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Exchange between *Escherichia coli* polymerases II and III on a processivity clamp

James E. Kath[1], Seungwoo Chang[1], Michelle K. Scotland[2,3], Johannes H. Wilbertz[1], Slobodan Jergic[4], Nicholas E. Dixon[4], Mark D. Sutton[2,3,5] and Joseph J. Loparo[1,*]

[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA, [2]Department of Biochemistry, University at Buffalo, State University of New York, Buffalo, NY 14214, USA, [3]Witebsky Center for Microbial Pathogenesis and Immunology, University at Buffalo, State University of New York, Buffalo, NY 14214, USA, [4]Centre for Medical & Molecular Bioscience, Illawarra Health & Medical Research Institute and University of Wollongong, New South Wales 2522, Australia and [5]Genetics, Genomics and Bioinformatics Program, University at Buffalo, State University of New York, Buffalo, NY 14214, USA

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ABSTRACT

***Escherichia coli* has three DNA polymerases implicated in the bypass of DNA damage, a process called translesion synthesis (TLS) that alleviates replication stalling. Although these polymerases are specialized for different DNA lesions, it is unclear if they interact differently with the replication machinery. Of the three, DNA polymerase (Pol) II remains the most enigmatic. Here we report a stable ternary complex of Pol II, the replicative polymerase Pol III core complex and the dimeric processivity clamp, β. Single-molecule experiments reveal that the interactions of Pol II and Pol III with β allow for rapid exchange during DNA synthesis. As with another TLS polymerase, Pol IV, increasing concentrations of Pol II displace the Pol III core during DNA synthesis in a minimal reconstitution of primer extension. However, in contrast to Pol IV, Pol II is inefficient at disrupting rolling-circle synthesis by the fully reconstituted Pol III replisome. Together, these data suggest a β-mediated mechanism of exchange between Pol II and Pol III that occurs outside the replication fork.**

INTRODUCTION

DNA synthesis occurs in many different cellular contexts, from high fidelity genome duplication at replication forks to error-prone synthesis across from sites of DNA damage. Most organisms have multiple polymerases specialized for particular tasks, requiring proper polymerase selection and exchange.

Escherichia coli, which serves as a powerful model for deciphering the mechanism of polymerase exchange, has five DNA polymerases, polymerase (Pol) I through Pol V. The majority of chromosomal DNA synthesis on the leading and lagging strands is performed by Pol III, a heterotrimeric complex of a polymerase subunit (α), a proofreading subunit (ϵ) and a third subunit (θ) that moderately stimulates proofreading activity (1). The complex (αϵθ) is commonly referred to as the Pol III core. Pol I functions on the lagging strand during Okazaki fragment maturation (2).

E. coli additionally has two Y-family DNA polymerases, Pol IV and Pol V, which are regulated by the SOS DNA damage response. Both Pols IV and V lack proofreading domains and can perform translesion synthesis (TLS) across from bulky DNA lesions, although with different specificities (3). While these polymerases can alleviate damage-induced replication stalling, as a consequence of their TLS activity they have higher error rates on undamaged DNA relative to Pols I and III (3).

Although Pol II was the second *E. coli* DNA polymerase to be discovered, its cellular role remains enigmatic (4). It is encoded by the gene *polB*, which is non-essential (5). Since Pol II is regulated by the SOS response and has lesion bypass activity, it is considered to be a TLS polymerase (6–9). In contrast to Pols IV and V, however, it is a B-family DNA polymerase with 3′–5′ proofreading activity, two classifications that are shared by high fidelity replicative polymerases in other organisms (6,10,11). Additionally, lesion bypass by Pol II is inefficient, with 20% bypass of an abasic site analog over 30 min, for example; in comparison, Pol IV bypasses >90% of the cognate N^2-furfuryl-guanine lesion over 15 min (12–14). Additionally, *polB* mutant cells have minor or negligible survival defects when treated with DNA damaging agents (15,16). Other activities attributed to Pol II are stationary phase adaptation (17,18) and proofreading misinsertion errors, especially on the lagging strand (19).

*To whom correspondence should be addressed. Tel: +1 617 432 5586; Fax: +1 617 738 0516; Email: joseph_loparo@hms.harvard.edu
Present address: J. H. Wilbertz, Friedrich Miescher Institute of Biomedical Research, CH-4058 Basel, Switzerland.

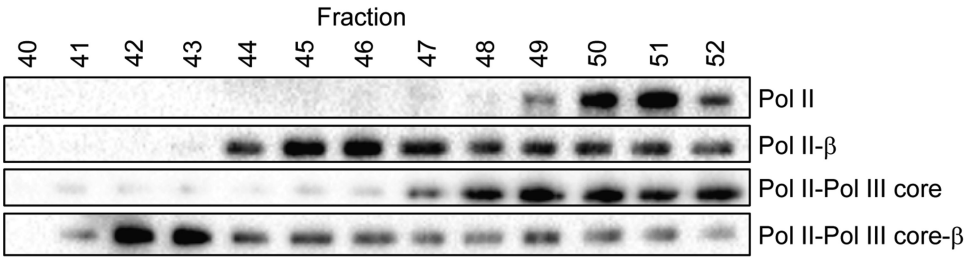

Figure 1. Complexes of Pol II (90 kDa), Pol III core (166 kDa) and the β dimer (81 kDa) were observed by incubating combinations of the three proteins, separating by size-exclusion chromatography, and blotting against Pol II in fractions. When chromatographed alone, the majority of Pol II was localized to fractions 50–52. In the presence of β clamp, 54.0% (± a range between replicates of 4.0%) of the Pol II was shifted to fractions 44–49. When incubated with Pol III core, 33.8% (± 0.6%) of the Pol II shifted to fractions 47–49, and in the presence of Pol III core and β clamp 61.7% (± 12.4%) of the Pol II was shifted to fractions 41–49, co-migrating with the majority of the Pol III core–β complex (Supplementary Figure S1).

