The γ complex (γδ′χψ) subassembly of DNA polymerase III holoenzyme transfers the β subunit onto primed DNA in a reaction which requires ATP hydrolysis. Once on DNA, β is a "sliding clamp" which tethers the polymerase to DNA for highly processive synthesis. We have examined β and the γ complex to identify which subunit(s) hydrolyzes ATP. We find the γ complex is a DNA dependent ATPase. The β subunit, which lacks ATPase activity, enhances the γ complex ATPase when primed DNA is used as an effector. Hence, the γ complex recognizes DNA and couples ATP hydrolysis to clamp β onto primed DNA. Study of γ complex subunits showed no single subunit contained significant ATPase activity. However, the heterodimers, γβ and γβ′, were both DNA-dependent ATPases. Only the γδ ATPase was stimulated by β and was functional in transferring the β from solution to primed DNA. Similarity in ATPase activity of DNA polymerase III holoenzyme accessory proteins to accessory proteins of phage T4 DNA polymerase and mammalian DNA polymerase δ suggests the basic strategy of chromosome duplication has been conserved throughout evolution.

DNA polymerase III holoenzyme is the principle replicase of the Escherichia coli chromosome (1). In common with chromosomal replicates of phages T4 and T7, yeast, Drosophila, mammals, and their viruses, the E. coli replicase is composed of a DNA polymerase subunit accompanied by accessory proteins. DNA polymerase III holoenzyme (holoenzyme) contains at least 10 subunits (α, ε, β, τ, β′, γ, δ′, χ, ψ, η) (2). The holoenzyme hydrolizes two molecules of ATP to form a tightly bound initiation complex on a primed template (3). The DNA substrate often used in holoenzyme studies is a bacteriophage single-strand (ss) DNA circular genome (5.4-8.6 kb) "coated" with the E. coli SSB protein and primed with a single oligonucleotide (3-6). Upon formation of the initiation complex, the holoenzyme is rapid in synthesis (>500 nucleotides/s) and replicates the entire template without dissociating from the DNA even once (i.e. it is highly processive) (4-6).

The remarkable processivity of the holoenzyme requires its accessory proteins (4). The three subcore subassembly of the holoenzyme contains the DNA polymerase subunit (α) (7), the proofreading 3'-5' exonuclease subunit (τ) (8), and the δ subunit (9). The core polymerase synthesizes DNA at a rate of approximately 20 nucleotides/s (10) and is only processive for approximately 11 nucleotides (4). However, the highly processive character of the holoenzyme can be reconstructed upon mixing the core polymerase with both the β subunit and the δ protein γ complex (γδ′χψ subunits) (3, 11, 12).

Reconstitution of the processive polymerase activity of the holoenzyme can be divided into two distinct stages (3, 11, 12). In the first stage, the γ complex and β subunit hydrolyze ATP to form a preinitiation complex on primed DNA. In the second stage, the core polymerase assembles with the preinitiation complex to form the highly processive enzyme. Thus, it is the preinitiation complex which confers such remarkable processivity onto the holoenzyme. Further study of the first stage in which the preinitiation complex forms showed the γ complex acts catalytically to transfer the β subunit from solution to primed DNA (2, 11, 33). Only the γ and δ subunits of the γ complex are essential to transfer β onto primed DNA (20). Once on DNA, the β subunit slides freely along the duplex portion of the primed template (33). The β subunit also directly binds to α, the DNA polymerase subunit (33). Hence, the preinitiation complex is a "sliding clamp" of the β subunit on DNA, which tethers the polymerase to the primed template and thereby confers onto it highly processive synthesis.

To further our understanding of highly processive synthesis, in this report, we examine the β subunit and γ complex to identify which subunit(s) hydrolyze the ATP.

