

The Mutagenesis Protein UmuC Is a DNA Polymerase Activated by UmuD', RecA, and SSB and Is Specialized for Translesion Replication*

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Replication of DNA lesions leads to the formation of mutations. In *Escherichia coli* this process is regulated by the SOS stress response, and requires the mutagenesis proteins UmuC and UmuD'. Analysis of translesion replication using a recently reconstituted *in vitro* system (Reuven, N. B., Tomer, G., and Livneh, Z. (1998) *Mol. Cell* 2, 191–199) revealed that lesion bypass occurred with a UmuC fusion protein, UmuD', RecA, and SSB in the absence of added DNA polymerase. Further analysis revealed that UmuC was a DNA polymerase (*E. coli* DNA polymerase V), with a weak polymerizing activity. Upon addition of UmuD', RecA, and SSB, the UmuC DNA polymerase was greatly activated, and replicated a synthetic abasic site with great efficiency (45% bypass in 6 min), 10–100-fold higher than *E. coli* DNA polymerases I, II, or III holoenzyme. Analysis of bypass products revealed insertion of primarily dAMP (69%), and to a lesser degree dGMP (31%) opposite the abasic site. The UmuC104 mutant protein was defective both in lesion bypass and in DNA synthesis. These results indicate that UmuC is a UmuD', RecA-, and SSB-activated DNA polymerase, which is specialized for lesion bypass. UmuC is a member of a new family of DNA polymerases which are specialized for lesion bypass, and include the yeast *RAD30* and the human *XP-V* genes, encoding DNA polymerase η .

Mutagenesis caused by UV light and by many other DNA damaging agents in *Escherichia coli* is under control of the SOS response, a highly regulated stress response, which functions to increase cell survival under adverse environmental conditions that cause DNA damage (1). Genetic analysis has uncovered four genes, whose products are required for SOS mutagenesis. Two of these, DNA polymerase III (pol-III)¹ and RecA, participate also in replication and recombination, respectively.

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¹ The abbreviations used are: pol-I, -II, -III, polymerase I, II, and III, respectively; PCR, polymerase chain reaction; bp, base pair(s); ss, single-stranded; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis.

The other two, UmuD and UmuC, are specifically required for the mutagenic reaction. It was found that UmuD is processed into a shorter form, UmuD', which is the form active in SOS mutagenesis (reviewed in Ref. 2).

Based on *in vivo* and *in vitro* data, UmuD' and UmuC were thought to be accessory proteins, which assist DNA polymerase III in replicating DNA lesions which usually block replication (2–5). According to this mechanism, the mutations occur by misinsertion opposite the DNA lesion by the DNA polymerase, a result of the miscoding nature of most DNA lesions. Recently SOS mutagenesis was reconstituted with purified components in two laboratories (6, 7). The results, which confirmed an earlier study (4), provided strong biochemical evidence that SOS mutagenesis occurs by replication through DNA lesions, in a reaction which depends on UmuC, UmuD', RecA and SSB. Moreover, it was shown that there is a qualitative difference in the specificity of bypass when translesion replication was compared in the absence or presence of SOS proteins. DNA polymerase III holoenzyme bypassed an abasic site via a misalignment mechanism, resulting in skipping over the lesion, and the formation of –1 frameshifts (7, 8). In contrast, in the presence of UmuC, UmuD', RecA, SSB, and pol-III holoenzyme, the abasic site was replicated, with an A usually inserted opposite it (7). Here we report that *in vitro* SOS translesion replication occurs in the absence of added DNA polymerase, and that UmuC is a DNA polymerase, which is activated by UmuD', RecA, and SSB, and performs very effective lesion bypass.

