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The Frequency and Accuracy of Replication Past a Thymine-Thymine Cyclobutane Dimer Are Very Different in *Saccharomyces cerevisiae* and *Escherichia coli*

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We have compared the mutagenic properties of a T-T cyclobutane dimer in baker's yeast, *Saccharomyces cerevisiae*, with those in *Escherichia coli* by transforming each of these species with the same single-stranded shuttle vector carrying either the *cis-syn* or the *trans-syn* isomer of this UV photoproduct at a unique site. The mutagenic properties investigated were the frequency of replicational bypass of the photoproduct, the error rate of bypass, and the mutation spectrum. In SOS-induced *E. coli*, the *cis-syn* dimer was bypassed in ~16% of the vector molecules, and 7.6% of the bypass products had targeted mutations. In *S. cerevisiae*, however, bypass occurred in about 80% of these molecules, and the bypass was at least 19-fold more accurate (~0.4% targeted mutations). Each of these yeast mutations was a single unique event, and none were like those in *E. coli*, suggesting that in fact the difference in error rate is much greater. Bypass of the *trans-syn* dimer occurred in about 17% of the vector molecules in both species, but with this isomer the error rate was higher in *S. cerevisiae* (21 to 36% targeted mutations) than in *E. coli* (13%). However, the spectra of mutations induced by the latter photoproduct were virtually identical in the two organisms. We conclude that bypass and error frequencies are determined both by the structure of the photoproduct-containing template and by the particular replication proteins concerned but that the types of mutations induced depend predominantly on the structure of the template. Unlike *E. coli*, bypass in *S. cerevisiae* did not require UV-induced functions.

Vectors which carry a defined and uniquely placed mutagenic lesion, particularly single-stranded constructs of this kind, are powerful tools for investigating mutagenic mechanisms in vivo (2, 12). Studies that use such vectors have for the most part been carried out with *Escherichia coli*, but the extent to which information from this species can be used to understand mutagenesis in other, particularly eukaryotic, organisms is not yet known. We have examined this issue by introducing samples of the same single-stranded shuttle vector construct, carrying either a *cis-syn* or a *trans-syn* T-T cyclobutane dimer, into both *E. coli* and baker's yeast, *Saccharomyces cerevisiae*. We had two aims for these experiments: (i) to investigate the reasons why a particular type of mutagenic DNA damage is in fact mutagenic, a question that is of both theoretical and practical interest; and (ii) to examine bypass mutagenesis in *S. cerevisiae* and to determine whether UV-induced gene products were needed for bypass.

Central to the first problem is the question of the relative influence of the structure of the mutagen-altered template on the one hand and of the properties of the replication complex on the other in determining the mutagenic properties of the lesion. It was originally suggested that cyclobutane dimers and many other replication-inhibiting lesions were noninstructive or nonpairing (16) and that the majority of mutagenic events that occur opposite the lesion reflected properties of the DNA polymerase, such as a preference for binding purine nucleotide triphosphates, particularly dATP (the A rule; [11]). Although subsequent analysis of the nucleotide sequence changes induced by UV (10) and other mutagens did not appear to support this view, such data were difficult to interpret because of uncertainties about the identities of the various mutagenic lesions and the targeting of the mutations and because of the lack of information specific to any given lesion. More recently, experiments with a cyclobutane dimer, pyrimidine (6-4) pyrimidone adduct, or abasic lesion, each placed at the same target site within the same single-stranded vector (1, 2, 7–9), have shown that individual lesions exhibit unique mutagenic properties, implying that the structure of the modified template itself influences these properties. However, such data do not demonstrate that this structure is the only determinant of all or some of its mutagenic properties; the properties may result from a unique interaction between the particular template structure and the particular replication proteins and conditions. If the latter is the case, limitations are placed on the use of molecular modelling in the investigation of mutagenic properties, making it unlikely that this approach could be used, in the long term, to accelerate and simplify genotoxic assessment.

