Biochemical basis of SOS-induced mutagenesis in *Escherichia coli*: Reconstitution of *in vitro* lesion bypass dependent on the UmuD₂C mutagenic complex and RecA protein

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ABSTRACT Damage-induced SOS mutagenesis requiring the UmuD/C proteins occurs as part of the cell’s global response to DNA damage. *In vitro* studies on the biochemical basis of SOS mutagenesis have been hampered by difficulties in obtaining biologically active UmuC protein, which, when overproduced, is insoluble in aqueous solution. We have circumvented this problem by purifying the UmuD/C complex in soluble form and have used it to reconstitute an SOS lesion bypass system *in vitro*. Stimulated bypass of a site-directed model abasic lesion occurs in the presence of UmuD/C, activated RecA protein (RecA*), β-sliding clamp, γ-clamp loading complex, single-stranded binding protein (SSB), and either DNA polymerases III or II. Synthesis in the presence of UmuD/C is nonprocessive on damaged and undamaged DNA. No lesion bypass is observed when wild-type RecA is replaced with RecA1730, a mutant that is specifically defective for Umu-dependent mutagenesis. Perhaps the most noteworthy property of UmuD/C resides in its ability to stimulate both nucleotide misincorporation and mismatch extension at aberrant and normal template sites. These observations provide a biochemical basis for the role of the Umu complex in SOS-targeted and SOS-untargeted mutagenesis.

*Escherichia coli* normally replicates its DNA accurately, but the fidelity of replication decreases dramatically after cells are exposed to a variety of DNA-damaging agents that induce the SOS response (1–3). Given a choice, *E. coli* will evoke a damage avoidance pathway that in all likelihood involves polymerase strand switching to a nondamaged DNA template (4, 5). Nevertheless, situations arise where the DNA replication machinery encounters a lesion and the only recourse is the direct replication of the damaged template.

Genetic characterization of the error-prone translesion DNA synthesis pathway shows that it depends on the UmuD/C complex, activated RecA protein (RecA*) and DNA polymerase (pol) III (6–10). A prevailing model for translesion DNA synthesis, based on genetic experiments, suggests that it can be separated into two steps: nucleotide misincorporation directly opposite the lesion, believed to require pol III and RecA (11, 12), and lesion bypass believed to involve pol III and UmuD/C proteins (12–14).

A major roadblock in the path toward understanding the phenomenon of Umu-dependent translesion DNA synthesis has been the inability to purify biologically active UmuC. By using a denatured-renatured form of UmuC, Echols and colleagues (15) were able to purify UmuC and demonstrate that together with UmuD* and RecA*, DNA pol III was able to facilitate limited translesion DNA synthesis of a synthetic abasic site (16). We recently have succeeded in purifying the native UmuD/C complex directly, in soluble form (17). We showed that this complex binds cooperatively to single-stranded DNA (17), having similar affinities to damaged and undamaged DNA, and effectively blocks recombinational strand exchange *in vitro* (W. M. Rehrauer, I.B. R.W., M.F.G., and S. C. Kowalczykowski, unpublished data). In the present study, we use the soluble UmuD/C complex to reconstitute an *in vitro* lesion bypass assay dependent on UmuD/C and RecA*.

A key finding is that in the presence of UmuD/C, the fidelity of DNA synthesis is compromised markedly at both damaged and undamaged template sites, thus providing a framework for studying the biochemical mechanisms governing SOS targeted and untargeted mutagenesis.

MATERIALS AND METHODS

**Materials.** The following reagents were all purchased: T4 polynucleotide kinase (United States Biochemical/Amer sham), T4 DNA ligase (Promega), EcoRI restriction enzyme (New England Biolabs), *E. coli* single-stranded binding protein (SSB; Pharmacia), ultrapure ATP and dideoxynucleoside triphosphates (Pharmacia), and [γ-32P]ATP (4000 Ci mmol−1 Cl− = 37 GBq) (ICN). Purification of pol III and its accessory proteins (18) and pol II (19) were carried out as described. RecA protein and pol I antibody were generous gifts from Stephen Kowalczykowski (University of California, Davis) and Lawrence Loeb (University of Washington, Seattle), respectively.