MATERIALS AND METHODS

Proteins—UmuD', UmuD, and the MBP-UmuC fusion protein were overexpressed and purified as described previously (7). The UmuC was further purified by heparin-Sepharose CL-6B chromatography (Amersham Pharmacia Biotech). A gradient of 80–1000 mM NaCl was used, and UmuC was eluted at 600 mM NaCl. The *umuC104* allele was constructed by PCR-based site-directed mutagenesis, introducing the 720GAT→AAT mutation (9). Using plasmid pMAC as a template, the 5'-terminal portion of *umuC* was amplified using the primers 5'-ATG GGG TAA ACC GGT GGT TGT-3' (primer 338) and 5'-CTC ATT AAT ACT GTA AAT CTC-3' (primer 342), and the 3'-terminal portion of *umuC* was amplified using the primers 5'-CCG GAA TTC TTT ATT TGA CCC TCA GTA AAT C-3' (primer 131) and 5'-GTA TTA ATG AGG CAT TCT GCG-3' (primer 341). The resulting fragments (241 and 983 bp, respectively) contained a sequence overlap of 11 nucleotides spanning the *umuC104* mutation. The DNA fragments were gel-purified, mixed, and used in a final PCR step with primers 338 and 131 to construct the entire *umuC104* gene. The PCR product (1214 bp) was cut with *AgeI* and *EcoRI* and subcloned into pMAC, which was previously cleaved with the same nucleases. The resulting plasmid was termed pMAC104. The sequence of the *umuC104* gene was verified by DNA sequence analysis. The MBP-UmuC104 protein was purified as described for MBP-UmuC. SSB and RecA were purified as described (Refs. 10 and 11, respectively), except that a phosphocellulose purification step was added for RecA. Restriction nucleases, T4 DNA ligase and T4 polynucleotide kinase were from New England Biolabs. T7 gp6 exonuclease was from Amersham Pharmacia Biotech, and Pwo DNA polymerase, polymerase I (pol-I), and exonuclease III were from Roche Molecular Biochemicals.

DNA Substrates—The preparation of the gapped plasmid carrying a site-specific lesion was recently described (8, 12). Throughout this study we used gapped plasmid GP21, which contained a site-specific synthetic (tetrahydrofuran) abasic site, and a ssDNA region of approximately 350 nucleotides (Fig. 1). The undamaged gapped plasmid (a plasmid with no base lesion) was prepared by nicking plasmid pOC2 (13) with *AatII* in the presence of 0.1 mg/ml ethidium bromide (14). The site-specific nicks were converted into gaps using exonuclease III, in a reaction mixture (300 μ l) containing 30 μ g of FII pOC2 and 300 units of exonuclease III,

for 25 min at 37 °C. The size of the gap was deduced to be approximately 350 nucleotides, based on the electrophoretic migration of the DNA after digestion of the ssDNA region with S1 nuclease. The primed and the gapped oligonucleotides were prepared as described previously (15, 16). Briefly, a ^{32}P 5'-labeled synthetic 19-mer (5'-TGCTGCAAGGC-GATTAAGT-3') was annealed to the template 5'-GGAAAACCTG-GCGTTAGCCGACTTAATCGCCTTGCA-3' (40-mer) to generate the primed template. The gapped duplex oligonucleotide was prepared in a similar way, except that an additional oligonucleotide, 16 nucleotides long (5'-AACGCCAGGGTTTCC-3') was annealed to the template, such that a duplex with a 5-nucleotides ssDNA gap was formed (Fig. 3).

Translesion Replication Assay—The translesion replication reaction was performed as described previously (7, 8), with minor changes. The reaction mixture (25 μl) contained 20 mM Tris-HCl, pH 7.5, 8 $\mu\text{g/ml}$ bovine serum albumin, 5 mM DTT, 0.1 mM EDTA, 4% glycerol, 1 mM ATP, 10 mM MgCl_2 , 0.1 mM each of dATP, dGTP, dTTP, and dCTP, 0.1 μg (2 nM) of gapped plasmid, 0.6 μM SSB, 4 μM RecA, 2.5 μM UmuD' or UmuD, and 10–230 nM MBP-UmuC. Reactions were carried out at 37 °C for the indicated periods of time. Analysis of the bypass products was modified as follows: prior to cleavage the reaction mixture was treated with calf intestine alkaline phosphatase (0.2 units, 1 h, 37 °C), to hydrolyze remaining dNTPs. This step was introduced because some restriction nuclease preparations were contaminated with DNA polymerase. The DNA was then digested with *Asp700* (5 units) and *MspA11* (5 units) to produce radiolabeled DNA bands which were four nucleotides longer than with the original *XmnI*/*BstXI* cleavage (Fig. 1). The DNA samples were fractionated by 15% PAGE-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. The specificity of bypass was determined by DNA sequence analysis of bypass products, as described previously (7).

