Analysis of the Stimulation of DNA Polymerase V of *Escherichia coli* by Processivity Proteins†

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ABSTRACT: Bypass of replication-blocking lesions in *Escherichia coli* is carried out by DNA polymerase V (UmuC) in a reaction that requires UmuD′, RecA, and single-strand DNA-binding protein (SSB). The activity of this four-component basic bypass system is a low-fidelity and low-processivity activity. Addition of the processivity subunits of pol III, the β subunit sliding DNA clamp, and the five-subunit γ complex clamp loader increased the rate of translesion replication approximately 3-fold. This stimulation was specific to the lesion bypass step, with no effect on the initiation of synthesis by pol V. The β subunit and γ complex increased the processivity of pol V from 3 to approximately 14−18 nucleotides, providing a mechanistic basis for their stimulatory effect. Stimulation of bypass was observed over a range of RecA and SSB concentrations. ATPγS, which strongly inhibits translesion replication by pol V, primarily via inhibition of the initiation stage, caused the same inhibition also in the presence of the processivity proteins. The in vivo role of the processivity proteins in translesion replication was examined by assaying UV mutagenesis. This was done in a strain carrying the *dnaN59* allele, encoding a temperature-sensitive β subunit. When assayed in an excision repair-defective background, the *dnaN59* mutant exhibited a level of UV mutagenesis reduced up to 3-fold compared to that of the isogenic *dnaN*$^+$ strain. This suggests that like in the in vitro system, the β subunit stimulates lesion bypass in vivo.

Genomic DNA accumulates lesions as a consequence of the action of external and internal DNA-damaging agents, like UV radiation, chemical mutagens, etc. Lesions that have escaped repair block replication, and thereby jeopardize genome integrity. When replication stops at lesions, ssDNA regions carrying the damaged nucleotides are formed. In *Escherichia coli*, these gap-lesion structures can be filled in with patches from the sister chromatids by recombinational repair, in an error-free manner. Alternatively, the gap can be filled in by DNA synthesis [translesion replication (TLR)], a process which is mutagenic due to the miscoding nature of most damaged nucleotides. Both mechanisms restore the double-stranded configuration of the DNA at the damaged site, thereby enabling a second attempt of excision repair (*1*, *2*).

The reconstitution of translesion replication with purified components (*3*, *4*) revealed that lesions are bypassed by DNA polymerase V (UmuC) in the presence of UmuD′, RecA, and SSB (*5*, *6*). Pol V can bypass a synthetic abasic site, a cyclobutyl TT dimer, and a 6−4 TT adduct, usually in a mutagenic manner (*6*, *7*). It is also highly mutagenic when replicating undamaged DNA, and preferentially forms transversion mutations (*7*, *8*). Lesion bypass by pol V requires assembly of a RecA nucleoprotein filament (*9*), which targets the polymerase to the primer terminus. SSB facilitates the formation of the RecA nucleoprotein filament and the unloading of RecA molecules (*10*), which is a necessity for pol V replication activity at both the initiation (*9*) and elongation and bypass stages (*10*). UmuD′ may serve as a bridge between pol V (UmuC) and the RecA nucleoprotein, since it interacts with both proteins, while direct interaction between UmuC and RecA was not observed (reviewed in ref *11*).

The processivity subunits of pol III, the β subunit sliding clamp and the γ complex clamp loader, were reported by Goodman and co-workers to be essential for lesion bypass by pol V (*4*, *5*). Such a requirement was not observed in our system, where significant bypass was obtained in the absence of these processivity factors (*6*, *9*, *12*). We attributed this difference to a difference in the stability of the RecA nucleoprotein filament formed on the two different substrates used in the two laboratories (*9*, *12*). A stable RecA filament could be assembled on the gapped plasmids used in our laboratory, thereby enabling effective loading of pol V on DNA, and high activity. Nevertheless, the reported effect of the processivity proteins on pol V-promoted lesion bypass raises the possibility that although not required, the β subunit and the γ complex may have a stimulatory effect on lesion bypass of pol V in our system. Another critical question is whether the processivity proteins affect TLR in vivo.

Here we show that the processivity proteins increase the level of lesion bypass 3-fold, by enhancing the processivity of pol V, and without affecting the initiation of replication. Moreover, we present evidence that the β subunit enhances in vivo TLR, as indicated by the in vivo stimulation of UV mutagenesis.

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EXPERIMENTAL PROCEDURES

Proteins. UmuD′ and the MBP−UmuC fusion (a fusion of UmuC and the maltose binding protein) were overexpressed and purified as previously described (*3*, *6*). Their degree of purity was estimated to be >95% for UmuD′ and 90−95% for the MBP−UmuC fusion. SSB, RecA, and the β subunit of pol III were purified as described previously (*13*−*15*), except that a phosphocellulose purification step was added for RecA. Their degree of purity was estimated to be >95%. The γ complex was a generous gift from M. O'Donnell. T4 DNA ligase, T4 polynucleotide kinase, and restriction nuclease were from New England Biolabs, except Asp700, which was from Boehringer Mannheim. T7 gp6 exonuclease was from Amersham, and ATPγS, creatine kinase, and creatine phosphate were from Sigma.

DNA Substrates. The preparation of the gapped plasmid GP21 carrying a site-specific lesion was described previously (*16*, *17*). GP21 contains a site-specific synthetic (tetrahydrofuran) abasic site, and a ssDNA region, approximately 350 nucleotides long. The processivity was tested on oligonucleotide-primed ϕx174 ssDNA. This DNA substrate was prepared by annealing 5.6 pmol of ϕx174 virion DNA (New England Biolabs) to 8.2 pmol of 5′-end-labeled primer in a 30 μL annealing mixture which contained 0.15 M NaCl.

Bacterial Strains. The bacterial strains used in this study were *E. coli* WBA3 *argE3 HisG4 leuB6 proA2 thr1 ara14 galK2 lacY1 mtl1 xyl5 thi1 tsx33 rpsL31 supE44 tna300*::Tn10, Δ*uvrC*::Cm, and WBA4, which is similar to WBA3, except that it is also *dnaN59*. They were constructed by P1 transduction (*18*), moving the Δ*uvrC*::Cm allele from *E. coli* CS5430 (*19*) into *E. coli* ZTR10 and ZTS10, an isogenic pair of *tna300*::Tn10 derivatives of AB1157, where ZTS10 is also *dnaN59*.

