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Intrinsic Polymerase Activities of UmuD′$_2$C and MucA′$_2$B Are Responsible for Their Different Mutagenic Properties during Bypass of a T-T *cis-syn* Cyclobutane Dimer

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In wild-type *Escherichia coli*, translesion replication is largely dependent upon the UmuD′$_2$C complex (DNA polymerase V [polV]) or its plasmid-encoded homologs, such as MucA′$_2$B. Interestingly, both the efficiency of translesion replication of a T-T *cis-syn* dimer and the spectra of mutations observed are different in Umu- and Muc-expressing strains. We have investigated whether the polIII core is responsible for these differences by measuring the frequency of dimer bypass, the error rate of bypass, and the resulting mutation spectrum in mutants carrying a deletion of *dnaQ* (ε subunit) or *holE* (θ subunit) or carrying the *dnaQ* allele *mutD5*, which is deficient in proofreading but is competent in the structural function of ε, or the *dnaE* antimutator allele *spq-2*. The chromosomal copy of the *umuDC* operon was deleted in each strain, and the UmuDC, UmuD′C, MucAB, or MucA′B proteins were expressed from a low-copy-number plasmid. With only few exceptions, we found that the characteristically different mutation spectra resulting from Umu- and Muc-mediated bypass are maintained in all of the strains investigated, indicating that differences in the activity or structure of the polIII core are not responsible for the observed phenotype. We also demonstrate that the MucA′$_2$B complex is more efficient in promoting translesion replication than the UmuD′$_2$C proteins and show that, contrary to expectation, the T-T dimer is bypassed more accurately by MucA′$_2$B than by UmuD′$_2$C. These results are consistent with the view that in a wild-type cell, the polV-like enzymes are responsible for the spectra of mutations generated during translesion replication and that polIII may simply be required to fix the misincorporations as mutations by completing chromosomal replication. Our observations also show that the mutagenic properties of a lesion can depend strongly on the particular enzyme employed in bypass.

It has been inferred, on the basis of a variety of genetic evidence obtained over the last 20 years, that translesion replication and DNA damage-induced mutagenesis (SOS mutagenesis) in *Escherichia coli* are dependent on the activity of DNA polymerase III (polIII) holoenzyme or a modified form of it (4, 5, 14, 26, 27; reviewed in reference 10). Although polIII is usually unable to perform lesion bypass by itself, it can do so with the aid of what were previously thought to be accessory factors, the chromosomally encoded UmuDC proteins or their plasmid-encoded homologs, such as MucAB (16, 22, 30). However, the recent discovery that the UmuD′$_2$C protein complex, now called polV, itself possesses intrinsic DNA polymerase activity (24, 25, 32, 33) raises the question of which enzyme is responsible for mutagenesis. Is polV solely responsible, or do some or all of the polIII subunits also influence SOS mutagenesis in vivo? In vitro studies show that polV is capable of both nucleotide incorporation opposite an abasic site and extension from this terminus in the absence of the polIII core (composed of the catalytic α subunit, the 3′-5′ exonucleolytic proofreading ε subunit, and the θ subunit, whose function is not yet known). The addition of a low level of polIII to the in vitro reaction mixture nevertheless stimulates elongation by some as yet undefined mechanism (33), raising the possibility that one or more of the polIII core subunits might influence efficiency of lesion bypass in vivo.

Although most attention has been given to translesion replication employing the Umu proteins, many homologs encoded on naturally occurring R plasmids (30) are capable of providing a substitute function, which presumably is also a DNA polymerase activity. These include the *mucAB* operon from the incN plasmid R46 and its better-characterized deletion derivative pKM101 (22) and the *rumAB* operon from the incJ plasmid R391 (18). Although these homologs are clearly related structurally and belong to the same branch of the UmuC/DinB/Rev1/Rad30 superfamily of DNA polymerases (21), they are not entirely equivalent with respect to their mutagenesis-promoting properties (1, 18, 35). A particularly clear example of this lack of equivalence is the difference in the predominant type of mutation that occurs during Umu- or Muc-facilitated replication past a site-specific T-T *cis-syn* cyclobutane dimer. Earlier studies (2, 19) using SMH10, a *uvrA6* derivative of AB1157 which contains a normal chromosomal copy of the *umuDC* operon, showed that almost all of the errors induced were 3′ T→A and 3′ T→C mutations and that there were about fivefold (130:28) more transversions than transitions. A similar result was found in experiments using RW82, another *uvrA6* derivative of AB1157 in which the chromosomal copy of the *umuDC* operon is deleted (37) and in which the *umu* genes were carried on a low-copy-number plasmid (31). However, the ratio of transitions to transversions was reversed when Muc

