Interaction between the \( \tau \) subunit of the DNA polymerase III holoenzyme and the DnaB helicase is critical for coupling the replicase and the primosomal apparatus at the replication fork (Kim, S., Dallmann, H. G., McHenry, C. S., and Marians, K. J. (1996) Cell 84, 643–650). In the preceding manuscript, we reported the identification of five putative structural domains within the \( \tau \) subunit (Gao, D., and McHenry, C. (2000) J. Biol. Chem. 275, 4433–4440). As part of our systematic effort to assign functions to each of these domains, we expressed a series of truncated, biotin-tagged \( \tau \) fusion proteins and determined their ability to bind DnaB by surface plasmon resonance on streptavidin-coated surfaces. Only \( \tau \) fusion proteins containing domain IV bound DnaB. The DnaB-binding region was further limited to a highly basic 66-amino acid residue stretch within domain IV. Unlike the binding of immobilized \( \tau \) to the DnaB hexamer, the binding of monomeric domain IV to DnaB\(_6\) was dependent upon the density of immobilized domain IV, indicating that DnaB\(_6\) is bound by more than one \( \tau \) protomer. This observation implies that both the leading and lagging strand polymerases are tethered to the DnaB helicase via dimeric \( \tau \). These double tethers of the leading and lagging strand polymerases proceeding through the \( \tau \)-\( \tau \) link and an additional \( \tau \)-DnaB link are likely important for the dynamic activities of the replication fork.

The DNA polymerase III holoenzyme is responsible for the replication of the \textit{Escherichia coli} chromosome. Like other replicases from eukaryotes and prokaryotes, the holoenzyme contains three functional subassemblies (for reviews see Refs. 1–3): the DNA polymerase III (a\( \varphi \)\( \theta \)) core, the \( \beta \) sliding clamp processivity factor, and the DnaX complex, a clamp assembly apparatus. The DNA polymerase III core contains the \( \alpha \), \( \epsilon \), and \( \theta \) subunits and provides the polymerase function. The DnaX complex (\( \tau \)\( \gamma \)\( \delta \)\( \delta ^* \)\( \chi \)\( \psi \)) is a multiprotein ATPase that recognizes the primer terminus and loads the \( \beta \) processivity factor onto DNA.

The \( \tau \) and \( \gamma \) subunits are different translation products of the \textit{dnaX} gene (4–7). The \( \tau \) subunit plays central roles in the structure and function of the holoenzyme. It interacts with the core polymerase to coordinate leading and lagging strand synthesis (8, 9). \( \tau \) also interacts with DnaB helicase to couple the replicase with the primosome and mediate rapid replication fork movement (10, 11). These two important functions of \( \tau \) reside in C-\( \tau \), a proteolytic fragment consisting of its unique C-terminal 213 amino acid residues. \( \tau \) binds tightly to the \( \alpha \) subunit; the shorter translation product \( \gamma \) does not. C-\( \tau \) is a monomer and binds \( \alpha \) with a 1:1 stoichiometry as determined by sedimentation equilibrium analyses (12). Results from a recent study indicated that C-\( \tau \) binds DnaB, can partially replace full-length \( \tau \) in reconstituted rolling circle replication reactions, and effectively couples the leading strand polymerase with DnaB helicase at the replication fork (12). DnaB helicase is composed of six identical subunits and is a stable hexamer over a wide range of concentrations in the presence of magnesium ions (13, 14).

In the preceding manuscript, we reported that \( \tau \) comprises five potential structural domains (15). Domains I, II, and III are common to both \( \gamma \) and \( \tau \). Domain IV includes 66 amino acid residues of the C-\( \tau \) sequence and the C-terminal 17 residues of \( \gamma \). Domain V corresponds to the 147 C-terminal residues of the \( \tau \) subunit. Based on these assignments, biotin-hexahistidine-tagged \( \tau \) proteins lacking specific domains were produced. Results from binding studies employing these truncated fusion proteins indicated that the binding site of \( \tau \) for \( \alpha \) subunit lies within its C-terminal 147 amino acid residues (domain V).