**MATERIALS AND METHODS**

Sources—Radioactive nucleotides were from DuPont-New England Nuclear; unlabeled nucleotides were from Pharmacia LKB Biotechnology Inc.; DNA modification enzymes were from New England Biolabs; RNAs were from Sigma; and pure proteins were prepared as described: SSB (16), γ (7), ε (8), α complex (17), γ (18), β (19) γχψ (20), δ (20), β′ (20). The γ complex was purified as described (21) with the following modifications: chromatography on Mono Q was performed in place of the DEAE-Trisacryl step, and the second heparin-agarose chromatography step was replaced by chromatography on an ATP-agarose column and a phosphocellulose column. The concentration of β was determined by absorbance using an ε260 value of 17,900 M-1 cm-1 (19). The concentration of γ was determined by amino acid analysis (Protein Microchemistry Facility, Biological Chemistry Department, University of Michigan). Concentrations of α, ε, SSB, γ complex, and γχψ were determined by the method of Bradford (22) using BSA as a standard. The concentrations of δ and
Isoo therms, which were determined by comparison of their Coomassie Blue staining intensity using a laser densitometer in a SDS-polyacrylamide gel with standard curves of γ, β, and BSA of known concentration analyzed in the same gel (21). This was done because 50% of the isoenzymes were not different from the major forms upon incubating
0.92 µg of y with either 24 ng of 6, 24 ng of 
317-mer (Fig. 1). Stimulation by 
complex ATPase was as active at 2 nM primed 317-mer (Fig. 1) or on the unprimed 317-mer (not shown). However, addition of β to the γ complex resulted in approximately 3-fold more ATPase activity (Fig. 1). We presume the β subunit stimulated the ATPase activity of the γ complex. However, it remains possible that a latent ATPase activity intrinsic to β becomes active upon interaction with the γ complex and DNA. The β stimulation of the γ complex ATPase was specific to the primed 317-mer (Fig. 1); β did not stimulate the γ complex ATPase on the unprimed 317-mer (Fig. 1). Stimulation by β of the γ complex ATPase was observed over a wide range of γ complex concentration (over a 60-fold range, not shown).

The γ complex ATPase activity on the primed 317-mer showed β elevated the γ complex ATPase (from 25 to 80 mol of ATP hydrolyzed/min/mo1 of γ complex) and increased the Kₐ for ATP 2-fold (from 26 to 49 µM) (Fig. 3). The γ complex ATPase was as active at 2 nM primed 317-mer as at 20 nM primed 317-mer (not shown). Hence the Kₐ of the γ complex ATPase for primed DNA is lower than 2 nM. The concentration of 3' primer ends used in these studies was above 15 nM.

Various nucleic acids were examined for their ability to induce the γ complex ATPase (Table I). Although poly(dA) was a poor effector of the γ complex ATPase, both poly(dA)-oligo(dt) and oligo(dT) were good effectors, suggesting the importance of 3' ends whether hybridized to ssDNA or not. Linear DNA (RFIII) was also an effector of the γ complex ATPase. Unprimed ssDNAs of natural sequence were all effectors of the γ complex ATPase. Secondary structure within ssDNA was probably the inducer of the γ complex ATPase since coating natural sequence ssDNAs with SSB diminished their effectiveness to near the level of poly(dA). However, the γ complex ATPase is not as active at 2 nM primed 317-mer. The γ complex ATPase was as active at 2 nM primed 317-mer as at 20 nM primed 317-mer (not shown). Hence the Kₐ of the γ complex ATPase for primed DNA is lower than 2 nM. The concentration of 3' primer ends used in these studies was above 15 nM.

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The β subunit stimulated the γ complex ATPase on poly(dA)-oligo(dt), but not on poly(dA) alone or oligo(dT)
Initiating ATPase of Polymerase III Holoenzyme

TABLE I
ATPase activity of the γ complex on various nucleic acid effectors

| Nucleic acid | γ complex | γ complex, β | γ complex, SSB | γ complex, SSB, β |
|--------------|-----------|-------------|--------------|------------------|
| No DNA       | 0.3       | 0.1         | 0.5          | 0.4              |
| Poly(dA)     | 0.3       | 0.1         | 0.5          | 0.7              |
| Oligo(dT)    | 4.1       | 2.9         | 1.3          | 1.4              |
| Poly(dA)-oligo(dT) | 6.5       | 10.8        | 4.0          | 11.4             |
| Unprimed 317-mer | 8.5       | 9.6         | 3.2          | 3.1              |
| Primed 317-mer | 4.5       | 10.9        | 13.1         | 39.5             |
| φX174 ssDNA  | 6.7       | 14.8        | 2.9          | 0.5              |
| M13mp18 ssDNA | 6.8       | 13.2        | 2.0          | 0.6              |
| M13mp18 RFIII(SmaI cut) | 3.4       | 9.6         | 4.0          | 11.8             |
| E. coli tRNA | 0.8       | 0.9         | 0.6          | 0.4              |
| Yeast RNA    | 1.2       | 0.6         | 0.9          | 0.2              |