Translesion Replication Assay. The translesion replication reaction was performed as previously described (*3*, *6*), with minor changes. The standard reaction mixture (25 μL) contained 20 mM Tris-HCl (pH 7.5), 8 μg/mL bovine serum albumin, 5 mM DTT, 0.1 mM EDTA, 4% glycerol, 1 mM ATP, 10 mM $MgCl_2$, dATP, dGTP, dTTP, and dCTP (0.1 mM each), 0.1 μg (2 nM) of gapped plasmid, 50 nM SSB, 2 μM RecA, 400 nM UmuD′, and 100 nM pol V (MBP−UmuC). When indicated, 40 nM (as a dimer) β subunit and 20 nM γ complex were added to the reaction mixture. Reactions were carried out at 37 °C as follows. RecA and the DNA substrate were incubated in the assay buffer for 2 min at 37 °C in the presence of the β subunit and γ complex; then SSB was added, and reaction mixtures were incubated for an additional 4 min at 37 °C. A mixture of umuD′ and the MBP−UmuC fusion was then added, and incubation continued for the indicated periods of time. When indicated, an ATP regeneration system was included, by adding creatine phosphate (10 mM) and creatine kinase (0.2 mg/mL) to the reaction mixture. Reactions were stopped by adding 20 mM EDTA and 0.2% SDS (final concentrations) followed by heat inactivation (65 °C for 10 min), and the DNA was purified from the proteins by proteinase K digestion followed by phenol/chloroform extraction and ethanol precipitation. The purified DNA mixture was treated with calf intestine alkaline phosphatase (0.2 unit, 1 h, 37 °C) to hydrolyze the remaining dNTPs. The DNA was then digested with *Asp*700 (5 units) and *Msp*A1I (5 units). This produced radiolabeled DNA fragments 19, 30, and 47 nucleotides long, representing the unextended primer, the replication stop at the lesion, and the bypass product, respectively. Bands 17- and 18mer correspond to exonuclease activity present in the restriction enzymes. The DNA samples were fractionated by 15% PAGE with urea, followed by phosphorimager analysis (Fuji BAS 2500). The extent of bypass was calculated by dividing the amount of bypass products by the amount of the extended primers ("lesion bypass"). When indicated, the extent of bypass was also calculated differently, by dividing the amount of bypass products by the total amount of DNA [translesion replication (TLR)]. Initiation of synthesis was calculated by dividing the amount of extended primers by the total amount of DNA. The bypass figures shown below represent single experiments; however, some of them were performed twice, and they were planned to be overlapping such that reliable and coherent results were obtained.

Processivity Assay. The processivity assay was performed by analyzing the length of replication products under decreasing concentrations of pol V (*20*). Reactions were carried out with an oligonucleotide-primed ϕx174 circular ssDNA, using the same reaction conditions that were used for the translesion replication assay, and with pol V concentrations of 0.25−200 nM. After the replication step, the products were fractionated by 15% PAGE with urea, followed by phosphorimager analysis. The processivity was calculated by averaging the length of the DNA products.

In Vitro Replication Fidelity Assay. The assay was performed as previously described (*8*), in the absence or presence of the processivity proteins, β subunit at 200 nM (as dimer), and the γ complex at 100 nM.

In Vivo Mutagenesis. The mutagenesis assay system, which monitors the UV-induced conversion of histidine auxotrophy to prototrophy, was performed as previously described (*21*). An overnight culture grown at 30 °C was diluted to an OD_{595} of 0.05 and grown at 30 °C to midlog phase (OD_{595} = 0.4−0.5). Cells were washed and concentrated 5-fold in minimal medium containing histidine. Cells (1.5 mL) were UV-irradiated at the indicated UV doses in a small Petri dish (60 mm × 15 mm), after which they were incubated at either 30 or 42 °C for 1 h. After incubation, the cells were washed with 10 mM Tris-HCl (pH 7.5) and 0.15 M NaCl and plated on minimal plates containing limiting amounts of His (2 mg/L). His^+ revertants appeared after incubation for 2−3 days at 30 °C as colonies over a faint lawn of His^- cells. The total number of colonies was determined by plating the appropriate dilution of the culture on the same plate. The mutation frequency was calculated by dividing the number of mutants by the total number of colonies per plate. Each experiment was performed three times.

RESULTS

The β Subunit and the γ Complex Stimulate Lesion Bypass by Pol V. The experimental system for assaying lesion bypass was described previously (*3*, *6*). It is based on a gapped plasmid carrying a site-specific synthetic abasic site in a ssDNA region, and a ^{32}P-radiolabeled phosphate in the primer strand. TLR is assayed as the extension of the primer strand across the lesion, detected by the change in the electrophoretic mobility of the DNA, which was pretreated with

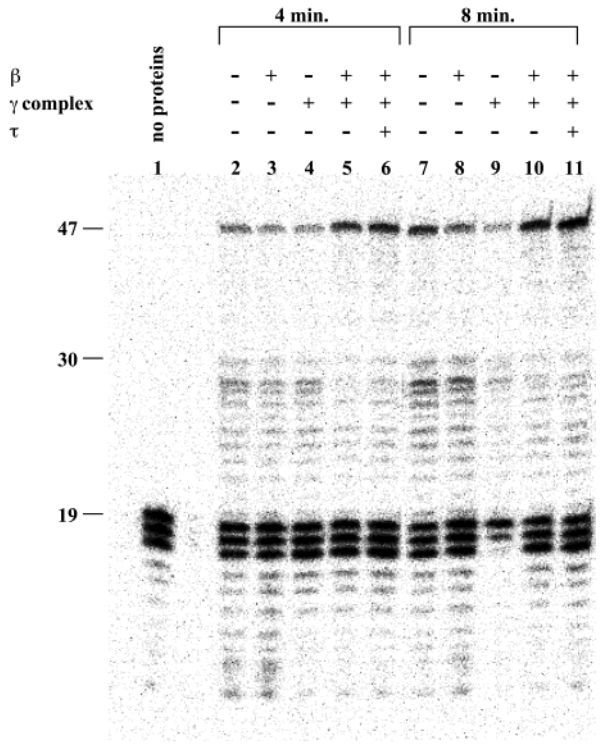

FIGURE 1: β subunit and γ complex stimulate lesion bypass by pol V. A translesion replication assay was performed as described in Experimental Procedures for 4 (lanes 2−6) or 8 min (lanes 7−11). The reactions were carried out with pol V, UmuD′, RecA, and SSB, in the presence or absence of the processivity proteins (indicated at the top of the lanes). Lane 1 shows a reaction without any proteins. Lanes 6 and 11 also included the τ subunit of the pol III holoenzyme. The reaction products were fractionated by 15% PAGE with urea followed by phosphorimager analysis: 19mer, unextended primer; 30mer, site of the lesion; and 47mer, full bypass product. The bands that are 18 and 17 nucleotides long were formed by partial excision of the primer strand during processing of the reaction products.

