Mechanism of Loading the *Escherichia coli* DNA Polymerase III β Sliding Clamp on DNA

*BONA FIDE PRIMER/Templates Preferentially Trigger the γ Complex to Hydrolyze ATP and Load the Clamp***

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The *Escherichia coli* DNA polymerase III γ complex clamp loader assembles the ring-shaped β sliding clamp onto DNA. The core polymerase is tethered to the template by β, enabling processive replication of the genome. Here we investigate the DNA substrate specificity of the clamp-loading reaction by measuring the pre-steady-state kinetics of DNA binding and ATP hydrolysis using elongation-proficient and deficient primer/template DNA. The ATP-bound clamp loader binds both elongation-proficient and deficient DNA substrates either in the presence or absence of β. However, elongation-proficient DNA preferentially triggers γ complex to release β onto DNA with concomitant hydrolysis of ATP. Binding to elongation-proficient DNA converts the γ complex from a high affinity ATP-bound state to an ADP-bound state having a 10-fold lower affinity for DNA. Steady-state binding assays are misleading, suggesting that γ complex binds much more avidly to non-extendable primer/template DNA because recycling to the high affinity binding state is rate-limiting. Pre-steady-state rotational anisotropy data reveal a dynamic association-dissociation of γ complex with extendable primer/templates leading to the diametrically opposite conclusion. The strongly favored dynamic recognition of extendable DNA does not require the presence of β. Thus, γ complex uses ATP binding and hydrolysis as a mechanism for modulating its interaction with DNA in which the ATP-bound form binds with high affinity to DNA but elongation-proficient DNA substrates preferentially trigger hydrolysis of ATP and conversion to a low affinity state.

In the absence of processivity proteins, synthesis by DNA polymerases is relatively inefficient because of repeated DNA dissociation and rebinding events. Sliding clamps alleviate this problem by encircling DNA and tethering polymerases to the DNA template to dramatically enhance the processivity of synthesis. The *Escherichia coli* β sliding clamp is composed of two identical crescent-shaped subunits that form a ring in solution (1, 2). The clamp does not spontaneously load onto DNA but requires the activity of a clamp loader for assembly. Clamp loaders perform the mechanical task of opening clamps and depositing them on DNA templates (3). The binding and hydrolysis of ATP drive this assembly reaction.

The *E. coli* clamp loader is composed of five different subunits, three copies of the dnaX gene product (γ and/or τ), δ, δ’, χ, and ψ (4–7). The dnaX gene produces two polypeptides, a full-length gene product, τ, and a truncated gene product, γ, approximately two-thirds the length of τ (8–10). The C-terminal region of τ interacts with the α subunit of the polymerase and supports the formation of a DNA polymerase III holoenzyme complex in *vitro* that contains two core polymerases and a clamp loader consisting of τγδδ’χψ (11–13). Both τ and γ contain ATP binding sites and are capable of functioning in fully active clamp-loading complexes referred to as the (τγδδ’χψ) and (γδδ’χψ) complexes, respectively (5, 14). ATP binding by the clamp loader induces a conformational change that exposes the binding sites for the β clamp (15) and DNA (16). Hydrolysis of ATP is coupled to the release of the clamp on DNA and most probably produces a conformational change that masks binding sites for β and DNA (17, 18). Thus, ATP binding and hydrolysis modulate γ complex-β and γ complex-DNA interactions during the clamp-loading cycle.

This paper addresses a key question regarding the *E. coli* clamp-loading reaction. How does the *E. coli* clamp loader target the β clamps to the correct sites on DNA? Ideally, DNA replication would be most efficient if the clamp loader assembled clamps onto primed templates at primer 3’ ends. This DNA substrate specificity could be achieved if the clamp loader had a high affinity for ss/ds1 junctions at primer 3’ ends. However, the DNA polymerase must also bind to these ss/ds junctions so the clamp loader would compete with the polym-

