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# The β Subunit of the *Escherichia coli* DNA Polymerase III Holoenzyme Interacts Functionally with the Catalytic Core in the Absence of Other Subunits*

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**We have previously demonstrated that the addition of a stoichiometric excess of the β subunit of *Escherichia coli* DNA polymerase III holoenzyme to DNA polymerase III* or holoenzyme itself can lead to an ATP-independent increase in the processivity of these enzyme forms (Crute, J. J., LaDuca, R. J., Johanson, K. O., McHenry, C. S., and Bambara, R. A. (1983) *J. Biol. Chem.* 258, 11344–11349). Here, we show that the β subunit can interact directly with the catalytic core of the holoenzyme, DNA polymerase III, generating a new form of the enzyme with enhanced catalytic and processive capabilities. The addition of saturating levels of the β subunit to the core DNA polymerase III enzyme results in as much as a 7-fold stimulation of synthetic activity. Two populations of DNA products were generated by the DNA polymerase III•β enzyme complex. Short products resulting from the addition of 5–10 nucleotides/primer fragment were generated by DNA polymerase III in the presence and absence of added β subunit. A second population of much longer products was generated only in β-supplemented DNA polymerase III reactions. The DNA polymerase III-β reaction was inhibited by single-stranded DNA binding protein and was unaffected by ATP, distinguishing it from the holoenzyme-catalyzed reaction. Complex formation of the DNA polymerase III core enzyme with β increased the residence time of the enzyme on synthetic DNA templates. Our results demonstrate that the β stimulation of DNA polymerase III can be attributed to a more efficient and highly processive elongation capability of the DNA polymerase III•β complex. They also prove that at least part of β's normal contribution to the DNA polymerase III holoenzyme reaction takes place through interaction with DNA polymerase III core enzyme components to produce the essential complex necessary for efficient elongation *in vivo*.**

The DNA polymerase III holoenzyme[1] is a multisubunit complex of proteins which is responsible for most of the replicative DNA synthesis in *Escherichia coli* (for a review see Kornberg, 1982; McHenry, 1985). The exact contribution of individual holoenzyme protein subunits to the replication process is still unclear. The holoenzyme contains a DNA polymerase III catalytic core[1] composed of the α, ε, and θ subunits and at least four additional protein subunits; γ, δ, τ, and β (McHenry and Kornberg, 1977; McHenry and Crow, 1979; McHenry, 1982). Three stable subassemblies of DNA polymerase III have also been isolated which lack one or more of the protein subunits found in the holoenzyme. In each subassembly of DNA polymerase III holoenzyme, a correlation exists between the absence of individual subunits and the loss of one or more of the properties associated with the holoenzyme. These properties include ATP-dependent formation of stable initiation complexes with DNA (Wickner and Kornberg, 1974; Wickner, 1976; Johanson and McHenry, 1982; Burgers and Kornberg, 1982b), the ability to use single-stranded DNA templates coated with polyamines and DNA binding proteins for highly processive synthesis (Fay *et al.*, 1981, 1982), and the ability of the more complex forms of the enzyme to progress through sites on natural DNA templates which would otherwise cause the enzyme to pause or terminate synthesis (LaDuca *et al.*, 1983).

Recent studies have led to valuable insights concerning the role of individual holoenzyme subunits in the natural DNA replication process. The α subunit, the *dnaE* gene product (Welch and McHenry, 1982), contains the active site for synthesis (Spanos *et al.*, 1981). Mutations in the *dnaQ* (*mutD*) locus, the coding region for the ε subunit, affect the 3′ to 5′ exonuclease activity of DNA polymerase III and the fidelity of DNA synthesis (Echols *et al.*, 1983). The presence of τ causes the core DNA polymerase III to dimerize (McHenry, 1982) perhaps forming an asymmetric structure of distinguishable leading and lagging strand polymerases (Johanson and McHenry, 1984). Finally, the τ, γ-δ, and β subunits of the holoenzyme have been shown to increase the processivity of the core DNA polymerase III from 10 to more than 5000 (Fay *et al.*, 1981, 1982). These results define subunit roles and also indicate that the determination of the mechanism of a

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[1] DNA polymerase III holoenzyme is the term used for the complex of proteins including the core DNA polymerase III and several auxiliary proteins which act as the natural replicative enzyme in *E. coli*. DNA polymerase III is the name used for the catalytic core of holoenzyme and is composed of the α, ε, and θ subunits. DNA polymerase III′ is a complex of DNA polymerase III core and τ. DNA polymerase III* is a complex form of DNA polymerase III which contains τ, γ-δ in addition to α, ε, and θ. DNA polymerase III* requires addition of β to reconstitute holoenzyme activity.

particular subunit must take into account whether or not its associated function resides solely in a specific subunit, or arises from other subunits once complexes are formed.

Earlier studies have demonstrated that β could increase the processivity of more complex enzyme forms in the absence of ATP (Crute *et al.*, 1983). Normally, with the full complement of holoenzyme subunits, ATP leads to a tight, highly processive complex between polymerase and primed DNA (Fay *et al.*, 1981). This complex can replicate an entire 5000 nucleotide G4 molecule in less than 15 s without dissociation and remains firmly bound for greater than 20 min at room temperature (Fay *et al.*, 1981; Johanson and McHenry, 1980, 1982). Thus, if the dynamic affinity of the polymerase during the elongation reaction is the same as the static affinity when bound to a primer or product, the holoenzyme may have a processivity sufficient to replicate the entire leading strand of the *E. coli* chromosome without dissociation. In our previous study of the incomplete reaction in the presence of β (Crute *et al.*, 1983), we learned that although the processivity increases markedly, complexes of sufficient stability to permit isolation are not formed.

In the present study we sought additional information regarding subunit function by examining the functional changes that occur in DNA polymerase III in the presence of β in the absence of other holoenzyme subunits. We present evidence that the β subunit can interact directly with DNA polymerase III generating a functionally unique form of the enzyme. The predominant effect of β on the reactivity of DNA polymerase III is to increase the processive capabilities of the resulting complex. This is the first report of a direct interaction between β and the core DNA polymerase III. This study also provides direct evidence for a role of the β subunit in the elongation process.