We have investigated this question by determining whether any of the three basic parameters of mutagenesis (bypass frequency, bypass error frequency, and mutation spectrum) are similar in *S. cerevisiae* and *E. coli*, as would be expected if replication proteins and conditions were unimportant, by using the same vector construct for the two species. We find that bypass and error frequencies can be very different in the two organisms, indicating that these properties depend both on the nature of the replication complex and on the template structure. However, the mu-

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tation spectra appear to be very similar, at least for the one isomer yielding data that could be compared, and we conclude that, at least for this particular UV photoproduct, the kinds of mutations induced depend largely on the chemical structure of the lesion-containing template. We also find that bypass replication in *S. cerevisiae*, in contrast to this phenomenon in *E. coli*, does not require UV-inducible gene functions; in the former, the uptake or replication of the vector molecules, whether they contain a photoproduct or not, is merely uniformly reduced by the UV irradiation.

MATERIALS AND METHODS

Construction of the dimer-containing vector. Single-stranded vectors carrying a uniquely placed T-T cyclobutane dimer were constructed by the method described by Banerjee et al. (1, 2) (see Fig. 1), modified as follows. Photoproduct-free oligomers, with the sequence 5′ GCAAGTTGGAG 3′, or such 11-mers containing either a *cis-syn* or a *trans-syn* dimer located at the unique T-T target site (2) were inserted into the shuttle vector pYMV1. These oligomers were >99.5 and >99% pure, respectively. The pYMV1 shuttle vector is M13mp7L1 (1) that contains, at the *Fsp*I site, a 2-kb insert composed of the 0.9-kb *Hin*cII-*Xba*I fragment from the yeast endogenous 2μm plasmid origin of replication joined by adapters to a 1.1-kb *Hin*dIII fragment carrying the yeast *URA3* gene. pYMV1 viral DNA was digested with *Bam*HI as well as with *Eco*RI to destroy the polylinker hairpin and inhibit reformation of the uncut vector at the later ligation step. Annealing and recircularization of the linearized DNA with the 51-mer scaffold were carried out in 50 mM NaCl at a vector concentration of 25 ng/μl by heating at 70°C for 15 min, followed by slow cooling to room temperature overnight. The annealing mix was concentrated by centrifugation through a Centricon-30 microconcentrator (Amicon, Danvers, Mass.) and divided into three equal parts for ligation with a 100-fold M excess of 11-mer carrying either a *cis-syn* or a *trans-syn* dimer or no photoproduct, carried out at a vector concentration of 167 to 200 ng/μl. The scaffold was removed immediately before transformation by adding a 10-fold M excess of anti-51-mer, the complement of the scaffold, and heating to 85°C for 5 min. About 30 to 50% of the linearized vector is converted to covalently closed circular molecules. Linear molecules transform yeast cells at a lower frequency and generate plasmids lacking the 11-mer insert that are easily detected by sequence analysis; transformants of this type, together with those resulting from uncut vector molecules and scaffold priming (G-G type [see reference 1]), can therefore be excluded from the final data. On average, the background frequency of such nonconstruct events was about 10% in *S. cerevisiae* rather than 1 to 2%, as in *E. coli*. Yeast strains PRY43 (*MAT***a** *rad1*Δ::*LEU2 his3*Δ*1 leu2-3,112 trp1-289 ura3-52*), kindly supplied by Louise Prakash, University of Rochester, and PG6-5B (*MAT* **a** *rad1*Δ::*LEU2 phr1-1 his ura3-52*) were made competent with LiCl (4), transformed with 1 μg of construct, and plated on synthetic complete medium lacking uracil. To analyze the sequence of replicated vectors, DNA was extracted from the yeast transformants by a modification of the method described by Hoffman and Winston (3), in which phenol-chloroform was replaced by chloroform alone and the sodium dodecyl sulfate was precipitated with KAc (122 mM final concentration). These modifications increased the yield of plaques by about fivefold when the DNA was used to transfect competent cells of JM101. Samples from each batch of construct DNA were used not only to transform yeast cells but also to transfect cells of *E. coli* SMH10, an F^+ Δ(*pro-lac*) derivative of AB1886, which is itself an isogenic *uvrA6* derivative of AB1157. Bacterial cells were made competent with calcium chloride (1), and all other methods with SMH10 were as described previously (1), with the exception that, as noted above, a 10-fold M excess of anti-51-mer was added to the construct before denaturation. pYMV1 phage DNA, obtained directly from SMH10 transfectants or indirectly from yeast transformants via JM101, was analyzed either by hybridization to detect nonmutants, followed by sequence analysis of all vectors that failed to hybridize (1), or by sequencing alone by the dideoxy method. Autoradiograms were read over a region extending from ~25 nucleotides 3′ to ~100 nucleotides 5′ to the photoproduct target site.