DNA Synthesis Assays—Gap-filling DNA synthesis was performed with unlabeled gapped plasmid pOC2, which contained no nucleotide lesions. The reaction mixture (25 μl) was performed under conditions similar to those of the translesion replication reaction, except that it contained 5 nM gapped plasmid pOC2, 0.1 mM each of dATP, dCTP, and dGTP, 10 μM [$\alpha\text{-}^{32}\text{P}$]dTTP, 0.6 μM SSB, 4.2 μM RecA, 4.8 μM UmuD', 500 nM fusion UmuC protein, and 8 units/ μl of T4 DNA ligase. Reactions were incubated for 5–20 min at 37 °C, after which the reaction products were analyzed by agarose gel electrophoresis followed by phosphorimaging. Primer extension assays by UmuC were performed with ^{32}P end-labeled primed oligonucleotide or gapped duplex oligonucleotide. The reaction mixture (25 μl) was similar to that of the translesion replication assay except that oligonucleotide substrates were at 55 nM.

RESULTS AND DISCUSSION

The SOS translesion replication reaction that was reconstituted in our laboratory with purified components included a gapped plasmid carrying a site-specific lesion in the ssDNA region, pol-III holoenzyme, a UmuC fusion protein, UmuD', RecA, and SSB (7). The substrate used, termed GP21, was a gapped plasmid containing a ssDNA region of ~350 nucleotides, a synthetic abasic site in the ssDNA region, and an internal radiolabeled phosphate in the primer terminus strand (7, 8). A portion of GP21, including the vicinity of the lesion, is shown in Fig. 1 (upper panel). Addition of a DNA polymerase led to extension of the primer up to the lesion, and when lesion bypass occurred, synthesis continued past the lesion. The analysis of replication products was done by cutting the products with restriction nucleases *MspA11*, which cuts four nucleotides upstream to the radiolabel, and *Asp700*, which cuts downstream to the lesion, followed by urea-PAGE (Fig. 1, upper panel). The products were then visualized by phosphorimaging.

In an attempt to define the minimal requirement of this lesion bypass reaction, we examined whether all 10 subunits of pol-III holoenzyme were required for Umu-dependent translesion replication. Surprisingly, we found that translesion replication occurred in the absence of any added pol-III holoenzyme subassembly. This suggested that one of the components other than pol-III contained DNA polymerase activity. The prime candidate was UmuC because, in contrast to UmuD' (12 kDa), it is large enough to be a DNA polymerase (48 kDa). Fig. 1

