Replication forks formed in the absence of the \( \tau \) subunit of the DNA polymerase III holoenzyme produce shorter leading and lagging strands than when \( \tau \) is present. We show that one reason for this is that in the absence of \( \tau \), but in the presence of the \( \gamma \)-complex, leading-strand synthesis is no longer highly processive. In the absence of \( \tau \), the size of the leading strand becomes proportional to the concentration of \( \beta \) and inversely proportional to the concentration of the \( \gamma \)-complex. In addition, the \( \beta \) in the leading-strand complex is no longer resistant to challenge by either anti-\( \beta \) antibodies or poly(dA):oligo(dT). Thus, \( \tau \) is required to cement a processive leading-strand complex, presumably by preventing removal of \( \beta \) catalyzed by the \( \gamma \)-complex.

The replication fork of Escherichia coli is extraordinarily processive. Two replication forks form at oriC and synthesize roughly 2.2 megabase pairs of DNA before they meet again in the terminus region. The enzymatic machinery at the replication fork accomplishes this task while supporting two distinct modes of DNA synthesis. Whereas the leading strand is synthesized in a continuous fashion that reflects the overall processivity of the replication fork, the lagging strand is synthesized discontinuously in short Okazaki fragments 2 kb in length (1). This issue is compounded by the fact that the mechanism responsible for maintaining the processivity of the replicative polymerase on either strand is the same, a complex between the \( \beta \) subunit and the polymerase core of the DNA polymerase III holoenzyme (Pol III HE) (2, 3).

The core (4), the catalytic polymerase/exonuclease subassembly of the Pol III HE, is essentially a distributive enzyme, synthesizing only a few nucleotides per primer binding event (5). For conversion to a processive enzyme, the core must be clamped onto DNA by associating with the \( \beta \) subunit (2, 3), a dimer that encircles double-stranded DNA (6, 7). \( \beta \) can be loaded onto the primer terminus via the action of five other HE subunits that themselves associate, forming the \( \gamma \)-complex (\( \gamma \), \( \delta \), \( \delta' \), \( \chi \), and \( \psi \)) (8).

Using rolling circle DNA replication supported by a tailed form II DNA template (TFII) and the \( \psi \)X-type primosomal proteins (PriA, PriB, PriC, DnaT, DnaB, DnaC, and DnaG), the single-stranded DNA-binding protein (SSB), and either bona fide Pol III HE or HE reconstituted from purified subunits, we have reconstituted the coordinated leading- and lagging-strand synthesis of the E. coli replication fork (9-13). These replication forks were shown to have processivities of at least 0.5 megabase, yet they also made Okazaki fragments whose average length was about 1.8 kb (9). This suggested that the required protein elements for establishing processivity and triggering lagging-strand polymerase cycling were present.

The mechanism for inducing polymerase release on the lagging strand is yet to be established firmly. We have proposed that protein-protein interactions between a primase synthesizing the primer for the next Okazaki fragment and the lagging-strand polymerase initiates termination of synthesis and keys recycling of the polymerase to the new primer (11, 13). In the bacteriophage T4 system, Hacker and Alberts (14) have argued that the lagging-strand polymerase will dissociate spontaneously from the gene 45 protein clamp when it hits the 5'-end of the previous Okazaki fragment. Stukenberg et al. (15) have made similar arguments for the E. coli fork.

Challenging experiments have shown that both the polymerase and helicase on the leading-strand side of the fork are processive (16). Our recent studies have demonstrated that protein-protein interaction between the \( \tau \) subunit of the Pol III HE and DnaB is required to mediate rapid replication fork movement. We show here that \( \tau \) contributes in yet another way to processivity on the leading strand by protecting \( \beta \) in the leading-strand polymerase complex from being removed by the action of the \( \gamma \)-complex.