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proteins were substituted and the predominant mutation was 3′ T→C. The MucA′$_2$B and UmuD′$_2$C complexes also differed with respect to bypass efficiency; the MucAB proteins promoted bypass more efficiently than their Umu counterparts, in keeping with their known ability to enhance DNA damage-induced mutation frequencies above those usually seen in strains expressing the *umuDC* operon (1, 18, 35). Even so, the error rate of MucA′$_2$B-assisted bypass did not appear to be higher; indeed, if anything, it was lower.

A variety of mechanisms might cause differences in the mutagenic properties of a T-T dimer when Muc rather than Umu proteins are employed. They might arise from different inherent error-making properties of the DNA polymerase that inserts nucleotides opposite the lesion. However, if this is the case, such a phenomenon would be highly unusual because the same predominant type of mutation appears to be determined by the structure of the lesion rather than the enzyme; even though the error rate may vary, the same major type of mutation is normally induced by a given lesion, even when introduced into very different enzymatic environments, such as those found within yeast and *E. coli* cells (2, 3, 11, 12, 19, 20). Alternatively, if the polIII holoenzyme is involved in translesion replication in vivo, it might, according to whether the Umu or Muc proteins were involved, elongate differentially from the T · T and T · G mispairs which are responsible for the mutations observed. Moreover, these mismatches might be subject to differential proofreading by the 3′-5′ exonuclease activity of the polIII ε subunit encoded by *dnaQ*.

The aim of the work described in this report was, therefore, to reassess the respective roles of the polIII core and polV or its functional homolog MucA′$_2$B in SOS mutagenesis in vivo. To this end, we have examined the frequency of translesion replication past a single T-T *cis-syn* cyclobutane dimer, together with the error frequency of bypass and the mutation spectra, in various *E. coli* strains containing mutations or deletions of each of the polIII core subunits (α, *dnaE*; ε, *dnaQ*; θ, *holE*) in the presence of the *E. coli* UmuDC proteins or their highly active plasmid-encoded homologs MucAB. We found that the mutation spectra characteristic of Umu and Muc proteins are maintained in almost all of the strains and under almost all of the conditions investigated, indicating that differences in the activity or structure of the polIII core are not responsible for the observed phenotype. We also found that the MucA′$_2$B complex is more efficient at promoting translesion replication than the UmuD′$_2$C proteins and that, contrary to expectation, bypass of the T-T dimer dependent on MucA′$_2$B is more accurate than that mediated by UmuD′$_2$C. Such findings show that a lesion's mutagenic parameters can be greatly influenced by the properties of the polymerase employed in translesion replication.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. The strains are all isogenic with *uvrA6* mutant strain TK603 (17). Most have been described before, and details of their construction are given in reference 31. The exception is DV01, which was made by transducing the Δ*holE202*::*cat* allele from RM4193 into EC8 and selecting for chloramphenicol resistance (29). The construction of plasmids expressing the UmuDC or MucAB proteins or their mutagenically active counterparts UmuD′C and MucA′B is described in reference 31.

Preparation of vectors with a site-specific T-T *cis-syn* cyclobutane dimer. Single-stranded vectors based on the hybrid phage M13mp7L2 (2) and carrying a specifically located T-T *cis-syn* cyclobutane dimer were constructed as described previously (2, 3). In this method, viral DNA from M13mp7L2 is linearized by digestion with *Eco*RI, which cuts within the small hairpin region, and the linear DNA is recircularized by annealing with a 51-mer scaffold oligomer. The ends of the scaffold are complementary to the terminal 20 nucleotides at each of the ends of the linearized vector, which are therefore separated by 11 nucleotides. An 11-mer with a unique T-T dimer is then ligated efficiently into this gap, and the scaffold is removed by heat denaturation in the presence of a large excess of an antisense 51-mer which has a sequence complementary to that of the scaffold. The control vector is constructed from equal aliquots of the recircularized material in identical reactions carried out with the unmodified 11-mer. Under the conditions used, ligation efficiencies are >90% and equal for the control and modified materials. Molecules containing the dimer are produced by exposing 50 μg of the oligomer 5′ GCAAGTTGGAG 3′ in 100 μl of anoxic aqueous acetophenone (2×10^{-2} M) to ~250 kJ of filtered sunlamp radiation (>315 nm) per m^2. The desired species is purified by high-performance liquid chromatography and repeatedly repurified to achieve >99.5% purity.