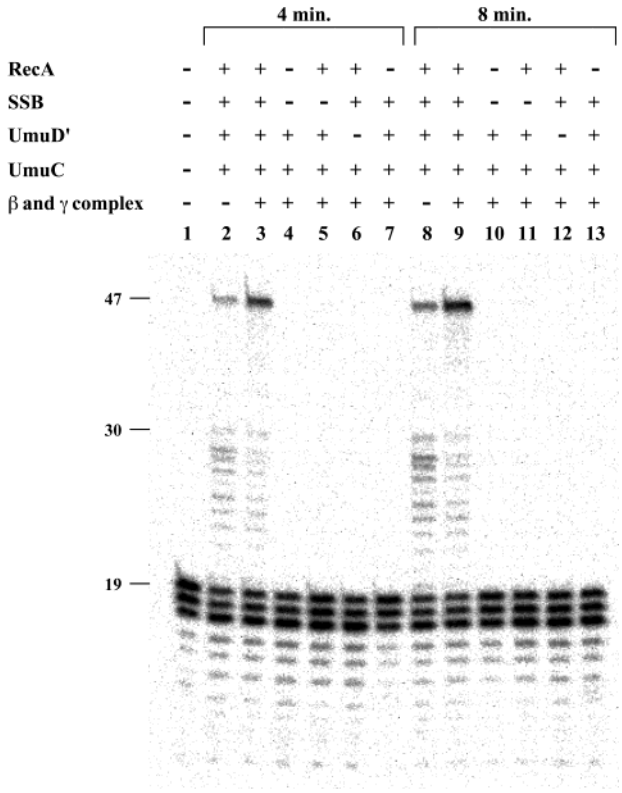

FIGURE 2: RecA, SSB, and UmuD′ are each required for TLR in the presence of the processivity proteins. A translesion replication assay was performed for 4 (lanes 2−7) or 8 min (lanes 8−13): lane 1, no proteins in the reaction; lanes 2 and 8, standard TLR reaction; lanes 3 and 9, TLR reaction in the presence of the processivity proteins; and lanes 4−7 and 10−13, TLR reaction without one or more of the accessory proteins (UmuD′, RecA, and SSB). The reaction products were fractionated by 15% PAGE with urea followed by phosphorimager analysis.

restriction nucleases to reduce the size of the products. To examine the effect of processivity proteins on lesion bypass, a TLR assay was performed with the four basic proteins, namely, pol V, UmuD′, RecA, and SSB, either with or without the β subunit and the γ complex. As can be seen in Figure 1, addition of the β subunit and γ complex stimulated bypass, as indicated by the increase in the intensity of the full bypass product (47mer). In addition, there was less pausing of synthesis at the site of the lesion (30mer) when the processivity proteins were used (Figure 1, lanes 5 and 10). No stimulation was observed when the β subunit or the γ complex was each added alone (Figure 1, lanes 3, 4, 8, and 9). No additional stimulation was observed upon addition of the τ subunit, which is responsible for dimerization of the pol III holoenzyme (Figure 1, lanes 6 and 11).

To examine whether the three pol V accessory proteins required in the basic TLR reaction are still required in the presence of the processivity proteins, a bypass experiment was performed in which UmuD′, RecA, and SSB were each omitted from the reaction, one or more at a time. As can be seen in Figure 2, each of these proteins was essential in the TLR reaction, and no polymerization was detected in their absence.

The β Subunit and the γ Complex Stimulate Lesion Bypass but Have No Effect on the Initiation of Synthesis by Pol V. The effect of the β subunit and the γ complex was examined using either a linear or a circular gap-lesion DNA substrate. As can be seen in Figure 3, the processivity proteins increased TLR on both types of substrates. They caused 3−4-fold stimulation on the linear gap-lesion substrate (Figure 3A, lanes 2, 3, 6, and 7) and an approximately 2-fold stimulation on the circular gap lesion (Figure 3A, lanes 4, 5, 8, and 9). Notice that overall TLR was higher on the circular substrate than on the linear substrate. The reason for this phenomenon might be a higher stability of the RecA filament on the circular substrate.

The gel image of the TLR reaction products contains information about both the ability to bypass the lesion and the ability to initiate synthesis at the primer. The extent of bypass is calculated as the percent of products that have transversed the lesion, out of all the synthesis events (i.e., of all the primers that were extended). Initiation of synthesis is calculated as the percentage of the primers that were extended (of all the primers). The multiplication of the two yields the percentage of the molecules on which the lesion was bypassed, or TLR. TLR can also be calculated by dividing the amount of bypass products by the total amount of primers. Figure 3B shows in graphical form the results of the experiment shown in Figure 3A (4 min time point). Remarkably, the stimulatory effect of the β subunit and the γ complex was exclusively at the lesion bypass stage. There was no effect on the initiation of synthesis by pol V, on either

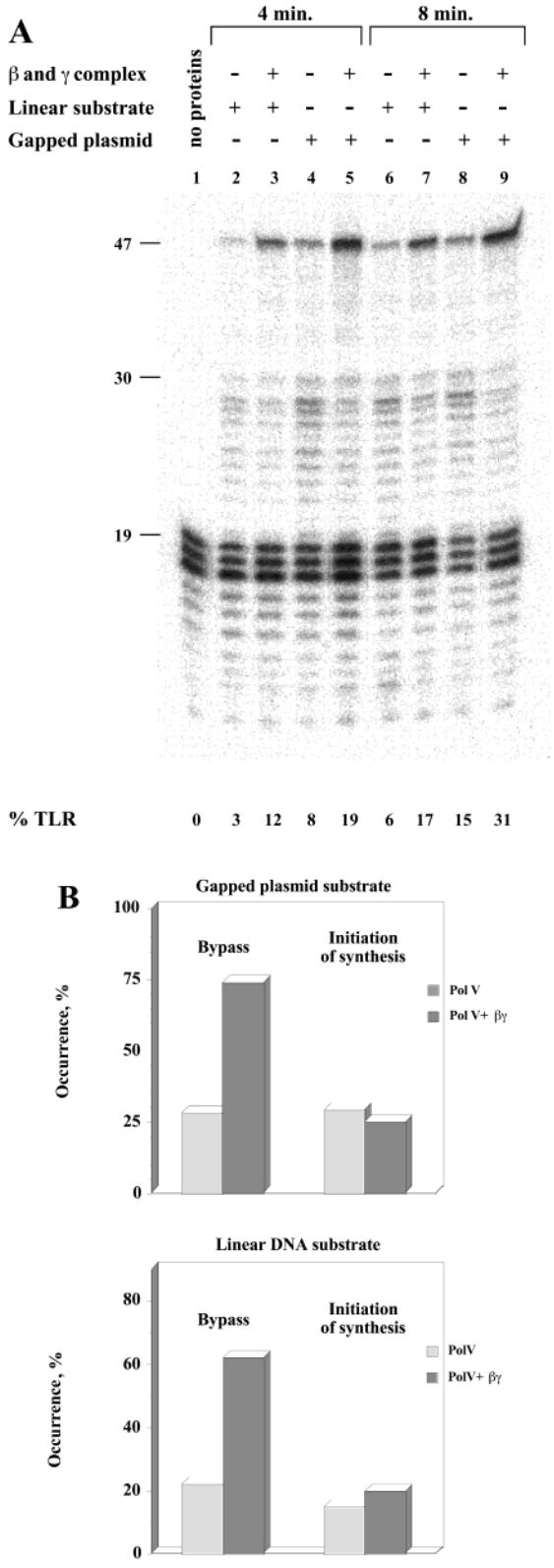

FIGURE 3: β subunit and γ complex stimulate lesion bypass, but have no effect on the initiation of synthesis by pol V. (A) A gel image of TLR experiments, performed on a linear or a circular gap-lesion DNA substrate. The reactions were carried out for 4 (lanes 2−5) or 8 min (lanes 6−9): lane 1, no proteins; lanes 2, 3, 6, and 7, linear substrate; and lanes 4, 5, 8, and 9, circular substrate. The reaction products were fractionated by 15% PAGE with urea followed by phosphorimager analysis. (B) Effect of the processivity proteins on bypass and on initiation of synthesis. The data were taken from the 4 min time point in panel A.