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1 The abbreviations used are: ss/ds, single-stranded/double-stranded; p/t(s), primer/template(s); PBP, *E. coli* phosphate-binding protein; MDCC-PBP, PBP covalently labeled at Cys-197 with N'-[2-(1-maleimidoethyl)glycyl]-5-diethylaminoxycoumarin-3-carboxamide; RhX, X-rhodamine; ATPγS, adenosine 5’-O-(thiotriphosphate); DTT, dithiothreitol; BSA, bovine serum albumin.
erase for binding and reduce the efficiency of DNA synthesis. This competition would be particularly detrimental on the lagging strand where clamps must be loaded for every one- to two-kilobase Okazaki fragment that is synthesized. The clamp loader does in fact have a high affinity for ss/ds DNA junctions at primer 3’ ends, but these sites trigger a change in the γ complex causing it to release DNA (16). The clamp loader then exists in a state with reduced affinity for DNA. This site-induced decrease in affinity provides a mechanism that prevents the clamp loader from competing with the polymerase for loaded clamps. It also could provide a dynamic mechanism for recognition of sites for loading β where only the appropriate sites trigger the loading reaction. In this paper, we investigate the DNA structural features required to trigger γ complex to release the clamp and DNA and show that the presence of an elongation-proficient p/t DNA substrate is absolutely necessary to trigger γ complex to hydrolyze ATP and release the clamp.

### EXPERIMENTAL PROCEDURES

#### Enzymes—
All of DNA polymerase III proteins were purified, and γ complex was reconstituted as described previously (1, 5) and stored in 20 mM Tris-HCl, pH 7.5, 2 mM DTT, 0.5 mM EDTA, and 10% glycerol. Assay buffers for all of the experiments contained 8 mM MgCl2 in 20 mM 392 DNA synthesizer using standard chemistry and reagents from Glen Research (Sterling, VA). DNA was purified by denaturing polyacrylamide gel electrophoresis. A 105-nucleotide template with the sequence shown as follows was used in all of the experiments: 5’-GAG CGT CAA GAT GTA GGT ATT TCC A 3’.

#### Amino modifiers—
Amino modifiers were incorporated into this template to allow for site-specific labeling with X-rhodamine isothiocyanate (catalog number X-491, Molecular Probes) as described previously (19, 20). These amino modifiers were positioned at three separate sites for control experiments to demonstrate that binding results were not affected by the position of the X-rhodamine (RhX) probe. Two modifiers were located on T at positions 26 and 39 (amino modifier C2dT, Glen Research) indicated by lowercase letters, and the third modifier was located on the 5’-terminal hydroxyl (amino modifier C6, Glen Research).

Three 30-nucleotide primers that are complementary to different sites on this 105-mer (see Fig. 1) were annealed separately by incubating 1.2 equivalents of primer with 1 equivalent of template in 20 mM Tris-HCl, pH 7.5, and 50 mM NaCl at 80 °C for 5 min and then slowly cooling to room temperature. Annealed p/t’s were used without further purification because we previously demonstrated that a small excess of primer had no effect on loading reactions (16).

#### Steady-state Fluorescence Anisotropy Measurements—
Steady-state anisotropy measurements were taken using a QuantaMaster QM-1 fluorometer (Photon Technology International, London, Ontario, Canada) as described previously (16). Titration experiments were performed by the addition of a constant volume of γ complex solution to a cuvette containing a solution of RhX-labeled DNA and assay buffer. Assays contained variable amounts of γ complex and 50 mM RhX-labeled DNA and 0.5 mM ATP in assay buffer. Binding experiments were performed with RhX probes located at three different sites, and the results were not influenced by the site of labeling (data not shown). The binding of γ complex to a mixture of labeled and unlabeled DNA was initiated by the addition of ATP as described previously (16). After the addition of ATP, solutions contained 50 mM RhX-labeled ss 50-mer, 400 mM γ complex, 0–500 mM unlabeled competitor DNA, and 0.5 mM ATP in assay buffer.