TABLE 1. Strains and plasmids used in this work

Strain or plasmid	Relevant genotype or characteristics	Source or reference
Strains		
TK603	*uvrA6*	17
RW82	Same as TK603 but Δ*umuDC*595::*cat*	37
EC8	Same as TK603 but Δ*umuDC*596::*ermGT*	9
RM4193	Δ*holE202*::*cat*	29
DV01	Same as EC8 but Δ*holE202*::*cat*	This work
DV02	Same as EC8 but *dnaQ*903::*tet spq-2 zae*::Tn*10d*-Cam	34
DV03	Same as EC8 but *spq-2 zae*::Tn*10d*-Cam	34
DV05	Same as EC8 but *mutD5 zaf-13*::Tn*10*	34
DV12	Same as TK603 but *mutD5 zaf-13*::Tn*10*	34
DV13	Same as EC8 but *mutD5 zaf-13*::Tn*10 lexA3* (Ind$^-$)	34
Plasmids		
pRW154	Low-copy-number plasmid expressing *umuDC*; Spc^r	31
pRW134	Low-copy-number plasmid expressing *umuD′C*; Spc^r	31
pRW144	Low-copy-number plasmid expressing *mucAB*; Spc^r	31
pRW294	Low-copy-number plasmid expressing *mucA′B*; Spc^r	31

Other methods. Transfection procedures and analysis of the replicated vector sequence were done as previously described (2, 3). Where indicated, cells were irradiated with 4 J of 254-nm UV per m^2 immediately before being made competent with $CaCl_2$, to induce the SOS regulon. Five hundred microliters of irradiated or unirradiated competent cells was transfected with 5 ng of control or lesion-containing construct DNA, and the resulting plaques were counted. Only 1.5 ng of DNA was used with strain DV02 because it was highly transformable. Conversely, 7.5 ng of DNA was used with the poorly transformable DV05 strain. The number of plaques from the dimer-containing construct normalized to the control numbers was used to estimate the frequency of translesion replication. Replication events at the dimer target site were determined by hybridization and sequence analysis (2, 3).

RESULTS

Influence of mutations in each of the three subunits of the polIII core on Umu- and Muc-dependent spectra. The difference between the mutation spectra of cells expressing the Umu and Muc complexes is well illustrated by the data from RW82 (Table 2). When this Δ*umuDC* strain contained an episomal copy of either *umuDC* or *umuD′C*, 3′ T→A transversions were always severalfold more abundant than the transition, whether the cells were UV irradiated or not. In contrast, when the strain expressed either the MucAB or MucA′B proteins, the reverse was true in all cases. Results from strains carrying an episomal *umuDC* operon are closely similar to those obtained with the intact chromosomal genes, even though there are probably three to five plasmids per cell, and thus the cells are likely to contain more Umu protein. The ratios of transitions to transversions were not significantly heterogeneous ($\chi^2_{[3]} = 3.37$; $P = 0.5$ *to* 0.1) in UV-irradiated cells of RW82 with either *umuDC* or *umuD′C* on the plasmid, compared to those from SOS-induced cells of the isogenic parent strain TK603 and its

TABLE 2. Number of replicated vector molecules with a normal or mutant sequence at the T-T dimer site, ratio of 3′ T→A to 3′ T→C mutations, and bypass error rate in *uvrA6* strains expressing (where indicated) UmuDC or MucAB proteins or their mutagenically active counterparts UmuD′C or Muc A′B from low-copy-number plasmids