The objective of this study was to determine the domain(s) of the \( \tau \) subunit involved in binding DnaB. Biotin-hexahistidine-tagged \( \tau \) proteins lacking specific domains were expressed and purified. Analysis of DnaB binding to these truncated \( \tau \) proteins by surface plasmon resonance permitted the assignment of the DnaB-binding domain of \( \tau \).

**EXPERIMENTAL PROCEDURES**

**Strains—**E. coli DH5\( \alpha \) and HB101 were used for initial molecular cloning procedures and plasmid propagation. \textit{E. coli} BL21(DE3) was used for protein expression.

**Buffers—**Buffer L, Buffer W and HKGM Buffer were prepared as previously described (15).

**Chemicals and Reagents—**SDS-polyacrylamide gel electrophoresis protein standards were obtained from Amersham Pharmacia Biotech, and precasted molecular mass markers were from Bio-Rad or Life Technologies, Inc. N\( \varnothing \)\( \varnothing ^* \)-NTA\(^1\) resin, QiAquick Gel extraction kits, QiAquick PCR purification kits, and plasmid preparation kits were purchased from Qiagen. The Coomassie Plus protein assay reagent and ImmunoPure Streptavidin were from Pierce. CM5 sensor chips (research grade), P-20 surfactant, N-hydroxysuccinimide, I-ethyl-5-(3-dimethylamino)propyl) carbodiimide, and ethanolamine hydrochloride were obtained from BIAcore Inc.

**Proteins—**Three biotin-tagged \( \tau \) proteins C(0)\( \varnothing \), C-\( \Delta \)147\( \varnothing \), and

\(^1\) The abbreviations used are: NTA, nitrotriacetic acid; PCR, polymerase chain reaction; RU, resonance unit.
The pET11 vector is under the control of the T7 promoter. N-Δ413γ as well as holoenzyme subunits were prepared as previously described (15).

Construction of the Fusion Plasmids—Plasmids PΔ1-C-Δ213γ and pETT11-N-Δ430γ were constructed to express the fusion proteins C-Δ213γ and N-Δ430γ, respectively. Fusion protein C-Δ213γ corresponds to γ, the shorter of the two potential dnaX products. In this construct, the 213 C-terminal residues found exclusively in the γ subunit are replaced by a peptide tag, which includes hexahistidine and a 15-aminoc acid residue biotinylation sequence. N-Δ430γ corresponds to C-γ, the N-terminal 430 amino acids found in both γ and γ are replaced by the hexahistidine/biotinylation tag. PΔ1 is a semi-synthetic E. coli RNA polymerase-dependent promoter containing two lac operators (16). The pET11 vector is under the control of the T7 promoter.

The starting material for construction of plasmid PΔ1-C-Δ213γ was PΔ1(C0)γ, which encodes the C-terminal tagged full-length γ protein (15). PCR primer C-213P1 (Table I) is complementary to a 110-nucleotide stretch upstream of the RsrII site within dnaX. Primer C-213P2 (Table I) corresponded to dnaX codons 423–430 preceded by a noncomplementary SpeI restriction site. PΔ1(C0)γ was digested with RsrII and SpeI. The PCR product generated by use of primers C-213P1/C-213P2 was cleaved with RsrII and SpeI and then ligated into the linearized vector to generate plasmid PΔ1-C-Δ213γ.

Primers N-430P1 and N-430P2 (Table I) and plasmid PΔ1-N-Δ1γ, which encodes an N-terminal tagged γ protein (15), were used to generate a PCR product for the construction of pETT11-N-Δ430γ. The resultant PCR product consisted of a PstI restriction site within the noncomplementary 5′ region followed by dnaX codons 431–436 and a KpnI site near the 3′ end. The KpnI site located downstream of the natural dnaX stop codon. After digestion with PstI and KpnI, the resultant 929-base pair fragment was used to replace the dnaE gene of vector pETT11-N0 (16) to produce plasmid pETT11-N-Δ430γ.