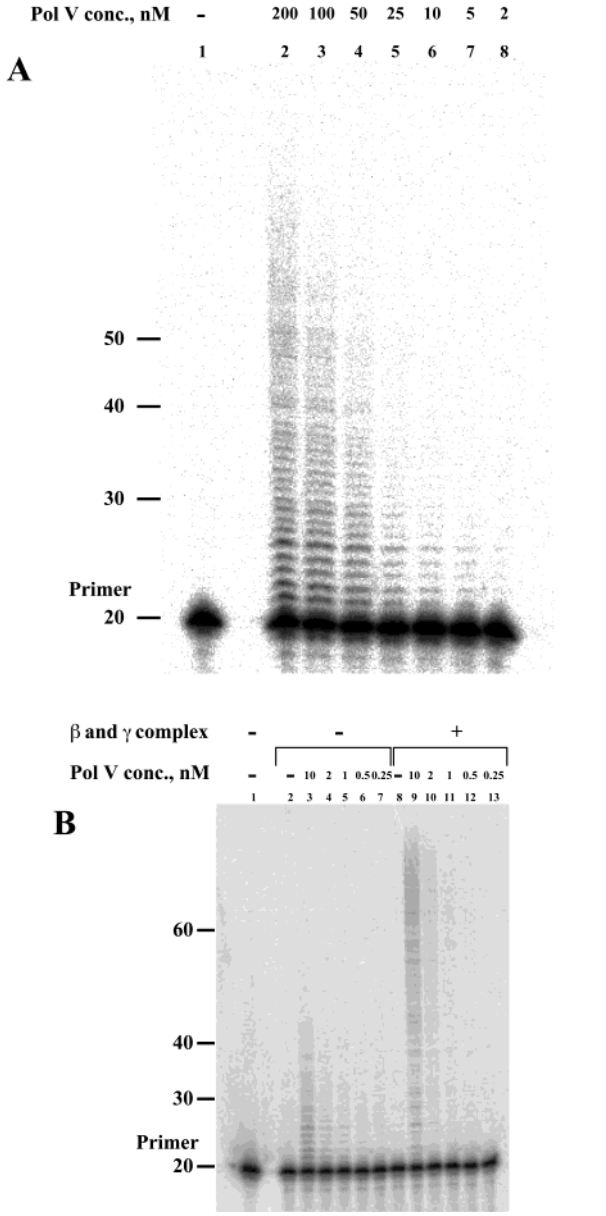

FIGURE 4: β subunit and γ complex increase the processivity of pol V. (A) DNA synthesis was performed by pol V, UmuD′, RecA, and SSB on an oligonucleotide-primed $\phi\chi$174 substrate for 4 min. The polymerase concentration was decreased from 200 to 2 nM (lanes 2−8). Lane 1 shows a reaction without any proteins. The processivity of pol V was determined by averaging the length of the DNA products, at the lowest concentrations of the polymerase. The reaction products were fractionated by 15% PAGE with urea followed by phosphorimager analysis. (B) A similar experiment was performed at polymerase concentrations of 10−0.25 nM, in the presence (lanes 8−13) or absence (lanes 2−7) of the β subunit and the γ complex.

the linear or circular DNA substrate (Figure 3B). Initiation of synthesis by pol V was unaffected by the presence of processivity proteins also on a gapped plasmid with no lesion (data not shown).

The β Subunit and the γ Complex Increase the Processivity of Pol V. The most likely mechanistic explanation for the increased level of bypass observed in the presence of the β subunit and the γ complex is an increased processivity of pol V. We examined the processivity of pol V in both the absence and presence of processivity proteins on a primed ϕX174 substrate. Figure 4A shows DNA synthesis with