## MATERIALS AND METHODS

*Proteins and Enzymes*—*E. coli* DNA polymerase III, DNA polymerase III′, DNA polymerase III*, and DNA polymerase III holoenzyme were prepared by the methods of McHenry and Crow (1979), McHenry (1982), Fay *et al.* (1982), and McHenry and Kornberg (1977), respectively. Homogeneous β subunit was prepared from an overproducing strain in a procedure to be described.[2] *E. coli* single-stranded DNA binding protein (SSB[3]) was prepared by the method of Chase *et al.* (1980).

Assays of DNA polymerase III* and DNA polymerase III holoenzyme were performed as described (Johanson and McHenry, 1980, 1984). One unit of either DNA polymerase III*, when supplied with saturating levels of the β auxiliary subunit, or DNA polymerase III holoenzyme is defined as 1 pmol of (total) deoxynucleoside monophosphate incorporated/min on an M13Gori-1 DNA template with priming by *dnaG* primase *in situ*. One unit of DNA polymerase III or DNA polymerase III′ is the amount of enzyme catalyzing the incorporation of 1 pmol of (total) deoxynucleotide/min on an activated salmon sperm DNA template.

Assays for β activity measure the reconstitution of the holoenzyme from DNA polymerase III* and the β subunit by its function in the conversion of single-stranded G4 DNA to the duplex replicative form in the presence of appropriate substrates (Johanson and McHenry, 1980). One unit is defined as the incorporation of 1 pmol of total deoxynucleotide/min at 30 °C.

*Nucleotides and Polynucleotides*—Unlabeled dNTPs were purchased from ICN K & K Laboratories, Inc. Dideoxynucleoside thymidine triphosphate (ddTTP) was purchased from P-L Biochemicals. [$^3$H]dTTP (78.7 Ci/mmol), [α-$^{32}$P]dATP, [α-$^{32}$P]dTTP, and [γ-$^{32}$P]ATP (all > 3000 Ci/mmol) were purchased from New England Nuclear. Poly(dA)$_{500-700}$ and Poly(dA)$_{3000-4000}$ was purchased from The Midland Certified Reagent Co. Oligo(dT)$_{10}$ and oligo(dT)$_{16}$ were purchased from Pharmacia/P-L Biochemicals. Poly(dA)·oligo(dT)$_{10}$ and poly(dA)·oligo(dT)$_{16}$ complexes (containing adenine and thymine in molar ratios of from 2:1 to 30:1) were prepared by annealing poly(dA)$_{3000-4000}$ and either oligo(dT)$_{10}$ or oligo(dT)$_{16}$ at 37 °C for 5 min in 0.1 mM EDTA and 10 mM Tris-HCl (pH 7.5). All concentrations of DNA are given as total nucleotide.

*Quantitation of DNA Synthesis*—A new method was developed for the efficient quantitative analysis of DNA synthesis on synthetic poly(dA)·oligo(dT) primed templates. Synthetic reactions were terminated by the addition of EDTA to a final concentration of 100 mM at 4 °C. Radiolabeled oligonucleotide samples (5–25 μl) were diluted in 1 ml of 0.3 M ammonium formate (pH 7.8), 10 mM sodium pyrophosphate and kept on ice prior to filtration. Samples were filtered onto DEAE-nitrocellulose discs (2.4-cm diameter and 0.45-μm pore size, Schleicher and Schuell) which were then rinsed with 50–75 ml of cold 0.3 M ammonium formate (pH 7.8), 10 mM sodium pyrophosphate, dried, and counted in 1.0 ml of a toluene-based scintillation fluor (4 g/liter Omnifluor, New England Nuclear). Short, single-stranded oligonucleotides could be recovered in high yields using this method with a minimum of length or nucleotide composition dependence.

*Preparation of Natural DNA Templates*—Calf thymus DNA (Sigma) and salmon sperm DNA (Sigma) was activated with pancreatic DNase I to a level of 2.5% acid solubility using the method of Uyemura and Lehman (1976). Bacteriophage fd RF I DNA was isolated according to Blair *et al.* (1972) and subsequently nicked and gapped as described (Wierowski *et al.*, 1983). Average gap size was 100 nucleotides with approximately 14 3′-OH termini/fd RF molecule as determined by methods previously described (Hockensmith and Bambara, 1981). Single-stranded bacteriophage fd DNA strands were specifically primed with denatured *Sau*3A1-D fragment DNA as previously described (LaDuca *et al.*, 1983). Origin-RNA primed M13Gori-1 DNA (0-M13 Gori) was prepared with *dnaG* primase *in situ* as described (Johanson and McHenry, 1982). Single-stranded M13Gori-1 DNA is an M13 chimera of 8623 nucleotides containing the G4 complementary origin (Kaguni and Ray, 1979).

*Measurement of the Length of Products Synthesized on Poly(dA)·Oligo(dT) Templates*—The length of products made on poly(dA)·oligo(dT) primer-templates were determined for the four subassemblies of DNA polymerase III holoenzyme using a method that permits sizing of the extended oligo(dT)$_{10}$ or oligo(dT)$_{16}$ primers (Fay *et al.*, 1981). Reaction mixtures (50 μl) contained 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 200 μg/ml of bovine serum albumin, 20% glycerol (v/v), 0.1 mM EDTA, and 25 mM dithiothreitol. For determinations using DNA polymerase III, reaction mixtures contained 200 μM poly(dA)·oligo(dT)$_{10\text{ or }16}$ (in varying molar ratios), 50 μM [$^3$H]dTTP (10 Ci/mol), and approximately 23 units of enzyme. Determinations using DNA polymerase III holoenzyme, DNA polymerase III*, and DNA polymerase III′ were performed as above with approximately 50–100 units, respectively, of each polymerase unless otherwise noted. The reaction mixtures were incubated at 30 °C for the times indicated in the figure legends; then EDTA was added to 50 mM and the tubes placed on ice. An aliquot (5 μl) was removed to determine the extent of [$^3$H]dTMP incorporation. Variations in the reaction mixtures are indicated in the figure legends. Generally, less than 1 [$^3$H]dTMP was incorporated/3′ terminus. In cases where incorporation exceeded 1 nucleotide/3′ terminus, the average product size was sufficiently great that less than 5% of the available termini had sustained synthesis. Thus, the extension of each reacted oligo(dT) primer terminus was the result of one binding event of the polymerase, and the length of extension is an indicator of the processivity of the enzyme. The length of the extended oligo(dT) primer was determined by gel filtration under alkaline conditions as previously described (Fay *et al.*, 1981). Quantitative values of oligonucleotide size were obtained for products ranging from 1 to approximately 200 nucleotides. Products of greater length were excluded from the resin, precluding an exact size measurement.