Strain	Relevant genotype	Plasmid	UV light fluence (J/m^2)	Sequence at T-T dimer site (no.)				A/C ratio[a]	Error rate (%)
				T-T	T-A	T-C	Other		
TK603	*umuDC*$^+$	None	0	204	0	1	0		0.5
			4	540	27	3	1	9.0	5.4
DV12	*umuDC*$^+$ *mutD5*	None	0	183	2	4	0	0.5	3.2
			4	355	18	1	1	18.0	5.3
RW82	Δ*umuDC*	UmuDC	0	627	26	10	2	2.6	5.7
			4	1,860	95	21	5	4.5	6.1
		UmuD′C	0	1,011	40	15	1	2.7	5.3
			4	1,041	72	7	3	10.3	7.3
		MucAB	0	1,758	9	46	3	0.20	3.2
			4	2,040	9	39	1	0.23	2.4
		MucA′B	0	1,214	1	40	6	0.03	3.7
			4	1,202	5	38	3	0.13	3.7
DV02	Δ*umuDC* Δ*dnaQ spq-2*	UmuDC	0	535	14	23	3	0.61	7.0
			4	740	43	7	4	6.1	6.8
		UmuD′C	0	874	34	24	2	1.4	6.4
			4	894	39	11	1	3.5	5.4
		MucAB	0	722	1	18	1	0.06	2.7
			4	730	2	11	1	0.18	1.9
		MucA′B	0	1,016	7	59	4	0.12	6.5
			4	1,032	8	39	5	0.21	4.8
DV03	Δ*umuDC dnaQ*$^+$ *spq-2*	UmuDC	0	62	3	1	0	3.0	6.1
			4	317	26	5	1	5.2	9.2
		MucAB	0	763	4	25	6	0.16	4.4
			4	389	1	10	1	0.10	3.0
DV05	Δ*umuDC mutD5*	UmuDC	0	89	1	4	0	0.25	5.3
			4	387	19	5	0	3.8	5.8
		MucAB	0	380	1	8	0	0.13	2.3
			4	624	0	12	1		2.0
DV01	Δ*umuDC* Δ*holE*	UmuDC	0	122	5	2	0	2.5	5.4
			4	120	7	5	0	1.4	9.1
		UmuD′C	0	141	8	1	0	8.0	6.0
			4	172	10	0	0	10.0	5.5
		MucAB	0	274	0	3	0		1.1
			4	377	0	9	0		2.3
		MucA′B	0	59	0	0	0		0.0
			4	115	0	5	0		4.2

[a] Ratio of 3′ T→A to 3′ T→C mutations.

mutD5 derivative DV12, both of which carry intact chromosomal operons. Results obtained with unirradiated TK603 and DV12 cells appear to be different, but this is probably misleading because of the very low bypass frequencies. When bypass frequencies are appreciable, replication of virtually all vector molecules entails dimer bypass. However, when bypass frequencies are very low, as in unirradiated TK603 and DV12 cells, a significant fraction of replicated vectors can result from the small trace of vectors lacking the dimer. The purity of the dimer-containing oligonucleotides used to construct the vector was >99.5%, but the remaining <0.5%, most of which is probably dimer-free oligomer, becomes significant when bypass frequencies in lesion-containing vectors are only a few percent.

The characteristically different ratios of 3′ T→A and of 3′ T→C mutations in strains expressing Muc rather than Umu proteins are, for the most part, also found when the proofreading function of the DNA polIII holoenzyme is disabled by deletion of *dnaQ* or by the *dnaQ* allele *mutD5* (Table 2). In the Δ*dnaQ spq-2* background, a substantial excess of 3′ T→C mutations was observed in *mucAB* or *mucA′B* cells, whether UV irradiated or not. Conversely, a substantial excess of 3′ T→A mutations was found in irradiated cells expressing UmuDC or UmuD′C. However, for unknown reasons, only a small excess of 3′ T→A mutations was observed in unirradiated cells expressing UmuD′C and in UmuDC cells there was even a modest excess of 3′ T→C mutations. Such a result is not attributable to the *dnaE* allele *spq-2*, which is also present in the strain; in a *dnaQ*$^+$ *spq-2* strain, the ratios of the two mutant classes were as expected (Table 2). Nevertheless, the disparate result obtained with the unirradiated cells may well be real, since it also appeared to occur in *mutD5* mutant strain DV05; more 3′ T→C than 3′ T→A mutations were found in unirradiated cells containing the UmuDC proteins, although the numbers were too small for a firm conclusion on this point. In other respects, the data from the *mutD5* strain are as expected. Finally, within the constraint that the number of samples analyzed was small,

the results obtained with Δ*holE* mutant strain DV01 appear to resemble approximately those from the RW82 background.

