DNA Polymerase III Accessory Proteins

II. CHARACTERIZATION OF δ AND δ'*

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The γ complex subassembly (γδδ'χψ) of DNA polymerase III holoenzyme couples ATP to assemble the ring-shaped β subunit around DNA forming a DNA sliding clamp. This β clamp is needed for highly processive synthesis by the holoenzyme. Here, the δ and δ' subunits of the γ complex are studied for their structural and functional interaction with each other and with the γ subunit. Both δ and δ' are monomeric in their native state, and they bind each other tightly to form a 1:1 complex. Neither δ nor δ' alone binds tightly to the γ subunit. However, as a complex, δδ' binds γ tightly to form a γδδ' complex. The fact that all three subunits, γ, δ, and δ', are needed to form a tight complex correlates well with activity assays which show that γ and δ are capable but inefficient in assembly of the β ring onto DNA and δ' is needed for an efficient reaction.

The polymerase that duplicates the genetic material of Escherichia coli, DNA polymerase III holoenzyme, is comprised of at least 10 subunits (α, ε, η, γ, δ, δ', x, ψ, and β) (1) and in light of the δ' doublet produced by holoB (δ'1 and δ'2), may have 11 subunits in all (2). As a polymerase, the holoenzyme is one of the most rapid (750 nucleotide/s) and highly processive (hundreds of kilobases) (3–5). This speed and processivity derive from the ring-shaped β subunit which completely encircles DNA to form a “sliding clamp” and acts to continuously tether the polymerase to DNA for highly processive synthesis (6–8). To get onto DNA, β requires the γ complex (γδδ'χψ) which recognizes a primed template and couples ATP hydrolysis to assemble the β ring around DNA (9–12).

One of our goals is to identify the function of each subunit in the γ complex. In our initial studies using a few micrograms of δ and δ' purified from several kilograms of cells we found that a mixture of δ and γ was sufficient to assemble the β clamp onto primed DNA (13); the δ', x, and ψ subunits stimulated the reaction (12). Further study requires a rich source of these individual subunits. To that end, this series of reports identifies the genes encoding the δ, δ', x, and ψ subunits, and the genes have been used to obtain each subunit in quantity. In this report we use the abundant supply of δ and δ' (2) to corroborate and extend the earlier findings and begin new structural studies on these subunits. Biochemical study of the χ and ψ subunits is the subject of the fourth report (14).

EXPERIMENTAL PROCEDURES

Materials and Methods—Methods, materials, and sources not described here were described in the first report of this series (2). Buffer C is 20 mM Tris-HCl (pH 7.5), 10% glycerol, 2 mM dithiothreitol, 0.5 mM EDTA, and 100 mM NaCl.

Replication Assays—Assays of δ, δ', and δδ' complex with either γ or ρ were as described for the δ', ρ' assay in the accompanying report (2) except the amount of δ, δ', γ, and ρ was as indicated in the figure legends.

ATPase Assays—ATPase assays were performed in 20 µl containing 20 mM Tris-HCl (pH 7.5), 8 mM MgCl2, and 839 ng of M13mp18 ssDNA. Assays of γ, δ, δ', χ, and γ' with and without δ contained 100 µM [γ-32P]ATP and when present, 752 ng of γ (8 pmol as dimer), 310 ng of δ (8 pmol as monomer), 296 ng of δ' (8 pmol as monomer), and 1.28 µg of β (8 pmol as dimer). Proteins were added on ice, shifted to 37°C for 30 min, and then 0.5 µl was spotted onto a thin layer chromatography (TLC) sheet coated with Cel-300 polyethyleneimine (Brinkmann Instruments Co.). To assay the more active ATPase activity of γδδ' and ρ we used more ATP, less protein, and less time to assess the initial rate of reaction. Therefore ATPase assays of γδδ', ρδ, ρδ', and γδ' contained 300 µM [γ-32P]ATP and, when present, 94 ng of γ (1 pmol as dimer), 142 ng of ρ (1 pmol as dimer), 38 ng of δ (1 pmol as monomer), 27 ng of δ' (1 pmol as monomer), and 160 ng of β (2 pmol as dimer). Proteins were added on ice, shifted to 37°C for 10 min, and then spotted onto the TLC sheet. TLC sheets were developed in 0.5 M lithium chloride, 1 M formic acid. An autoradiogram of the TLC chromatogram was used to visualize Pi at the solvent front and ATP near the origin which were then cut from the TLC sheet and quantitated by liquid scintillation.