As with the other *E. coli* polymerases, processive DNA synthesis by Pol II requires an interaction with the β sliding clamp via a clamp-binding motif (CBM) at the polymerase's C-terminus (20,21). β is a head-to-tail dimer with both monomers presenting a protein-binding cleft on the same face, inspiring the proposal that it serves as a molecular 'toolbelt' by simultaneously binding a replicative polymerase and a TLS polymerase to facilitate rapid exchange (22). This exchange may also occur between polymerases at a single cleft involving secondary polymerase–clamp (23) or polymerase–polymerase interactions (24). An alternative model is one where a single polymerase occludes binding of others, requiring the full dissociation of one followed by association of another from solution. The precise details of exchange involving the three TLS polymerases likely reflect each of their cellular roles and the need to regulate access to replication intermediates.

We and others have reconstituted competition between Pol III core and Pol IV during primer extension using biochemical (23,25–27) and single-molecule (28) approaches to show that both can simultaneously bind β and exchange. We have also shown that that the exchange of Pol III core with Pol II was less efficient than with Pol IV (29). Here, we further clarify the cellular role of Pol II by characterizing the interactions of Pol II, Pol III core and β, and Pol II–Pol III core exchange during primer extension and within the full replisome. These results support the model that Pols II and III can exchange on a single β dimer, and additionally show that Pol II has preferential access outside of replication forks.

MATERIAL AND METHODS

Protein purification

E. coli proteins were purified with published protocols and were expressed without affinity tags unless otherwise noted: Pol II, Pol II^{C} (30) and Pol IV (31); the Pol III holoenzyme subunits α, δ and δ' (32); ε and θ (33); τ and refolded ψ within the χψ complex (34); β (35) and β^{+}/β^{C}, a stable dimer purified from mixed Myc-tagged β and His_6 and heart muscle kinase-tagged β(Δ5) (36). Pol III core (αεθ) and clamp loader complex with stoichiometry $\tau_3\delta\delta'\chi\psi$ were isolated from combined subunits by ion exchange chromatography (34). Pol III core and β used in gel filtration experiments were purified with alternative protocols (30,37). The helicase DnaB (38), primase DnaG (39) and single-stranded DNA-binding protein SSB (40) were also purified as previously described.

Characterization of protein interactions by size-exclusion chromatography

Gel filtration experiments were conducted using a Superose 12 10/300 column (GE Healthcare) equilibrated in Buffer A (20 mM HEPES [pH 7.5], 0.5 mM EDTA, 150 mM NaCl). Samples consisting of the indicated proteins (Pol II, Pol III core and/or β, 1.78 nmol each in 300 μL; see legend of Figure 1) were incubated at room temperature for 10 min prior to Superose 12 filtration. Each Superose 12 filtration experiment was performed in duplicate. Aliquots of each 250 μL Superose 12 fraction were electrophoresed in 12% SDS-PAGE gels, transferred to a polyvinylidene difluoride (PVDF) membrane using the Trans-Blot Turbo system (Bio Rad), and processed as a western blot using a polyclonal Pol II antibody (1:10,000 dilution), generated by Sigma by injecting His-tagged Pol II into New Zealand White rabbits, and a secondary horseradish peroxidase goat anti-rabbit antibody (Bio-Rad, 1:50,000 dilution). Pol II was imaged using a ChemiDoc MP (Bio-Rad), equipped with Image Lab Software version 5.2.1. The total amount of Pol II present in each fraction was determined using the quantity tool feature of the Image Lab software; the mean plus or minus the range of these values between the replicates is reported.

Single-molecule polymerase exchange experiments

Single-molecule flow stretching experiments were performed as previously described (28). Briefly, 7249 nucleotide M13mp18 (New England Biolabs) single-stranded (ss) DNA was linearized at the SalI restriction site and end-labeled with 5′-digoxigenin and 3′-biotin-containing oligonucleotides. M13 substrates were attached by one end to the streptavidin-coated surface of a custom microfluidic flow cell, and on the other to a 2.8 μm diameter, anti-digoxigenin-coated paramagnetic bead.

Laminar flow was used to exert a ∼3 pN force on the bead and, therefore, uniformly throughout the DNA tether. At this force, ssDNA is entropically coiled and double-stranded (ds) DNA is nearly extended to its crystallographic length, a length contrast that allows for observation of DNA synthesis. As the primer is extended, the DNA

molecule under tension lengthens, which can be tracked by observing the motion of the attached bead. Beads were imaged by dark-field microscopy through a 10X objective (Olympus) with a QIClick CCD camera (Q-Imaging).

Primer extension reactions were performed in replication buffer (50 mM HEPES-KOH [pH 7.9], 12 mM $Mg(OAc)_2$, 80 mM KCl, 0.1 mg mL^{-1} BSA) with 5 mM DTT, 1 mM ATP, 760 μM dNTPs, 15 nM clamp loader complex (with stoichiometry $\tau_3\delta\delta'\chi\psi$), 30 nM clamp (β or β^+/β^C, as dimers and Pol II and/or Pol III core at the indicated concentrations. Primer extension experiments omitted SSB to maximize the length contrast between ssDNA and dsDNA under force. Reactions were observed for 2750 frames at 2 Hz and recorded using the MicroManager software package (www.micro-manager.com).