*Analysis of the Length of Products Synthesized on Poly(dA)·Oligo(dT)$_{10}$ (20:1) Templates by High Resolution Gel Electrophoresis*—Product size determinations of DNA polymerase III synthetic reactions on poly(dA)·oligo(dT)$_{10}$ (20:1) templates were also made by polyacrylamide gel electrophoresis. Reaction mixtures were as described above, except that synthesis was conducted in the presence of 50 μM [α-$^{32}$P]dTTP (20 Ci/mmol). Synthetic reactions were terminated after 5 min at 30 °C with the addition of EDTA to 50 mM. Two volumes of 95% formamide containing 10 mM EDTA, and 0.1% xylene cyanol FF were added, and the mixture was boiled for 5 min to separate DNA strands. Aliquots (5 μl) were then subjected to

[2] K. O. Johanson and C. S. McHenry, manuscript in preparation.

[3] The abbreviations used are: SSB, *E. coli* single-stranded DNA binding protein; dNTPs, deoxynucleoside triphosphates; ddNTP, dideoxynucleoside triphosphate; RF, replicative form.

electrophoresis on 8% polyacrylamide gels (40 × 34 × 0.04 cm) containing 7 M urea, 100 mM Tris-borate buffer (pH 8.3), and 2 mM EDTA. Electrophoresis was continued for approximately 2 h at 1500 V at room temperature. Polyacrylamide gels were covered with Saran Wrap, and overlayed with Kodak X-Omat RP film, and exposed at −70 °C for 12 h.

*DNA Sequencing*—DNA sequencing using the *Sau*3A1-D fragment primed fd DNA was performed as described by Sanger *et al.* (1977) with the following alteration. Products generated by DNA polymerase I large fragment (New England Nuclear) were reacted with sufficient *Sau*3A1 nuclease during the dATP chase reaction to allow for complete cleavage of regenerated restriction sites. Electrophoresis of products to serve as size markers was conducted as previously described.

*End Labeling Reactions*—Nucleotide standards were labeled at the 5′ end as described by Maxam and Gilbert (1980). Polynucleotide kinase (Bethesda Research Laboratories) and [γ-$^{32}$P]ATP (3200 Ci/mmol, New England Nuclear) were used to label the 5′ ends of poly(dA)$_{700}$, oligo(dT)$_{10}$, and the *Sau*3A1-A, B, C, and D restriction fragments of fd RF DNA after prior dephosphorylation of the DNA with bacterial alkaline phosphatase (Bethesda Research Laboratories).

## RESULTS

*Stimulation of DNA Polymerase III in the Presence of Added β Subunit*—The addition of stoichiometric excesses of purified β subunit to the DNA polymerase III core enzyme resulted in stimulation of DNA synthetic activity on poly(dA)·oligo(dT) primer-templates with interprimer distances in excess of 20 nucleotides (Fig. 1). Maximal synthetic activities were achieved in the presence of 40,000–60,000 units (8–12 × $10^5$ units/ml; a 5.4–8.2 × $10^3$ molar excess) of β. In the presence of such saturating levels of the β subunit, DNA polymerase III activity was stimulated 7-fold on poly(dA) templates, having oligo(dT)$_{16}$ primers spaced 290 nucleotides apart, whereas little or no stimulation was observed on templates containing primers spaced only 20 nucleotides apart. The increase in synthetic activity is attributable to β subunit-induced alterations in DNA polymerase III activity since the β subunit alone has no synthetic activity.

Synthetic activities of both DNA polymerase III and the enzyme complex formed between the core polymerase and the β subunit (DNA polymerase III-β) were highly sensitive to salt (Fig. 2). Addition of NaCl to 75 mM, bringing the ionic strength of synthetic reactions from 75 to 150 mM, resulted in a 25-fold inhibition of DNA polymerase III and DNA polymerase III-β activity. At all NaCl concentrations, however, greater synthetic activity was found in the β-supplemented DNA polymerase III reactions. Addition of ATP to 500 μM had no stimulatory or salt stabilizing effects on the catalytic activities of DNA polymerase III or DNA polymerase III-β. In contrast, improved salt stability and enzymatic activity in the presence of ATP has been reported for reactions catalyzed by the more complex holoenzyme form of DNA polymerase III (Burgers and Kornberg, 1982b; Crute *et al.*, 1983). These observations distinguish the DNA polymerase III-β activity from that of the holoenzyme. They indicate that β-induced stimulation of DNA polymerase III is not a consequence of reconstitution of the core polymerase to the complete holoenzyme form of DNA polymerase III arising from τ, γ, and δ subunit contamination of the β preparation.

*Sizes of Nascent Products Synthesized Processively by DNA Polymerase III in the Presence or Absence of β Subunit*—Homopolymeric templates were utilized to permit the direct determination of processivity by measurement of the length of the products made from primers of known length after one polymerase binding event. Products made during single interations of DNA polymerase III or DNA polymerase III-β with poly(dA) templates containing primer fragments spaced between 20 and 290 nucleotides apart were analyzed (Fig. 3).