TABLE 1. Frequency of dimer bypass in *S. cerevisiae* and *E. coli*[a]

Organism	Strain	UV irradiation (J/m^2)	% Transformants or transfectants		
			Control	*cis-syn*	*trans-syn*
S. cerevisiae	PRY43	0	100	88	13
	PG6-5B	0	100	74	18
E. coli	SMH10	0	100	0.2	6
		4	101	16	16

[a] Values are the averages of numbers of transformants or transfectants, which are normalized to those for the photoproduct-free control in unirradiated cells, resulting from transformation or transfection with pYMV1 vectors carrying either a *cis-syn* or a *trans-syn* T-T cyclobutane dimer. All values are for transformation or transfection with construct. Events due to uncut, scaffold-primed (G-G), or other nonconstruct vectors have been excluded by sequence analysis. Data for *S. cerevisiae* PRY43 (*rad1*Δ) are the averages of four independent experiments with the *cis-syn* dimer and one experiment with the *trans-syn* dimer. Data for PG6-5B (*rad1*Δ *phr1-1*) are the averages of six independent experiments with both isomers simultaneously. The data for SMH10 (*uvrA6*) are the averages of seven independent experiments with both isomers.

RESULTS

Dimer bypass frequencies in *S. cerevisiae* and *E. coli*. The proportion of vector molecules in which the cyclobutane dimer was bypassed was estimated from the relative numbers of transformants or transfectants obtained with the photoproduct-containing vector, normalized to the numbers found with the lesion-free construct and unirradiated cells (Table 1). This frequency should accurately estimate bypass events, because control and dimer-containing 11-mer were ligated into equal samples of the same scaffold-recircularized material (Fig. 1) (1), because both normal and modified 11-mer were purified from the same photochemical reaction mix, and because ligation efficiencies for each type of oligomer are equal (1, 9). Similarly, it is unlikely that transformation frequencies are significantly influenced by selective uptake of modified or unmodified vector molecules. A variety of evidence, which is discussed below, suggests that transformation-transfection frequencies depend on efficiency of replication, not uptake, and in general the nonspecific mechanism by which DNA molecules enter cells does not appear to offer opportunities for discriminating between virtually identical 9.25-kb vector molecules that differ only with respect to the presence or absence of a single photoproduct. Finally, since pYMV1 is a shuttle vector that can be replicated in both *S. cerevisiae* and *E. coli*, identical samples of construct can be introduced into the two species, ensuring