Single-molecule data analysis

The positions of individual beads were fit to 2D Gaussians and tracked in movies with Diatrack (Semasopht). A representative immobile bead was used to subtract drift uniformly from all tethers, and trajectories of bead displacement in nanometers (nm) were converted into the number of base pairs (bp) synthesized using the calibration factor 3.9 bp nm^{-1}. Primer extension was defined as single or multistep motion in the direction of flow. Rapid jumps perpendicular to the flow were interpreted as the bead sticking or unsticking to the surface and those trajectories were excluded.

Synthesis trajectories were fit to segmented lines with custom optimization code written in MATLAB. For synthesis steps, the processivity (the amount of primer extension per binding event) was defined as the rise of the step, and the rate was defined as the slope. To be determined significant, synthesis steps were required to have a rise of greater than 3σ of the noise, determined for each trajectory individually, but typically ∼200 bp. All other segments were defined as pauses.

A cutoff of 45 bp s^{-1} was used in experiments to distinguish significantly processive events as either Pol II (slower) or Pol III (faster). This cutoff captured 93% of Pol III events and 94% of Pol II events in experiments with each polymerase alone. Pauses between events by different polymerases were defined as the time of exchange; if no such pause resulted from trajectory fitting, the time was defined to be zero. Exchange time data sets were compared using the two-sided Wilcoxon test (using the MATLAB function *ranksum*). The Bonferroni correction was used for multiple sample comparison where applicable. Distributions for processivities, rates, and pauses were normalized and presented as probability densities by dividing the raw counts in each bin by the total summed counts and the bin width. Where applicable, fits to single exponentials were made. In experiments with Pol II alone, the first bin of the processivity and pause distributions were excluded from exponential fits due to undersampling below the experimental spatial resolution (for a more detailed discussion, see (28)).

Rolling circle synthesis experiments

A rolling-circle dsDNA template was prepared as previously described using T7 DNA polymerase (New England Biolabs) to extend a tailed oligonucleotide primer annealed to M13mp7(L2) ssDNA, synthesizing the complementary strand and generating a fork structure (41). Substrates were purified with phenol/chloroform extraction. Rolling-circle replication reactions with the *E. coli* replisome were performed as previously described (42), with: 30 nM DnaB (as hexamer), 40 nM Pol III core, 6.75 nM $\tau_3\delta\delta'\chi\psi$, 30 nM β (as dimer), 600 nM DnaG and 500 nM SSB (as tetramer); 60 μM dNTPs supplemented with α-^{32}P-labeled-dATP, 250 μM UTP, GTP and CTP, and 1 mM ATP.

The Pol III replisome was loaded onto the fork structure by mixing Pol III core, β, clamp loader and helicase with dCTP, dGTP, ATP and 375 pM DNA substrate and incubating at 37°C for 5 min. Synthesis at 37°C was initiated by adding the dATP and dTTP, SSB and primase. Ten seconds after initiation, the indicated concentrations of Pol II or Pol IV were added. Reactions were quenched after 10 min by adding 25 mM EDTA and replication products were separated on a denaturing alkaline agarose gel (0.6%). The dried gel was exposed to a phosphor screen and imaged with a Personal Molecular Imager. The image displayed in Figure 8 is representative of two experiments.

RESULTS AND DISCUSSION

Detection of a Pol II–Pol III–β ternary complex

To determine if Pol II, the Pol III core and β can form a complex, we incubated equimolar quantities of the three proteins in different combinations and isolated the resulting complexes with size-exclusion chromatography. Probing with a specific Pol II antibody allows for sensitive detection in dilute fractions (Figure 1). Coomassie blue staining was also used to verify protein co-migration (Supplementary Figure S1).

In isolation, the majority of Pol II elutes in fractions 50–52, while when incubated together, Pol II and β elute together in earlier fractions, indicative of a higher molecular weight complex. A comparison to standards suggests that Pol II and β are in a 1:1 complex (Supplementary Figure S2), in agreement with surface plasmon resonance experiments (43). When mixed with the Pol III core in the absence of β, a small shift in Pol II mobility occurs (33.8 ± a range between replicates of 0.6% in fractions 44–49), suggesting a low-affinity interaction between the two polymerases; however, when β was added, the shift is larger and more striking (61.7 ± 12.4% within fractions 41–49), corresponding to co-migration of Pol II with the Pol III–β complex (Supplementary Figure S1).

Rapid exchange of Pol II and Pol III bound to a single β dimer

Although these data demonstrate a novel ternary complex of Pol II, Pol III core and β, they do not necessarily support the model that β-mediated polymerase exchange occurs during DNA synthesis. In addition to a high-affinity β-binding CBM within its α polymerase subunit, Pol III has a second, lower affinity CBM in its proofreading subunit, ε (44–46). This second CBM is not required for processive Pol III synthesis, but it increases its processivity and rate. This has led to a proposed model that the Pol III core binds