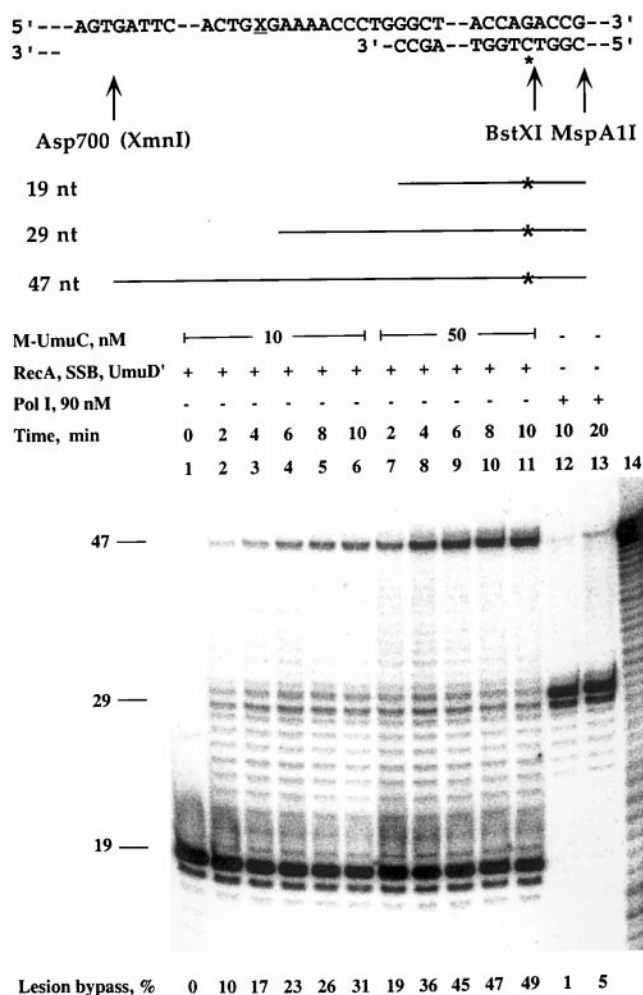


FIG. 1. Translesion replication by the UmuC fusion protein in the presence of UmuD', RecA, and SSB. Upper panel, the DNA substrate used in the lesion bypass assay. The DNA sequence in the vicinity of the site-specific synthetic abasic site in gapped plasmid GP21 is shown. The asterisk marks an internal radiolabeled phosphate. The cleavage sites of restriction nucleases *Asp700* (*XmnI*), *BstXI*, and *MspA11* are indicated. The replication products obtained after cleavage with restriction nucleases *Asp700* and *MspA11* were 19, 29, and 47 nucleotides long, for the unextended primer, the product arrested at the lesion, and the bypass product, respectively (shown underneath the sequence). Lower panel, a time course of translesion replication was performed as described under "Materials and Methods" with 10 or 50 nM MBP-UmuC (*M-UmuC*), as indicated. DNA polymerase I in the control reactions was at 90 nM. The reaction products were restricted with *Asp700* and *MspA11*, followed by urea-PAGE fractionation and phosphorimager analysis. Lane 14 contains a ^{32}P -labeled 47-mer marker oligonucleotide, representing the expected bypass product.

shows translesion replication by the UmuC fusion protein, in the presence of UmuD', RecA, and SSB, without added DNA polymerase. Notice that lesion bypass occurred with a UmuC concentration as low as 10 nM, arguing against the presence of a contaminating DNA polymerase in the UmuC preparation. Interestingly, initiation of replication by the UmuC polymerase was not very effective, as indicated by the amount of unextended primer (Fig. 1, lower panel). However, once polymerization started, it progressed without much inhibition at the lesion. For comparison, Fig. 1 contains reactions with DNA pol-I. As can be seen (Fig. 1, lanes 12 and 13), pol-I was strongly inhibited at the abasic site, and bypass was 50-fold lower than with UmuC/UmuD'/RecA/SSB although a higher concentration of pol-I was used. These results indicate that one of the proteins, most likely UmuC, is a DNA polymerase specialized for translesion replication.