Growth and Induction of Expressing E. coli Strains—E. coli strain BL21 (DE3) containing the expression plasmids pETT11-N-Δ430γ or PΔ1-C-Δ213γ was grown at 37 °C in 2 and 6 liters, respectively, of F medium (17) containing 100 µg/ml ampicillin. Cells were induced with isopropyl-β-D-thio-galactoside, biotin-treated, and harvested as described (15).

Protein Purification—The procedures for purification of C-Δ213γ and N-Δ430γ were similar to those described for other truncated γ fusion proteins (15). Briefly, induced cells (14 g for C-Δ213γ or 22 g for N-Δ430γ) were lysed in the presence of lysozyme (2.5 mg/ml), EDTA (5 mM), and phenylmethylsulfonyl fluoride (1 mM) for 2 h at 4 °C and 6 min at 37 °C. For purification of C-Δ213γ, 0.226 g of ammonium sulfate was added to each milliliter of the resulting supernatant, and the precipitate was collected by centrifugation at 23,300 g for 1 h. The pellets were then resuspended in Buffer L. Each suspension was mixed with 1 ml of Ni2+-NTA resin and pre-equilibrated with Buffer L, and the slurries were then packed into 1 ml columns. Columns were washed with ~30 column volumes of buffer W containing 23 mM imidazole. Bound proteins were then eluted with buffer W containing 150 mM imidazole in a single step. 13 mg of C-Δ213γ were obtained in the preparation used for these studies. The purification of N-Δ430γ was as that for C-Δ213γ except that: 1) the supernatant proteins were precipitated with 65% ammonium sulfate, 2) 3 ml of pre-equilibrated Ni2+-NTA resin were used, 3) the columns were washed with buffer W containing 10 mM imidazole, and 4) elutions were effected by a 10–100 mM imidazole gradient in buffer W. 61 mg of purified N-Δ430γ were obtained in the preparation used for these studies.

Surface Plasmon Resonance—A BIACore instrument was used for protein-protein binding studies. Research grade CM5 sensor chips were used in all experiments. Streptavidin was captured onto sensor chips by N-hydroxysuccinimide/1-ethyl-3-[3-dimethylaminopropyl]carbodiimide coupling as previously described (15). The biotinylated γ proteins were then injected over the immobilized streptavidin sensor chip. Binding analyses of γ to DnaB (0.025–1 µM) were performed in HKGM buffer at 20 °C. Kinetic parameters were determined using the BIAlcalulationTM2 software.

Other Procedures—DNA polymerization assays, protein determinations, and SDS-polyacrylamide gel electrophoresis were performed as described in the preceding paper (15).

Results

Expression and Purification of the Truncated γ Fusion Proteins—The γ subunit binds to DnaB helicase and is the only subunit within the holoenzyme shown to interact with DnaB (10). The unique C terminus of γ (C-γ) bound DnaB in a coupled immunoblotting method (12). To confirm this observation and more precisely map the DnaB binding region of γ, a series of truncated γ proteins lacking specific domains were produced, and their interactions with DnaB helicase were quantified using BIACore methodology. The γ fusion proteins employed in this study included C(0)γ (domains I-V), C(Δ147)γ (domains I-IV), C(Δ213)γ, which was equivalent to γ (domains I-III plus 17 amino acids of domain IV), N-Δ413γ (domains IV and V), and N-Δ430γ, which was equivalent to C-γ (the C-terminal 66 residues of domain IV plus domain V in its entirety). The truncated terminus of each fusion protein was tagged with a peptide containing both a hexahistidine sequence to aid in purification as well as a short biotinylation sequence. The biotinylation sequence enabled oriented immobilization of the fusion proteins onto BIACore sensor chips via biotin-streptavidin binding. C(0)γ, C(Δ147)γ, and N-Δ413γ were expressed and purified as previously described (15). C(Δ213)γ and N-Δ430γ were expressed in the BL21 (DE3) strain by induction with isopropyl-β-D-thio-galactoside and reached similar expression levels (2–5% of total cell proteins). Both C(Δ213)γ and N-Δ430γ were purified by Ni2+-NTA affinity chromatography. After Ni2+-NTA purification, C(Δ213)γ was obtained at 80% purity, and N-Δ430γ at 90% purity as determined by SDS-polyacrylamide gel electrophoresis analysis (Fig. 1). The activities of the fusion proteins were ascertained by their ability to replace γ or γ in DNA polymerase III reconstitution assays (15). The specific activity of C(Δ213)γ was 5.5 × 10⁶ units/mg, similar to that of full-length C(0)γ(5.7 × 10⁶ units/mg). As expected, no holoenzyme reconstitution activity was detected for N-Δ430γ, which lacks the γ sequence required for assembly of the β processivity factor on DNA.