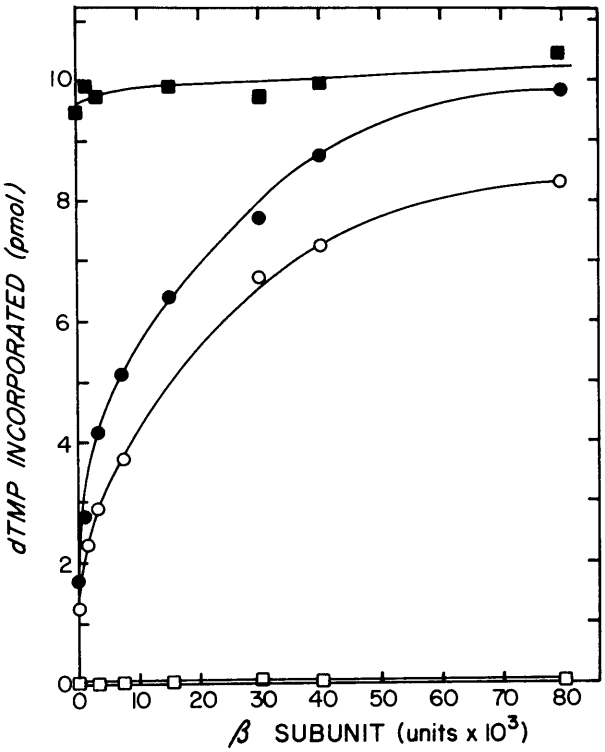


FIG. 1. **β stimulation of DNA polymerase III on homopolymeric templates.** The rate of polymerization by DNA polymerase III was measured on homopolymeric templates in the presence of varying concentrations of the β subunit. Reaction mixtures (50 μl) contained 50 μM poly(dA)·oligo(dT)$_{16}$, 50 μM [$^3$H]dTTP (10 Ci/mmol) and approximately 8 units of DNA polymerase III. Aliquots (20 μl) were removed after a 5-min incubation at 30 °C and radioactive incorporation determined as described under "Materials and Methods." DNA polymerase III activity profiles are shown for reactions catalyzed on poly(dA)·oligo(dT)$_{16}$ in the ratio of 2.25:1 (■), 11.3:1 (●), or 19.1:1 (○). Interprimer distances on reacted templates were 20, 165, and 290 nucleotides, respectively. Reaction rate of the β subunit in the absence of DNA polymerase III (□) was measured on poly(dA)·oligo(dT)$_{16}$ (11.3:1). Addition of 10,000 units of the β subunit is equivalent to a final concentration of 2.0 × $10^5$ units/ml.

In the absence of added β subunit, the majority of products synthesized by DNA polymerase III on each of the primer-template combinations were less than 25 nucleotides in length (approximately 1–10 nucleotides added to the oligo(dT)$_{16}$ primer). DNA polymerase III reactions supplemented with 15,000 units (3.0 × $10^5$ units/ml) of β on poly(dA) templates containing primers spaced 20, 165, or 290 nucleotides apart, resulted in a 1.2-, 2.6-, and 3.0-fold stimulation, respectively, in total synthetic activity compared to reactions catalyzed in the absence of β. The enhanced synthetic activity observed in the presence of β on each of the poly(dA) templates is accompanied by an increase in the length of processive synthesis by the DNA polymerase III-β enzyme complex. The reactions generated two populations of DNA products, a population of short DNA chains characteristic of DNA polymerase III in the absence of β, and a class of longer DNA products (Fig. 3). The longer products are only observed if the interprimer distance is sufficiently large (Fig. 3, *panels B* and *C*). At an interprimer distance of 20 nucleotides, primers in front of the advancing DNA polymerase appear to interfere with elongation (Fig. 3, *panel A*). Increasing the amount of added β subunit present in DNA polymerase III reaction resulted in corresponding changes in the relative number of long products generated (Fig. 3, *panel B*).

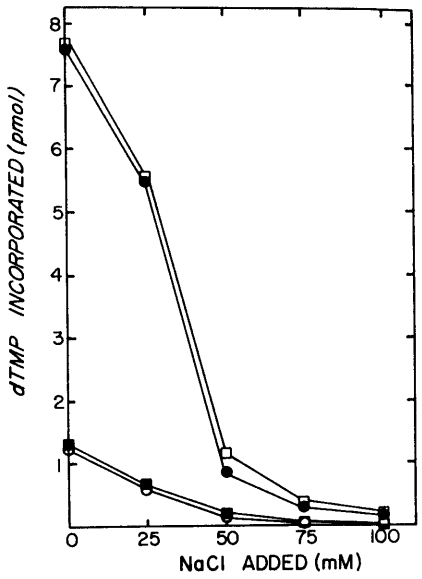


FIG. 2. **Salt sensitivity of DNA polymerase III activity in the presence of excess β and ATP.** A poly(dA)·oligo(dT)$_{16}$ template (50 μM) in the ratio of 11.3:1 (nucleotides) was reacted under conditions outlined under "Materials and Methods" in a total volume of 50 μl. NaCl was added to reaction mixtures at a final concentration of 0, 25, 50, 75, or 100 mM. Reactions without added NaCl had a conductivity equivalent to 75 mM NaCl. Aliquots (20 μl) were removed after a 5-min incubation at 30 °C and radioactive incorporation determined. Activity profiles are shown for DNA polymerase III alone (○), DNA polymerase in the presence of 40,000 units ($8.0 \times 10^5$ units/ml) of β (●), DNA polymerase III plus 500 μM ATP (■), and DNA polymerase III in the presence of both 500 μM ATP and 40,000 units ($8.0 \times 10^5$ units/ml) of β (□).

To identify the discrete products made by DNA polymerase III in the presence of β at higher resolution, we examined the reaction products separated by polyacrylamide gel electrophoresis (Fig. 4). Reactions were catalyzed on poly(dA)·oligo(dT)$_{10}$ (20:1) templates in the presence of [α-$^{32}$P]dTTP. The products generated in each reaction were denatured and separated by polyacrylamide gel electrophoresis. The distribution of products synthesized by DNA polymerase III in the absence of β ranged in size from 1 nucleotide added to the oligo(dT)$_{10}$ primer fragment to approximately 20 (Fig. 4, *lane 2*). Reactions catalyzed in the presence of 1500 units ($6.0 \times 10^4$ units/ml) of the β subunit resulted in the production of a second population of products which were 300–700 nucleotides in length (*lane 4*). When higher levels of the β subunit were added (*lanes 5* and *6*), more long products were generated. Short DNA products, characteristic of DNA polymerase III, were observed in all reactions regardless of the amount of β subunit added. The addition of ATP (500 μM) to the DNA polymerase III reactions catalyzed in the absence or presence of the β subunit had no effect on product profiles (*lanes 3* and *7*, respectively). The very long DNA products generated by DNA polymerase III-β were, as expected, shorter than the poly(dA) template DNA (*lane 10*) but were longer than the average interprimer distance of 190 nucleotides on the poly(dA)·oligo(dT) (20:1) template. The synthesis of products exceeding the length between adjacent primer fragments may result from primer displacement as a result of active involvement of the enzyme-β complex or by passive reaction processes.