Although, with only a few exceptions, an excess of transversions occurred in strains containing Umu proteins, whereas an excess of transitions was found in Muc-containing strains, the extent of these biases varied considerably. Part of this variability seems to be dependent on whether the cells were UV irradiated or not; irradiated cells tended to produce more transversions than their unirradiated counterparts. Among the 12 sets of results obtained in total from the RW82, DV02, DV03, and DV05 strains, all but 2 showed a higher proportion of transversions in UV-irradiated cells. However, the effect of UV irradiation was significant only in cells containing the Umu proteins ($\chi^2_{[1]} = 36.18$; $P \ll 0.001$). Although there was a small trend in the same direction in Muc-expressing strains, the effect was not significant ($\chi^2_{[1]} = 1.19$; $P = 0.5$ to 0.1).

Bypass accuracy is greater with Muc than with Umu proteins. In addition to the ratios of the different types of mutations, another important parameter of mutagenesis is the overall error rate of translesion replication. Because the Muc proteins vigorously promote mutagenesis (18, 35), it might be expected that dimer bypass replication facilitated by these proteins would be less accurate than bypass using the Umu gene products. Previous results failed to detect the expected increase in error rate, and indeed, if anything, the error rate seemed lower, although the data were inadequate to establish this point. The present results (Table 2), however, clearly indicate that this is the case. Using the data from RW82, DV02, DV03, and DV05, the overall error rate in strains expressing the Muc proteins is only a little over half of that in strains containing the Umu gene products (3.4% in Muc strains, 6.3% in Umu strains). For the most part, the difference is seen consistently with all of the strains and conditions, although the results from strain DV02 with the *mucA′B* plasmid are, for unknown reasons, anomalous. Nevertheless, Muc- and Umu-dependent error rates are, on average, very significantly different in both UV-irradiated ($\chi^2_{[1]} = 82.4$; $P \ll 0.001$) and unirradiated ($\chi^2_{[1]} = 20.6$; $P \ll 0.001$) cells. Again, the results from the Umu-containing strains seem quite typical of those from strains with a normal chromosomal copy of *umuDC*. The average error rate in the plasmid-containing UV-irradiated strains is a little higher than the average value from TK603 and DV12 (Table 2), in which *umuDC* is chromosomally expressed, but the difference is short of significance ($\chi^2_{[1]} = 3.73$; $P = 0.1$ to 0.05). Moreover, an error rate of 6.3% in the *umu* plasmid-containing strains was identical to that previously observed in a large set of data from a strain with genomic *umuDC* (19). These results indicate not only that the error rate is independent of the location of the *umuDC* operon but also that it is independent of the level of Umu or Muc proteins and the amount of bypass.

Effect of proofreading or the ε subunit on lesion bypass frequency in cells containing Umu or Muc proteins. A variety of evidence suggests that the 3′-5′ exonucleolytic proofreading activity of the polIII ε subunit plays little or no role in induced mutagenesis and lesion bypass (8, 36, 38), but the high spontaneous mutability of strains lacking this activity has made it difficult to obtain decisive results. Slater and Maurer (28) have clearly shown, using a Δ*dnaQ spq-2* strain, that the dependence on MucAB for efficient replication of UV-irradiated ϕX174 phage DNA cannot be relieved by absence of the ε subunit. Their experiments did not differentiate between the structural and proofreading roles of ε, however, or examine possible effects of the *spq-2* mutation, a *dnaE* allele also present in their strain, and the system used was relatively insensitive to small changes in bypass frequency. Experiments using vectors uniformly carrying a single lesion have the advantage that they provide direct measures of bypass frequency and are unaffected by high spontaneous mutation rates.