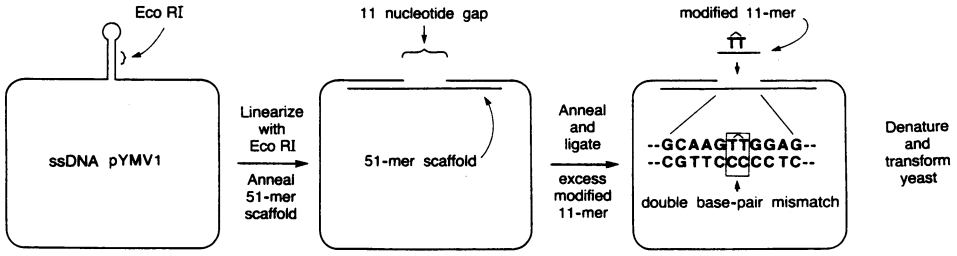

FIG. 1. Method for constructing single-stranded shuttle vector molecules that carry a specifically located *cis-syn* or *trans-syn* T-T cyclobutane dimer. Samples of each batch of construct were used to transfect *E. coli* as well as to transform *S. cerevisiae*. The double-basepair mismatch at the photoproduct target site is a genetic marker used to detect the small proportion of construct molecules that retain the scaffold oligomer. Replication of these molecules does not require bypass, because the scaffold oligomer can prime minus strand replication in vivo. Such events are detected by the presence of a G-G sequence in the plus strand. ssDNA, single-stranded DNA.

that the results from the two species are exactly comparable. pYMV1 is M13mp7L1, used in previous studies with *E. coli* (1, 2, 7–9), into which a yeast origin of replication and *URA3* gene have been inserted (see Materials and Methods). Only a single type of dimer, together with the control, was studied in experiments with *S. cerevisiae* PRY43, but both isomers were investigated simultaneously in experiments with *S. cerevisiae* PG6-5B and *E. coli* SMH10 (Table 1).

The data given in Table 1 show that the frequency of bypass can be very different in *S. cerevisiae* and *E. coli*; bypass occurred in 70 to 90% of molecules carrying a T-T *cis-syn* dimer when these vectors were replicated in *S. cerevisiae* but only at most in 16% of such molecules when they were replicated in *E. coli*. In contrast, bypass of a T-T *trans-syn* dimer was about equally frequent in the two species. The results given in Table 1 also suggest that efficient bypass in *S. cerevisiae*, unlike that in *E. coli*, can occur without UV irradiating the cells. As shown previously with the M13mp7L1 vector, bypass of the *cis-syn* dimer in *E. coli* is almost completely SOS dependent, and the same is true of T-T pyrimidine (6-4) pyrimidone adducts and abasic sites (8, 9). Uniquely, the T-T *trans-syn* dimer is bypassed at low frequency in unirradiated cells, although again the frequency is enhanced by SOS induction (1). The values given in Table 1 for the *cis-syn* isomer in pYMV1 are very similar to those observed in these earlier experiments with the M13mp7L1 vector but, for unknown reasons, the values for the *trans-syn* isomer are only about half of those observed previously.

The reduction in the transforming or transfecting ability of some photoproduct-carrying vector molecules, relative to that of control constructs, is likely to reflect inefficient replication rather than inefficient uptake of the DNA molecules by the cells. The efficiency of uptake into unirradiated yeast cells of construct molecules carrying the *cis-syn* dimer is clearly very similar to that of control molecules, because relative transformation frequencies are 70 to 90% of those of the control. Further, in *E. coli* the number of transfectants is modulated by the level of SOS induction, which controls the efficiency of bypass replication but not uptake; a photoproduct-free construct transfects SOS-induced and uninduced cells with equal efficiency. Uninduced cells transfected with a photoproduct-carrying construct usually yield less than 1% of the control frequency of plaques, whereas exposure of the *uvrA6* strain SMH10 to 4 J of 254-nm UV per m^2 typically raises this frequency to about 20%. Exposure of a *uvr*$^+$ strain to 40 J/m^2 can increase this frequency to 45% of that of the control, and in strains with a genetically derepressed SOS regulon, the frequency can be 60 to 70% (unpublished data). These observations support the view that transfection efficiency depends on bypass efficiency rather than variable uptake. Finally, similar yields of plaques are found when vectors carrying a single abasic site or uracil are transfected into *ung*$^+$ but not *ung* cells (8), indicating that the presence of the lesion before replication, rather than before uptake, is the important variable.

The suggestion from the data given in Table 1 that efficient bypass of photoproducts in *S. cerevisiae* may not require UV-inducible gene functions was further investigated in experiments with both unirradiated and irradiated cells of strain PG6-5B (Table 2). Transformation frequencies with each construct, whether it contained photoproduct or not, were found to be uniformly decreased by about fivefold in cells exposed to a fluence of 2 J/m^2 immediately before they were made competent; therefore, the data provide no evidence for UV-stimulated bypass.