a The level of detection in these assays was 0.1–0.3 mol P, released/mol γ complex/min.

TABLE II
γ complex utilization of (deoxy)ribonucleoside triphosphates

| (d)NTP   | γ complex | γ complex + β |
|----------|-----------|--------------|
| ATP      | 7.6       | 14.6         |
| CTP      | 0.3       | 0.3          |
| GTP      | 0.3       | 0.3          |
| UTP      | 0.3       | 0.3          |
| dATP     | 7.3       | 13.8         |
| dCTP     | 0.3       | 0.3          |
| dGTP     | 0.3       | 0.3          |
| dTTP     | 0.3       | 0.3          |

FIG. 1. The γ complex ATPase depends on primed DNA and is stimulated by β. ATPase assays were performed as described under "Materials and Methods" except the volume was 20 μl and contained 44 ng of γ complex, 980 ng of SSB, and one of the following: 22 ng of unprimed 317-mer (+), 22 ng of unprimed 300-mer and 86 ng of β (open circles), 22 ng of primed 317-mer (closed squares), or 20 ng of primed 317-mer and 86 ng of β (open circles). The open squares were reactions that contained 20 ng of primed 317-mer, 980 ng of SSB, and 86 ng of β.

FIG. 2. Thermal inactivation rates of γ complex ATPase and replication activities. The γ complex (440 ng) was incubated at 45 °C in 80 μl of 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 2 mM DTT, and 20% glycerol. At the times indicated, 5 μl was withdrawn and assayed for ATPase activity at 37 °C on poly(dA)-oligo(dT) (circles) as described under "Materials and Methods," and 0.5 μl was assayed for replication activity (squares) as described under "Materials and Methods" except for use of 2.2 μg of αC17 ng of β, and a 2-min incubation at 37 °C before the 20-s pulse of DNA synthesis.

FIG. 3. Steady state kinetic analysis of the γ complex ATPase. ATPase assays were performed as described under "Materials and Methods" except the assays contained 22 ng of primed 318-mer, 490 ng of SSB, and the indicated amount of ATP. Squares, without β; circles, plus 43 ng of β alone, indicating β needs a 3' end hybridized to ssDNA to stimulate the γ complex ATPase. Interestingly, the γ complex ATPase was stimulated by β on bacteriophage ssDNAs in the absence of SSB but not in the presence of SSB, indicating secondary structure within the ssDNA was the substrate effector for β to stimulate the γ complex ATPase. β also stimulated the γ complex ATPase in the presence of RFIII duplex DNA (Table I) (β was not an ATPase in the presence of duplex DNA, not shown).

The γ Complex Contains at Least Two ATPases—To identify the ATPase subunit(s) of the γ complex, the γ, δ, and δ' alone, indicating β needs a 3' end hybridized to ssDNA to stimulate the γ complex ATPase. Interestingly, the γ complex ATPase was stimulated by β on bacteriophage ssDNAs in the absence of SSB but not in the presence of SSB, indicating secondary structure within the ssDNA was the substrate effector for β to stimulate the γ complex ATPase. β also stimulated the γ complex ATPase in the presence of RFIII duplex DNA (Table I) (β was not an ATPase in the presence of duplex DNA, not shown).

The γ Complex Contains at Least Two ATPases—To identify the ATPase subunit(s) of the γ complex, the γ, δ, and δ'
ATPase activity of the γδ was negligible (Fig. 4, B and D).