*Effect of Added β on Reactions Catalyzed by DNA Polymerase III Holoenzyme and Subassemblies*—The effect of addition of excess β on the catalytic and processive capabilities of DNA polymerase III, DNA polymerase III′, DNA polymerase III*, and DNA polymerase III holoenzyme are compared in Table I. Despite the differences in subunit composition, the synthetic rates of the holoenzyme and each of the three subassemblies of DNA polymerase III were enhanced approximately 3-fold by 15,000 units ($3.0 \times 10^5$ units/ml) of added β

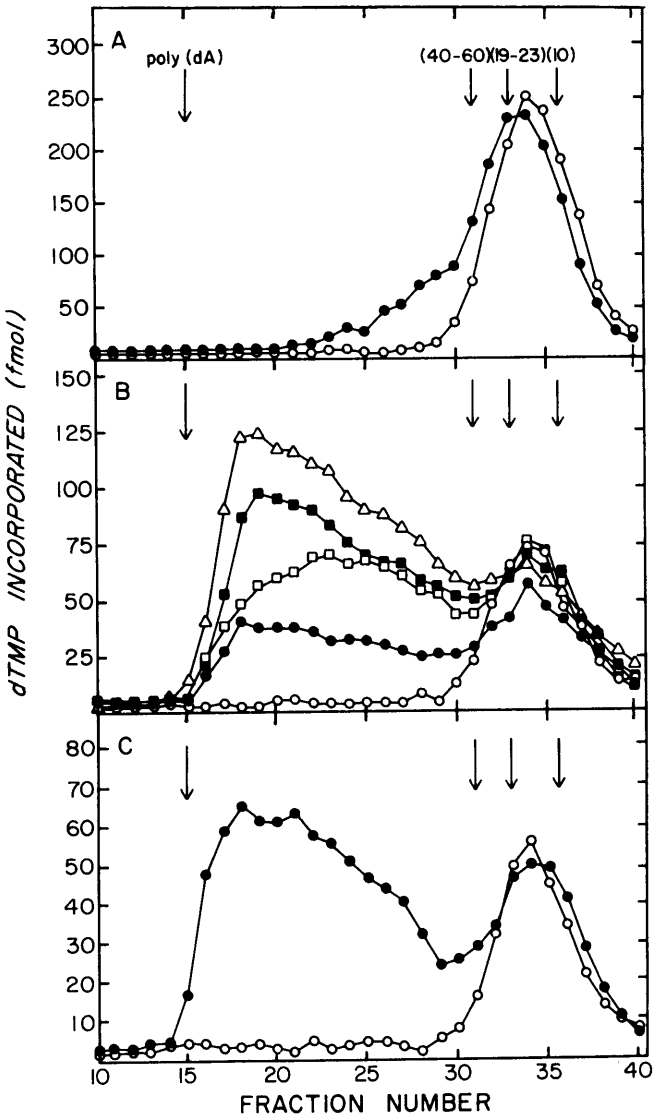


FIG. 3. **Products made by DNA polymerase III in the presence of excess β.** Products generated by DNA polymerase III on homopolymeric templates following a 2-min incubation at 30 °C were analyzed. *A*, primers were extended under the standard reaction conditions described under "Materials and Methods" on poly(dA)·oligo(dT)$_{16}$ (2.25:1) templates having an interprimer distance of approximately 20 nucleotides. Synthesis by DNA polymerase III alone (○) resulted in 0.045 pmol of dTMP incorporated/3′-OH terminus, the addition of 15,000 units ($3.0 \times 10^5$ units/ml) of the β subunit (●) resulted in 0.054 pmol of dTMP incorporated/3′-OH terminus. *B*, primers were extended on poly(dA)·oligo(dT)$_{16}$ (11.3:1) templates having an interprimer distance of approximately 165 nucleotides. Synthesis by DNA polymerase III in the presence of 0 (○); 7,500 (●); 15,000 (□); 20,000 (■) or 40,000 (△) units of the β subunit resulted in 0.052, 0.084, 0.134, 0.156, and 0.190 pmol of dTMP incorporated/3′-OH terminus, respectively. *C*, primers were extended on poly(dA)·oligo(dT)$_{16}$ (19.1:1) templates having an interprimer distance of approximately 290 nucleotides. Synthesis by DNA polymerase III alone (○) resulted in 0.049 pmol of dTMP incorporated/3′-OH terminus, the addition of 15,000 units ($3.0 \times 10^5$ units/ml) of the β subunit (●) resulted in 0.146 pmol of dTMP incorporation/3′-OH terminus. Positions of elution of oligonucleotide standards are shown by the *arrows*.