Error frequency and mutation spectrum in *S. cerevisiae* and *E. coli*. The error frequency and mutation spectrum associated with replication past the T-T cyclobutane dimer were determined by hybridization and sequence analysis or by sequence analysis alone (1). Hybridization to detect the normal sequence was employed when low mutation frequencies were expected. Under the conditions used, the 15-mer probe fails to hybridize with phage carrying a mutation anywhere within the 11-mer sequence, and no false-positive results were detected either in a sample of 476 randomly picked plaques (1) or in the sets of seven negative controls (1) used on each hybridization membrane. The sequences of all phages failing to hybridize were then analyzed by the dideoxy method. Autoradiograms were examined in a region starting ~25 nucleotides 3′ and ending ~100 nucleotides 5′ to the 11-mer insertion site. In the yeast experiments, DNA

TABLE 2. Frequency of dimer bypass in unirradiated and UV-irradiated *S. cerevisiae*[a]

UV irradiation (J/m^2)	% Transformants[b]		
	Control	*cis-syn*	*trans-syn*
0	100	70	23
2	18	13	5
	(100)	(67)	(26)

[a] Yeast cells were transformed with pYMV1 carrying a *cis-syn* or a *trans-syn* T-T cyclobutane dimer or the photoproduct-free control sequence. The results are corrected as described in footnote *a* to Table 1 and are the averages of three independent experiments with strain PG6-5B.

[b] Values in parentheses are normalized to data for the control construct in UV-irradiated cells.

TABLE 3. pYMV1 replication products from unirradiated *S. cerevisiae* or SOS-induced *E. coli*[a]

Sequence at T-T target site	No. observed				
	S. cerevisiae			*E. coli*	
	Control	*cis-syn*	*trans-syn*	*cis-syn*	*trans-syn*
T-T	555	686	100	667	457
A-T	0	1	41	0	16
C-T	0	0	0	0	4
G-T	0	0	0	0	0
T-A	0	0	0	49	0
T-C	0	0	1	6	12
T-G	0	0	1	0	1
ΔT	0	0	19	0	42[b]
Other[c]	1	3	4	2	3[d]
Total	556	690	166	724	535

[a] Data are numbers of normal or mutant pYMV1 phages resulting from replication past a *cis-syn* or a *trans-syn* T-T cyclobutane dimer or from the photoproduct-free control sequence.

[b] Includes one each of ΔT7 and G8 and ΔT6 or T7 plus A3 A4→AAA.

[c] All other mutations in the 11-mer sequence 5′ G1 C2 A3 A4 G5 T6 T7 G8 G9 A10 G11 3′. By this numbering scheme, the mutations were as follows: yeast control, A3→C; yeast *cis-syn*, T6 T7→AG, A3 A4→AAA, A3 A4→AAA plus T6→A; yeast *trans-syn*, A4→T, G8 G9→GGG, G5→GG, T6 T7→TTT; *E. coli cis-syn*, T7 G8→TAG, G5→A; *E. coli trans-syn*, T6 T7→TAC, T6 T7→AG, G5→GG. No vector sequence mutations were found in a region starting ~25 nucleotides 3′ and ending ~100 nucleotides 5′ to the 11-mer insertion site.

[d] An additional 39 vectors had the sequence G5→T, which was found with only one preparation of modified 11-mer, and may therefore be an error of oligonucleotide synthesis.