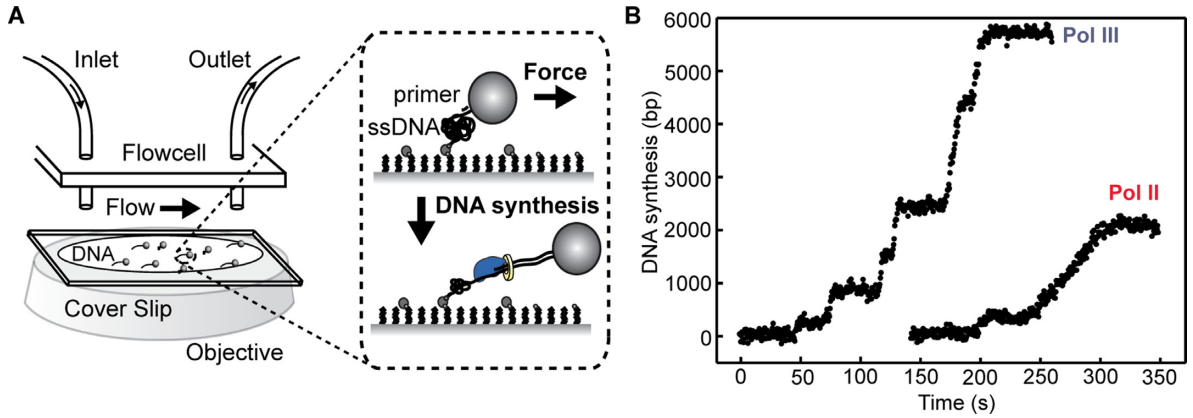

Figure 2. (**A**) A single-molecule primer extension assay uses the differential extension of ssDNA and dsDNA molecules under tension to observe DNA synthesis. Schematic is adapted from (28). (**B**) Synthesis by Pol II or Pol III core on individual molecules, shown here in example trajectories, occurs in processive events interspersed by pauses.

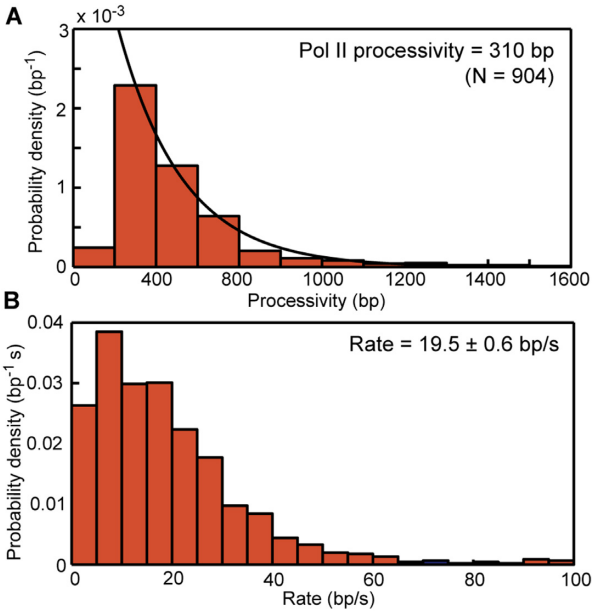

Figure 3. Primer synthesis by individual Pol II molecules. (**A**) The distribution of the processivity of synthesis steps, fit to a single exponential with a constant of 310 bp (95% confidence bounds: 250 bp, 410 bp). The first bin was excluded from the fit due to undersampling below the experimental spatial resolution (see Methods). (**B**) Rate distribution for steps, with mean and standard error of the mean (s.e.m.).

both clefts on the β dimer until a lesion-induced stall, after which the ϵ–β contact is broken, allowing for TLS polymerase access to the clamp (45). Alternatively, TLS polymerases could associate with the clamp or the Pol III core directly and capture a β-binding cleft during synthesis, allowing for a more dynamic mechanism of exchange.

To directly test these models for exchange between Pol II and Pol III core, we used a single-molecule flow stretching assay that we have previously used to study synthesis by Pol IV and Pol III core, and exchange between the two (28). In this assay, primed ssDNA templates are coupled to micron-scale beads within a microfluidic flow cell (Figure 2A). Laminar flow is used to exert a constant, ∼3 pN force on the bead which extends the ssDNA tether. The differential extension of ssDNA and dsDNA at this force results in lengthening of the tethers during primer extension, which is measured for individual molecules by observing bead displacement in dark-field microscopy.

Primer extension by Pol II or Pol III core individually in the presence of β loaded by the clamp loader occurs in processive synthesis steps interspersed by pauses (Figure 2B). The processivity (∼300 bp) and rate (19.5 bp s^{-1} on average) of Pol II (Figure 3) are in agreement with bulk biochemical measurements of primer extension (20). A direct comparison to an equivalent single-molecule analysis of Pol IV (28), and bulk experiments with Pol V (47), further reveals that Pol II is the fastest *E. coli* translesion polymerase, although all three are significantly slower than Pol III core, which can extend a primer at ∼220 bp s^{-1} (28).

Pauses observed between processive synthesis steps in single-molecule primer extension represent polymerase dissociation from β and the diffusion-limited recruitment of a new polymerase from solution, for both Pol III (28,34) and Pol II (Supplementary Figure S3). The timescale of exchange between Pol II and III should also be diffusion-limited, unless the two polymerases associate within a Pol II–Pol III core–β complex prior to exchange. In the latter scenario, the exchange timescale would likely be much faster, limited by the conformational dynamics of exchange.