DnaB Binding to γ Proteins Containing Domain IV—The interaction between DnaB and C(0)γ was first characterized via use of BIACore technology. C(0)γ (2025 RU) was immobilized onto a streptavidin sensor chip. DnaB solutions of varying concentrations were passed over the immobilized C(0)γ, and binding activity was monitored (Fig. 2A). Attempts to fit the dissociation phase to a single first-order dissociation equation were unsuccessful, suggesting that a more complex mechanism was operative. To simplify the kinetic analysis, a limited interval (35–125 s following the starting point of dissociation) was analyzed from each binding curve and fit to a model in which two simultaneous independent dissociation processes occur. The two apparent dissociation rate constants koff major and koff minor (Table II) corresponded to 70–80% and 20–30% of the dissociating species, respectively. koff major was used to calculate the apparent association rate (k on). The apparent Kd was calculated from k on and k off. The interaction between DnaB and C(0)γ had an apparent Kd of 4 nM (Table II). Under the conditions employed in these studies, DnaB is known to exist as a

### TABLE I

Oligonucleotides used for construction of truncated γ fusion proteins

<table>
<thead>
<tr>
<th>Oligonucleotide number</th>
<th>Use</th>
<th>Sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-213p1</td>
<td>C-Δ213γ</td>
<td>TAGACATTGCGCTGCTGAGCTG</td>
</tr>
<tr>
<td>C-213p2</td>
<td>C-Δ213γ</td>
<td>GGCATACATGCTTITGTCTGTTGCTCC</td>
</tr>
<tr>
<td>N-430p1</td>
<td>N-Δ430γ</td>
<td>AACTGCAAGAGATGAACCGCGAACCC</td>
</tr>
<tr>
<td>N-430p2</td>
<td>N-Δ430γ</td>
<td>CTGGCATGGGAGACCCACAC</td>
</tr>
</tbody>
</table>

* The underlined regions are complementary to the DnaX gene sequence on the template.
hexamer (14), and C(0)τ is a tetramer (18). The binding ratio of the DnaB hexamer (DnaB₆) to the C(0)τ tetramer [C(0)τ₄] was 0.72, indicating that these multimers likely interact with a 1(DnaB₆): 1[C(0)τ₄] stoichiometry.

C-D²₁₃τ, equivalent to C-terminally tagged γ, was captured onto the streptavidin-derivatized sensor chip (3400 RU), followed by injection of DnaB (1 mM). No interaction between C-D²₁₃τ and DnaB was detected (Fig. 2B), consistent with the previous finding that γ does not interact with DnaB helicase (10).

Next, DnaB samples (0.05–0.5 mM) were injected over immobilized C-D¹⁴⁷τ (2860 RU) (Fig. 2C). An apparent $K_d$ of 5 nM was obtained, which is similar to that of the C(0)τ-DnaB interaction (Table II). This suggests that C-Δ147τ contains elements sufficient for binding to DnaB at the same level observed for the intact τ subunit. C-D¹⁴⁷τ (domains I-IV) bound DnaB, but C-D²₁₃τ (domains I-III) did not, localizing the region required for DnaB binding to somewhere within domain IV.