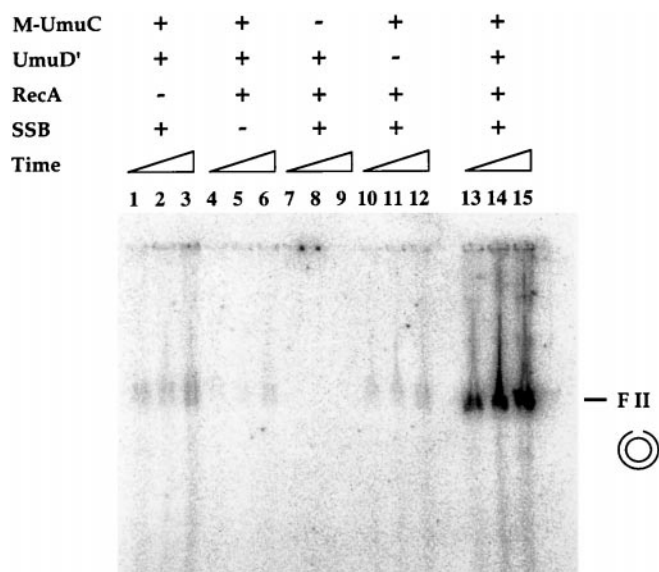


FIG. 2. UmuC DNA polymerase activity is stimulated by UmuD', RecA, and SSB. The DNA synthesis assay was performed as described under "Materials and Methods" with an unlabeled gapped plasmid, radiolabeled dTTP, and unlabeled dCTP, dATP, and dGTP, and the protein compositions are indicated on top of the figure. After incubation for 5, 10, and 20 min at 37 °C, the samples were treated with proteinase K, fractionated by agarose gel electrophoresis, dried, and visualized by phosphoimaging.

The ability of the UmuC/UmuD'/RecA/SSB proteins to carry out DNA synthesis on undamaged DNA was assayed by the incorporation of radiolabeled dTTP into unlabeled gapped plasmid with no lesions. The replication products were fractionated by agarose gel electrophoresis, followed by phosphoimaging. Fig. 2 (lanes 13–15) shows that the UmuC/UmuD'/RecA/SSB proteins catalyze DNA synthesis on undamaged DNA. To establish the identity of the DNA polymerase, each of the components was omitted, one at a time, and DNA synthesis was examined in the same way. As can be seen in Fig. 2, omission of each of RecA, SSB, or UmuD' caused a strong reduction but not complete elimination of DNA synthesis. In contrast, omission of the UmuC fusion protein completely abolished DNA synthesis (Fig. 2, lanes 7–9). This indicates that UmuC is a DNA polymerase and that UmuD', RecA, and SSB cause a strong stimulation of its activity.

In an attempt to directly demonstrate the DNA polymerase activity of UmuC, a synthetic oligonucleotide template, 40-nucleotides long, primed with a ³²P end-labeled 19-mer oligonucleotide was used as a substrate. In addition, a gapped duplex oligonucleotide was prepared, by annealing an additional 16-mer oligonucleotide to the same primer template, such that a 5-nucleotides single-stranded gap was formed (Fig. 3). As can be seen in Fig. 3, MBP-UmuC alone had a very weak DNA polymerase activity (lane 4), but it was slightly stronger on the gapped duplex (lane 9). We subjected the MBP-UmuC protein to an additional purification step on a heparin-Sepharose affinity column. As can be seen in Fig. 3, the DNA polymerase activity of this preparation was higher (lane 2), as compared with the previous preparation (lane 4). Again, activity on the gapped duplex was higher than on the primed template (Fig. 3, compare lane 7 to lane 2). Adding UmuD' did not change the activity of UmuC (Fig. 3, lanes 1, 3, 6, and 8). The ability of UmuC to bypass an abasic site was tested with the same set of oligonucleotides, which contained a synthetic abasic site in the template strand at position 20 (15). It was found that UmuC alone, or together with UmuD', were unable to bypass the lesion (data not shown). The same result was ob-

