCUSSION

The results presented above are summarized in Fig. 3 and suggest the following mechanism of DNA elongation of a primed DNA template: (*i*) the *dnaZ* protein and DNA EF III form a protein complex in the absence of DNA EF I and ATP; (*ii*) The *dnaZ* protein–DNA EF III complex catalyzes the transfer of DNA EF I to a primed DNA template in a reaction dependent on ATP or dATP; (*iii*) DNA polymerase III binds to the complex of DNA EF I and primed DNA template without further participation of ATP, *dnaZ* protein, or DNA EF III and without additional DNA EF I; and, (*iv*) DNA synthesis occurs upon addition of dNTPs, also without additional ATP as a cofactor.

The claim by others that this DNA elongation reaction could be catalyzed by "DNA polymerase III holoenzyme" containing two (15) or three (16) protein subunits is not supported by the results presented here or by previous data which show that (*i*) the four elongation proteins are separated from each other in active form by methods which would not normally separate protein subunits (1) and (*ii*) *dnaZ* protein, DNA EF I, and DNA EF III can function with other DNA polymerases, including *E. coli* DNA polymerase II (1, 8) and *B. subtilis* DNA polymerase III (17), in addition to *E. coli* DNA polymerase III. Thus, "DNA polymerase III holoenzyme" may be simply a mixture of the four DNA elongation proteins.

The involvement of *E. coli dnaZ* and *dnaE* proteins in the *in vitro* DNA elongation reaction studied here suggests the possibility that this reaction mechanism may share similarities with the *in vivo* mechanism.

I wish to thank J. Hurwitz for his help and also M. Gellert and G. Felsenfeld for their support.

1. Wickner, S. & Hurwitz, J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1053–1057.
2. Filip, C. C., Allen, J. S., Gustafson, R. A., Allen, R. G. & Walker, J. R. (1974) *J. Bacteriol.* **119**, 443–449.
3. Truitt, C. L. & Walker, J. R. (1974) *Biochem. Biophys. Res. Commun.* **61**, 1036–1042.
4. Taketo, A. (1975) *Mol. Gen. Genet.* **139**, 285–291.
5. Gefter, M. L., Hirota, Y., Kornberg, T., Wechsler, J. & Barnoux, C. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 3150–3153.
6. Wickner, S. & Hurwitz, J. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4120–4124.
7. Wickner, S. & Hurwitz, J. (1975) in *DNA Synthesis and Its Regulation*, eds. Goulian, M. M., Hanawalt, P. C. & Fox, C. F. (W. A. Benjamin, Inc., Menlo Park, Calif.), pp. 227–238.
8. Hurwitz, J. & Wickner, S. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 6–10.
9. Wickner, S., Wright, M., Berkower, I. & Hurwitz, J. (1974) in *DNA Replication*, ed. Wickner, R. B. (Marcel Dekker, Inc., New York), pp. 195–215.
10. Livingston, D. M., Hinkle, D. C. & Richardson, C. C. (1975) *J. Biol. Chem.* **250**, 461–469.
11. Sigal, N., Delius, H., Kornberg, T., Gefter, M., & Alberts, B. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3537–3541.
12. Hurwitz, J., Wickner, S. & Wright, M. (1973) *Biochem. Biophys. Res. Commun.* **51**, 257–267.
13. Jovin, T. M., Chrambach, A. & Naughton, M. A. (1964) *Anal. Biochem.* **9**, 351–369.
14. Conway, T. W. & Lipmann, F. (1964) *Proc. Natl. Acad. Sci. USA* **52**, 1462–1469.
15. Wickner, W. & Kornberg, A. (1974) *J. Biol. Chem.* **249**, 6244–6249.
16. McHenry, C. S. (1976) *Fed. Proc.* **35**, abstr. 1840.
17. Low, R., Rashbaum, S. & Cozzarelli, N. (1976) *J. Biol. Chem.* **251**, 1311–1325.