TABLE 3. Effects of Umu-like proteins on translesion replication in Δ*umuDC uvrA6* strains carrying *dnaQ*$^+$, Δ*dnaQ*, *spq-2*, or *mutD5* alleles

Genotype	UV light applied to cells (J/m^2)	% Replication past a T-T *cis-syn* dimer			
		pUmuDC	pUmuD′C	pMucAB	pMucA′B
Δ*umuDC dnaQ*$^+$	0	3.7	64.5	46.0	83.6
	4	71.5	79.7	98.2	80.2
Δ*umuDC* Δ*dnaQ spq-2*	0	13.9	80.2	94.4	91.2
	4	80.3	83.3	99.4	86.7
Δ*umuDC dnaQ*$^+$ *spq2*	0	3.3		42.3	
	4	64.9		95.0	
Δ*umuDC mutD5*	0	17.5		48.7	
	4	72.0		112.0	

As shown in Table 3, which gives the average results of five replicate experiments with each strain and condition, the frequencies of dimer bypass in *dnaQ*$^+$ and Δ*dnaQ spq-2* strains containing episomal *umuDC*, *umuD′C*, *mucAB*, or *mucA′B* genes were, in most cases, fairly similar. As first pointed out by Slater and Maurer (28), this indicates that absence of the ε subunit does not alleviate the need for the plasmid-encoded proteins. Nevertheless, it is also evident from Table 3 that translesion replication frequencies were consistently higher in the Δ*dnaQ spq-2* background, although the extent of the difference varies from as little as an additional 1% bypass to as much as an additional 48% bypass, according to the particular proteins expressed and whether the cells were UV irradiated or not. On average, bypass occurred in an additional 20% of vector molecules replicated in unirradiated Δ*dnaQ spq-2* cells and an additional 5% in UV-irradiated cells. Analysis of variance showed that the difference between the two strains was statistically significant ($P < 0.001$) and that the particular proteins used and the irradiation treatment were also significant ($P < 0.001$). The largest difference occurred in the unirradiated strains with the *mucAB* plasmid, whereas the smallest differences generally occurred under conditions in which the bypass frequency was high, as found in the UV-irradiated cells. Results obtained with the *dnaQ*$^+$ *spq-2* strain were closely similar to those from the wild type, suggesting that the *spq-2* mutation is not responsible for the differences observed in the Δ*dnaQ spq-2* strain. The comparison with data from the *mutD5* strain, intended to determine whether the increased bypass was the result of the loss of proofreading or of the structural function of the ε subunit, was not so decisive, however. Although the bypass frequencies in *mutD5* strains containing the *mucAB* plasmid were much more similar to those in the wild type than to those in the Δ*dnaQ spq-2* strain, this was not true for unirradiated cells containing the *umuDC* plasmid; the bypass frequency was 3.7% in the wild-type background but 17.5% in the *mutD5* strain, even greater than the frequency of 13.9% observed in the Δ*dnaQ spq-2* strain. This discrepancy is probably explained by our earlier observation (34) that unirradiated *mutD5* cells (although not Δ*dnaQ spq-2* strains) are partially SOS induced. It is therefore likely that the increased bypass frequencies observed in Δ*dnaQ spq-2* strains resulted from the loss of the structural function of the ε subunit and not from the absence of proofreading.

DISCUSSION

The chief aim of this work has been to investigate whether the *E. coli* DNA polIII core plays a role in vivo in determining the differences observed between the mutagenic properties of polV (UmuD′$_2$C) and the plasmid-encoded homolog of polV (MucA′$_2$B) during replication past a T-T dimer. Results presented in this and an earlier paper (31) show that Muc-dependent bypass differs from that mediated by polV in three respects. First, substituting Muc for Umu proteins drastically alters the ratio of the two major classes of mutation induced by the dimer. In strains with a normal chromosomally located *umuDC* operon, almost all mutations occur at the site of the 3′ thymine and about 80% are T→A transversions, with the remainder being T→C transitions (19). The same mutations and ratio were also found in strains in which the chromosomal operon was deleted and the Umu proteins were produced from genes carried on a low-copy-number plasmid (Table 2 and reference 31). However, substitution of *mucAB* for *umuDC* on the plasmid reversed the ratio of the mutant classes, with the transition now being the major class of mutations. Second, T-T dimer bypass with Muc rather than Umu proteins is nearly twice as accurate; the average error rate was 3.4%, compared to 6.3% in Umu-containing strains (Table 2). Third, the Muc proteins are generally more efficient at promoting bypass and result in a higher proportion of the vector molecules being fully replicated (Table 3; reference 31). Investigation of the mechanisms responsible for these differences has the potential of increasing our understanding of the enzymology of bypass in vivo.