The mol of P, released per mol of protein per min was calculated assuming that the concentration of protein equaled the concentration of γ or ρ (as dimer) except for assays lacking γ or ρ in which case the amount of protein was assumed to be the number of mol of either δ (as monomer) or δ' (as monomer) or of b, δ', γ, or ρ' complex.

Gel Filtration—Gel filtration of δ, δ', and δδ' complex was performed using an HR 10/30 Superdex 75 column equilibrated in buffer C. Either δ (30 µg, 0.78 nmol as monomer), δ' (30 µg, 0.61 nmol as monomer), or a mixture of δ and δ' (60 µg each) was incubated for 30 min at 15°C in 100 µl of buffer C, and the entire sample was injected onto the column. The column was developed with buffer C at 0.3 ml/min and after the first 6 ml, fractions of 170 µl were collected. Fractions were analyzed by 13% SDS-polyacrylamide gel (100 µl) lane stained with Coomassie Blue. Denaturation of ssDNA gels was performed using a Pharmacia LKB Biotechnology Inc. Ultrascan XL laser densitometer.

Gel filtration of γ or ρ mixed with either δ, δ', or both δ and δ' was performed using an HR 10/30 Superose 12 column equilibrated in buffer C as described above. The amount of protein used in each analysis is described in the figure legends. Replication assays of column fractions were performed as described above except γ was omitted, and 2 µl of fraction was added to the assay after 50-fold dilution using 20 mM Tris-HCl (pH 7.5), 10% glycerol, 2 mM dithio-
each in 100 μl of buffer C. The K,,
Superdex 75 and Superose 12 columns was 24 ml (manufacturer's
specifications), and the V,, determined using M13mp18 DNA sat-
urated with SSB (total molecular mass = 25 Mda), was 5.8 ml for
Superose 12 and 6.5 ml for Superdex 75.

Glycerol Gradient Sedimentation—Sedimentation analysis of δ, δ',
and a mixture of δ and δ' was performed using 11.6 ml of 10-30%
glycerol gradients in buffer C. Either δ (57 μg, 1.5 nmol as monomer),
δ' (56 μg, 1.5 nmol as monomer), or a mixture of δ and δ' (57 and 56
μg, respectively) was incubated at 15 °C for 30 min in 100 μl of buffer
C and then layered onto separate gradients. Protein standards (50 μg
each in 100 μl of buffer C) were layered onto another gradient, and
the gradients were centrifuged at 270,000

Fractions of 170 μl were collected from the bottom of the tube and
analyzed (100 μl/ lane) in a 13% SDS-polyacrylamide gel stained with
Coomassie Blue.

Light Scattering—Diffusion coefficients of δ, δ', and δδ' complex
were determined by dynamic light scattering at 780 nm in a fixed
angle

The observed diffusion coefficient of δδ' in the presence of
0.3 M NaCl. The observed diffusion coefficient of δδ' (56 μg, 1.7 nmol as monomer),
δδ' (56 μg, 1.5 nmol as monomer), δδ' (56 μg, 1.5 nmol as monomer), δδ' (56 μg, 1.5 nmol as monomer), and δδ' (56 μg, 1.5 nmol as monomer) was in 400 μl of 20 mM Tris-HC1 (pH 7.5), 100 mM NaCl, and 1.2% glycerol. The mixture of δ and δ' (100 μg each) was in 400 μl of 20 mM Tris-HC1 (pH 7.5) and 100 mM NaCl. The observed diffusion coefficient of δ' in the presence of 1.2% glycerol was 0.6% higher than in the absence of glycerol. Hence, the 1.2% glycerol in the δ and δ' samples had little effect on the observed diffusion coefficient.

RESULTS

Native Mass of δ and δ'—As one step toward a larger goal
determining the holoenzyme structure we have determined
whether δ and δ' are monomeric, dimeric, or higher order
structures. In a gel filtration analysis, the δ and δ' subunits
migrated in essentially the same position as one another (Fig.
1A, panels 1 and 2). Comparison with protein standards
yielded a Stokes radius of 26.5 Å for δ and 25.8 Å for δ',
slightly smaller than ovalbumin (43.5 kDa, 27.5 Å) (Fig. 1A,
panel 4), indicating that both δ and δ' are monomeric (their
gene sequences predict 38.7 kDa

δδ' complex was also performed (Table 1). The Stokes radii
calculated from the diffusion coefficients obtained from light
scattering were within 6% of the Stokes radii of δ, δ', and δδ'
determined by gel filtration.