#### Pre-steady-state Fluorescence Anisotropy Measurements—
Assays were performed using a BIOMIC SPM-4 stopped-flow (Molecular Kinetics, Pullman, WA) equipped with four independently driven reagent syringes and a 30-μl cuvette (model FC-15) with a 1.5-mm path length as described previously (16). One stopped flow syringe was loaded with 480 mM γ complex, 0.5 mM ATP, and 1 μM β dimer (when present) in assay buffer. The second syringe was loaded with 100 mM DNA and 0.5 mM ATP in assay buffer. Reactions were initiated by mixing 80 μl of γ complex solution with 80 μl of DNA solution at a flow rate of 10 ml/s at 20 °C. The reaction dead time under these mixing conditions was 3.7 ± 0.7 ms as determined using the method described by Peterman (21). Vertically and horizontally polarized emission intensities were measured at 1-ms intervals, and 16–24 stopped flow runs were signal-averaged. Raw anisotropy data were fit to sums of exponentials and used in kinetics simulations. Data shown in Fig. 3 have been smoothed over 3 data points. As a control, pre-steady-state experiments with the 3’ center p/t were done with the probe at three different sites to demonstrate that the results were not influenced by the site of labeling (data not shown).

#### Pre-steady-state ATPase Assays—
E. coli phosphate-binding protein covalently labeled at Cys-197 with N-[2-(1-maleimidyl ethyl)-7-diethyl-

#### RESULTS

### Binding of γ Complex to DNA Substrates with Primers Located at Different Sites—
The anisotropy of RhX covalently attached to DNA substrates was used to “report” on binding interactions with the γ complex. In these experiments, the increase in anisotropy of RhX in the presence of increasing concentrations of γ complex was measured under steady-state binding conditions. Three partially duplex DNA substrates were made by annealing three separate primers, 30 nucleotides in length, to different sites on a 105-nucleotide-long template (see Fig. 1). Each of these DNAs were covalently labeled with RhX at position 5 of a template T located 39 nucleotides from the 5’ end. Two substrates, the 5’-blunt p/t and center p/t, have 5’-template overhangs and support primer extension by a DNA polymerase (elongation-proficient). The third substrate, 3’-blunt p/t, positions the primer 3’ end at a blunt duplex end and cannot be extended by a DNA polymerase (elongation-deficient).

When γ complex (0–1600 nM) was titrated into a solution containing 50 nM DNA substrate and 500 μM ATP, a relatively large increase in anisotropy was observed for the 3’-blunt p/t substrate, whereas smaller increases were observed for the 5’-blunt and center p/t substrates (Fig. 1). These results seem to indicate that the apparent affinity of γ complex is greater for the 3’-blunt p/t substrate that is not extendable by DNA polymerases. However, earlier work (16) with the center p/t substrate revealed that γ complex does in fact bind with high affinity to this substrate under pre-steady-state conditions. This interaction with the center p/t substrate converts γ complex from a high affinity to a low affinity binding state so that

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[Further text and references are included in the original document.]

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only a small population of DNA is bound at steady state.

**Competition Binding Assays**—Competition binding assays were used to measure the ability of each of the p/t substrates to compete with a single-stranded DNA substrate for γ complex binding. Increasing concentrations of unlabeled p/t competitor were added to a solution of γ complex (400 nM) and ss 50-mer DNA (50 nM) covalently labeled with RhX. As the concentration of competitor DNA was increased, the fraction of γ complex bound to the ss 50-mer decreased in each case (Fig. 2). However, the 5'-blunt and center p/ts more effectively competed with the ss 50-mer for γ complex than the 3'-blunt p/t substrate. These results apparently contradict those of direct binding assays (Fig. 1), which suggested that γ complex binds the 5'-blunt and center p/ts more weakly. Together, these steady-state binding assays indicate that the γ complex interacts with the 5'-blunt and center p/t substrates in the similar manner but interacts differently with the 3'-blunt substrate.