Based upon earlier genetic work (4, 5, 14, 26, 27; reviewed in reference 10), we initially hypothesized that such differences would result from a modification of polIII. However, given the finding that UmuD′$_2$C is an error-prone polymerase, polV, and that MucA′$_2$B almost certainly possesses a similar activity, our observations are most easily explained by the fact the characteristic mutation spectra of Umu- and Muc-dependent dimer bypass simply reflect the inherent properties of DNA polV and its MucA′$_2$B counterpart. An enzyme-dependent difference in error propensity of this magnitude is unusual because previous studies suggested that the mutation spectrum is largely determined by the structure of the particular lesion concerned. Even though the overall error frequency might vary, the same predominant type of mutation was induced by a given lesion when introduced into the very different enzymatic environments found within yeast and *E. coli* cells. Thus, a 3′ T→C mutation is the major type of event induced by a T-T pyrimidine (6-4) pyrimidinone adduct in both species (12, 20) and in each of them, a 5′ T-to-A mutation is the predominant event induced by a T-T *trans-syn* cyclobutane dimer (3, 11). Whether the mutations induced by a *cis-syn* dimer are similar in the two species is hard to determine, since almost no mutations are induced by this photoproduct in yeast (11). Thus, it is unusual if, as seems likely, the differences in mutation spectrum are caused by the inherent error propensities of polV and its MucA′$_2$B counterpart. Structural studies of the two polymerases cocrystallized with primed T-T dimer-containing templates are likely to be useful in finding molecular mechanisms for such a difference.

The data in Table 2 clearly demonstrate that the 3′-5′ exonuclease proofreading function of the ε subunit of the polIII holoenzyme has no influence on the mutation spectrum in Umu- and Muc-containing cells. Our present data are also incompatible with models that explain the difference in mutation spectra based on differential extension from T · T and T · G mismatches, because the difference between the mutation patterns induced in the presence of Umu or Muc proteins was independent of the proportion of vector molecules in which translesion replication occurred. Assuming a frequency of mispair formation independent of Umu or Muc, elongation preference will have the greatest capacity to change the ratio of the mutations recovered when few bypass events occur. It will have a decreasing effect as the frequency of bypass increases and will not occur at all if 100% of the molecules are fully replicated. Contrary to this expectation, the ratios characteristic of Umu- and Muc-expressing cells were maintained even when translesion replication occurred in 80% of the vector molecules in Umu-containing cells and in 100% of the vector molecules in Muc-containing cells (Tables 2 and 3).

The finding that bypass using the Muc proteins is nearly twice as accurate as bypass with polV is surprising in view of the known capability of the *muc* operon to promote higher levels of DNA damage-induced mutagenesis than those found with *umu*. Although it is possible that this result is restricted to dimers, with higher error rates being found with other lesions, the increased ability of the Muc proteins to elevate induced mutation frequencies more probably resides in their superior capacity to promote translesion replication. Part of this superior capacity probably reflects a greater susceptibility of the MucA protein to posttranslational processing by activated RecA (15). However, a recent study (33) suggests that much lower levels of MucA′$_2$B than polV are sufficient for high levels of mutagenesis, indicating that MucA′$_2$B is likely to possess inherently greater activity for promoting nucleotide insertion and extension opposite any lesion.