DnaB Recognizes a 66-Amino Acid Sequence within Domain IV—To confirm that domain IV was the DnaB-binding domain, N-D⁴₃⁰τ (1200 RU) was captured onto a BIAcore sensor chip, and its interaction with DnaB was assessed (Fig. 3A). The dissociation phase did not fit to a single first-order dissociation equation, so the binding data were fit to the model that assumes two parallel dissociation processes. The apparent $K_d$ was about 45 nM, which is similar to that of the interaction between DnaB and C(0)τ (Table II). The sum of these results indicates that the DnaB binding site is located within the unique C-terminal 66 residues of the τ subunit.

The C-terminal 17 amino acid residues of domain IV are lacking in N-Δ430τ. To investigate whether these 17 residues provide additional binding energy for the τ-DnaB interaction, DnaB binding studies using fusion protein N-Δ413τ were per-
formed. However, the DnaB/N-Δ413r interaction was characterized by an apparent $K_d$ of 5 nM, which is similar to that observed for the interaction of DnaB with N-Δ430r (Fig. 3B and Table II). Thus, it is unlikely that the C-terminal 17 residues of domain IV contribute significantly to DnaB binding interactions.

More than One $\tau$ Protomer Binds a DnaB Hexamer—In the preceding experiment, the binding ratio of DnaB$_6$ to the monomeric N-Δ430r was less than 0.1. This value is significantly different from 0.72, the observed ratio for the interaction between DnaB$_6$ and C(0)$\tau$. One potential underlying cause of the low binding ratio for the former interaction is the binding of DnaB$_6$ to more than one immobilized N-Δ430r molecule. To test this hypothesis, we examined the interactions of DnaB (1 μM) with sensor chips bearing differing amounts of N-Δ430r (146 RU-2700 RU). The corresponding densities of the six different levels of N-Δ430r tested are shown in Table III. Binding of DnaB to immobilized N-Δ430r at 146 RU was not observed. Increased binding ratios of DnaB to N-Δ430r were observed for surfaces bearing greater densities of N-Δ430r (Fig. 4). If we assume that each DnaB$_6$ binds two (N-Δ430r)$_2$ molecules, then the binding ratio of DnaB to N-Δ430r is increased from 0.04 to 0.24 within the range of the amount of immobilized N-Δ430r tested (Table III). The same apparent dissociation and association rate constants for the DnaB and N-Δ430r interaction were obtained at different N-Δ430r density as reported in Table II. These results are consistent with the multivalent binding of DnaB and N-Δ430r. The observed $K_d$ is the product of the individual $K_d$ values for single site binding interactions. No binding was observed at low N-Δ430r density, suggesting that the monomeric $\tau$-DnaB$_6$ interaction is too weak to be observed with the BIAcore methodology. The apparent $K_d$ values of the DnaB/N-Δ430r interaction and DnaB/C(0)$\tau$ interaction were the same, suggesting that the interaction between DnaB and C(0)$\tau$ is also multivalent; more than one C(0)$\tau$ monomer binds each DnaB$_6$. The binding ratio between DnaB and N-Δ413r was also N-Δ413r density-dependent and increased with increased immobilized N-Δ413r density (data not shown).

To ensure that the observed binding ratio of the hexameric C(0)$\tau$ to the tetrameric C(0)$\tau$ was not density-dependent, the binding ratio of DnaB$_6$ to C(0)$\tau$ was examined at an increased density (4262 RU) of C(0)$\tau$ on a sensor chip. In a previous experiment, 2025 RU of C(0)$\tau$ was used (Fig. 2A), and a binding ratio of 0.72 DnaB$_6$ to C(0)$\tau$ was observed. The C(0)$\tau$ concentrations in these two different experiments corresponded to 568
and 270 μM of C(0)τ as monomer, within the density range of N-4340τ used in the density dependence experiment (Table III). The observed binding ratio of DnaB₆ to C(0)τ was 0.69, which was not significantly different from the ratio obtained when using with 2025 RU of C(0)τ (Table III).