In our earlier work we found that γβ' was not active in reconstituting a processive polymerase with the αε and β subunits (20). Hence, we were somewhat surprised to find ATPase activity upon mixing γ with δ' (δ and δ' are distinct polypeptides). The γβ' ATPase was most active on poly(dA)-oligo(dT) (Fig. 4A), about one-third as active on oligo(dT) (Fig. 4C), and was without activity on poly(dA) (Fig. 4B) and in the absence of DNA (Fig. 4D).

A mixture of γ, δ, and β' subunits yielded more ATPase activity on poly(dA)-oligo(dT) than summation of the γδ and γβ' activities, indicating formation of a γδβ' subassembly (Fig. 4A). Mixture of δ and δ' (without γ) did not yield ATPase activity (Fig. 4A). The γδβ' ATPase was quite active on oligo(dT) (Fig. 4C) but was less active on poly(dA) (Fig. 4B) and in the absence of DNA (Fig. 4D).

The γχψ complex exhibited very slight DNA-dependent ATPase activity (Fig. 4A), although the preparation has not been studied for a slight ATPase contaminant (i.e., the γχψ preparation is limited in availability). Nevertheless, significant ATPase activity was only gained upon mixing the γχψ complex with either δ or δ' (Fig. 4A). Also, mixture of both δ and δ' with γχψ produced more ATPase activity than summation of γχψδ and γχψδ', indicating formation of a γχψδδ' complex (Fig. 4A). The reconstituted 5 subunit γ complex was approximately 1.4 times more active than γβ', indicating the χψ subunits (also distinct genes) stimulate the ATPase activity of γβ' or have ATPase activity of their own. The ATPase activity of the reconstituted γ complex on poly(dA)-oligo(dT) was approximately 30% more active than the γ complex purified intact (Fig. 4A). This is within experimental error of the protein concentration measurements.

The ATPase activities of all the subunit combinations were as low on poly(dA) as in the absence of DNA (Fig. 4, B and D, respectively). Oligo(dT) induced nearly the same level (~70–80%) of ATPase activity as poly(dA)-oligo(dT) (Fig. 4C). The exceptions were the γβ' and γχψβ' ATPases which were only 20–30% as active on oligo(dT) relative to poly(dA)-oligo(dT). Perhaps γδ interacts with ssDNA ends more efficiently than γβ'.

β Specifically Stimulates the ATPase Activity of the γδ Subassembly—The γδβ' ATPase was stimulated by β (Fig. 5A) much like β stimulated the γ complex (Fig. 1). Hence, the χψ subunits are not required for β to stimulate the γ complex ATPase. The β stimulation of the γδβ' ATPase was specific to the poly(dA)-oligo(dT) template and was not supported by poly(dA) alone or by oligo(dT) alone (Fig. 5A). Furthermore, β did not stimulate the γβ' ATPase on any of these DNA substrates (Fig. 5B). However, β stimulated the γδ ATPase 10-fold specifically on the primed template, poly(dA)-oligo(dT) (Fig. 5C). The greater β stimulation of the γδ ATPase was due to the higher amount of β-independent ATPase in γδβ'. The β subunit affected the γχψδ, γχψδ', and γχψδδ' ATPases in similar fashion as the γδ, γδ', and γδδ' ATPases, respectively (not shown). Furthermore, β did not stimulate the low level ATPase of γχψ or of γ (not shown). Nor did β reveal an ATPase upon mixture with δ, δ', or δ' (not shown).

The δ' Subunit Stimulates γδ in Replication—Previous studies showed γδ was capable of reconstituting a fully pro-
cessive polymerase with β and the α complex (20). The δ′ subunit was not active in the reconstitution assay with β and α (20). We have found here that γδ′ is a DNA-dependent ATPase and δ′ stimulates the γδ ATPase. This led us to examine whether the δ′ subunit would stimulate γδ in the replication assay with β and α. To test this, the γδ′, γδ6′, and γ complex were individually titrated into reactions containing β, α complex, and γ complex as determined in Ref. 21.