The γδδ' Complex—In the γ complex, the γ, δ, and δ' subunits are
bound together along with χ and δ. Does δ or δ' (or both) bind to γ or do they need the χ and/or δ subunits to bind to γ? The physical interaction among δ, δ', and γ was examined by gel filtration (Fig. 2).

The γ subunit (47 kDa) was mixed with a 4-fold molar excess of each of δ and δ' and then was gel filtered. A complex of γδδ' was formed as indicated by coigration of both δ and δ' with γ (fractions 24-28 in Fig. 2A); excess δδ' complex eluted much later (fraction 40-48). Hence χ and δ are not needed for δδ' to bind to γ. The γ subunit elutes abnormally
early in gel filtration for a dimer (20, 22) but becomes a dimer upon binding the δδ' complex explaining why the γδδ' complex elutes in a similar position as γ by itself.

To determine which subunit, δ or δ', binds directly to γ, the γ subunit was mixed with either δ or δ' and then gel filtered.

The γ + δ mixture showed that a gel-filterable γδ complex was not formed as indicated by the absence of δ in fractions 24-32 containing γ (Fig. 2C). The γ + δ' mixture did not form a gel-filterable complex either, as indicated by the lack of coelution of γ and δ' (Fig. 2B) (the large amount of δ' used in the experiment of Fig. 2B prevented resolution of δδ' and δδ'). Therefore both δ and δ' must be present with γ to form a gel-filterable γδδ' complex (i.e. γ + δδ' → γδδ').

The gel filtration column fractions of the γδδ' complex were active in assembly of the β clamp on primed DNA leading to processive DNA synthesis by the polymerase (panel D, Fig. 2). The δδ' complex is not active without γ (data not shown), and therefore the slight activity in fractions 40-42 was probably a result of some γ which trailed into the peak of the δδ' complex. The column fractions of the γ + δ and γ + δ' mixtures were essentially inactive.

An earlier sedimentation analysis detected a complex of γ with δ (13). However, using pure cloned δ we see no complex with γ in gel filtration (or in glycerol gradient analysis (data not shown)). As discussed in detail below, the δ preparation used in the earlier study contained a slight amount of δδ', and therefore the complex observed by assays in the earlier study was most likely the γδδ' complex.

The δδ' Complex—The γ subunit contains all the sequences of γ plus an additional 24 kDa of protein at the carboxyl terminus. Hence one might not be surprised with the finding that a γδδ' complex can be isolated by gel filtration as indicated by coelution of δ and δ' with γ (in fractions 20-

2 The Stokes radius was calculated from the diffusion coefficient using the equation: Stokes radius = kT/6πηD, where k is Boltzmann's constant, T is absolute temperature, η is viscosity, and D is the diffusion coefficient.

3 R. O'martins and M. O'Donnell, unpublished observations.
FIG. 1. $\delta$ and $\delta'$ form a complex. Native mass of $\delta$, $\delta'$, and $\delta\delta'$ complex. A, gel filtration analysis of $\delta$ (panel 1), $\delta'$ (panel 2), and a mixture of $\delta$ and $\delta'$ (panel 3) was performed as described under “Experimental Procedures.” Column fractions are identified above and below the Coomassie-stained SDS-polyacrylamide gels. The first lane of each gel contains protein standards (MW), and their molecular masses are indicated to the left. The $\delta$, $\delta_1$, and $\delta_2$ subunits are identified to the right of each gel. Panel 4 shows the elution of $\delta$, $\delta'$, and $\delta\delta'$ relative to protein standards of known Stokes radius calculated from their diffusion coefficients (23) as explained in Footnote 2. B, glycerol gradient sedimentation analysis of $\delta$ (panel 1), $\delta'$ (panel 2), and $\delta\delta'$ (panel 3) was performed as described under “Experimental Procedures.” Panel 4 compares the migration of protein standards of known S value (23) with the migration of $\delta$, $\delta'$, and $\delta\delta'$. BSA, bovine serum albumin (67 kDa, 34.9 Å, 4.41 S); Ova, chicken ovalbumin (45.5 kDa, 27.5 Å, 3.6 S); Ti, soybean trypsin inhibitor (24 kDa, 23.8 Å); Myo, horse myoglobin (17.5 kDa, 19.0 Å, 2.0 S); Met, horse kidney metallothionein (9.7 kDa, 17.3 Å).
we have reexamined their activity with y in detail and find that the polymerase to bind the preinitiation complex (see scheme in Fig. 1). Native mass and frictional coefficient were calculated from the Stokes radius and S value as described (15). These calculations require the partial specific volume of δ and δ', which were calculated by summation of the partial specific volumes of the individual amino acids for each δ and δ' (24). Molecular masses of δ, δ', and the δδ' complex (assuming a composition of 1:1:1) were calculated from the gene sequences of δ and δ' (2).