DISCUSSION

In the preceding paper, we detailed our use of limited proteolysis studies to identify five putative structural domains of the τ protein (15). Domains I–III are common to both τ and γ. Domain IV is composed of 17 amino acid residues from the C-terminal end of γ plus 66 amino acids from the unique C terminus of τ. Domain V is located at the C-terminal end of τ. One function of τ is to bind DnaB, coupling the holoenzyme with the primosome at the replication fork. C-τ, the unique C terminus of τ, bound DnaB in a coupled immunoprecipitation method (12). In reconstituted rolling circle replication reactions, C-τ can partially replace full-length τ in coupling the leading strand polymerase with the DnaB6 helicase at the replication fork (12).

This study further defined the DnaB binding domain of τ by analyzing the interactions of DnaB with several truncated τ proteins. N-Δ1413τ, N-Δ4340τ, C-Δ1417τ, and C(0)τ bound DnaB with similar apparent Kᵦ values. Because complicated binding kinetics were operative, the apparent Kᵦ values we obtained in this study were not the true constants. However, the resulting apparent Kᵦ values presumably contain the same systematic errors and therefore permit a quantitative comparison of relative affinities. The relative binding affinities of the different τ fusion proteins for DnaB indicate that τ amino acid residues 431–496 are sufficient for DnaB binding. This 66-residue stretch corresponds to the C-terminal portion of τ domain IV.

Although similar apparent Kᵦ values were obtained for the interactions of DnaB₆-C(0)τ and DnaB₆-(N-Δ430τ), the binding ratios for the DnaB₆-(N-Δ430τ) were density-dependent. We conclude that more than one N-Δ430τ monomer is required to bind DnaB₆ and that the interactions between DnaB and τ involved multivalent binding. Thus, the true microscopic Kᵦ for binding of DnaB₆ to a single N-Δ430τ was too weak to observe using a BIAcore. At higher N-Δ430τ densities, binding was observed between DnaB₆ and two or more N-Δ430τ molecules; the observed macroscopic Kᵦ is roughly equal to the product of each of the constituent microscopic Kᵦ values.²

² Assuming that the 8 nM apparent Kᵦ resulted from (N-Δ430τ)₆ DnaB₆ interactions, the affinity between DnaB and each monomeric and N-Δ430τ were in the 900 μm range provided that there was no cooperativity involved (8 μm)^6 = 900 μm. This low (900 μm) affinity range is consistent with the lack of detected interaction.

N-Δ430τ would be the high density dependence of binding of DnaB. The left panel shows the interactions of DnaB with six N-Δ430τ derivatized sensor chips: A, 146 RU; B, 368 RU; C, 720 RU; D, 1156 RU; E, 1998 RU; F, 2700 RU, respectively. A streptavidin-derivatized sensor chip lacking the bound N-Δ430τ provided a blank control, and the subtracted data are shown. DnaB at 1 μM diluted in HKGM buffer was injected over the six N-Δ430τ-immobilized sensor chips for 4 min at 5 μl/min. The schematic at right indicates that the density dependence of binding on a sensor chip is so low that only one N-Δ430τ molecule binds each DnaB₆ molecule, the resultant interaction is too weak to be observed using this methodology. However, at higher densities of immobilized N-Δ430τ, multiple N-Δ430τ molecules bind each DnaB₆ molecule, and the resultant interaction is strong enough to be detected.

FIG. 5. Both the leading and lagging strand polymerases couple the DnaB helicase via τ at the replication fork. The dimeric τ protein binds the leading and lagging strand polymerases through domain IV. The two domain IVs of the dimeric τ protein also bind a hexameric DnaB molecule at the replication fork. This double polymerase tether proceeding through τ-τ and τ-DnaB interactions helps keep the lagging strand associated with the replication fork and may serve to help target the dissociated lagging strand polymerase to the next primer synthesized at the replication fork.