MATERIALS AND METHODS

Reagents, DNAs, and Enzymes

NTPs, dNTPs, and poly(dA) were from Pharmacia Biotech Inc. \([\alpha-32P]dATP \) was from Amersham, Bio-Gel A-150m was from Bio-Rad. Oligo(dT)\(_{20}\) was synthesized using an Applied Biosystems 380A DNA Synthesizer and was used after gel purification. Alkaline phosphatase was from Boehringer Mannheim. DNAs from bacteriophages \( \phi\)A-Y7M and \( \phi\)IR229-A33 were prepared as described previously (17).

Replication Proteins

SSB was prepared according to Minden and Marians (18). PriA, PriB, PriC, DnaT, DnaB, DnaC, and DnaG were prepared according to Marians (19). Pol III HE subunits and subassemblies were prepared as follows: core (4) and to be described, \( \psi \)X (22).

Rolling Circle DNA Replication

TFII DNA template was prepared as described by Mok and Marians (16) using [\(^{3}H\)JTP. Rolling circle reaction mixtures (12 \( \mu \)l final volume)

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**RESULTS**

Replication Forks Formed in the Absence of \( \tau \) Synthesize Shorter Leading and Lagging Strands—In the course of our studies on the roles of the subunits of the Pol III HE at the replication fork, we noted that replication forks formed in the absence of \( \tau \) synthesized shorter leading and lagging strands than those reconstituted with \( \tau \) (Fig. 1). We found that \( \tau \) played a central role in ensuring the generation of a processive replication fork. A protein-protein interaction between \( \tau \) and the replication fork helicase DnaB was required to mediate rapid fork movement 2 and, as described here, in the absence of \( \tau \), the leading-strand side of the fork becomes nonprocessive.

The processivity of the E. coli replication fork in vitro is reflected in its high rate of speed (9, 16), the inaccessibility of \( \beta \) on the leading strand to challenge (16, 23), and the insensitivity of the length of the leading strand to the concentration of \( \beta \) (9). Forks that lacked \( \tau \) and free \( \gamma \)-complex were still processive. 2 We examined whether this held true in the presence of added \( \gamma \)-complex as well.

The length of the leading strand synthesized by \( \tau \)-less replication forks was dependent on the concentration of the \( \beta \) subunit (Fig. 2). This is strikingly different from the situation in the presence of \( \tau \) where, although overall DNA synthesis is dependent on \( \beta \), the size of the leading strand is independent of the \( \beta \) concentration (9). This is consistent with the leading-strand \( \beta \)-core complex needing to form only once for synthesis of a long continuous DNA product. This observation suggested that multiple \( \beta \) subunits were required in the absence of \( \tau \) in order to synthesize the leading strand. That is, \( \beta \) was being cycled in and out of the leading-strand complex.

The only group of proteins available in these reactions that could affect \( \beta \) loading onto 3'-ends was the \( \gamma \)-complex. It followed that it was the \( \gamma \)-complex that was removing \( \beta \) from the leading-strand complex as well. If this were true, then the size of the leading strand synthesized in the absence of \( \tau \) should also be affected by the concentration of the \( \gamma \)-complex. This proved to be the case.

In the presence of \( \tau \), the concentration of the \( \gamma \)-complex had no effect on leading-strand synthesis (Fig. 3A), whereas in the absence of \( \tau \), the size of the leading strand synthesized decreased progressively as the concentration of \( \gamma \)-complex in-

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**Fig. 1. Replication forks formed in the absence of \( \tau \) synthesize shorter leading and lagging strands.** Standard rolling circle replication reactions in the presence of primase and either in the presence or absence of \( \tau \) as indicated were performed, processed, and analyzed, as described under “Materials and Methods.” Total DNA synthesis in the reactions shown here were 186 pmol + \( \tau \) and 19 pmol - \( \tau \) of \([\alpha^{-32}P]dAMP\) incorporated into acid-insoluble product.