TABLE 4. Numbers of normal and mutant pYMV1 vectors replicated in unirradiated (−UV) or UV-irradiated (+UV) *S. cerevisiae*[a]

Sequence at T-T target site	No. observed					
	Control		*cis-syn*		*trans-syn*	
	−UV	+UV	−UV	+UV	−UV	+UV
T-T	168	113	172	145	60	38
A-T	0	0	0	0	7	1
C-T	0	0	0	0	1	0
G-T	0	0	0	0	0	0
T-A	0	0	0	0	0	0
T-C	0	0	0	0	2	0
T-G	0	0	0	0	1	0
ΔT	0	0	0	0	5	5
Other[b]	1	0	0	0	2	0
Total	169	113	172	145	78	44

[a] Data are numbers of normal or mutant pYMV1 phages resulting from replication past a *cis-syn* or a *trans-syn* T-T cyclobutane dimer or from the photoproduct-free control sequence.

[b] By the notation given in footnote *c* of Table 3, these were as follows: control, C2→G; *trans-syn*, two G5→T mutations.

extracted from individual transformant colonies was used to transfect *E. coli* JM101, and a single plaque derived from each transformant was picked for analysis. Only one plaque was needed for analysis because, as shown by the data discussed below, vector molecules derived from a single yeast transformant were not detectably heterogeneous. In addition, virtually all of the mutations detected are likely to have arisen in *S. cerevisiae* rather than secondarily in *E. coli*, because samples of the shuttle vector extracted from transformants were replicated with high accuracy in the bacterium (Table 3). Similarly, few mutations were found when pYMV1 constructs containing photoproduct-free 11-mer were directly transfected into *E. coli*; a single mutant, a deletion of the 5′ G of the oligomer, was found in a set of 986 plaques from uninduced cells, and a single G→A substitution of the 3′ G was found in a set of 426 plaques from SOS-induced cells.

The results from the sequence analysis (Table 3) show that the error rate accompanying dimer bypass, like the bypass frequency, can also differ in the two species. The *cis-syn* T-T dimer was bypassed with very little error in *S. cerevisiae* (0.4% targeted mutations), whereas in *E. coli* the frequency of targeted mutations induced by this photoproduct was at least 19-fold higher (7.6%), a difference that is highly significant ($P \ll 0.001$). Further, the difference between the two species is in fact much greater, because none of the types of mutations typically induced by this photoproduct in *E. coli* were found in 1,007 yeast transformants analyzed (Tables 3 and 4), and each of the yeast mutations was a single, unique event; it cannot be ruled out that such low-frequency events were induced by contaminating photoproducts rather than by the *cis-syn* dimer. However, bypass replication in *S. cerevisiae* is not uniformly more accurate; on the contrary, the *trans-syn* isomer induces more mutations in this species (37% targeted mutations) than in *E. coli* (13% targeted mutations). Although an independent set of experiments (Table 4) gave a somewhat lower estimate for *S. cerevisiae* (21% targeted mutations), it is clear that the frequency of errors due to replication past the *trans-syn* dimer is no lower in *S. cerevisiae* than in *E. coli*; it is probably higher.

Although the overall error frequencies in *S. cerevisiae* and *E. coli* can be quite different, the types of mutations induced in the two species were almost identical, at least with regard to the *trans-syn* dimer; the *cis-syn* dimer induces too few mutations in *S. cerevisiae* for a valid comparison to be made. In both species, the chief mutations produced by the *trans-syn* dimer were targeted single T deletions and substitutions at the 5′ T of the T-T photoproduct site. However, the relative frequencies of these induced sequence changes were different. About twice as many substitutions as deletions were found in *S. cerevisiae*, but only half as many were found in *E. coli*. In addition, four times as many 5′ T→A as 5′ T→C substitutions were observed in the bacterium, but almost all of the 5′ substitutions were T→A in *S. cerevisiae*; no T→C substitutions were detected in the first set of data, and only a single example was detected in the second set (Table 4). Similarly, the relative frequency of 3′ T→C mutations was also lower in *S. cerevisiae* than in *E. coli*; however, the status of this result is unclear, because only a very low frequency for this mutation was found previously in *E. coli* with the M13mp7L1 vector (1). In other respects, *E. coli* data acquired with the two vectors were qualitatively and quantitatively similar.