Consistent with this interpretation, the number of DnaB₆ binding to a BIAcore chip surface increases with the density of immobilized N-Δ430τ. Increases in N-Δ430τ density would result in increased numbers of N-Δ430τ molecules becoming located within each DnaB₆ binding sphere. The DnaB₆ binding sphere is a function of the diameter of the distance between two binding sites within each DnaB₆ molecule. Within each binding sphere, a certain number (n) of N-Δ430τ molecules can be accommodated; n is equal to the maximum potential binding stoichiometry of N-Δ430τ to DnaB₆.

Recently, a model for quantifying the principal aspects of multivalent binding was developed (19). We used this model to estimate the probability of more than one N-Δ430τ molecules binding DnaB simultaneously. The proportion of spheres containing a given number of N-Δ430τ molecules was calculated assuming a binomial distribution. The DnaB-binding sphere was defined as the volume within which binding of the DnaB by two N-Δ430τ molecules can occur, and it was calculated using the following equation: Vₛ = 4/3πR^3, where D is the distance between two binding sites on DnaB. The DnaB hexamer is a cyclic structure and contains six chemically identical subunits (14, 20, 21). Based on hydrodynamic and electron microscopic studies, the cyclic structure of the DnaB hexamer has an outside diameter of ~140 Å and an inner channel of ~40 Å.

FIG. 4. N-4445 density dependence of binding of DnaB. The left panel shows the interactions of DnaB with six N-Δ430τ derivatized sensor chips: A, 146 RU; B, 368 RU; C, 720 RU; D, 1156 RU; E, 1998 RU; F, 2700 RU, respectively. A streptavidin-derivatized sensor chip lacking the bound N-Δ430τ provided a blank control, and the subtracted data are shown. DnaB at 1 μM diluted in HKGM buffer was injected over the six N-Δ430τ-immobilized sensor chips for 4 min at 5 μl/min. The schematic at right indicates that if the N-Δ430τ density on a sensor chip is so low that only one N-Δ430τ molecule binds each DnaB₆ molecule, the resultant interaction is too weak to be observed using this methodology. However, at higher densities of immobilized N-Δ430τ, multiple N-Δ430τ molecules bind each DnaB₆ molecule, and the resultant interaction is strong enough to be detected.

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expected DnaB binding sphere would be in the range from $4/3 \pi (40 \text{Å})^3$ to $4/3 \pi (140 \text{Å})^3$ (268–11480 nm$^3$, respectively).

If we assume that the interaction between $\tau$ and DnaB involves two N-Δ430$\tau$ molecules, the calculated DnaB binding sphere is 2500 nm$^3$, within the possible range for an interaction between two $\tau$ protomers and DnaB$_6$.

The notion that two $\tau$ protomers bind each DnaB hexamer is consistent with the presence of a $\tau$ dimer at the replication fork (Fig. 5). $\tau_2$ functions to dimerize the DNA polymerase III core to enable simultaneous synthesis of leading and lagging strands. We already know that the leading strand polymerase is tethered to DnaB (12). The findings presented in this report indicate that the same DnaB molecule couples both of the leading and lagging strand polymerases. Thus, a double tethers exists between the leading and lagging strand polymerase, one is through the $\tau$-$\tau$ link and the additional one through the $\tau$-DnaB link. This second tether might help keep the lagging strand associated with the replication fork and may serve to help re-target the dissociated lagging strand polymerase to the next primer synthesized at the replication fork (Fig. 5). Our mapping results demonstrate that the DnaB helicase binds $\tau$ domain IV and that the polymerase $\alpha$ subunit binds domain V (15). These findings indicate important roles that the C terminus $\tau$ plays in DNA synthesis.

REFERENCES