**Purification of UmuD/C Complex.** Purification was carried out as reported (17) with the following modifications: after polyetheleneimine precipitation, proteins were extracted by stirring the pellet in R-buffer (20 mM Tris-HCl, pH 7.5/0.1 mM EDTA/1 mM DTT/20% glycerol) containing 1 M NaCl. Ammonium sulfate was added to reach 50% of saturation, followed by centrifugation of the suspension. The pellet was dissolved in R-buffer containing 1 M NaCl, dialyzed against R-buffer with 1 M NaCl, followed by a second ammonium sulfate precipitation step in which UmuD/C complex precipitated at 30% saturation. The spun pellet was again dissolved

Abbreviations: RecA*, activated RecA protein; pol, polymerase; SSB, *E. coli* single-stranded binding protein; p/t DNA, primer-template DNA.

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in R-buffer with 1 M NaCl and dialyzed against R-buffer containing 50 mM NaCl before chromatography using DEAE and phosphocellulose as described (17). Phosphocellulose fractions containing UmuD/C were concentrated and applied separately onto either Superdex 75 (17), Sephadex G100, or Sephadex G150 columns (Pharmacia) and eluted in R-buffer containing 1 M NaCl, resulting in a purity of >95%.

**Primer/Template Construction.** All oligonucleotides were synthesized on an Applied Biosystems model 392 DNA/RNA synthesizer. The template used in the replicative bypass assay, a 7.2-kb linear single-stranded DNA with an abasic lesion located 50 bases from the 5’ end, was constructed as described (16). Two 5’-end 32P-labeled 30-mer oligonucleotides were used as primers. In the “running-start” assay, the first primer is annealed to the template such that its 3’ end is 46 nt from the lesion. In the “standing-start” assay, the 3’ end of the primer is located one base before the abasic lesion.

**Replicative Bypass Assay.** The reaction mixture (10 μl) contains 20 mM Tris-HCl (pH 7.5), 8 mM MgCl2, 5 mM DTT, 0.1 mM EDTA, 25 mM sodium glutamate, 1 mM ATP, and 4% (vol/vol) glycerol. Running-start reactions were performed as follows: 2 nM primed DNA substrate was incubated for 2 min at 37°C with 40 nM β protein dimer, 10 nM γ-complex, 1 μM RecA, 200 nM UmuD/C complex, and 100 μM each of dATP and dCTP. SSB (300 nM as tetramer) was then added to the mixture and incubated for another 2 min. Replication was initiated by addition of dGTP and dTTP (100 μM each) and either pol III core, pol III α-subunit, or pol II, at concentrations between 0 and 20 nM. Reactions were carried out at 37°C for 10 min, then quenched by adding 20 μl EDTA (20 mM) in 95% formamide. The product DNA was heat denatured run on a 10% polyacrylamide denaturing gel. Replication products were quantitated by using a PhosphorImager (Molecular Dynamics). Standing-start reactions were carried out in a similar manner as running-start reactions, except that all four dNTPs were added after preincubation with UmuD/C, RecA, SSB and β,γ-complex.

**Reconstituting Lesion Bypass in Vitro By Using Purified UmuD/C, RecA, SSB, and Components of Pol III Holoenzyme.** A linearized M13 DNA template containing a single, site-directed abasic lesion was copied after using different combinations of pol III core, pol III accessory proteins (β,γ-complex), RecA, SSB, and UmuD/C (Fig. 1 Left). The primer-template (Fig. 1 Upper) was designed to permit loading of the β processivity clamp by the γ-complex (21) and to allow binding of UmuD/C, RecA, and SSB (17). Relativley weak, nonprocessive synthesis is catalyzed by pol III core alone (Fig. 1, lane 1). In the presence of β,γ-complex plus SSB, synthesis by pol III core becomes much more processive, terminating one base before reaching the abasic site (Fig. 1, lane 2). Addition of RecA, either to pol III core (Fig. 1, lane 3) or to pol III core, β,γ-complex, and SSB (Fig. 1, lane 4) stimulates each reaction, but primer elongation still terminates at the X – 1 position, one base before the lesion.