**Kinetics of γ Complex Binding to Different DNA Substrates**—Pre-steady-state anisotropy measurements were made to examine the dynamic nature of the binding interactions between γ complex and various p/t DNA substrates including ss DNA. In the absence of β clamp, initial rapid increases in anisotropy indicated that γ complex bound rapidly to each of the four DNA substrates. However, two different types of binding kinetics were observed for elongation-proficient and deficient substrates. For the elongation-proficient 5'-blunt p/t (Fig. 3A, −β) and center p/t (Fig. 3B, −β) substrates, a rapid increase in anisotropy over the first 50 ms was followed by a slower decrease in anisotropy over approximately the next 250 ms. At steady state in the absence of the β clamp, the anisotropy was barely above that of free DNA (light gray), indicating that only an extremely small fraction of DNA was bound. These binding kinetics are consistent with a reaction cycle in which an activated γ complex rapidly binds DNA; the interaction with DNA inactivates γ complex, converting it to a state with reduced affinity for DNA; and the γ-complex slowly reverts back to the activated DNA binding state (16).

In contrast, time courses for binding reactions with the elongation-deficient 3'-blunt p/t (Fig. 3C, −β) and ss 105-mer DNA (Fig. 3D, −β) showed a rapid increase in anisotropy but little or no decrease in anisotropy. The reaction with the 3'-blunt p/t showed a slight decrease in anisotropy but not nearly the magnitude observed for the 5'-blunt or center p/t DNA. The higher anisotropy values seen in the steady-state regime of these reactions indicate that a greater fraction of DNA was bound by γ complex. These DNA substrates do not seem to efficiently trigger the γ complex to release DNA and convert to a low affinity DNA binding state.

This dynamic interaction with elongation-proficient DNA substrates is not an artifact of performing assays in the absence of the β clamp but part of the clamp-loading reaction. Similar results were obtained in loading reactions where the β clamp was present. In assays containing β and the elongation-proficient 5'-blunt (Fig. 3A, +β) and center (Fig. 3B, +β) p/t DNA, a rapid increase in anisotropy attributed to βγ complex binding followed by a slower decrease attributed to the release of the clamp on DNA and dissociation of γ complex was observed. Because the clamp has been loaded onto DNA, anisotropy values remain higher at steady state than in reactions...
without the clamp. In reactions with the 3'-blunt p/t (Fig. 3C, +β) and ss 105-mer (Fig. 3D, +β), a rapid increase in anisotropy to a constant value was observed, which is consistent with an equilibrium binding interaction of the βγ complex with these DNA substrates rather than DNA-triggered release of the clamp on DNA. Time courses for reactions containing elongation-deficient DNA substrates and ATP (Fig. 3, C and D) resemble those done previously with elongation-proficient DNA substrates in the presence of non-hydrolyzable ATPγS where clamp release does not occur (23). The magnitude of the increase in anisotropy was greater in reactions with β than without β. This difference in amplitudes is consistent with the model presented below where an equilibrium population of approximately 40% of the γ complex is present in a conformation that is active for DNA binding in the absence of β.

Kinetics of ATP Hydrolysis in the Presence of Different DNA Substrates—The anisotropy data show that DNA substrates suitable for polymerase extension induce a change in γ complex that reduces its affinity for DNA, whereas elongation-deficient DNA substrates do not. The kinetics of ATP hydrolysis in assays with each of the four DNA substrates were measured to determine whether they also differed for extendable DNA substrates compared with non-extendable substrates. A real time fluorescence-based assay was used to quantitate the concentration of inorganic phosphate released on hydrolysis of ATP (24).

This assay uses E. coli phosphate-binding protein (PBP), which avidly binds inorganic phosphate. When PBP is covalently labeled with a coumarin fluorophore (MDCC-PBP), phosphate binding produces an increase in fluorescence of the probe.

DNA substrates capable of supporting synthesis by a DNA polymerase gave different time courses for ATP hydrolysis than those that could not. In assays without the β clamp, the first turnover of ATP was rapid relative to the steady-state turnover for the extendable 5'-blunt and center p/ts (Fig. 4A). The first turnover was biphasic with the combined amplitudes indicating that three ATP molecules were hydrolyzed for every molecule of γ complex present. The kinetics of ATP hydrolysis in assays with the non-extendable 3'-blunt p/t and ss 105-mer differed significantly. They lacked a "burst" of ATP hydrolysis and showed only a linear increase in P_i release at a rate of 0.92 μmol/s.