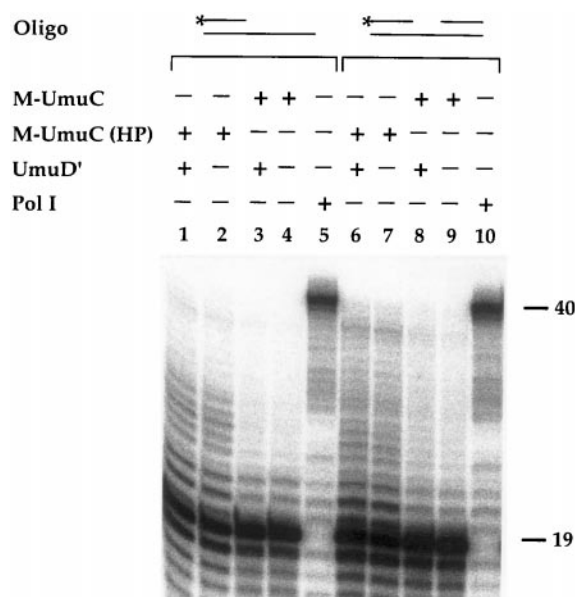


FIG. 3. DNA polymerase activity of UmuC in the absence of RecA and SSB. The reactions were performed with a primed oligonucleotide, or a gapped duplex oligonucleotide, as described under "Materials and Methods," with 230 nM MBP-UmuC and 2.5 μM UmuD'. Pol-I (90 nM) was used as a control. The reactions were conducted for 30 min at 37 °C. M-UmuC (HP) is the MBP-UmuC protein purified by an additional heparin-Sepharose column.

tained with the gapped plasmid GP21 (data not shown). Therefore, although UmuC is a DNA polymerase, its remarkable lesion bypass ability depends on UmuD', RecA, and SSB.

To provide further evidence that UmuC is a DNA polymerase, the UmuC104 mutant protein was overexpressed and analyzed. This protein contains a Asp¹⁰¹ → Asn amino acid substitution, which renders it non-mutable by UV light *in vivo* (9, 17). This mutation is in the SIDE motif, which is conserved among all homologues of UmuC (1, 18). A side-by-side comparison of translesion replication activity revealed that the mutant protein was completely defective in lesion bypass, consistent with its *in vivo* phenotype (Fig. 4, compare lanes 2 and 3 to lanes 4 and 5; and lanes 9 and 10 to lanes 11 and 12). UmuC104 also lost its ability to extend the primer (Fig. 4, lanes 6 and 7 and lanes 13 and 14), indicating that Asp¹⁰¹ is essential for both polymerase and lesion bypass activities.

The specificity of bypass by UmuC/UmuD'/RecA/SSB was examined by determining the DNA sequence of the newly synthesized bypass products. Analysis of 16 isolates revealed that A was most frequently inserted opposite the synthetic abasic site (69%, 11/16), whereas G was inserted less frequently (31%, 5/16). This specificity is in agreement with the *in vivo* mutagenic specificity of abasic sites (19–21).

In addition to the results presented above, it was found that: (1) the DNA polymerase activity of the UmuC fusion protein was retained when it was purified from an *E. coli* strain lacking pol-II, therefore eliminating the possibility of a contamination of pol-II. (2) Adding pol-I or pol-III core to translesion replication reactions did not increase lesion bypass; in fact it caused some inhibition, probably because of competition for the primer-template terminus (data not shown). Taken together these results indicate that UmuC is a lesion bypass DNA polymerase whose activity requires UmuD', RecA, and SSB.

The most striking property of the UmuC polymerase, is its remarkable ability to replicate the synthetic abasic site with high efficiency. When compared with other *E. coli* DNA polymerases acting on the same substrate (Ref. 12, and Fig. 1), UmuC/UmuD'/RecA/SSB are 50–100-fold more efficient than