Reassessment of the role of the polIII core in SOS mutagenesis. Our observation that polV and MucA′$_2$B exhibit substantially different mutation spectra and a twofold difference in error rate clearly indicates that these enzymes play an important role at the site of the 3′ thymine in the dimer, the first encountered by polymerase, and this places limitations on a possible role for the polIII core in translesion replication. Moreover, the mutagenic properties are essentially unchanged over a wide range of expression levels of polV and MucA′$_2$B (for example, UmuDC without SOS induction and UmuD′C or MucA′B with SOS induction), conditions that should substantially change the levels of the polV-like enzymes relative to that of polIII. If both polIII and the polV-like enzymes were active in bypass, the mutagenic properties would be expected to vary, but this is not observed. Moreover, the absence of any influence of proofreading on the mutagenic properties of the dimer and earlier evidence indicating the absence of an effect of proofreading on UV-induced mutagenesis (38) argue against extension by polIII. To date, no plausible molecular mechanism has been suggested for the hypothesized suppression of the ε subunit's proofreading activity during translesion replication and its restoration immediately beyond the lesion. In contrast, replication by polV or its counterparts (which appear to lack proofreading activity) is a much more persuasive explanation. Thus, we believe that in a wild-type cell, polV and its plasmid-encoded homologs perform both the misincorporation and extension steps in translesion replication. Indeed, recent biochemical analysis of polV supports such an assumption (32). As polV is a distributive enzyme (32, 32a, 33), it is almost certainly replaced by the more processive enzyme polIII once the kinetic block to replication has been alleviated and allows polIII to fix the polV-dependent misincorporated base as a mutation.

How, then, can this conclusion be reconciled with the previous genetic data indicating a major and essential role of polIII in SOS mutagenesis? The chief evidence indicating a direct role for polIII in this process comes from the experi-

ments of Hagensee et al. (14) and, in particular, those of Bridges and Bates (4), who examined induced mutagenesis in a strain carrying a temperature-sensitive allele of *dnaE* and the *pcbA1* suppressor (which increases replication by polI and permits growth at the restrictive temperature). As no DNA damage-inducible mutagenesis was observed at the restrictive temperature, it was concluded that at least the α catalytic subunit of polIII was required for SOS mutagenesis (4, 14), an entirely reasonable interpretation at that time. However, these experiments suggest that all DNA damage-induced mutagenesis is dependent on polIII, a result that seems inconsistent with our results. When coupled with the recent findings that polV is an inessential DNA polymerase that lacks proofreading activity and is capable, at least in vitro, of performing translesion replication in the absence of polIII (33), it seems more likely that the lack of mutagenesis in these strains at 43°C resulted from some indirect effect of the *dnaE* temperature-sensitive mutation. These may include effects on SOS induction or on bypass; in the latter case, polI or the mutant DnaE protein may inhibit access of polV to the 3′ OH terminus or facilitate replication by some relatively accurate enzyme, such as polII, that is not usually employed in translesion replication. Alternatively, as the *dnaEts pcbA1* mutant strains used in these experiments are extremely slow growing, the proteolytic degradation of polV is likely to be much faster than in a wild-type strain (9), which could lead to the observed decrease in mutagenesis.

Although we did not observe an effect of proofreading itself on any of the mutagenic parameters measured, we nevertheless saw a small but reproducible increase in the frequency of bypass in the Δ*dnaQ* background that is probably the result of the deficiency in the structural, rather than the proofreading, function of the ε subunit (Table 3). Since the θ subunit binds only to ε, replication is carried out by the α catalytic subunit alone in Δ*dnaQ* strains and this form of the enzyme is likely to compete less well for free 3′ OH termini than the intact polIII core. As shown by Tang et al. (33), polIII and polV compete for 3′ termini and the α subunit presumably competes less well than the core for these binding sites, resulting in a greater frequency of productive incorporation and extension by polV or MucA′$_2$B and thus bypass. Binding by polIII, on the other hand, is unlikely to result in nucleotide incorporation or extension, precluding bypass. Results of both in vitro reconstruction (33) and in vivo experiments (this report) therefore indicate that under normal conditions, misincorporation and bypass are carried out principally by polV or its homologs rather than polIII and that polIII is probably only required for the completion of replication and thus for the production of the fully replicated genomes that are detected in our experimental system.

Of course, that does not mean that in the complete absence of polV or its homologs, polIII cannot perform translesion replication. Limited bypass of an abasic site, a *cis-syn* T-T dimer and a 6-4 photoproduct, is, in fact, observed in vitro with the polIII holoenzyme (32a). We have previously reported relatively efficient bypass of a *cis-syn* T-T dimer in proofreading-deficient Δ*umuDC* strains (34), and such replication may well be polIII dependent. The delayed-photoreversal experiments of Bridges and Woodgate (7) also indicated that in vivo misincorporation can occur in the absence of the Umu proteins and experiments done by Sharif and Bridges (27) with a temperature-sensitive *dnaE* allele suggested that these misincorporations are dependent on the polIII α subunit.

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