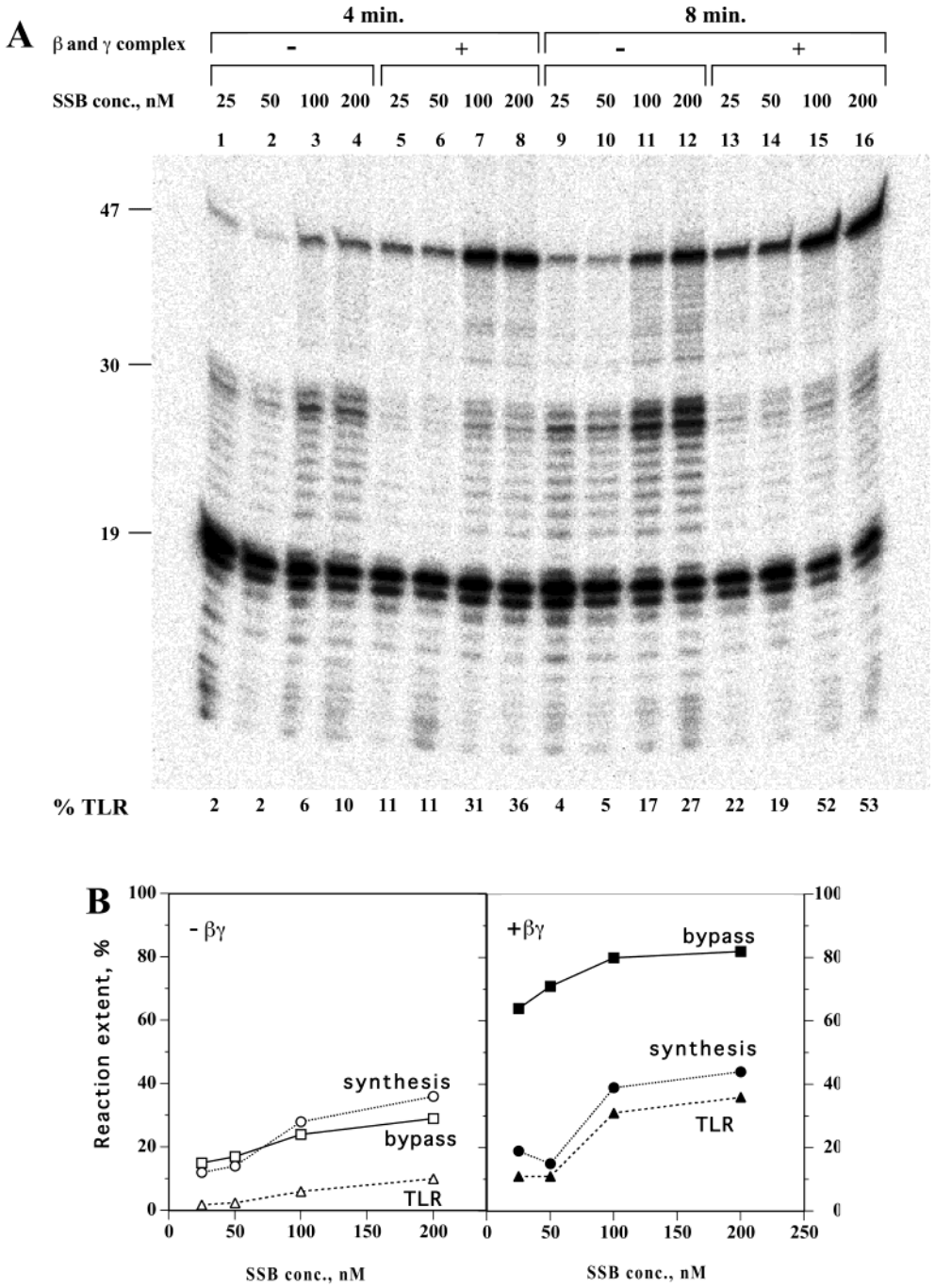

FIGURE 5: Effect of SSB concentration on initiation and bypass activities of pol V, in the absence and presence of the processivity proteins. (A) A TLR assay was performed with SSB concentrations of 25−200 nM as indicated at the top of the lanes. The reactions were carried out in the absence (lanes 1−4 and 9−12) or in the presence (lanes 5−8 and 13−16) of the β subunit and the γ complex for 4 (lanes 1−8) and 8 min (lanes 9−16). The reaction products were fractionated by 15% PAGE with urea followed by phosphorimager analysis. (B) Analysis of the percentage of bypass (squares), initiation of synthesis (circles), and TLR (triangles) in the absence (left, empty symbols) and presence (right, filled symbols) of the β subunit and the γ complex, at 4 min.

decreasing concentrations of pol V, in the presence of UmuD′, RecA, and SSB. In this method of determining processivity (*20*), the concentration of the polymerase is decreased until there is no change in the extension of the primer. The processivity of the polymerase is estimated by calculating the average extended DNA length. On the basis of these data, the processivity of pol V is 3. When a similar experiment was performed in the presence of the β subunit and the γ complex, the processivity was 14−18, representing an increase of 5−6-fold (Figure 4B).

Increased SSB Concentrations Stimulate Initiation by Pol V in both the Absence and Presence of Processivity Proteins. We have previously argued that the inability to observe lesion bypass in the absence of SSB implies that SSB has a direct role in bypass, aside from loading RecA on the DNA (*9*). It was suggested that this role is to facilitate dissociation of RecA from DNA during polymerization by pol V (*10*). We examined whether the presence of the β subunit and the γ complex would change the effect of SSB on bypass and on synthesis by pol V. First, a titration of SSB was performed

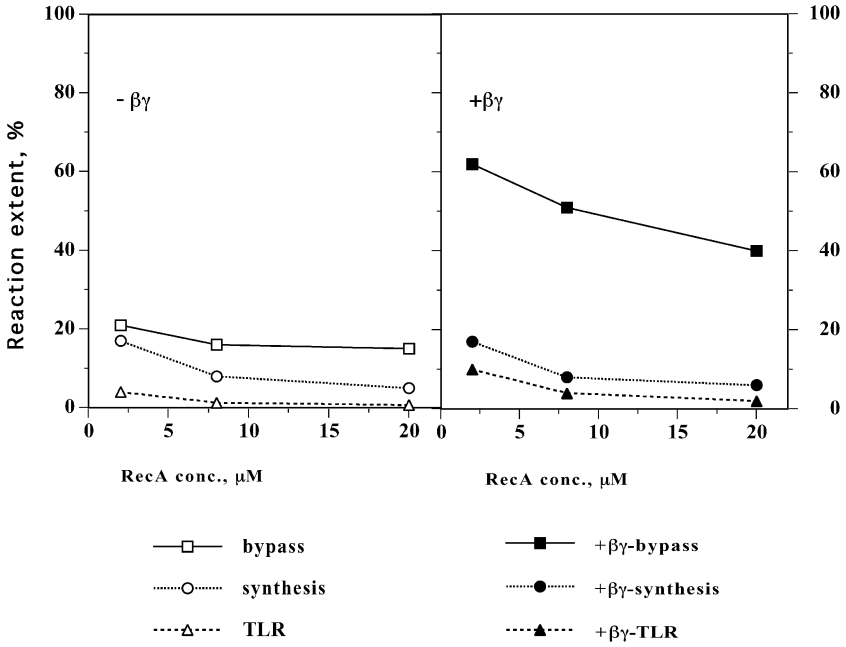

FIGURE 6: Effect of RecA concentration on initiation and bypass activities of pol V, in the absence and presence of the processivity proteins. A TLR assay was performed with RecA concentrations of 2, 8, and 20 μM, in the absence (left, empty symbols) and presence (right, filled symbols) of the β subunit and the γ complex. These reactions were performed in the presence of an ATP regeneration system, consisting of creatine phosphate and creatine kinase. The extent of bypass (squares), initiation of synthesis (circles), and TLR (triangles) were determined for the 4 min reaction, as described in Experimental Procedures.

in the absence of the β subunit and the γ complex. As can be seen in Figure 5A and quantified for the 4 min time point in Figure 5B, increasing the concentration of SSB from 25 to 200 nM caused a 3-fold increase in the initiation of synthesis (○) and a 2-fold increase in bypass (□), resulting in an overall increase of 5−6-fold in TLR (△). Still, under these conditions, TLR reached no more than 10% at 4 min. The effect of increasing concentrations of SSB on synthesis in the presence of the β subunit and the γ complex (●) was very similar to that observed without the processivity subunits. This is consistent with previous results, where the β subunit and the γ complex had no effect on synthesis by pol V (Figure 3). The effect of SSB on bypass in the presence of the β subunit and the γ complex (■) was identical to that observed without the processivity protein (□), as can be seen from the similar patterns of the two graphs. However, the effect of the processivity proteins on bypass is greater than the effect of SSB. Bypass was increased 3−4-fold over the entire range of SSB concentrations (■), as compared to bypass without the processivity proteins reaching a very high level of 70−80%. Under these conditions, the extent of TLR (▲) is determined by the slow process of initiation of synthesis, as can be clearly seen by the nearly overlapping curves of synthesis and TLR (Figure 5B). Hence, in the presence of the β subunit and the γ complex, the absolute level of TLR, namely, the fraction of molecules on which bypass occurred, increases with increasing amounts of SSB, up to 53% at 8 min (Figure 5A, lane 16).