The key observation is that addition of UmuD/C enables significant bypass of the lesion, with continued synthesis to the end of the template (Fig. 1, lane 5). The presence of an intense pause band at X – 1 suggests that incorporation opposite the lesion is still rate limiting, although it is possible that this band could arise by excision of a nucleotide incorporated opposite the abasic site by the e proofreading exonuclease of pol III core. The polymerization reaction leading up to the lesion and beyond is essentially nonprocessive in the presence of UmuD/C (Fig. 1, lane 5), although pol III and β,γ-complex are both present along with ATP. A summation of integrated band intensities beyond the lesion (summed from site X +1 to the end of the template) relative to the bands extended up to and including the lesion (summed from site 1 to X) shows that the amount of bypass observed after a 10 min incubation is ~20%; i.e., the amount of lesion bypass corresponds to 20% of the total synthesis.

Either ATP or the nonhydrolyzable ATP analog adenosine 5’-[(γS)32P]triphosphate (ATPγS) is required for lesion bypass (data not shown). ATP or ATP-[γS] is needed in the reaction for loading β on DNA by γ-complex (22), and also for binding RecA to DNA (23), thus converting it to RecA*. ATP is hydrolyzed throughout the reaction, resulting in a reduction to 60% of its initial level after a 10-min reaction (data not shown). To verify that the initial input concentration of ATP (1 mM)
is sufficient to sustain the conversion of RecA → RecA*, we added LexA protein to the reaction at 10 min and observed RecA*-mediated cleavage of LexA (data not shown).

Optimal stoichiometries for each of the mutasomes components in catalyzing lesion bypass were determined to be: M13 DNA (2 nM), UmuDΔC (200 nM), RecA (1 μM), pol III core (in a range of 1–10 nM), β-splaying clamp (40 nM), γ-clamp loading complex (10 nM), SSB (300 nM as tetramer), ATP (1 mM). The conditions leading to efficient lesion bypass differ in two important ways from those previously reported by using renatured UmuC (16, 24). First, a molar ratio of 26UmuD/ UmuC was required in the earlier study to observe a small amount of bypass compared with the physiologically correct value of 2 UmuD/ UmuC found for the soluble UmuDΔC complex used in the current study. This 13-fold difference suggests that perhaps most of the renatured UmuC was not biologically active. Polyethylene glycol (PEG) was required to observe lesion bypass in the earlier study, perhaps as a crowding agent to concentrate UmuC at the site of the lesion. Our bypass assay is insensitive to PEG.

**UmuDΔC Catalyzes RecA*-Dependent Lesion Bypass in the Absence of Exogeneous Pol III Core.** Unexpectedly, lesion bypass was observed to occur in a “control” reaction containing UmuDΔC, RecA, β, γ-complex, and SSB, in the absence of exogeneous pol III core (Fig. 1, lane 9). Lesion bypass does not occur in the absence of either RecA (Fig. 1, lane 7) or β, γ-complex plus SSB (Fig. 1, lane 8). However, one interesting difference in the gel band patterns is the appearance of two adjacent bands just before the lesion (at X – 1) and directly opposite the abasic site (X) (Fig. 1, lanes 7 and 8). Thus, a band corresponding to stable incorporation opposite the lesion persists in the absence of added pol III core (in contrast to the presence of pol III core, compare with Fig. 1, lanes 2–4).