<table>
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<th>δ</th>
<th>δ'</th>
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<tr>
<td>Stokes radius (Å)</td>
<td>26.5</td>
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<td>Sedimentation coefficient (S)</td>
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<td>3.0</td>
<td>3.9</td>
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<td>Partial specific volume (cm³/g)</td>
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<td>Native mass from gene sequence (Da)</td>
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<td>36,934</td>
<td>75,638</td>
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<td>Frictional coefficient (f/f₀)</td>
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<td>Radius calculated from D (Å)</td>
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<td>26.3</td>
<td>32.5</td>
</tr>
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42 of Fig. 3A) and by activity assays which show that most of the activity is in these fractions (Fig. 3B). A smaller peak of activity elutes with the δδ' complex because of some τ that trails into these fractions. We also analyzed mixtures of τ with either δ or δ' separately with the result that τδ and τδ' complexes were not observed in the column fractions (data not shown, but the conditions and results were similar to Fig. 2 using τ instead of γ).

Replication Activity of δ, δ', and δδ' with γ—We showed previously that a mixture of γ and δ was active in assembling δ onto DNA leading to rapid and processive synthesis by the αε polymerase which matched that of the entire holoenzyme (12, 13, 19). The δ' subunit did not substitute for the δ subunit but did stimulate the γ + δ activity in formation of the δ clamp (12, 13). Now with large amounts of δ and δ' available, we have reexamined their activity with γ in detail and find that the amount of activity using δ and the extent of stimulation of γ + δ upon adding the δ' subunit are highly dependent upon the amount of γ one includes in the assay. In these replication assays, the substrate is M13mp18 ssDNA primed with a synthetic oligonucleotide and "coated" with SSB. The γ and δ (or δ') subunits are added to the DNA along with the β subunit and the αε polymerase for 8 min giving time for these proteins to assemble the preinitiation complex on the DNA (this consists of a β dimer and may contain other subunits under the conditions used here) and for the αε polymerase to bind the preinitiation complex (see scheme in Fig. 4). DNA synthesis is initiated upon addition of dNTPs, and the reaction is stopped after 20 s, which is sufficient time for the processive polymerase to complete the circular DNA.

The structural and functional data correlate. The activity assays show that δδ' is much more efficiently utilized by γ than δ is utilized by γ. This observation is consistent with the

**TABLE I**

Native molecular mass of δ, δ', and δδ' complex

The Stokes radii and S values of δ, δ', and δδ' complex were determined from the gel filtration and glycerol gradient analysis in Fig. 1. Native mass and frictional coefficient were calculated from the Stokes radius and S value as described (15). The structural and functional data correlate. The activity is in these fractions (Fig. 2A), but did stimulate the activity elutes with the δδ' complex because of some τ that trails into these fractions. We also analyzed mixtures of τ with either δ or δ' separately with the result that τδ and τδ' complexes were not observed in the column fractions (data not shown, but the conditions and results were similar to Fig. 2 using τ instead of γ).

**Fig. 2. Constitution of a γδδ' complex.** The γ subunit was mixed with either δ, δ', or both δδ' and was then gel filtered (Superose 12) as described under "Experimental Procedures." Panel A, mixture of γ (23 µg, 0.24 nmol as dimer), δ (38 µg, 1 nmol as monomer), and δ' (37 µg, 1 nmol as monomer). Panel B, mixture of γ (94 µg, 1 nmol as dimer) with δ' (74 µg, 2 nmol as monomer). Panel C, mixture of γ (94 µg, 1 nmol as dimer) with δ (76 µg, 2 nmol as monomer). Column fractions are indicated above and below the set of three gels. The first lanes of each gel are standards (μmol), and their molecular masses are indicated to the left. The γ, δ, and δ' subunits are identified to the right. Panel D shows the activity assays of the column fractions performed as described under "Experimental Procedures." Gel filtration fractions of γδδ' were assayed using comparable amounts of proteins as in panels B and C.