DISCUSSION

The studies of this report show the γ complex is a DNA-dependent ATPase. The best DNA effector was primed DNA of natural sequence and coated with SSB. The β subunit showed no detectable ATPase with or without DNA. However, the γ complex and primed DNA yielded approximately 3-fold more ATPase activity in the presence of β than in its absence. Presumably, β stimulated the ATPase activity of the γ complex, although the possibility that β becomes an ATPase in the presence of the γ complex and primed DNA can not be ruled out. Study of the γ, δ, δ′ subunits and γδ6′ subassembly of the γ complex showed none of these alone were significant ATPases. However, mixing experiments showed both γδ and γδ′ were DNA-dependent ATPases. Only the γδ ATPase was stimulated by β. Furthermore, only γδ′ was active in reconstitution of the processive polymerase with β and α, although δ′ stimulated γδ in the replication assay.

These results suggest the function of the γ complex is to recognize DNA and couple ATP hydrolysis to clamp β onto the DNA as shown in Fig. 7. In the first diagram of Fig. 7, the γ complex is shown on the ss/dsDNA-primed junction with both ATP and β bound to it. Evidence for ability of the γ complex to bind ATP and primed DNA is its ATPase activity which depends on DNA and is maximally stimulated by primed DNA. Evidence the γ complex on primed DNA binds β is the stimulation of the γ complex ATPase by β in the presence of primed DNA. These experiments do not address whether the γ complex first binds DNA and then β, or whether the γ complex first binds β and then DNA.
early steps are under investigation. Furthermore, the natural primed template for the γ complex is a RNA-DNA heteroduplex made by primase during replication of the lagging strand. Thus, studies of the exchange of an RNA primer terminus from primase to the γ complex are necessary to further understand the role of the γ complex in lagging strand DNA synthesis.

In the second diagram of Fig. 7, the γ complex hydrolyzes ATP to clamp onto the duplex portion of the DNA. The subunit that binds the ATP coupled to the β clamp reaction must be γ, δ, or β since only these three are needed to form the preinitiation complex (20). The apparent lack of ATPase activity in β implies either γ or δ is the ATP-binding subunit. It is known that γ binds ATP tightly, making γ a favorite candidate (30). Similar studies of δ are precluded by the inability to obtain large amounts of δ. However, ultraviolet light cross-links ATP to both γ and δ subunits in the holoenzyme (28). Hence, unambiguous assignment of the subunit which binds the ATP to clamp β to DNA will require further study. Previous studies have shown the holoenzyme binds ATP (3) but does not hydrolyze it until it is presented with a primed template (5). Hence, the ATP hydrolysis step is at the point of the ternary complex of DNA-γ complex-β and not at an earlier stage requiring only one or two of the components.1 After transfer of β to DNA, the γ complex dissociates from the β-DNA clamp freeing it to transfer yet other β molecules to DNA (2, 11, 33). The β clamp slides freely along duplex DNA (33). The β sliding clamp binds the core polymerase, thereby tethering it to the template for highly processive DNA synthesis (33).

The γ complex and β accessory proteins of the holoenzyme are functionally analogous to the accessory proteins of bacteriophage T4 polymerase and human polymerase δ. The phage T4 DNA polymerase (gene 43 protein) has three accessory proteins which utilize ATP and confer processivity onto the polymerase (31). The phage T4 gene 44/62 accessory protein complex, like γδ, is a DNA-dependent ATPase stimulated by 3′ ends and is further stimulated by the T4 gene 45 accessory protein (like β) (13). The T4 system appears to lack the ATPase analogous to γδ and the proteins analogous to χ and ψ. Likewise the human δ polymerase is stimulated in processive synthesis by its accessory proteins, the multiprotein activator 1 (RF-C complex), and the PCNA protein (14, 32). Activator 1 (RF-C), like the γ complex, has five subunits (15), is a primed DNA-stimulated ATPase, and is stimulated additionally by PCNA (accessory protein with analogous function to β) (14, 15). The apparent conservation in function of polymerase accessory proteins spanning the spectrum from E. coli to humans suggests the basic solution to problems of replicating duplex DNA have gone unchanged throughout evolution.

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Addendum—Lee Fradkin and Arthur Kornberg have also studied the ATPase properties of the γ complex and its interaction with β. Their conclusions are similar to those described here.