Therefore, our UmuDΔC preparation contains a weak pol activity (Fig. 1, lane 6) which is stimulated in the presence of RecA (Fig. 1, lane 8). We estimate that UmuDΔC is at least 95% pure, based on the absence of contaminating bands on silver-stained or Coomassie-stained polyacrylamide gels (data not shown). The presence of pol I contamination can be ruled out because primer extension is unaltered when polymerization occurs in the presence of a potent neutralizing pol I antibody (data not shown). However, we cannot rule out the presence of trace levels of either pol III or pol II, despite the fact that the UmuDΔC complex was purified by gel filtration. We have verified that the UmuDΔC complex migrates with an expected molecular mass of 70 kDa, indicating that it is unlikely to be directly bound to a pol. It is therefore much more likely that pol II, whose molecular mass is 89.9 kDa (25), is present as a contaminating pol, rather than either pol III core (167.5 kDa) or pol III α-subunit (130 kDa) (26). However, we cannot yet eliminate the possibility that UmuDΔC might itself contain an intrinsic, low fidelity, pol activity. UmuDΔC and C exhibit no significant sequence similarity to any of the known pols, although UmuC is weakly homologous to yeast Rev1, which incorporates dCMP opposite abasic sites (27).

A strain of *E. coli* carrying the recA1730 mutation (SI17F) is proficient for most of RecA's activities but is specifically defective for Umu-dependent mutagenesis (8, 10, 28), probably because RecA1730 is unable to target the Umu proteins to lesion-containing DNA (29). Although DNA synthesis continues to take place when wild-type RecA protein is replaced with purified RecA1730, bypass of the abasic lesion no longer occurs (Fig. 1, lane 10). Instead, the primer is extended one base prior to the lesion, with a much smaller amount of incorporation occurring directly opposite the lesion. These data are in contrast to the lesion bypass promoted by wild-type RecA protein (Fig. 1, lanes 5 and 9), but are consistent with the activity of RecA1730 in vivo (8, 10), and in vitro (29).

**The Effect of Pol III and Pol II on Lesion Bypass.** We have measured lesion bypass as a function of the concentration of three DNA pols, pols III, II, and α-subunit, in the presence of a fixed concentration of UmuDΔC (Fig. 2). The primer 3' end is located one base before the abasic lesion allowing incorporation of a single nucleotide opposite X from a standing start (Fig. 2 Upper).

In reactions containing UmuDΔC, preincubations were carried out for 4 min in the presence of β, γ-complex, SSB, ATP (1 mM) and two dNTPs, dCTP (100 μM), and dATP (100 μM). dCTP is present to protect against primer degradation by pol-associated exonuclease activity; dATP can be incorporated opposite X, but can only be extended by formation of downstream mispairs (see Fig. 3A). UmuDΔC panel, lane A). After preincubiation, the three pols were added, along with dTTP (100 μM) and dGTP (100 μM), in separate reactions. An increase in the amount of intermediate and full-length products occurs with increasing concentrations of pol in the presence of UmuDΔC (Fig. 2). However, the fraction of primers extended opposite the lesion and beyond actually decreases from about 40% to roughly 25% as the concentrations of pol III core and pol II increase from 0 to 20 nM and 10 nM, respectively (Fig. 2). The decrease in the fraction of extended primers is caused by pol III core and pol II proofreading occurring primarily at the lesion site (X) and at the downstream X + 1 site (pol III core and pol II panels, lanes 3 and 4). In contrast, there is no reduction in gel band intensities at X and X + 1 by using nonproofreading pol III α (pol III α panel, lanes 3 and 4), whereas the fraction of primers extended opposite and beyond the lesion increases rather than decreases (Fig. 2).