Increased RecA Concentrations Inhibit the Initiation Stage of TLR. It has been previously shown in our laboratory that 2 μM RecA was required to reach a saturation level of bypass (*9*). A further increase in the level of RecA caused a small reduction in bypass and, more significantly, a reduction in primer utilization. This is presumably because access of pol V to the primer requires local unloading of RecA. We used this effect of RecA on the initiation of synthesis to probe again the specificity of the effect of the β subunit and the γ complex on bypass versus initiation. To that end, we have studied the effect of the β subunit and the γ complex on the initiation of synthesis and on bypass, in the presence of excess amounts of RecA. These experiments were performed in the presence of an ATP regeneration system, consisting of creatine phosphate and creatine kinase, to ensure that there is no shortage of ATP in the reaction, caused by the ATPase activity of RecA. As can be seen in Figure 6, there was a decrease in the initiation of synthesis with an increase in the amount of RecA (○). The same exact effect was seen in the presence of the β subunit and the γ complex (●). Hence, the β subunit and the γ complex did not influence the reduction in the initiation of synthesis with an excess of RecA. Again, this is consistent with the experiments described above indicating that the β subunit and the γ complex do not affect initiation. With regard to bypass, an increasing concentration of RecA (from 2 to 20 μM) caused a slight effect of inhibition, in the absence (□) or presence (■) of the β subunit and the γ complex. Over the entire range of RecA concentrations, the stimulatory effect of the β subunit and the γ complex on bypass was approximately 3-fold.

ATPγS Greatly Inhibits Translesion Replication in both the Presence and Absence of the Processivity Proteins. ATPγS is a nonhydrolyzable ATP analogue, which is known to strongly stabilize RecA nucleoprotein filaments (*22*). It has been shown that the presence of ATPγS reduced translesion replication by pol V (*9*). Under these conditions, the RecA filament extends to cover the entire DNA molecule, probably preventing the accessibility of pol V to the primer terminus. Indeed, inhibition is primarily at the initiation stage of synthesis (*9*). Since the β subunit and the γ complex stimulate bypass, we wondered if they can overcome the effect of ATPγS, and allow bypass in its presence. As can be seen in Figure 7, in the presence of ATPγS instead of

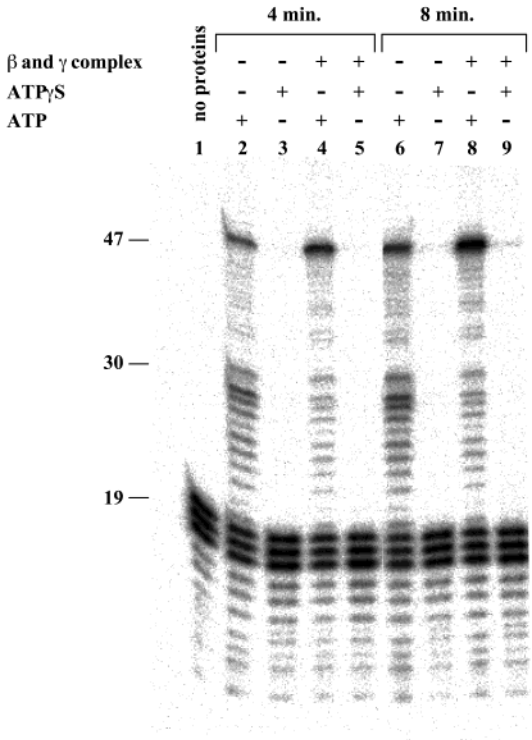

FIGURE 7: ATPγS greatly inhibits translesion replication in the absence or presence of the processivity proteins. A TLR assay was performed using either ATP (lanes 2, 4, 6, and 8) or ATPγS (lanes 3, 5, 7, and 9). The reactions were carried out for 4 (lanes 2−5) or 8 min (lanes 6−9), in the absence (lanes 2, 3, 6, and 7) or presence (lanes 4, 5, 8, and 9) of the β subunit and the γ complex. Lane 1 shows a reaction with no proteins. The reaction products were fractionated by 15% PAGE with urea followed by phosphorimager analysis.

ATP, TLR was severely inhibited, in the presence or absence of the β subunit and the γ complex. This indicates that the β subunit and the γ complex cannot interfere with the stable filament of RecA which is built in the presence of ATPγS.

The β Subunit Stimulates Mutagenesis in Vivo. The question of whether the processivity proteins are involved in TLR in vivo arises. Chromosomal TLR cannot be assayed directly, and therefore, UV mutagenesis, which is the result of TLR, was used instead. The mutagenesis assay system was the His reversion system, which monitors the UV-induced conversion of histidine auxotrophy to prototrophy. UV mutagenesis in this system is pol V-dependent, reflecting in vivo TLR by pol V (*2*). To examine the involvement of the β subunit in UV mutagenesis, we utilized an *E. coli* strain carrying the *dnaN59* mutation, a temperature sensitive mutation in the *dnaN* gene encoding the β subunit. At 42 °C, this mutation inactivates the β subunit, and as a result, DNA replication and cell division stop. At 30 °C, the mutant β subunit is fully functional in DNA replication, and cells grow normally (*23*). To eliminate indirect effects on mutagenesis caused by the β subunit via excision repair, we constructed a Δ*uvrC dnaN59* double mutant. The UvrC protein is an essential component of the Uvr(A)BC excinuclease (*24*), and in its absence, the cells are totally defective in nucleotide excision repair. To examine the effect of the *dnaN59* mutation on UV mutagenesis, Δ*uvrC dnaN59* cells were grown at 30 °C to midlogarithmic stage, UV-irradiated, and incubated at either 30 or 42 °C for 1 h. Then the cells were plated on low-His plates and incubated at 30 °C. As a control, the isogenic *uvrCdnaN*$^+$ parent was assayed in the same way. As can be seen in Figure 8A, at 30 °C the *uvrCdnaN59* double mutant exhibited lower UV mutagenesis, up to 3-fold, as compared the *uvrCdnaN*$^+$ strain. The simplest explanation for these results is that the β subunit stimulates UV mutagenesis, and that the *dnaN59* mutation, while not affecting replication at this temperature, diminishes the effect of the β subunit on UV mutagenesis. When cells were incubated at 42 °C after UV irradiation, the mutagenesis response of the *uvrC dnaN*$^+$ control cells was significantly decreased, and therefore, the effect of the *dnaN59* mutation was reduced (Figure 8B, empty symbols). The fact that UV mutagenesis of the control cell is reduced by post-UV treatment at 42 °C indicates the presence of a heat-labile component, which is required for UV mutagenesis.