To distinguish between these two models, we measured the timescale of exchange between Pol II (15 nM) and Pol III core (5 nM), concentrations at which exchange between the two polymerases readily occurs (Figure 4). The ratio of the concentrations, which influences competition between the two polymerases, was chosen to match the ratio in normal, replicating cells (48), but absolute concentrations were reduced by roughly fivefold so the diffusion-limited association of each polymerase from solution could be clearly measured. These diffusion times were determined by measuring the pause lengths in experiments with each polymerase alone and fitting to an exponential distribution (Figure 5A and B). That the association of Pol III core is faster despite a lower polymerase concentration likely reflects an increased association rate of Pol III (6.8×10^4 M^{-1} s^{-1}, for the α sub-

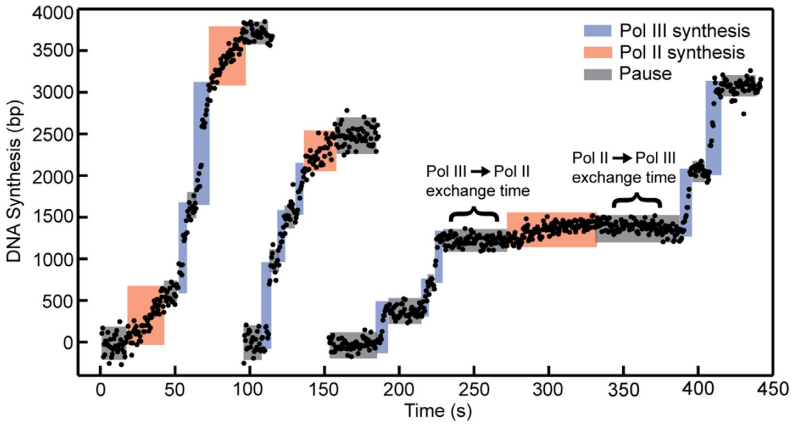

Figure 4. Examples of exchange between Pol III core (5 nM) and Pol II (15 nM) observed during synthesis on individual DNA molecules. Examples of exchange times between events by different polymerases are also highlighted.

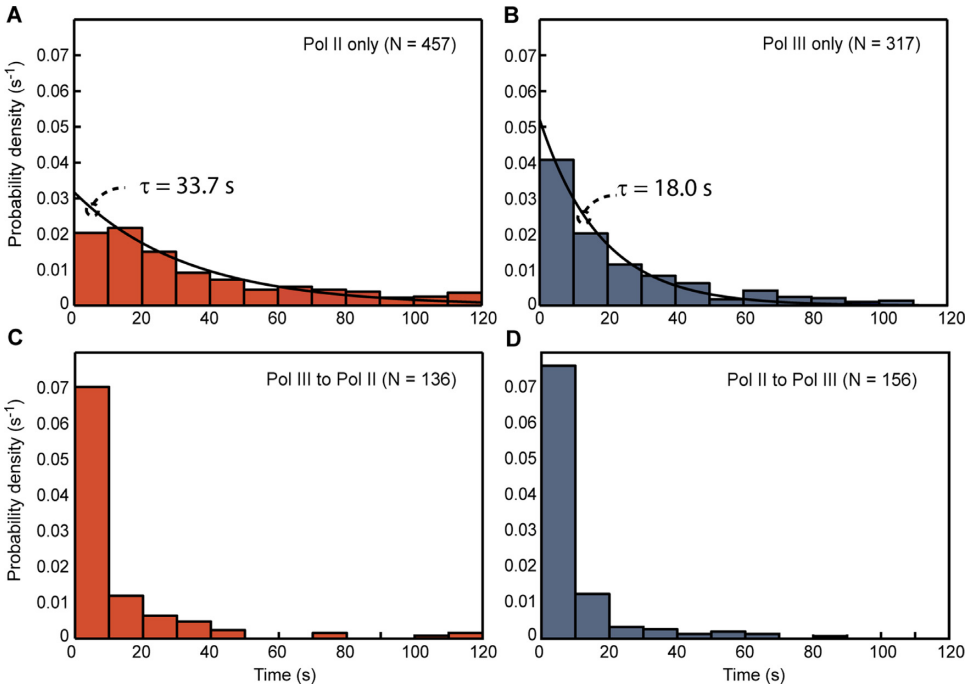

Figure 5. Quantification of exchange supports the toolbelt model for Pol II and Pol III core. Exchange by (**A**) Pol II (15 nM) or (**B**) Pol III alone (5 nM) represents dissociation of a polymerase followed by the diffusion-limited association of a new polymerase. Exchange timescales from (**C**) Pol III to Pol II or (**D**) Pol II to Pol III at matched concentrations are more rapid ($P < 0.001$), indicating β–mediated exchange.

unit) compared to Pol II (3.8×10^4 M^{-1} s^{-1}) for β clamp binding (36,43).

In comparison to the diffusion timescale of Pol II (Figure 5A), the timescale of exchange from Pol III to Pol II (Figure 5C) was significantly faster ($P < 0.001$), with the vast majority of exchange occurring in less than 10 s. These rapid rates prevented an accurate fit to an exponential distribution, and show that for exchange on most molecules, Pol II is not being recruited from solution after termination of synthesis by Pol III core. Similarly, the second half of the exchange reaction, from Pol II back to Pol III, was significantly faster than the diffusion timescale of Pol III alone ($P < 0.001$, Figure 5B versus D), also reflecting a diffusion-independent mechanism.

To determine if this rapid exchange involves Pol II associating at the second cleft of β weakly bound by the Pol III ϵ subunit, we used a mutant clamp that contains a single-binding cleft, β^+/β^C (36). For exchange with β^+/β^C from Pol III to Pol II, and from Pol II to Pol III, we found a significant increase in the timescale (Figure 6) compared to experiments with wildtype β ($P < 0.001$), supporting this model of exchange. Interestingly, the exponentially distributed exchange times with β^+/β^C remained faster than the diffusion-limited timescales. This suggests Pol II may engage in at least two modes of exchange, with the second involving capture of the β cleft tightly bound by the Pol III α subunit.