Bypass in the absence of UmuDΔC increases as the levels of pol are increased. For the case of pol III core and α-subunit, a small amount of bypass is observed at 20 nM enzyme concentrations (Fig. 2, lanes 7), whereas barely detectable full-length product DNA formed at 20 nM pol concentrations (Fig. 2). A larger amount of full-length product is made by pol II. The key point to emphasize is that synthesis opposite the lesion and beyond is reduced significantly for all three pols when UmuDΔC is absent from the reaction (Fig. 2, –UmuDΔC, lanes 5–7).

![Fig. 2. Effect of pol III and pol II on translesion replication.](image-url)
of UmuD pol concentration, depending primarily on the concentration of proteins. The abasic lesion containing and normal DNA templates are shown above each gel. A portion of each template sequence is shown on the right. UmuD pol measurements made in the presence of pol III (1 nM) and pol II (0.2 nM), resulted in banding patterns identical to those shown in the UmuD pol panel.

In a reciprocal experiment, in which the concentration of UmuD pol was varied (at fixed pol concentration), we observed an increase in bypass efficiencies as UmuD pol concentration is increased, with maximum bypass occurring at a molar ratio of 100 UmuD pol/1 primer-template DNA (p/t DNA) (data not shown). A shallow inhibition (~30%) in bypass occurs at higher concentrations of UmuD pol in the range of 200 nM to 1 μM, which is possibly caused by interactions taking place between UmuD pol and RecA bound to DNA downstream of the replication fork (ref. 17; W. M. Rehrauer, I.B. R.W., M.F.G., and S. C. Kowalczykowski, unpublished data). The data in Fig. 2 demonstrate that UmuD pol is able to stimulate incorporation opposite the lesion and the addition of one or more nucleotides beyond the lesion.

For α-subunit, the band intensities at X and X + 1 (one base downstream from the lesion) remain constant as α is varied between 0 and 20 nM (Fig. 2, α-subunit panel, + UmuD pol lanes). Therefore, in the absence of the ε-exonuclease subunit, the incorporation at X and X + 1 is essentially independent of pol concentration, depending primarily on the concentration of UmuD pol. An increase in full-length product at increased levels of α suggests that the pol displaces UmuD pol and continues synthesis downstream from the lesion. A similar conclusion can be drawn from the data with pol III core, and also from experiments in which the pols and UmuD pol are both present when the reaction is initiated by addition of all four dNTPs (data not shown).

UmuD pol-Stimulated Base Misincorporation and Mismatch Extension at Aberrant and Normal Template Sites. A clear illustration of the marked reduction in pol fidelity in the presence of UmuD pol is seen when incorporation at aberrant and normal template sites is carried out by using just one dNTP substrate (Fig. 3). A standing-start protocol is used to measure incorporation at an abasic lesion, X (Fig. 3A) or at a normal template T (Fig. 3B). All reactions contain RecA, βγ-complex, SSB, and ATP (1 mM), and either pol III core, pol II, or UmuD pol. The levels of pol III core (1 nM) and pol II (0.2 nM) were chosen to give similar (or greater) amounts of synthesis than UmuD pol (200 nM, in the absence of exogenous pol), on an undamaged DNA template with the four dNTPs present (Fig. 3B, compare lanes 4 for the three panels).

There is essentially no stable incorporation catalyzed by pol III (1 nM) opposite X by using either a single dNTP or in the presence of all four dNTPs (a faint band is seen in lanes A and 4, representing a small incorporation of dAMP). For pol II (0.2 nM), faint bands are observed for incorporation of dGMP, dAMP, and dTMP, but not dCMP. Pol II catalyzes a small amount of full-length product in the presence of all four dNTPs, consistent with our previous demonstration that pol II can copy past abasic lesions with a substantially higher efficiency than pol III (30).

Synthesis carried out with UmuD pol in the presence of only dGTP or dATP substrates results in the incorporation of five consecutive G’s or A’s, whereas synthesis with either dTTP or dCTP results in the incorporation of either three T’s or one C (Fig. 3A). The results are similar in the presence or absence of exogenous pol (data not shown).