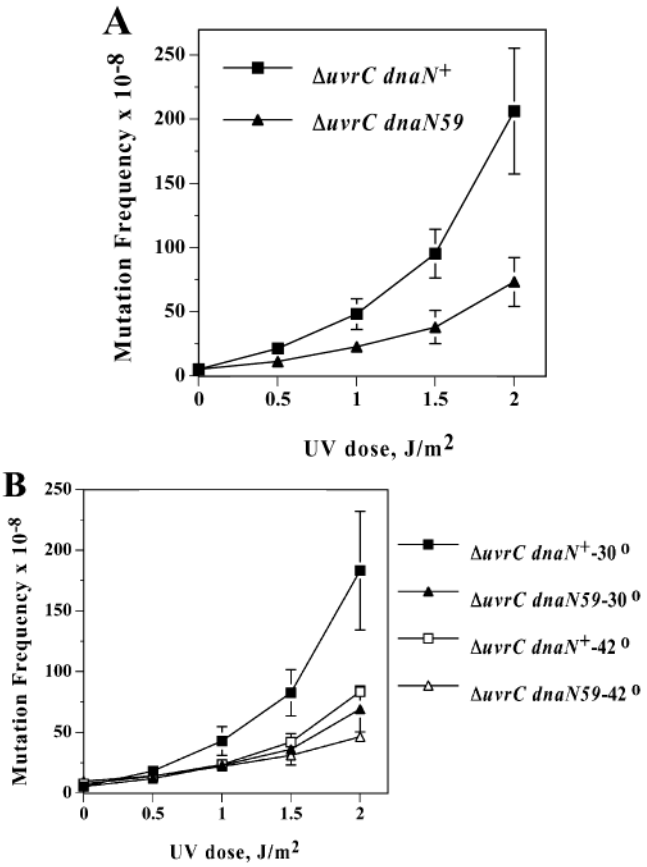

FIGURE 8: β subunit stimulates mutagenesis in vivo. UV-induced mutations from histidine auxotrophy to prototrophy, as a measure of TLR, were assayed in a *dnaN59* mutant, in a background of excision repair deficiency (Δ*uvrC*). Cells were grown at 30 °C to midlogarithmic stage, UV-irradiated, and incubated at either 30 or 42 °C for 1 h. Then the cells were plated on low-His plates, and incubated at 30 °C. (A) Comparison of the UV mutagenesis of *uvrCdnaN59* (▲) and *uvrCdnaN*$^+$ (■) strains at 30 °C. (B) Comparison of the UV mutagenesis in the two strains after treatment at the permissive temperature (30 °C, filled symbols) or the restrictive temperature (42 °C, empty symbols).

DISCUSSION

The processivity proteins of *E. coli*, the β subunit sliding DNA clamp and the γ complex, are essential for replication by pol III, endowing it with remarkable processivity (*25*). Moreover, in vitro experiments have shown that these proteins increase the processivity of other polymerases, including pol II (*26*, *27*), pol IV (*7*, *28*), and pol V (*7*; this

study), although the in vivo significance of these effects is not yet known. As far as pol V is concerned, it was reported that lesion bypass by UmuD′$_2$C, RecA, and SSB was negligible, unless the β subunit and the γ complex were added (*5*, *7*). This differed from our results, where significant TLR was observed with pol V, UmuD′, RecA, and SSB, in the absence of the β subunit and the γ complex (*6*, *9*, *12*). The reported requirement for the β subunit and the γ complex was attributed to the inability to form a stable RecA nucleoprotein filament at the lesion site in the substrate used by the Goodman group (*9*, *12*). Nevertheless, the reported positive effect of the β subunit and the γ complex on lesion bypass by pol V prompted us to examine the effect of these processivity proteins in our TLR system. Our in vitro results show that although the β and γ complexes are not essential for lesion bypass, they do stimulate bypass by 3-fold. These effects were observed over a range of SSB and RecA concentrations. Moreover, we present evidence, for the first time, suggesting that the β subunit is a stimulatory factor in UV mutagenesis in vivo.

Interestingly, the processivity proteins had no effect on the initiation of synthesis by pol V. We have previously suggested that initiation requires local dissociation of the RecA filament, to allow full access of the polymerase to the primer−template region (*9*, *11*). This might well be the rate-limiting step in initiation, as indicated by the effects of increasing concentrations of SSB and RecA on initiation (Figures 5 and 6). Increasing SSB concentrations from 25 to 200 nM stimulated initiation 3-fold, most likely due to the facilitated displacement of RecA from the primer−template region. This effect of SSB was observed in the presence or absence of the β and γ complex, and the extents of initiation were similar. Increasing the concentration of RecA, on the other hand, had an inhibitory effect. Increasing the RecA concentration from 2 to 20 μM inhibited primer extension 3.5-fold. This was caused, most likely, by stabilization of the RecA nucleoprotein filament at higher RecA concentrations, which inhibited access of pol V and SSB to the primer−template region. Again, this effect of RecA was observed in the absence and presence of the β and γ complex, and the extents of primer extension were similar. On the basis of these results, we conclude that the β subunit and the γ complex have no significant effect on the rate-limiting steps in primer extension by pol V. This is consistent with the dramatic inhibition of TLR, due to inhibition of initiation, caused by ATPγS in the presence of the β subunit and the γ complex.

The effect of the β and γ complex was strictly on the elongation step, including lesion bypass. This effect is caused, most likely, by the increased processivity of pol V in the presence of the β and γ complex. The processivity of pol V in the absence of the β and γ complex, on circular ssDNA, was estimated to be 3 nucleotides/initiation event. This increased to 14−18 nucleotides/initiation event upon addition of the processivity proteins. These results are in general agreement with the results of Goodman and co-workers, who reported that pol V has a processivity of 12 in the presence of the β subunit and the γ complex (*10*). In their case, processivity in the absence of the β subunit and the γ complex was determined only in the presence of ATPγS, and not in the presence of ATP (*10*). The higher processivity in the presence of processivity factors implies a longer residence time on DNA before dissociation, which enables a greater extent of lesion bypass. It is noteworthy that the processivity subunits were found to endow pol III with the ability to bypass a synthetic abasic site (*16*). In that case, however, the polymerase without the processivity machinery was absolutely blocked by the lesion. It seems therefore that increased processivity may be a general mechanism for increasing the efficiency of lesion bypass by DNA polymerases, regardless of their inherent ability to bypass lesions.