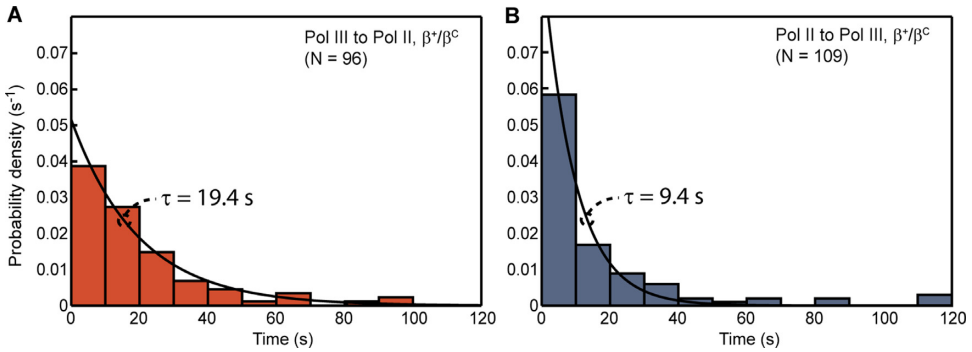

Figure 6. In experiments with the single-cleft clamp β^{+}/β^{C}, exchange from (**A**) Pol III to Pol II and (**B**) Pol II to Pol III are intermediate between a diffusion-limited timescale (Figure 5A and B) and the rapid, β-mediated exchange (Figure 5C and D) ($P < 0.001$ for all comparisons).

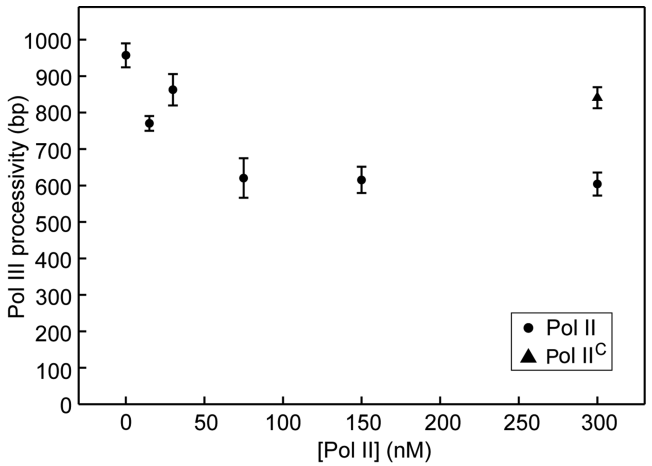

Figure 7. Increasing concentrations of Pol II significantly reduce the processivity of Pol III ($P < 0.01$ for 75–300 nM Pol II), indicative of dynamic processivity between the polymerases. Removing the Pol II cleft-binding motif in Pol II^{C} rescues the effect (*NS* versus Pol III alone). Values represent means with s.e.m., with sample sizes: 470 (Pol III alone), 848 (with 15 nM Pol II), 142 (30 nM), 38 (75 nM), 95 (150 nM), 209 (300 nM) and 384 (300 nM Pol II^{C}).

Displacement of Pol III by Pol II in primer extension but not within the full replisome

Exchange between two polymerases bound in a complex with β can either occur after the first polymerase terminates synthesis and fully dissociates from DNA, or through displacement of the first prior to termination of synthesis. In the latter case, the incoming polymerase gains a 'foothold' by binding a secondary binding surface from which it can capture a common binding site during a transient, partial dissociation of the replicating polymerase (49). In this scenario, the presence of the second polymerase at the secondary binding site can potentially lead to the premature dissociation of the first, reducing its processivity. This 'dynamic processivity' has been observed for polymerases bound to the same helicase within the T4 and T7 replisomes (50–52), and the capture of a binding cleft on β from Pol III by Pol IV following its association at a unique binding site on the β 'rim' (23,24,28,53).

To determine if dynamic processivity exists between Pol III core and Pol II, we performed single-molecule experiments with increasing concentrations of Pol II, simulating SOS induction. The ability to assign individual synthesis events to each polymerase allows us to unambiguously determine the effect of Pol II on the processivity of Pol III core, even as the relative contribution by each polymerase changes. As was previously shown for Pol IV, Pol II leads to the reduction of the Pol III processivity in a dose-dependent manner (Figure 7, $P < 0.01$). This reduction depends on the capture of a β cleft from Pol III, as Pol II^{C}, a mutant lacking the CBM, does not significantly affect the processivity distribution. As this mode of exchange disrupts Pol III synthesis, it likely represents capture of a β cleft bound by Pol III α, which is critical for synthesis (54).

To determine if Pol II has the ability to displace the fully reconstituted Pol III holoenzyme, we used a rolling-circle synthesis assay. This approach involves pre-loading Pol III core, β, the clamp loader complex $\tau_3\delta\delta'\chi\psi$, and the DnaB helicase onto a purified rolling-circle M13 template, constructed by synthesizing the complementary strand with T7 DNA polymerase using a tailed primer to generate a fork structure (41). After pre-loading, rolling-circle synthesis is initiated by adding nucleotides, including α-^{32}P-labeled-dATP, primase, and SSB; in the absence of TLS polymerases, Pol III rapidly makes several revolutions around the circular template, generating long leading strand products that are visualized following alkaline agarose gel electrophoresis. A DNA ladder was used to determine that the leading strand products were in excess of 25 kilobase pairs (kb), or more than two revolutions around the rolling-circle substrate. A template band at ~7 kb is the result of insertion by Pol III on a fraction of substrates that were incompletely filled in by T7 polymerase.