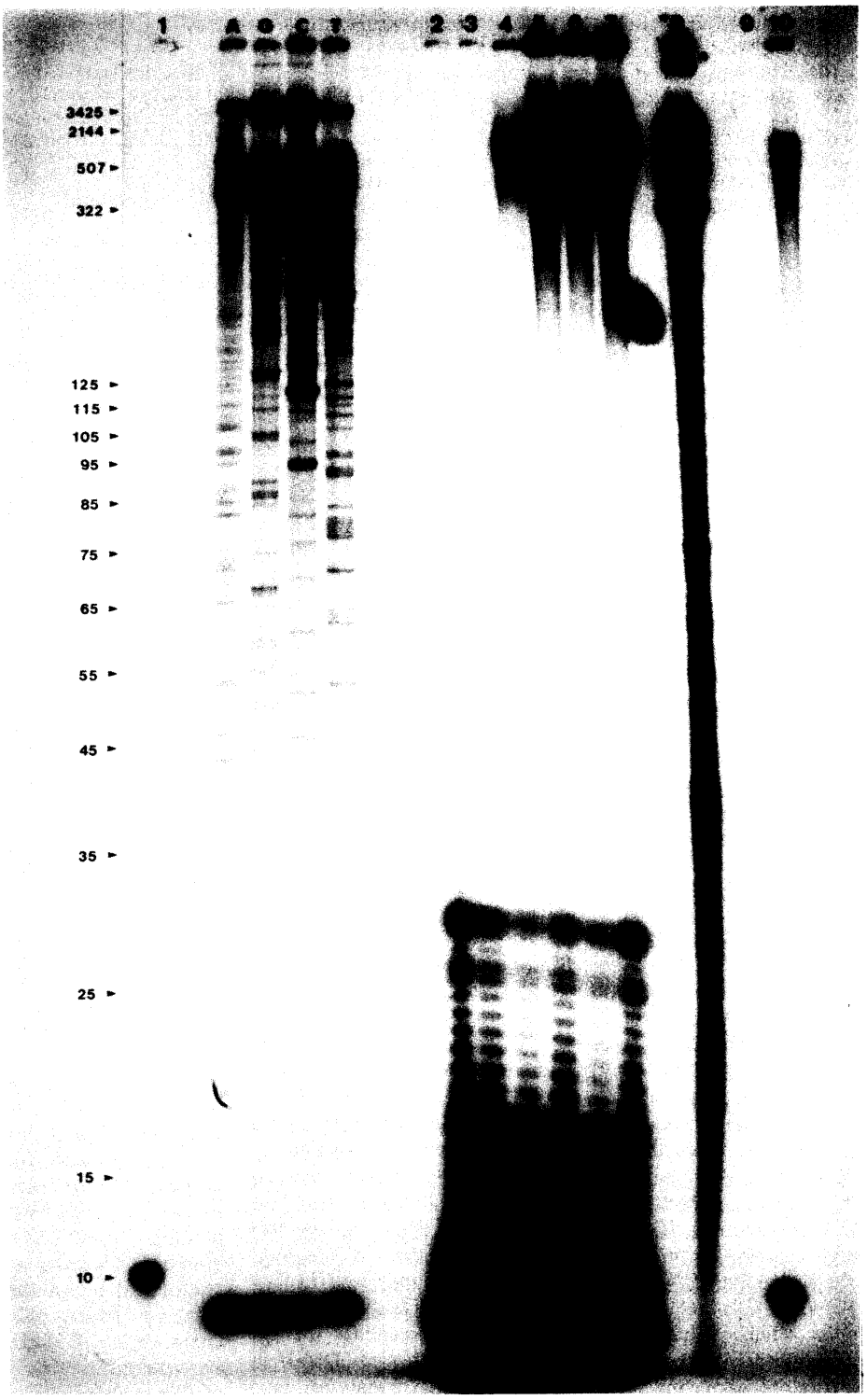


FIG. 4. **Examination of DNA polymerase III-β reaction products using polyacrylamide gels.** DNA polymerase III reactions were carried out as described under "Materials and Methods" on a poly(dA)·oligo(dT)$_{10}$ (20:1) template in a total volume of 25 μl. *Lanes 1, 8, 9,* and *10* contain oligonucleotide standards. *Lanes 1* and *9* contain oligo(dT)$_{10}$; *lane 8* contains *Sau*3A1 digestion products of bacteriophage fd RF DNA (322, 507, 2,144, and 3,425 nucleotides in length); *lane 10* contains poly(dA)$_{500-700}$ used as a template. Dideoxynucleotide chain termination reactions utilizing DNA polymerase I large fragment on bacteriophage fd templates specifically primed with the restriction endonuclease *Sau*3A1-D fragment were used to generate oligonucleotide size markers. Products terminated using dideoxyadenosine 5′-triphosphate, dideoxyguanosine 5′-triphosphate, dideoxycytidine 5′-triphosphate, and dideoxythymidine 5′-triphosphate are shown in *lanes A, G, C,* and *T,* respectively. The position of chain termination products 15–125 nucleotides in length are shown by the *arrows*. DNA polymerase III reaction products are shown in *lanes 2–7*; *lanes 2, 4, 5,* and *6* represent the addition of 0, 1,500, 7,500, and 15,000 units of β, respectively; *lane 3* represents the addition of 0 units of β and 500 μM ATP; *lane 7* represents the addition of 15,000 units of β and 500 μM ATP.

subunit, reflecting the ability of β to interact with each polymerase. Additionally, each enzyme form exhibited an increased capacity to processively synthesize DNA products with length in excess of 50 nucleotides, when reacted in the presence of added β subunit. Between 65–80% of the products generated by each of the polymerase-β mixtures were greater than 50 nucleotides in length. In the absence of added β subunit, only the holoenzyme form of DNA polymerase III (the only assembly which contains β) was capable of processively synthesizing products exceeding 50 nucleotides in length. This finding establishes the important role of β in contributing to the elongation reaction by increasing the processivity of the polymerase.

*DNA Polymerase III-β Reactions on Natural DNA Templates*—To test the ability of the β subunit to increase DNA polymerase III activity on natural DNAs, four templates were constructed which differed in primer composition and primer density. Three of the templates (gapped fd RF; *Sau*3A1-D′fd, and origin-RNA primed M13Gori-1) were similar in that each contained long stretches of single-stranded DNA and few primer fragments. DNA polymerase III activity was increased 2–4-fold on each of these templates when the reactions were catalyzed in the presence of 40,000 units ($1.6 \times 10^6$ units/ml)

TABLE I

*β effect on the reactivity of DNA polymerase III subassemblies*

Reactions were carried out under standard reaction conditions in 50-μl reaction volumes containing 200 μM poly(dA)·oligo(dT)$_{16}$ (11.3:1) at 30 °C. Reactions catalyzed by DNA polymerase III, DNA polymerase III′,DNA polymerase III*, and DNA polymerase III holoenzyme were terminated after 2, 5, 2, and 1 min of reaction respectively. Units of each enzyme form reacted are described under Materials and Methods.

| Enzyme form | pmol dTMP incorporated/ 3′-OH termini | -Fold stimulation | Fraction of products >50 nucleotides long[a] |
|---|---|---|---|
| DNA polymerase III | 0.05 | | 0 |
| DNA polymerase III-β[b] | 0.13 | 2.6 | 0.65 |
| DNA polymerase III′ | 0.14 | | 0 |
| DNA polymerase III′-β[b] | 0.38 | 2.8 | 0.71 |
| DNA polymerase III* | 0.11 | | 0 |
| DNA polymerase III*-β[b] | 0.40 | 3.6 | 0.83 |
| DNA polymerase III holoenzyme | 0.05 | | 0.19 |
| DNA polymerase III holoenzyme-β[b] | 0.17 | 3.5 | 0.81 |

[a] Based on fraction of total radioactivity recovered from the calibrated alkaline-agarose A5M sizing columns.

[b] β subunit was supplemented at a level of 15,000 units ($3.0 \times 10^5$ units/ml).

of the β subunit (Table II). Coating the single-stranded regions of each template with *E. coli* single-stranded DNA binding protein (4:1 ratio of SSB to DNA) to eliminate template secondary structures inhibited DNA polymerase III both in the presence and absence of added β subunit. In similar reactions catalyzed by the holoenzyme form of DNA polymerase III, SSB and β had an additive effect with regard to the level of enzyme stimulation observed (data not shown). The results confirm earlier observations (McHenry, 1982) that the capacity to utilize SSB-coated template strands is associated with one or more of the protein subunits found in the holoenzyme, which are not present in the core DNA polymerase III.