The in vivo experiments conducted with the *dnaN59* mutant strains suggest that the β subunit stimulates UV mutagenesis in vivo. To our knowledge, this is the first in vivo evidence that the β subunit participates in SOS mutagenesis, and the first in vivo evidence that the β subunit affects a polymerase other than pol III. The effect was evident when UV mutagenesis was compared in a pair of isogenic *uvrCdnaN59* and *uvrCdnaN*$^+$ strains at the permissive temperature. UV-induced His reversion mutations were induced in the *dnaN59* mutant at a frequency 3-fold lower than that in the *dnaN*$^+$ cells, which is strikingly similar to the in vitro effect of the processivity proteins on bypass. Notice that UV mutagenesis is caused primarily by TLR of cyclobutylpyrimidine dimers and 6−4 pyrimidine−pyrimidine adducts, whereas our in vitro system assays TLR of abasic sites. Nevertheless, the results were similar. It is interesting that the reduced UV mutagenesis in the *dnaN59* mutant is expressed at 30 °C, a temperature at which the β subunit is fully functional in replication (*23*). We interpret this result to suggest that the *dnaN59* mutation separates between the functions of the β subunit in replication and lesion bypass. This can be affected by differential effects on the interactions with DNA polymerases III and V; however, this point has yet to be established. The effect of the β subunit on UV mutagenesis might be even greater, since the *dnaN59* mutation might have some residual functionality in mutagenesis. Under our conditions, there was a 2−3-fold reduction in UV mutagenesis when the *uvrCdnaN*$^+$ cells were assayed at 42 °C. Under these conditions, the *uvrCdnaN59* cells exhibited an only marginal decrease compared to the *uvrCdnaN*$^+$ cells. Due to the overall decrease in the mutagenic response, it is difficult to draw conclusions from this experiment. However, it is noteworthy that even when the β subunit is inactivated, there is UV mutagenesis, suggesting that pol V performs translesion replication also in the absence of the processivity subunit.

In addition to the effect of the processivity proteins on the extent of bypass by pol V, their effect on the fidelity of pol V should be considered, since this may affect in vivo UV mutagenesis. We examined the effect of the processivity proteins on the fidelity of pol V in vitro, using a gap filling assay and the *cro* repressor gene as a reporter (*8*). The frequency of Cro$^-$ mutations caused by pol V-catalyzed DNA synthesis in the presence of the processivity proteins was $(2.7 \pm 1.2) \times 10^{-2}$/gene, similar to that obtained with pol V in the absence of processivity proteins [$(2.5 \pm 0.3) \times 10^{-2}$, consistent with the value obtained previously, namely, $(2.3 \pm 0.4) \times 10^{-2}$ (*8*)]. Thus, the processivity proteins had no effect on the fidelity of pol V in this assay system. Taken together, the in vivo results suggest that the β subunit stimulates lesion bypass by pol V, thereby enhancing UV mutagenesis. This is consistent with our in vitro results on

damaged and undamaged DNA substrates. However, more experiments are needed to prove this suggestion.

Although they were originally discovered as the processivity subunits of pol III, it is clear now that the β subunit and the γ complex increase the processivity of pol II (*26*, *27*), pol IV (*7*, *28*), and pol V (*7*; this study). There is also evidence for an interaction between the β clamp and pol I, and increased activity of pol I was observed in the presence of the processivity subunits, which is likely explained by an increase in the processivity of pol I (*29*). Thus, the processivity mechanism is shared by at least four, and possibly all five, known DNA polymerases of *E. coli*. This may offer a potential mechanism for DNA polymerases switching on DNA, e.g., at a site where the replication fork collapsed at a blocking lesion. The β subunit sliding clamp may stay on the DNA, and provide the platform for polymerase switching.

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REFERENCES

1. Livneh, Z., Cohen-Fix, O., Skaliter, R., and Elizur, T. (1993) *CRC Crit. Rev. Biochem. Mol. Biol. 28*, 465−513.
2. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA repair and mutagenesis*, ASM Press, Washington, DC.
3. Reuven, N. B., Tomer, G., and Livneh, Z. (1998) *Mol. Cell 2*, 191−199.
4. Tang, M., Bruck, I., Eritja, R., Turner, J., Frank, E. G., Woodgate, R., O'Donnell, M., and Goodman, M. F. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 9755−9760.
5. Tang, M., Shen, X., Frank, E. G., O'Donnell, M., Woodgate, R., and Goodman, M. F. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 8919−8924.
6. Reuven, N. B., Arad, G., Maor-Shoshani, A., and Livneh, Z. (1999) *J. Biol. Chem. 274*, 31763−31766.
7. Tang, M., Pham, P., Shen, X., Taylor, J. S., O'Donnell, M., Woodgate, R., and Goodman, M. F. (2000) *Nature 404*, 1014−1018.
8. Maor-Shoshani, A., Reuven, N. B., Tomer, G., and Livneh, Z. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 565−570.
9. Reuven, N. B., Arad, G., Stasiak, A. Z., Stasiak, A., and Livneh, Z. (2001) *J. Biol. Chem. 276*, 5511−5517.
10. Pham, P., Bertram, J. G., O'Donnell, M., Woodgate, R., and Goodman, M. F. (2001) *Nature 409*, 366−370.
11. Livneh, Z. (2001) *J. Biol. Chem. 276*, 25639−25642.
12. Goldsmith, M., Sarov-Blat, L., and Livneh, Z. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 11227−11231.
13. Lohman, T. M., and Overman, L. B. (1985) *J. Biol. Chem. 260*, 3594−3603.
14. Cox, M. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem. 256*, 4676−4678.
15. Johanson, K. O., Haynes, T. E., and McHenry, C. S. (1986) *J. Biol. Chem. 261*, 11460−11465.
16. Tomer, G., Reuven, N. B., and Livneh, Z. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 14106−14111.
17. Tomer, G., and Livneh, Z. (1999) *Biochemistry 38*, 5948−5958.
18. Miller, J. H. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Plainview, NY.
19. Moolenaar, G. F., Moorman, C., and Goosen, N. (2000) *J. Bacteriol. 182*, 5706−5714.
20. Bambara, R. A., Fay, P. J., and Mallaber, L. M. (1995) *Methods Enzymol. 262*, 270−280.
21. Tadmor, Y., Ascarelli-Goell, R., Skaliter, R., and Livneh, Z. (1992) *J. Bacteriol. 174*, 2517−2524.
22. Roca, A. I., and Cox, M. M. (1990) *CRC Crit. Rev. Biochem. Mol. Biol. 25*, 415−456.
23. Burgers, P. M., Kornberg, A., and Sakakibara, Y. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5391−5395.
24. Sancar, A. (1994) *Science 266*, 1954−1956.
25. Kelman, Z., and O'Donnell, M. (1995) *Annu. Rev. Biochem. 64*, 171−200.
26. Hughes, A. J., Bryan, S. K., Chen, H., Moses, R. E., and McHenry, C. S. (1991) *J. Biol. Chem. 266*, 4568−4573.
27. Bonner, C. A., Stukenberg, P. T., Rajagopalan, M., Eritja, R., O'Donnell, M., McEntee, K., Echols, H., and Goodman, M. F. (1992) *J. Biol. Chem. 267*, 11431−11438.
28. Wagner, J., Fujii, S., Gruz, P., Nohmi, T., and Fuchs, R. P. (2000) *EMBO Rep. 1*, 484−488.
29. Lopez de Saro, F. J., and O'Donnell, M. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 8376−8380.

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