Adding Pol IV to the reaction 10 s after Pol III initiation inhibits synthesis of long leading strand products in a dose-dependent manner; in contrast, the Pol III replisome is largely resistant to inhibition by Pol II (Figure 8). A study using a minicircle template also showed that Pol IV is more efficient than Pol II at inhibiting Pol III synthesis (55), although the lack of inhibition by Pol II in this assay is more striking. The authors also demonstrated that the rate of Pol II is not increased in the presence of the helicase, which eliminates the possibility that Pol II and Pol III synthesize at the same rate in the context of the replisome.

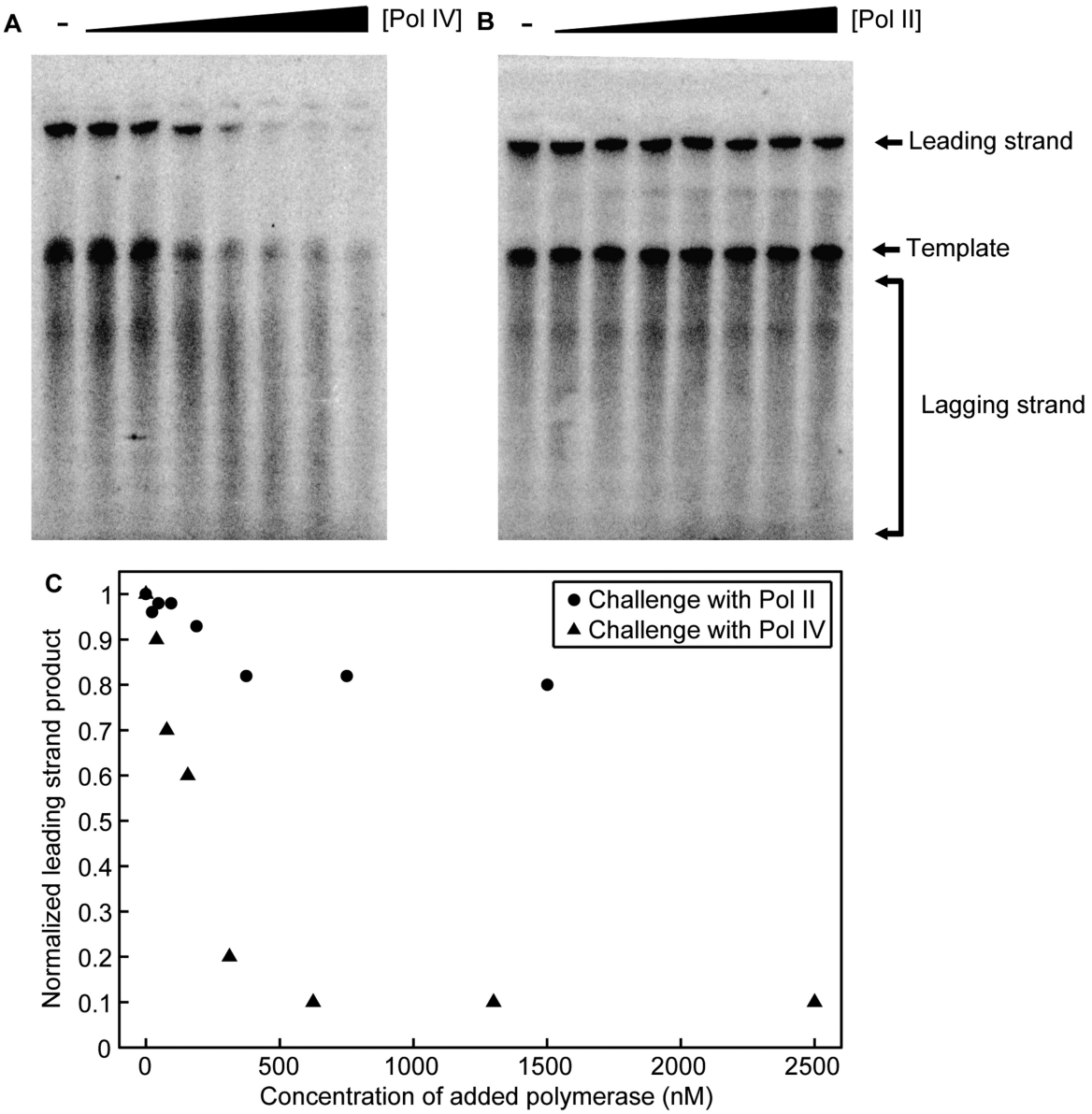

Figure 8. The fully reconstituted replisome is resistant to disruption by Pol II, but not Pol IV. Rolling circle replication by the Pol III holoenzyme is initiated, the indicated TLS polymerase is added 10 s later, and reactions are quenched after 10 min. Concentrations of polymerase added (left to right) for (**A**) Pol IV are: 0, 39, 78, 156, 312, 625, 1250 and 2500 nM; and for (**B**) Pol II: 0, 23, 47, 94, 188, 375, 750 and 1500 nM. (**C**) Quantification of the leading strand product, normalized to the intensity in the absence of challenging TLS polymerase.