The presence of excess levels of the β subunit had little stimulatory effect on the synthetic activity of DNA polymerase III when the reactions were catalyzed on activated calf thymus DNA templates. The activated calf thymus template is essentially a double-stranded DNA template with many single-stranded nicks and small gaps. Reactions catalyzed on this template reflect the inability of the β subunit to stimulate DNA polymerase III when 3′-OH primer densities are very high and elongation of DNA synthesis is restricted.

*Effect of β on DNA Synthesis by DNA Polymerase III when Extensive Elongation is Prevented by Use of a Chain Terminator*—The preceding results indicate that an excess of the β subunit enables the core polymerase to synthesize DNA by a highly processive mechanism, suggesting that β may facilitate tight coupling of the enzyme to the DNA template. Further effects of the β subunit on the DNA polymerase III reaction were investigated under conditions which were designed to restrict elongation during DNA synthesis. Primer extension on homopolymeric templates was prematurely terminated by addition of a DNA synthesis chain terminator, dideoxynucleoside thymidine triphosphate. A ratio of ddTTP to dTTP was chosen such that DNA chains synthesized by DNA polymerase III in the presence or absence of the β subunit would be of equivalent length, regardless of the intervening space between adjacent primer fragments on template strands. Under these conditions the synthetic activity observed in DNA polymerase III reactions, either supplemented with β or not,

TABLE II

*DNA polymerase III-β reactions on natural DNA templates*

Reaction mixtures (25 μl) contained 50 μM (0.4 μg) DNA, 8 units of DNA polymerase III and 50 μM each of dATP, dGTP, dCTP, and [$^3$H]dTTP. Reactions were incubated at 30 °C for 15 min, terminated with EDTA, and counted as described in "Materials and Methods."

| Template | β Subunits added | SSB[a] | pmol dTMP incorporated | -Fold stimulation[b] | -Fold inhibition[c] |
|---|---|---|---|---|---|
| | *units* | *μg* | | | |
| Sau3A1-D′fd | 0 | 0 | 0.20 | 1 | |
| | 7,500 | 0 | 0.56 | 2.8 | |
| | 41,000 | 0 | 0.68 | 3.4 | |
| | 0 | 1 | 0.05 | | 4 |
| | 0 | 2 | 0.04 | | 5 |
| | 41,000 | 1 | 0.14 | | 4.8 |
| Origin-RNA′ M13 Gori-1 | 0 | 0 | 0.18 | 1 | |
| | 7,500 | 0 | 0.64 | 3.5 | |
| | 41,000 | 0 | 0.74 | 4.1 | |
| | 0 | 1 | 0.05 | | 3.6 |
| | 0 | 2 | 0.04 | | 4.5 |
| | 41,000 | 1 | 0.17 | | 4.4 |
| Gapped fd RF[d] | 0 | 0 | 1.42 | 1 | |
| | 7,500 | 0 | 2.98 | 2.1 | |
| | 41,000 | 0 | 3.48 | 2.5 | |
| | 0 | 1 | 0.44 | | 3.2 |
| | 0 | 2 | 0.31 | | 4.6 |
| | 41,000 | 1 | 0.81 | | 4.3 |
| Activated calf thymus DNA | 0 | 0 | 19.50 | 1 | |
| | 7,500 | 0 | 21.84 | 1.1 | |
| | 41,000 | 0 | 25.98 | 1.3 | |
| | 0 | 1 | 8.86 | | 2.2 |
| | 0 | 2 | 4.75 | | 4.1 |
| | 41,000 | 1 | 7.25 | | 3.6 |

[a] SSB was added to DNA templates in ratios (μg) of 2:1 and 4:1 (SSB/DNA).

[b] β-Stimulation of DNA polymerase III activity.

[c] Inhibition of DNA polymerase III activity as a consequence of SSB or SSB plus β addition to reaction mixtures.

[d] The gapped fd RF DNA template contained approximately 14 gaps/circle of 100 nucleotides each.

would be attributable to fluctuations in the relative number of reacted 3′-OH termini rather than alterations in the processive capabilities of the enzyme.

As expected, in the absence of added ddTTP, DNA polymerase III activity increased as the 3′-OH primer concentration on the template increased (Fig. 5). In the presence of the β subunit (20,000 units, $8.0 \times 10^5$ units/ml), a further stimulation of DNA polymerase III activity was observed on each of the primer-template combinations. However, the magnitude of the β-induced stimulation of the enzyme decreased with increasing 3′-OH primer concentrations. Since the distance between adjacent primer fragments decreases with increasing 3′-OH concentration on the template, the reduced stimulation of the polymerase by β on high primer density templates is a measure of the limitation on the processive elongation of primers by the enzyme because of the close spacing of the primers.

In the presence of 1 mM ddTTP (20 ddTTP to 1 dTTP), the rate of the DNA polymerase III reaction in the absence of β was reduced by approximately 50% on each of the templates, whereas the reaction rate in the presence of both β and ddTTP was reduced by greater than 90%. Total incorporation on each of the templates reacted with DNA polymerase III-β in the presence of ddTTP was significantly reduced compared to similar ddTTP-restricted reactions catalyzed by DNA polymerase III (−β). Subsequent analysis of the prod-