REFERENCES

1. Kornberg, A. (1982) *1982 Supplement to DNA Replication*, pp. 512–512, Freeman Publications, San Francisco.
2. Maki, S., and Kornberg, A. (1988) *J. Biol. Chem.* 263, 6561–6569.
3. Burgers, P. M. J., and Kornberg, A. (1982) *J. Biol. Chem.* 257, 11468–11475.
4. Fay, P. J., Johansson, K. O., McHenry, C. S., and Bambara, R. A. (1981) *J. Biol. Chem.* 256, 976–983.
5. Burgers, P. M. J., and Kornberg, A. (1982) *J. Biol. Chem.* 257, 11474–11475.
6. O’Donnell, M., and Kornberg, A. (1985) *J. Biol. Chem.* 260, 12875–12883.
7. Maki, H., and Kornberg, A. (1988) *J. Biol. Chem.* 263, 11987–11992.
8. Schaefermann, R. H., and Echols, H. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 7747–7751.
9. McHenry, C. S., and Crow, W. (1979) *J. Biol. Chem.* 254, 1748–1753.
10. Maki, H., Maki, S., Ikekken, R. S., and Kornberg, A. (1986) in *Mechanisms of Replication and Recombination* (Kelly, T., and McMacken, R., eds.) Vol. 47, pp. 63–73, Alan R: Liss, Inc., New York.
11. Wickner, S. (1976) *Proc. Natl. Acad. Sci. U. S. A.* 73, 3511–3515.
12. O’Donnell, M. (1977) *J. Biol. Chem.* 262, 16558–16565.
13. Mace, D. C., and Alberts, B. M. (1984) *J. Mol. Biol.* 177, 279–293.
14. Tsurimoto, T., and Stillman, B. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 1023–1027.
15. Lee, S.-H., Kwong, A. D., Pan, Z.-Q., and Hurwitz, J. (1991) *J. Biol. Chem.* 266, 594–602.
16. Weiner, J. H., Bertsch, L. L., and Kornberg, A. (1975) *J. Biol. Chem.* 250, 1921–1930.
17. Studwell, P. S., and O’Donnell, M. (1990) *J. Biol. Chem.* 265, 1171–1178.
18. Maki, S., and Kornberg, A. (1986) *J. Biol. Chem.* 261, 6547–6554.
19. Johansson, K. O., Haynes, T. E., and McHenry, C. S. (1986) *J. Biol. Chem.* 261, 11460–11465.
20. O’Donnell, M., and Studwell, P. S. (1990) *J. Biol. Chem.* 265, 1179–1187.
21. Maki, S., and Kornberg, A. (1986) *J. Biol. Chem.* 261, 6555–6560.
22. Bradford, M. M. (1976) *Anal. Biochem.* 72, 248–254.
23. Ray, D. S. (1969) *J. Mol. Biol.* 43, 631–641.
24. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual*, pp. 90–91, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
25. Rowen, L., and Kornberg, A. (1978) *J. Biol. Chem.* 253, 758–764.
26. Studwell, P. S., Stuckenberg, P. T., Onrust, R., Skangalis, M., and O’Donnell, M. (1990) *UCLA Symp. Mol. Cell. Biol. New Ser.* 127, 153–164.
27. Deleted in proof.
28. Biwas, S. B., and Kornberg, A. (1984) *J. Biol. Chem.* 259, 7990–7993.
29. Deleted in proof.
30. Tsuchihashi, Z., and Kornberg, A. (1989) *J. Biol. Chem.* 264, 17790–17795.
31. Nossal, N. G., and Alberts, B. M. (1984) in *Bacteriophage T4* (Mathews, C., Kulzer, E., Moisig, G., and Berget, P., eds.) pp. 11–71, American Society for Microbiology, Washington, D.C.
32. Wei, S.-H., and Hurwitz, J. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 5672–5676.
33. Studenberg, P. T., Studwell-Vaughan, P. S., and O’Donnell, M. (1991) *J. Biol. Chem.* 266, 11325–11334.

5. L. Fradkin and A. Kornberg, manuscript in preparation.
6. L. Fradkin and A. Kornberg, personal communication.