While the rolling-circle assay cannot observe association of Pol II with the holoenzyme and stochastic exchange events, it shows that displacement of Pol III by Pol II is more strongly blocked than displacement by Pol IV in the context of coordinated synthesis within a replication fork. In contrast, displacement of Pol III core in primer extension experiments by either TLS polymerase occurs with comparable efficiencies (Figure 7 and (28)). These data are also consistent with the result that a roughly equivalent level of overexpression of Pol IV, but not Pol II, impedes growth in the strain lacking the Rep helicase, which makes cells more sensitive to replisome stalling (29).

Potential mechanisms of polymerase exchange and regulation

We have shown that Pols II and III form a complex with a single β dimer, a binding mode that promotes rapid exchange during primer synthesis. Our data could support several potential mechanisms (Figure 9). First, Pol II could capture a binding cleft on β from the Pol III ϵ subunit prior to exchange, which is supported by slower exchange kinetics observed with the single-cleft clamp, β^{+}/β^{C}. However, β^{+}/β^{C} does not fully eliminate rapid exchange, suggesting that Pol II can also capture the cleft bound by the Pol III polymerase subunit, α. A direct interaction between Pol II and Pol III core, suggested by our gel filtration data, or an as-yet-undiscovered secondary binding site on β, analogous

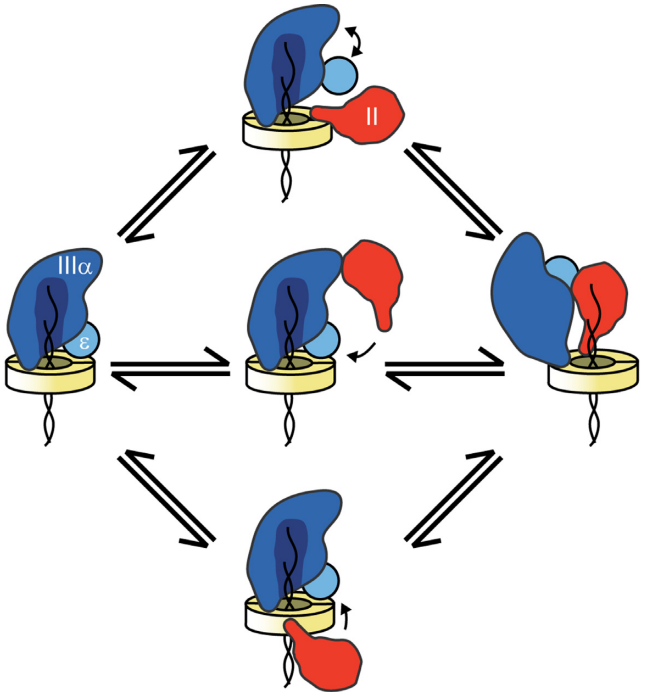

Figure 9. Potential mechanisms of exchange of Pol II with Pol III core on β. Pol II competes with the Pol III subunit ϵ directly for a β-binding cleft (top), or after an initial association with the Pol III core (middle) or a secondary binding site on β (bottom) prior to the handoff. Exchange may also occur by analogous mechanisms with the more strongly bound Pol III α subunit. The small Pol III subunit θ, which binds to ϵ, is not shown for clarity.

to the rim of the β dimer that is bound by Pol IV, could position Pol II to capture either β cleft and exchange with Pol III core. Detailed structural studies that map these interactions are needed to further clarify the mechanism of exchange.

Increasing concentrations of Pol II lead to the displacement of Pol III from primer extension reactions, suggesting that this may represent an increasing occupancy of Pol II at a lower affinity secondary binding site of the Pol III core–β complex. Binding at a secondary site could give Pol II a 'foothold,' from which it would be able to strip Pol III off the clamp through direct competition for common binding sites during transient dissociations, in particular the critical interaction of the Pol III α subunit with the binding cleft of β. Competition with Pol III for binding of this cleft would explain the requirement of the Pol II CBM.

In contrast, replication by Pol III within the full replisome significantly attenuates displacement by Pol II. Consistent with this observation, TLS by Pol II is inefficient in the presence of the stalled Pol III holoenzyme (56). This suggests that interactions with the helicase and the clamp loader complex within the full replisome that are absent in primer extension experiments either occlude secondary Pol II binding sites, or stabilize Pol III against displacement that could occur upon exchange. In a striking contrast, Pol IV remains able to displace Pol III despite a ∼15-fold lower affinity for β (43) and can readily carry out TLS in the context of the replisome (27,56). This, plus the longer time needed for expression and assembly of Pol V (57), argues that Pol IV has priority over other TLS polymerases for access to the replisome immediately following SOS induction.

Although Pol II may be excluded from the full replisome, there are other cellular contexts when it may exchange with Pol III. The Pol III primer extension reactions described here resemble incomplete Okazaki fragments that are prematurely released from the replisome before the termination of synthesis. Premature Okazaki fragment release can either occur when Pol III encounters a lesion on the lagging strand (58), or through stochastic loop release in the absence of a roadblock (59,60). A Pol III core–β complex at the resulting ssDNA gap could then exchange with Pol II or other TLS polymerases. That Pol II preferentially exchanges with Pol III within released, incomplete Okazaki fragments but not within an active replication fork is consistent with the observation that Pol II preferentially influences the fidelity on the lagging strand (19). In addition to structural studies, further clarification of the role of Pol II requires increasingly complex reconstitutions of polymerase exchange, and single-molecule imaging of Pol II dynamics.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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