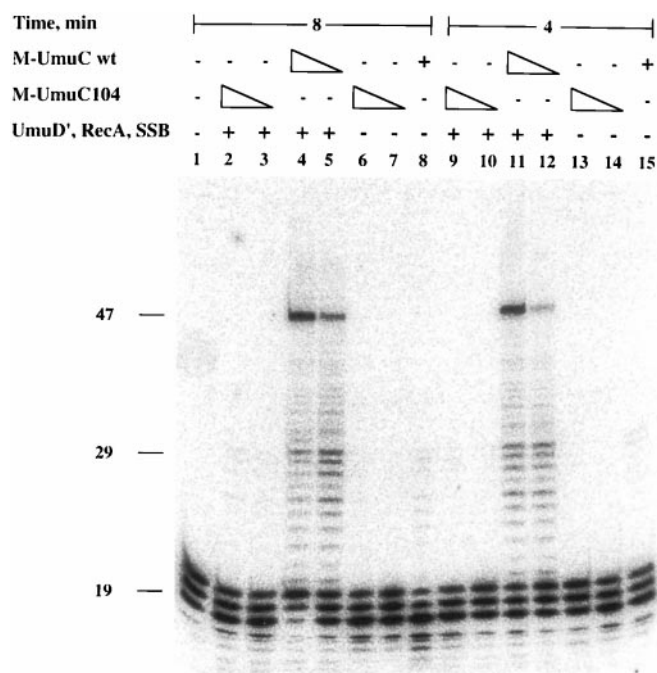


FIG. 4. **The UmuC104 protein is defective in both lesion bypass and DNA synthesis.** Translesion replication reactions were performed as described under "Materials and Methods" with MBP-UmuC (*M-UmuC*) or MBP-UmuC104 (*M-UmuC104*), 25 and 100 nM each, in the presence of UmuD', RecA, and SSB. When assayed in the absence of UmuD', RecA, and SSB (lanes 6–8 and 13–15), MBP-UmuC was at 100 nM, and MBP-UmuC104 was at 100 nM. Reaction products were restricted with *Asp700* and *MspA11*, followed by urea-PAGE fractionation and phosphoimaging.

pol-I or pol-II in lesion bypass. Pol-III holoenzyme was only 5–10-fold less effective than UmuC/UmuD'/RecA/SSB in bypassing the synthetic abasic site, but this bypass produced exclusively frameshifts, a lethal type of mutation (7, 8). In contrast, UmuC/UmuD'/RecA/SSB replicated the lesion, forming primarily base substitution, a milder type of mutation.

The initiation of polymerization by the UmuC DNA polymerase in the presence of UmuD', RecA, and SSB is slow under our reaction conditions, as indicated by the amount of unextended primer termini. This might indicate that loading of the UmuC DNA polymerase on DNA may require a special factor, although at this stage we cannot exclude the possibility that the fused MBP moiety interferes with initiation. Tang *et al.* have reported that SOS lesion bypass required the β subunit sliding DNA clamp, and the γ complex clamp loader, which together make up for six of the accessory subunits of pol-III holoenzyme (6). We have previously indicated that pol-III holoenzyme was required for lesion bypass, without establishing which of the ten subunits of pol-III holoenzyme were needed (7). It is clear from the results presented here that the actual replication of the abasic site did not require any of the subunits of pol-III holoenzyme. However, pol-III holoenzyme, or at least some of its subunits, may act along with UmuC to increase the overall efficiency of translesion replication. This can occur, for example, by stimulating the initiation stage of translesion replication or by facilitating the extension of products bypassed by UmuC/UmuD'/RecA/SSB. Such possibilities might explain the *in vivo* requirement for pol-III in SOS mutagenesis (22–24).

During preparation of this manuscript, Tang *et al.* (25) reported that the UmuD'₂C complex is a DNA polymerase and termed it DNA polymerase V. Our results generally agree with those of Tang *et al.* (25) and show directly that UmuC itself is the DNA polymerase. A major difference between the two lab-

oratories is in the protein requirements for translesion replication. In our system, lesion bypass required the UmuC fusion protein, UmuD', RecA, and SSB, whereas Tang *et al.* (25) reported that, in addition, six accessory subunits of pol-III holoenzyme, the 5-subunit γ complex (the clamp loader) and the β subunit processivity clamp, were required. This difference may stem from the differences between the two experimental systems utilized: (1) This study used a gapped circular DNA with a site-specific lesion in the ssDNA region, whereas Tang *et al.* (25) used a linear ssDNA with the lesion located 50 nucleotides from the DNA end. (2) We used an MBP-UmuC fusion protein and UmuD', whereas Tang *et al.* (25) used a complex of UmuD'₂C. Further experiments are needed to resolve the discrepancy in the requirement for pol-III accessory proteins in lesion bypass.