**Fig. 2. The concentration of \( \beta \) affects the size of the leading strand at \( \tau \)-less replication forks.** Standard rolling circle replication reactions performed in the absence of \( \tau \) and primase and containing the indicated concentrations of \( \beta \) were processed and analyzed as described under “Materials and Methods.” 5.0 pmol, 5.2 pmol, 5.6 pmol, and 5.3 pmol of \([\alpha^{-32}P]dAMP\) were incorporated into acid-insoluble product in the reactions shown from left to right. The DNA products with electrophoretic mobilities near the 6.4-kb marker and just slower than the 9.4-kb marker result from the addition of a limited number of nucleotides to the TFII DNA template and to dimer TFII, respectively.
creased (Fig. 3). At the highest concentrations of γ-complex tested, DNA synthesis was significantly inhibited. These observations suggested that, in the absence of τ, β was being cycled on and off the leading strand by the action of the γ-complex.

Replication Forks Formed in the Absence of τ Are Nonprocessive—We used two types of challenges in order to test the processivity of replication forks formed in the absence of τ; anti-β antibody and poly(dA):oligo(dT)20. The former is, of course, specific for β, whereas the latter serves to capture β, core, and probably DnaB, all of which are normally processive on the leading strand (9, 16).

Replication forks capable of synthesizing leading strands were formed using a β-TFII DNA complex, the preprimosomal proteins (the preprimosomal proteins minus DnaG), SSB, core, and the γ-complex either in the presence or absence of τ. After 1.5 min, the poly(dA):oligo(dT)20 challenge was added. Forks formed in the presence of τ were completely resistant to the challenge (Fig. 4). On the other hand, those formed in the absence of τ were sensitive, as evidenced by the sharply reduced size of the leading-strand product (Fig. 4). This demonstrated that, in the absence of τ, at least one of the normally processive enzymatic components on the leading strand was now acting distributively. The data described above suggested that this component was β. To assess this, we repeated the challenge experiment using anti-β antibody. β becomes inaccessible to anti-β antibody once it forms an initiation complex with core (24). We used this observation previously to show that the β on the leading strand was processive, i.e. it was insensitive to the presence of the antibody (16). We repeated those experiments here using standard rolling circle replication reactions reconstituted in either the absence or presence of τ (Fig. 5). In each case, replication was inhibited if the anti-β antibody was added before initiation. However, if the anti-β antibody was added 1.5 min after initiation, only replication forks formed in the presence of τ were resistant, those formed in its absence were inhibited almost completely.

These data show that τ-less replication forks are nonprocessive because the β in the leading-strand complex has now become vulnerable to disassembly catalyzed by the γ-complex.

**DISCUSSION**

During DNA replication, the processivity of the replication fork arises from two contributing sources: the DNA helicase and the leading-strand polymerase. We have recently shown that rapid fork movement requires a physical connection between the polymerase and the helicase that is mediated by a τ-DnaB protein-protein interaction. In the absence of this interaction, the polymerase follows behind the helicase at a rate equal to the slow (ca. 40 nt/s) unwinding rate of the helicase alone, whereas upon establishing a τ-DnaB contact, DnaB becomes a more effective helicase, increasing its translocation rate by more than ten-fold. We show here that τ also contributes directly to the processivity of the leading-strand polymerase by preventing the γ-complex-catalyzed removal of β from the leading strand.

In the absence of τ, the length of the leading strand was dependent on the concentration of β and inversely proportional
and it is while the polymerase is stalled that experiments. Thus, not only is 
the leading-strand synthesis (9). This suggested that, at a τ-less replication 
fork, β, and probably core, were being reused to synthesize the 
leading strand in short bursts. The distributive nature of the 
τ-less replication fork was demonstrated directly in challenge 
experiments were conducted using Pol III* and 
lagging-strand polymerase occurred. Thus, 
τ does not prevent recycling. This suggests that, in solution, and when a dimeric HE 
subassembly binds a primer end, the polymerase bound to the 
core will synthesize full-length product in 
line with an E, anti-β antibody was added 1.5 min after initiation of 
DNA synthesis, as indicated under "Materials and Methods." The 
distinct bands that appear at about 7 and 14 kb are inactive monomer and 
dimer templates that become labeled by the addition of a few nucleotide 
residues.