The first incorporation occurs opposite the abasic lesion. Synthesis taking place at template sites downstream from the lesion correspond mainly to the incorporation of mismatched nucleotides, followed by extension of mismatched termini and by additional misincorporations. A much larger amount of synthesis occurs in the presence of four dNTPs (Fig. 3A, lane 4). The weak primer extension band observed in the absence of dNTPs (Fig. 3, UmuD pol panel, lane 0) is caused by incorporation of ribo AMP. These results stand in marked contrast to the relatively weak primer extension catalyzed by pol III and pol II, in the absence of UmuD pol, and clearly demonstrate the remarkable effect of UmuD pol in relaxing the specificity of nucleotide incorporation at aberrant and normal template sites.
Given the paucity of incorporation by pol III and pol II on the abasic template, it is important to show that these pols carry out normal DNA synthesis on a normal template, in which X is replaced by T (Fig. 3B). As expected, the predominant reaction for pol III and pol II is the incorporation of dAMP opposite T when dATP is the only substrate present. A small amount of dTMP misincorporation is also occurring, possibly by a transient misalignment mechanism involving the downstream template A (31). In the case of UmuD

C, either four G’s, four A’s, three T’s, two C’s, or two ribo A’s are incorporated (Fig. 3B), indicating that the mutagenic Umu complex stimulates misincorporations and mismatch extensions on natural DNA templates as well as those containing DNA damage.

Although the data in Fig. 3 appear to suggest that the nucleotide specificities revealed in the single dNTP experiments for the UmuD

C complex is clearly distinct from either pol II or pol III, leaving open the possibility that Umu might contain a distinct polymerizing component, such altered pol specificities could also be accounted for by an alteration of pol II or pol III, leaving open the possibility that Umu might catalyze at greatly reduced efficiencies (about 10

4). Thus, contamination of UmuD

C with 0.5% pol III core or 0.2% pol II (Fig. 3B) caused incorporation of a nucleotide opposite a lesion, but cannot catalyze bypass in the absence of UmuDC and UmuC proteins (36). However, recent data show that replacement of umuDC by either mucAB or rumAB alters the mutational specificity at T–T cyclobutane dimers, suggesting that the UmuDC complex might also influence pol insertion specificities in addition to modulating bypass (37).

Requirement for RecA*, SSB, and β,γ-Complex During Lesion Bypass. RecA protein plays a direct role in catalyzing SOS mutagenesis beyond its involvement in cleavage of LexA and UmuD proteins (7–9, 29). Omission of wild-type RecA (Fig. 1, lanes 6 and 7) or the addition of RecA1730 (Fig. 1, lane 10), a missense mutant refractory to Umu-dependent mutagenesis, resulted in no detectable bypass. These observations are, therefore, entirely consistent with the genetic data and lend credence to the biological relevance of the reconstitution assay.

SSB protein was also essential for bypass. Such a requirement may be indirect because SSB eliminates DNA secondary structure, keeps the β-clamp from sliding off linear DNA (21), and helps RecA achieve its activated state (23). However, because MucB, a homolog of UmuC, was shown to interact directly with SSB in a yeast two-hybrid assay (38), perhaps lesion bypass is stimulated by a direct UmuC–SSB interaction. A requirement for β,γ-complex in the bypass assay might be to increase the residence time of the mutasome at the lesion site.

UmuDC-Stimulated Lesion Bypass in the Presence of Pol III and Pol II. Although our preparation of UmuDC appears to be >95% pure, we nevertheless observed significant primer elongation in the absence of exogenous DNA pol (Fig. 1, UmuDC). Optimal lesion bypass on the M13 DNA template requires the presence of a 100-fold molar excess UmuDC (200 nM UmuDC; 2 nM p/t DNA). We determined that the rate of DNA synthesis observed with UmuDC corresponds to about 1 nM pol III core or less than 0.2 nM pol II (Fig. 3B, lanes 4). Thus, contamination of UmuDC with 0.5% pol III core or <0.1% pol II, minute levels which would not show up on either Coomassie or silver-stained polyacrylamide gels, could account for DNA synthesis in the absence of exogenous pol. Contamination with pol I was ruled out by using a potent pol I neutralizing antibody. It is unlikely that UmuDC is bound to either pol III, pol I α-subunit, or pol II, because the Umu complex migrates with its expected molecular mass of 70 kDa based on Sephadex gel filtration in 1 M salt (17).