The addition of β to assays containing the extendable 5'-blunt and center p/ts increased the overall rate of the first ATP turnover by γ complex (Fig. 4B). Three molecules of ATP (3.2 based on amplitudes) per molecule of γ complex were hydrolyzed in a single rapid phase taking approximately 150–200 ms to complete compared with 600–700 ms in the absence of β (Fig. 4A). The increased rate was attributable primarily to the disappearance of the slower second pre-steady-state phase seen in assays with γ complex only. No burst and a linear increase in

FIG. 3. Kinetics of γ complex binding to DNA and loading β clamps on DNA. Solutions of γ complex were pre-incubated with ATP in the presence or absence of the β clamp prior to the addition of each of the three p/t DNA substrates and a ss 105-mer substrate. Binding kinetics are plotted in separate graphs for the 3'-blunt p/t (A), the center p/t (B), the 3'-blunt p/t (C), and the ss 105-mer (D) where each graph shows three plots of anisotropy as a function of time. In each case, the dark gray plot showing the greatest increase in anisotropy is free a DNA control generated by the addition of buffer only. Smooth black lines through the data represent empirical fits to the sums of exponentials. Final concentrations were 240 nM γ complex, 50 nM DNA, 500 μM ATP, 8 mM MgCl₂, and 500 μM β clamp when present in 20 mM Tris/HCl, pH 7.5, 50 mM NaCl, 40 μg/ml BSA, and 5 mM DTT. The three p/t substrates (A–C) were labeled with RhX on the template on T at position 26, and the ss 105-mer was 5'-end-labeled with RhX.
with the release of DNA, pre-steady-state kinetics of DNA binding and ATP hydrolysis were measured in assays under identical reaction conditions. The center rather than 5'-blunt p/t DNA was chosen for purely technical reasons to more closely examine the relationship of ATP hydrolysis to the γ complex-binding and release reactions. This center p/t is typically used in all of our assays and allows us to compare these results with those of other studies. These experiments were performed with an excess of DNA over γ complex so that every clamp loader could bind DNA and hydrolyze ATP in the first turnover.

Time courses for ATP hydrolysis and DNA binding showed similar features as those in Figs. 3 and 4. Rapid biphasic hydrolysis of ATP followed by a slower steady-state hydrolysis was observed in the ATPase assay. A concomitant rapid increase in anisotropy followed by a decrease to the level of free DNA was observed in the anisotropy binding assay (Fig. 5, A and B). A combined analysis of these two data sets allows us to determine the timing of ATP hydrolysis relative to DNA binding and release. DNA binding occurs prior to ATP hydrolysis, but ATP hydrolysis occurs prior to DNA release. A kinetic model for the binding of γ complex, hydrolysis of ATP, and release of the extendable p/t DNA including rate constants for each reaction (Fig. 5C) was used to simulate pre-steady-state anisotropy binding and ATP hydrolysis data (Fig. 5, A and B). The solid lines through the data points were obtained from simulation of this model using KINSIM (Fig. 5C) (22).

The rapid biphasic release of P_i by γ complex could be because of the presence of non-equivalent sites where individual γ subunits hydrolyze ATP at two different rates. Alternatively, the biphasic kinetics could result from two populations of γ complex in equilibrium where one population exists in a conformation that is active for DNA binding and rapidly hydrolyzes all three molecules of ATP at the same rate. The second population is initially inactive for DNA binding and ATP hydrolysis but can slowly convert to the active form, which gives rise to the slow phase of P_i release. Our model assumes the second case in which γ complex exists as an equilibrium mixture of two different conformational states as supported by unpublished data. Following ATP hydrolysis, the ADP-bound form of γ complex rapidly releases DNA.