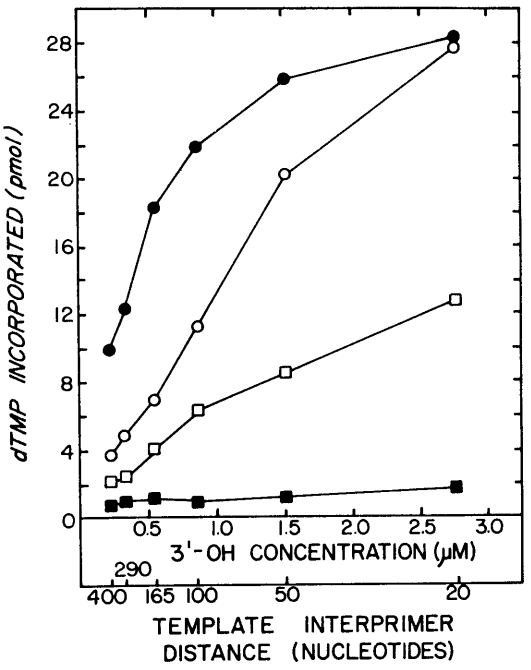


FIG. 5. **β Effect on the residence time of DNA polymerase III on DNA templates.** DNA polymerase III was reacted on poly(dA)·oligo(dT)$_{16}$ templates (50 μM) with nucleotide ratios (dA:dT) of 2.25:1, 4.1:1, 7.25:1, 11.3:1, 19.1:1, and 26:1 resulting in interprimer distances on the poly(dA) template of approximately 20, 50, 100, 165, 290, and 400 nucleotides, respectively. Activity profiles are shown for reaction mixtures (25 μl) containing: 8 units of DNA polymerase III (○); DNA polymerase III plus 1 mM ddTTP (□); DNA polymerase III plus 20,000 units ($8.0 \times 10^5$ units/ml) of the β subunit (●); and DNA polymerase III plus 1 mM ddTTP and 20,000 units of the β subunit (■). Enzyme reactions were incubated at 30 °C for 5 min as described under "Materials and Methods," terminated with the addition of EDTA to 100 μM and [$^3$H]dTMP incorporation determined. Enzyme reaction rate, as measured by dTMP incorporation in 5 min of reaction, is plotted as a function of 3′-OH concentration and corresponding interprimer distance on the poly(dA) template.

ucts synthesized by the core polymerase (±β) in the presence of ddTTP demonstrated the products to be elongated about one-half of the distance normally observed by the enzyme (−β) in the absence of the chain terminator (data not shown). Therefore, essentially all products are terminated by the ddTTP, and are the same range of length, whether β is present or not. If, in the presence of ddTTP, the DNA polymerase III-β enzyme complex had reacted with the same frequency on available 3′-OH termini as the core polymerase, then the reaction rate of either enzyme complex should have been equivalent. The much lower reaction rate observed in the reaction supplemented with β indicates that the rate of dissociation of the polymerase from a terminated template and rate of initiation of synthesis on a second 3′-OH terminus is decreased in the presence of the β subunit. This suggests that the residence time of the polymerase on one primer-template is greatly increased by the β subunit, even if significant elongation is not allowed.

## DISCUSSION

Interaction of purified β subunit with the catalytic core form of DNA polymerase III generates a synthetically more active and highly processive enzyme complex. The addition of the β subunit greatly enhances the synthetic rate of the core polymerase on a variety of synthetic and natural DNA templates. Maximal stimulations are observed on templates having relatively low 3′-OH primer concentrations and long interprimer distances. On templates which restrict DNA elongation (templates having interprimer distances of 20 nucleotides or less), no stimulation of DNA polymerase III activity is observed in the presence of β. Therefore, elevated synthetic rates in the presence of β are related to the enhanced ability of the core polymerase to synthesize longer DNA products.

As the level of β in the reaction is increased, the fraction of longer products generated increases. This length increase does not occur in a gradual fashion which produces intermediate length products, and then long products. Instead, two populations of elongated products are generated in these reactions, suggesting the activity of two distinct polymerases. One class of products characteristic of DNA polymerase III core (Fay *et al.*, 1981), is elongated only 5–10 nucleotides. The second class of products is elongated up to several hundred nucleotides and predominates at highest β concentrations. This class of products is evidently the consequence of a functional interaction between DNA polymerase III and the β subunit.

We considered the possibility that the addition of stoichiometric excesses of the β subunit to the core polymerase results in the reconstitution of holoenzyme activity arising from low level contamination of τ or γ-δ in the β and core preparations. However, our results eliminate this interpretation for several reasons. First, unlike the holoenzyme form of DNA polymerase III (Fay *et al.*, 1981), DNA polymerase III-β activity is inhibited on SSB-coated DNA templates. Second, the synthetic rate of DNA polymerase III-β reactions is unaltered in the presence of ATP, in contrast to similar holoenzyme catalyzed reactions in which synthetic activity is enhanced in the presence of ATP (Wickner and Kornberg, 1974; Crute *et al.*, 1983). Finally, the DNA polymerase III-β activity demonstrates a sensitivity to increased salt concentration similar to that of DNA polymerase III core polymerase and is much more sensitive than the holoenzyme (Burgers and Kornberg, 1982a,b; Crute *et al.*, 1983). The results suggest that DNA polymerase III-β is functionally unique.

The increased processive synthesis capacity of DNA polymerase III in the presence of the β subunit can be attributed to the enhanced stability of the enzyme·DNA interaction during synthesis in the presence of excess β subunit. Furthermore, reaction kinetics of the polymerase under conditions of restricted DNA synthesis elongation, in the presence of ddTTP, suggest that the residence time of the enzyme on the DNA template is increased in the presence of the β subunit. Formation of a stable enzyme·β·DNA complex presumably would lengthen the time interval between dissociation of the polymerase from a terminated template and initiation of synthesis on a second 3′-OH terminus. This effect would make the reaction rate very sensitive to inhibition by ddTTP, a phenomenon observed in Fig. 5. Thus, by stabilizing enzyme·DNA interaction when chain termination occurs, β increases the inhibitory effect of dideoxynucleoside triphosphate.

The addition of β to each of the four subassemblies of DNA polymerase III resulted in similar stimulations in synthetic rate and changes in enzyme processivity. Previous results have indicated that β is part of the elongation complex (Wickner, 1976; Johanson and McHenry, 1982) and that it is buried deep within this complex in a state that sterically prevents contact with antibody directed against β (Johanson and McHenry, 1981, 1982). Additionally, results showing that mutations in the *dna*N gene (the β structural gene) could complement mutations in the *dna*E gene (the α structural gene) also suggested some interaction of the two polypeptides (Kuwabara and Uchida, 1981). However, these results only demonstrated that β was in intimate contact with the internal

components of the elongation complex and did not prove that β was functionally involved in elongation. There had been no actual measurement of the subunit contacts through which β participates in the elongation complex. Since β has only been shown to interact with DNA polymerase III* until now, the possibility remained that β did not contact DNA polymerase III directly, that its effects were mediated through another subunit such as τ, γ or δ. The present study demonstrates (i) that β is directly involved in the elongation reaction, (ii) that one of its contributions is to increase the processivity of the polymerase, and (iii) that β contacts DNA polymerase III directly and brings about functional changes through this interaction.

These conclusions do not preclude either a functional interaction of β with other holoenzyme subunits or an involvement in the initiation process. We already know that the presence of β is required for the tight ATP-dependent formation of initiation complexes on primed templates. Further description of the mechanistic contributions of β must await the availability of the other holoenzyme components in large quantity in a homogeneous state.

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