to the concentration of the γ-complex. In the presence of τ, the 
concentrations of these subunits had no effect on leading-strand 
synthesis (9). This suggested that at a τ-less replication 
fork, β, and probably core, were being reused to synthesize the leading strand in short bursts. The distributive nature of the τ-less replication fork was demonstrated directly in challenge experiments. Thus, not only is τ essential for maintaining a high rate of fork movement, it is also essential for maintaining the leading-strand β-core complex in a form that is resistant to the action of the γ-complex.

This suggests that, at least on the leading strand, τ may contact β, perhaps covering a face of the protein that is essential for interaction with the γ-complex. This is consistent with the genetic finding that mutations in dnaX (encoding both τ and γ) can be suppressed by mutations in dnaN (encoding β) (24). Our recent studies show that the C-terminal domain of α binds both τ and β, thus placing them in proximity and presumably permitting direct protection. Alternatively, τ interacting core (probably via α) causes a rearrangement of the β-core complex.

It is not clear when, at a τ-less replication fork, β becomes available for attack by the γ-complex. In the absence of both τ and the γ-complex, the leading-strand β-core assembly is processive for at least 20 kb, following along on the DNA behind the slow moving helicase. It has also been shown that the combination of β and core will synthesize full-length product in a single binding event on poly(dA):oligo(dT) (5). Perhaps the τ-less leading-strand polymerase moves fitfully behind the helicase, occasionally stalling, and occasionally jumping ahead, and it is while the polymerase is stalled that β can be targeted by the γ-complex for recycling. On the other hand, in the absence of τ, β may be bound less tightly to the Pol III core, occasionally dissociating and diffusing away unidirectionally. Reassociation with the core would be rapid because of the constraints imposed by diffusion in two-dimensional space along the DNA fiber. It may be that β is removed by γ-complex while diffusing on the DNA.

In solution, τ dimerizes the core to give Pol III' (25). If this structure is similar at the fork, then the core-β complex on the lagging strand should be affected by τ in a similar fashion as the core-β complex on the leading strand. Thus, the lagging-strand polymerase should be refractory to recycling. Yet, this is clearly not the case. The lagging-strand core has been shown to cycle from the just-completed Okazaki fragment to the next primer terminus (12, 13). It seems likely, then, that mechanisms exist on the lagging strand that override the protective effect of τ.

These mechanisms could take one of many forms. Conformational rearrangements could occur upon termination of Okazaki fragment synthesis, by whatever mechanism, that expose the lagging-strand core-β to disassembly by the γ-complex. On the other hand, it may be that the protective effect of τ is specific to the leading-strand polymerase.

Stukenberg et al. (15) demonstrated that β would dissociate from core when the polymerase collided with the 5′-end of a DNA strand bound to a template, a situation similar to the lagging-strand polymerase colliding with the 5′-triphosphate end of the primer on the previous Okazaki fragment. These experiments were conducted using Pol III* and β on gapped duplex bacteriophage RF DNA. τ was present, yet disassembly of the core-β complex occurred. Thus, τ does not prevent recycling. This suggests that, in solution, and when a dimeric HE subassembly binds a primer end, the polymerase bound to the primer cannot sense, a priori, whether it is a leading- or a lagging-strand polymerase. Functional asymmetry that defines the leading- and lagging-strand polymerase may arise from a protein-protein interaction that is established between a properly oriented τ and DnaB. Establishment of this contact literally cements the replication fork together, activating the helicase and ensuring rapid fork movement, as well as causing a rearrangement that makes β refractory to the action of the γ-complex.

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