The kinetic simulation starts with an equilibrium mixture of γ complex that is active (40% γ,T-T,T) and inactive for DNA binding (60% γ,T,T,T). The exact nature of these two species is not defined by this experiment, and it is assumed that they both contain three molecules of ATP. However, it is also formally possible that binding one or more ATP molecules converts the inactive state to active state. Under these experimental conditions where saturating ATP concentrations were used, we cannot distinguish between these two possibilities. The active state of γ complex (γ,T-T,T,T) binds DNA and hydrolyzes all three molecules of ATP sequentially at the same rate before rapidly dissociating from DNA. The inactive form of γ complex

\[ \gamma_{inactive} \]

\[ \gamma_{active} \]

\[ P_i \]

\[ \text{DNA} \]

\[ \text{ATP} \]

\[ \text{MDCC-PBP} \]

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slowly (2.7 s⁻¹) converts to the active form where it binds DNA and hydrolyzes its ATP prior to releasing DNA.

These assays (Fig. 5) provide information regarding the kinetics of the first turnover but less about subsequent turnovers where the rates of ADP release from γ complex and ATP binding come into play. For this reason, we have combined these steps into one first-order reaction where the ADP-bound form of γ complex is converted to the ATP-bound form and used...
apparent rate constants to model the steady-state portion of the reaction. This first-order approximation is a reasonable approach because the ATP concentration is saturating and <1% ATP is converted to ADP over the time course of the reaction so that the ATP concentration effectively remains constant. Finally, because P_i release could not be observed until it bound MDCC-PBP, we included rate constants for this P_i binding step (24) in our model.

DISCUSSION

The E. coli clamp loader accomplishes the mechanical task of assembling the ring-shaped β clamp onto DNA. Our results show that the clamp loader uses a dynamic mechanism to target clamps specifically to DNA substrates that can serve as templates for synthesis by DNA polymerases (Fig. 6). Primed template DNA substrates that contain a ss/ds junction with the correct polarity for extension by a DNA polymerase, which is 3’-primer end with a 5’-template overhang, efficiently trigger hydrolysis of ATP and release of DNA by the clamp loader (Figs. 3, A and B, and 4, A and B). The γ complex is also able to bind non-extendable 3’-blunt-end p/t DNA and ss DNA in either the presence or absence of β but, in contrast, fails to release β on the DNA (Fig. 3, C and D) and fails to exhibit a pre-steady-state burst of ATP hydrolysis (Fig. 4, A and B). These data are completely consistent with our previous data showing that ATP hydrolysis is also associated with release of the clamp (17). Thus, the appropriate sites on DNA for clamp assembly trigger the clamp loader to hydrolyze ATP and release the clamp on DNA. These studies provide a mechanistic explanation for earlier observations that γ complex selectively loads clamps at ss/ds junctions with a 5’-ss overhang (25).

A key result is that steady-state measurements of direct binding interactions between γ complex and different DNA substrates can be misleading by apparently demonstrating that the γ complex binds with greater affinity to DNA substrates that are not extendable by DNA polymerase. Real time anisotropy binding assays were key to uncovering the dynamic nature of the interaction of γ complex with DNA constructs that can serve as substrates for DNA polymerases. These assays show a rapid increase in anisotropy followed by a slower decrease when γ complex binds to extendable DNA substrates containing ss/ds junctions with a 5’-ss overhang (Fig. 3, A and B). A “simple” equilibrium-binding reaction of γ complex to DNA would have shown an increase in anisotropy to a value that represented the equilibrium population of DNA bound by γ complex (i.e. a simple exponential rise) as observed in assays with ss DNA alone and 3’-blunt p/t DNA containing β (Fig. 3, C and D). The decrease in anisotropy in assays with elongation-proficient p/t substrates having a 5’-ss overhang indicated that a new state of γ complex with reduced affinity for DNA forms during the binding reaction. Because the rate-limiting step in the steady-state reaction is recycling of this low affinity DNA binding state to the high affinity state (Fig. 5C), very little DNA is bound, giving the appearance that the affinity of γ complex for elongation-proficient DNA is low. DNA p/ts that are not extendable do not efficiently trigger the conversion of γ complex to the low affinity state and thus give the appearance that they are bound with greater affinity in steady-state assays. It is likely that this low affinity DNA binding state is an ADP-bound form of γ complex based on the results from ATPase assays. This dynamic DNA binding interaction is also likely to occur with the eukaryotic clamp loader (replication factor C). Stronger binding interactions with an extendable p/t were seen in steady-state assays with non-hydrolyzable ATP–γS than in assays with ATP (26).