The weak, possibly adventitious, pol activity was titrated out by adding a large excess of purified pol in the assay, enabling us to examine the effect of UmuDC-stimulated bypass in the presence of pol III or pol II. By using up to a 20-fold excess of pol III core and pol II, we determined that the percentage of lesion bypass actually decreased by about a factor of two with increasing pol III activities (Fig. 2), attributable to proofreading at the lesion site and at a site one base downstream from the lesion. Despite the decrease in the percentage of lesion bypass with increasing concentrations of pol III core and pol II, there is a marked increase in the synthesis of intermediate- and full-length product DNA (Fig. 2), suggesting that pol III core or pol II can bind to primers that have been extended past the lesion for continued synthesis along an undamaged region of the template.

The principal conclusion to be drawn from the pol titration measurements is that both lesion bypass reactions, incorporation opposite the lesion and extension past the lesion, are determined by the action of the UmuDC and RecA* proteins, rather than by pol III, α-subunit, or pol II, because lesion bypass is reduced significantly for each pol in the absence of UmuDC (Fig. 2). Conversely, when the concentration of UmuDC is varied at a fixed pol concentration, the efficiency of bypass increases, reaching a maximum value at a ratio of about 100 UmuDC to 1 p/t DNA.
Effects of UmuDC on DNA Synthesis Fidelity and Processivity. Synthesis on undamaged DNA before encountering a lesion is stimulated in the presence of UmuDC and RecA* (Fig. 1). However, in contrast to processive synthesis carried out by pol III core plus βγ-complex, synthesis in the presence of UmuDC is distributive both upstream and downstream from the site of the lesion either in the presence or absence of exogenous pol III core (Fig. 1).

Perhaps the most noteworthy property of UmuDC is reflected in its ability to stimulate nucleotide misincorporation and mismatch extension at aberrant and normal template sites. This property is illustrated vividly in an experiment in which just a single dNTP is present for incorporation at an abasic site (Fig. 3A) or at a normal T (replacing the abasic site), in the same sequence context (Fig. 3B). UmuDC stimulates incorporation of each dNTP substrate opposite the lesion (including incorporation of riboA), and causes multiple misincorporations downstream from the lesion, whereas pol III core and pol II catalyze negligible incorporation either at the abasic site or beyond (Fig. 3A). In a similar vein, UmuDC stimulates misincorporations opposite a normal template T site and beyond, whereas pol III core and pol II predominantly incorporate only A opposite T (Fig. 3B).

These data illustrate how the Umu complex can be involved in both SOS-targeted and untargeted mutagenesis. In the case of mutagenesis targeted to the site of a lesion, UmuDC causes a reduction in polymerization fidelity leading to an increase in misincorporation opposite the lesion and to a stimulation of extension past the lesion by using an aberrant primer terminus. In a recent study, it was demonstrated that SOS-dependent spontaneous mutator activity reflects the processing of replication errors containing normal bases, rather than errors opposite cryptic lesions (39). Indeed, extension of natural base mispairs occurs with efficiencies of about 10^4 to 10^5 compared with extension of correctly matched base pairs (40, 41), and thus presents a strong kinetic block to further elongation. Therefore, the role of the UmuDC proteins in SOS mutagenesis may be to serve as generalized “elongation” factors that are used by the cell to extend any kinetically unfavorable primer terminus junction, not just those at a DNA lesion.

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