**D) Activity**

![Activity Graph](image-url)
weak complex which would not be isolable under the non-equilibrium conditions of gel filtration.

We previously studied the effect of δ and δ' on the γ DNA-dependent ATPase (12). Here we reexamine this using the pure cloned δ and δ' subunits and M13mp18 ssDNA, one of the best DNA effectors of the ATPase. We also extend this analysis to the τ subunit ATPase. Consistent with our previous study we find very little ATPase activity in δ, using the pure cloned δ and δ' subunits and M13mp18 ssDNA, one of the best DNA effectors of the ATPase. We also extend this analysis to the γ preparation for possible contaminants, and

gel filtration results in which the δδ' complex bound γ tight enough to survive the nonequilibrium conditions of gel filtration. A γδ complex must also form, as indicated by the replication assays, but the γδ interaction is probably weak, explaining the need for excess γ to obtain activity. A γδ complex was not observed in gel filtration as expected for a weak complex which would not be isolable under the non-equilibrium conditions of gel filtration.

Replication Activity of δ, δ', and δδ' with τ—We showed previously that δ', as well as δ, was active with τ in replication activity with β and ατ (13). However, we did not study the combined effects of both δ and δ' on τ activity. In Fig. 4B we present an analysis of τ activity with δ, δ', and δδ' complex performed in parallel, under the same conditions, as the analysis with γ in Fig. 4A. Consistent with the previous studies both δ and δ' are active with τ, and more τ (relative to γ) is required for activity. Only a small amount of τ is required for maximal activity with δδ'. In fact, the activity of τδδ' parallels that of γδδ'. Hence, both γ and τ are highly active with the δδ' complex in keeping with formation of γδδ' and τδδ' complexes stable enough to survive gel filtration.

DNA-dependent ATPase Activity of γ (and τ) with δ, δ', and δδ' Complex—We previously studied the effect of δ and δ' on the γ DNA-dependent ATPase (12). Here we reexamine this using the pure cloned δ and δ' subunits and M13mp18 ssDNA, one of the best DNA effectors of the ATPase. We also extend this analysis to the τ subunit ATPase. Consistent with our previous study we find very little ATPase activity in δ, δ', and γ (Fig. 5A). We have previously examined the slight ATPase activity in the γ preparation for possible contaminants, and

it appeared that this slight activity was truly inherent in γ (12), not a surprising finding since the related τ subunit is a known DNA-dependent ATPase (60-fold more active than γ) (17, 18). We have not rigorously examined the low level of ATPase activity in the δ and δ' preparations for a contaminating ATPase, and therefore we will assume here that δ and δ' are not ATPases until it is shown to be otherwise. A mixture of δ and δ' gave no increase in ATPase activity over the individual preparations. However, the γ ATPase was stimulated 3-fold by δ and 11-fold by δ'. When δ and δ' were mixed with γ, the ATPase activity was stimulated 140-fold (Fig. 5A). Thus the ATPase activity analysis correlates with analysis of the subunit interactions in which both δ and δ' are needed to form a stable complex with γ and for appreciable activity with γ.

The β subunit did not stimulate the γ, δ, δ', δδ', γδ, or γδ' ATPases. However, δ stimulated the γδδ' ATPase 2.5-fold. Previously we observed a greater stimulation by δ of the γ ATPase activity (10-fold), and β also stimulated the γδ ATPase 10-fold (12). The reason for the discrepancy with the results of this study is now clear. In the previous study, the δ subunit was prepared by resolving δ and δ' out of the γ
the y66' ATPase 2.5-fold, the 140-fold stimulation of y by M13mp18 ssDNA as described under "Experimental Procedures." The subunits in each assay are identified below the plot. Panel A, effect of δ, δ', and β on the γ ATPase. Panel B, effect of δ, δ', and β on the τ ATPase. The specific activity calculations are explained under "Experimental Procedures." ATPase assays were performed in triplicate, and the error bars represent one standard deviation.

complex, and then δ and δ' were separated on a Mono Q column. Now, with our experience using pure cloned δ and δ' we find they form a δδ' complex, and the δδ' complex comigrates with δ on Mono Q (data not shown). Therefore, we believe that our previous δ preparation may have been contaminated with a slight amount of δδ' complex. We have inspected our previous preparation of δ (purified from the γ complex) in an SDS gel and detect a low level (approximately 5%) of δδ' in it. We have repeated the ATPase assays under the conditions of the previous study using δ to which we add δ' to a level of 5% by weight; we find this δ (containing 5% δ') stimulates the γ ATPase 10-fold and is further stimulated by β as in our previous study (data not shown).