The γ complex has long been known to have DNA-dependent ATPase activity. More recently, the ATP requirements for individual steps in the clamp-loading reaction have been defined. ATP binding but not hydrolysis is required for DNA binding activity (16, 20) and clamp binding activity (15, 27) of the γ complex. A conformational change in the clamp loader that exposes sites for binding both DNA and the clamp is most probably produced by ATP binding. The hydrolysis of ATP is required for the release of the clamp on DNA (17, 18, 20). Here we show that hydrolysis of ATP is dependent on the structural features of the DNA substrate. DNA structures that can be extended by a DNA polymerase, the 5’-blunt p/t, and center p/t efficiently trigger a pre-steady-state burst of ATP hydrolysis, whereas those that cannot be extended, the 3’-blunt and ss DNA, do not (Fig. 4).

A combined analysis of DNA binding and ATP hydrolysis assays done under identical conditions in the absence of the β clamp revealed that γ complex initially binds DNA prior to ATP hydrolysis and subsequently hydrolyzes ATP before releasing DNA. This same sequence of events occurs in assays containing β that result in a productive loading reaction (17). Our results are consistent with a model (Fig. 5C) in which the γ-complex cycles through a reaction where it has a high affinity for DNA, binds DNA, and then converts to a lower affinity state and releases DNA. The high affinity DNA binding state is an ATP-bound form of γ complex, and the low affinity state is an ADP-bound form. The kinetic parameters derived from this model suggest that the ATP-bound form of γ complex binds DNA with an affinity that is 10^7 times greater (2 nm) than the ADP-bound form (100 μM). Thus, the clamp loader uses ATP binding and hydrolysis as a means for modulating its interaction with DNA so that it has a high affinity before clamp loading and a low affinity afterward.

Taken together, these results demonstrate the γ complex uses a dynamic mechanism for targeting the β sliding clamp to template sites where DNA synthesis is slated to begin. The clamp loader binds DNA with high affinity, and those sites with an ss/ds junction of the correct polarity to be extended by a DNA polymerase efficiently trigger the clamp loader to hydrolyze ATP and release the clamp on DNA (Fig. 6). DNA-triggered hydrolysis of ATP converts the γ complex to a state most probably ADP-bound with lower affinity for DNA, thus providing a mechanism for targeting the clamp to the proper sites on DNA and for preventing the clamp loader from competing with the polymerase for primer/template ends. Coordination between the clamp loader and the polymerase is critical for DNA synthesis on the lagging strand where a clamp must be loaded for each of the 1–2-kilobase Okazaki fragments synthesized every 1–2 s by the polymerase. Pre-steady-state kinetic data show that the γ complex-catalyzed β clamp loading and release reactions occur rapidly (~12 s^-1) (20) compared with the time scale for Okazaki fragment synthesis and, therefore, are not rate-limiting for Okazaki fragment synthesis. ATP binding and hydrolysis modulate the affinity of the clamp loader for DNA and the clamp so that it has a high affinity for both before the clamp is loaded and a low affinity for both after the clamp is loaded. This prevents competition between the clamp loader and core polymerase for clamps that have just been loaded on DNA. The dynamic nature of γ complex-DNA interactions is probably a common theme in DNA replication where many enzymes are required to work at the replication fork and each must have access to the DNA at the appropriate time.

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**DNA-triggered Clamp Loading**
DNA: REPLICATION REPAIR AND RECOMBINATION:
Mechanism of Loading the <i>Escherichia coli</i> DNA Polymerase III β Sliding Clamp on DNA: BONA FIDE PRIMER/Templates PREFERENTIALLY TRIGGER THE γ COMPLEX TO HYDROLYZE ATP AND LOAD THE CLAMP

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