The UmuC DNA polymerase is part of a novel family of DNA polymerases which function in lesion bypass. This includes the products of the *Saccharomyces cerevisiae* *RAD30*, and the human *XP-V* genes, which encode DNA polymerase η (26, 27). In addition, the *E. coli* *dinB* gene was recently shown to encode a DNA polymerase (*E. coli* DNA polymerase IV) (28). This gene is a *umuC* homologue, which functions in phage λ untargeted mutagenesis (29). The discovery of this class of DNA polymerases underscores the theme of DNA polymerases with specialized functions. There are DNA polymerases specialized for chromosome replication, for excision repair, and now also for translesion replication.

REFERENCES

- Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA Repair and Mutagenesis*, ASM Press, Washington, D. C.
- Walker, G. C. (1995) *Trends Biochem. Sci.* **20**, 416–420
- Banerjee, S. K., Christensen, R. B., Lawrence, C. W., and LeClerc, J. E. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 8141–8145
- Rajagopalan, M., Lu, C., Woodgate, R., O'Donnell, M., Goodman, M., and Echols, M. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 10777–10781
- Livneh, Z., Cohen-Fix, O., Skaliter, R., and Elizur, T. (1993) *CRC Crit. Rev. Biochem. Mol. Biol.* **28**, 465–513
- 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
- Reuven, N. B., Tomer, G., and Livneh, Z. (1998) *Mol. Cell* **2**, 191–199
- Tomer, G., Reuven, N. B., and Livneh, Z. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 14106–14111
- Koch, W. H., Ennis, D. G., Levine, A. S., and Woodgate, R. (1992) *Mol. Gen. Genet.* **233**, 443–448
- Lohman, T. M., and Overman, L. B. (1985) *J. Biol. Chem.* **260**, 3594–3603
- Cox, M. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem.* **256**, 4676–4678
- Tomer, G., and Livneh, Z. (1999) *Biochemistry* **38**, 5948–5958
- Cohen-Fix, O., and Livneh, Z. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 3300–3304
- Barzilai, R. (1973) *J. Mol. Biol.* **74**, 739–742
- Paz-Elizur, T., Takeshita, M., Goodman, M., O'Donnell, M., and Livneh, Z. (1996) *J. Biol. Chem.* **271**, 24662–24669
- Paz-Elizur, T., Takeshita, M., and Livneh, Z. (1997) *Biochemistry* **36**, 1766–1773
- Steinborn, G. (1978) *Mol. Gen. Genet.* **165**, 87–93
- McDonald, J. P., Levine, A. S., and Woodgate, R. (1997) *Genetics* **147**, 1557–1568
- Kunkel, T. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 1494–1498
- Lawrence, C. W., Borden, A., Banerjee, S. K., and LeClerc, J. E. (1990) *Nucleic Acids Res.* **18**, 2153–2157
- Takeshita, M., and Eisenberg, W. (1994) *Nucleic Acids Res.* **22**, 1897–1902
- Bridges, B. A., Mothershead, R. P., and Sedgwick, S. G. (1976) *Mol. Gen. Genet.* **144**, 53–58
- Brotcorne-Lannoye, A., Maenhaut-Michel, G., and Radman, M. (1985) *Mol. Gen. Genet.* **199**, 64–69
- Bridges, B. A., and Bates, H. (1990) *Mutagenesis* **5**, 35–38
- 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
- Johnson, R. E., Prakash, S., and Prakash, L. (1999) *Science* **283**, 1001–1004
- Masutani, C., Kusumoto, R., Yamada, A., Dohmae, N., Yokoi, M., Yuasa, M., Araki, M., Iwai, S., Takio, K., and Hanaoka, F. (1999) *Nature* **399**, 700–704
- Wagner, J., Gruz, P., Kim, S. R., Yamada, M., Matsui, K., Fuchs, R. P. P., and Nohmi, T. (1999) *Mol. Cell*, **4**, 281–286
- Brotcorne-Lannoye, A., and Maenhaut-Michel, G. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 3904–3908