The τ subunit, by itself, is an active DNA-dependent ATPase (17, 18) and turns over 54 times more ATP than γ (compare Fig. 5, A and B). The τ ATPase was essentially unaffected by β, or by δ with or without β, or by δ' with or without β. The presence of both δ and δ' stimulated the τ ATPase, although the effect was only 5-fold compared with the 140-fold stimulation of γ by δδ'. Whereas β stimulated the γδδ' ATPase 2.5-fold, the β subunit did not stimulate the τδδ' ATPase at all, yet the τδδ' complex is as active as γδδ' in reconstituting a processive polymerase with β and αε (compare Fig. 4, A and B).

DISCUSSION

No single subunit of the γ complex is active in assembling the β clamp on DNA (19). Presumably, this reaction is too complicated for just one protein. A mixture of γ and δ is capable of assembling β onto DNA (12, 13). The γ subunit binds ATP (20) and depends on DNA as a cofactor of its ATPase (12), and we have recently found that δ binds the β subunit. Therefore, one may speculate that γ binds the primed template, and δ brings in the β subunit, then ATP hydrolysis is coupled to assemble the ring-shaped β dimer around the DNA. Perhaps δ' increases the efficiency of γβ by physically bringing γ and δ together in the γδδ' complex, although it is also possible that δ' participates directly in the chemistry of the reaction. Further, δ' is active with τ in placing β onto DNA in the complete absence of δ, suggesting that within the holoenzyme, δ' may play a role specifically with τ in assembly of β clamps.

The physical studies described here show that neither δ nor δ', when separate, formed a gel-filterable complex with the γ subunit. Yet they most likely bind to γ (at least weakly) as indicated by activity assays in which γδ is active (without δ') in assembly of the β clamp, and δ' (without δ) stimulates the DNA-dependent ATPase activity of γ (12, 13). Gel filtration detects tightly bound protein-protein complexes, but since components are not at equilibrium during gel filtration, weak protein complexes will dissociate. The δ and δ' subunits formed a gel-filterable 1:1 δδ' complex, and when mixed with γ they efficiently formed a tight gel-filterable γδδ' complex. The structural requirement for both δ and δ' to form a tight complex with γ correlates with the activity requirements in which both δ and δ' are needed with γ for efficient ATPase activity and for efficient assembly of the β ring onto DNA. The τ subunit contains the sequence of γ plus an additional 24 kDa of protein at its carboxyl terminus. Since τ has all of the sequences of γ, it may be expected to bind the δ, δ', χ, and ψ subunits of the γ complex, unless the extra sequence in τ occludes the binding sites of the "γ region" within τ. This study shows that δ and δ' bind τ, and the fourth report (14) shows that χ and ψ also bind τ. We have since mixed all five proteins and they form a "τ complex" (τδδ'χψ). Hence, the holoenzyme may contain a τ complex. If the holoenzyme does contain a τ complex it would resolve the dilemma of the holoenzyme having one only γ complex (containing only one each of δ, δ', χ, and ψ), yet the holoenzyme has two each of δ, δ', χ, and ψ (i.e. γδδ'χψ: τδδ'χψ: τδδ'χψ: χψ). We have recently found that the holoenzyme has two each of δ, δ', χ, and ψ (i.e. γδδ'χψ: τδδ'χψ: τδδ'χψ: χψ).

In discussing the τ complex, it is important to note that a τ complex has never been purified from E. coli. The only known subassembly smaller than polIII' (holoenzyme lacking δ) that contains τ is polIII', in which a τ dimer binds two molecules of polIII core (21). The carboxyl-terminal 24-kDa sequence of τ is required for this interaction with polIII core, as γ cannot bind polIII core (22). If, in the holoenzyme, τ binds δ, δ', χ, and ψ, one would expect the polIII' subassembly to contain the δδ'χψ subunits, yet these subunits are not present in polIII'. Perhaps once τ binds to polIII core, the binding sites for χψδδ' are blocked, for example by steric hindrance. However, we have shown this is not so; a core-τδδ'χψ complex can be efficiently constituted. Perhaps the 1 M KCl used during purification of polIII' stripped the δδ'χψ off. The definitive answer as to whether τ acts with these γ complex subunits must await knowledge of the subunit associations between τ and these other proteins (δ, δ', χ, and ψ) within the holoenzyme.

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REFERENCES