It is unlikely that the results with the *cis-syn* dimer in yeast cells are the consequence of the repair of this lesion rather than efficient and accurate bypass replication, because single-stranded DNA provides few opportunities for repair. In addition, both strains used carried a deletion of the *RAD1* gene, and no detectable removal of dimers occurs in null mutants of this kind (15). Further, all cells were handled under nonphotoreactivating yellow light, and strain PG6-5B contained a mutation in the *PHR1* locus, the structural gene for yeast DNA photolyase.

Finally, there is no evidence to indicate that UV irradiation of the yeast cells before transformation changes the

error rate of bypass replication or the induced mutation spectrum (Table 4). The error rate for replication past the *trans-syn* isomer in unirradiated cells was, for unknown reasons, somewhat lower in this set of experiments than in the first set (Table 3), but in other respects the two sets of data are very similar.

Sequence homogeneity of vectors derived from individual transformants. Transformation is much less efficient in *S. cerevisiae* than in *E. coli*, and far more construct molecules than yeast cells must be used, rather than fewer as is the case with bacteria. At the same time, only a small subpopulation of the yeast cells are actually competent, increasing the possibility that two or more vector molecules might be taken up and replicated by any one transformant. If uptake and replication of more than one construct molecule were a common event, estimates of the mutagenic parameters might be seriously compromised. We examined this issue by the transformation of yeast cells with an equal mixture of two almost identical hybrid phage shuttle vector molecules, followed by the extraction of DNA from individual transformants, transfection of *E. coli*, and analysis of the resulting plaques. The two vector species used were derived from pYMV1 and differ only with regard to a tandem pair of bases located in an inessential region; they were made by replacing the polylinker region with the 11-mer sequence containing either a centrally placed G-G or T-T nucleotide pair. Sets of plaques from individual transformants were examined by hybridization and sequence analysis. Three sets of transformants were analyzed: sets of 32 and 13 colonies, from which up to 20 plaques each were sampled (averages, 15 and 18, respectively), and a set of 13 colonies, from which up to 10 plaques were sampled (average, 5). Overall, 46 colonies exclusively produced the G-G vector, and 19 exclusively produced the T-T vector. No evidence of heterogeneity was detected.

In addition to the experiment described above, 10 plaques from each of 15 yeast colonies that had been transformed with a pYMV1 construct carrying the *trans-syn* dimer were investigated by hybridization and sequence analysis. Five colonies contained a mutant sequence, and the remainder were normal; however, no heterogeneity was found in either the mutant or in the normal examples. Finally, no heterogeneity was detected in an additional 80 plaques analyzed from two normal colonies and one mutant colony from this set. We conclude that the frequency with which yeast cells take up and replicate multiple copies of the vector is low and that usually only a single mutational event occurs in any one cell, which presumably arises during the conversion of the single-stranded vector to double-stranded DNA.

DISCUSSION

By transforming *S. cerevisiae* and *E. coli* with the same single-stranded shuttle vector construct carrying either a *cis-syn* or a *trans-syn* T-T cyclobutane dimer, we have shown that the frequency with which the UV photoproducts are bypassed during replication and the error frequency of this bypass can be very different in these two organisms. Similarly, the relative frequencies of each kind of induced mutation were not the same in these two species. Qualitatively, however, the types of mutations induced were virtually identical, at least as far as the *trans-syn* isomer was concerned; the mutational spectra in the two organisms differed only with respect to a few single-occurrence events. The *cis-syn* isomer produced too few mutations in *S. cerevisiae* to make a comparison possible.

The virtually invariant natures of the mutational spectra imply that the types of mutations induced depend almost exclusively on the structure and properties of the modified template, and very little on the particular DNA polymerase, other proteins, or conditions under which replication takes place. However, studies with a greater variety of organisms will be needed to confirm this conclusion, and studies with a greater variety of lesions will be needed to establish the extent to which it is general. Template structure might influence the mutation spectrum in a number of ways. The base of the 5′ nucleotide in the *trans-syn* dimer is in *syn* with regard to the glycosyl bond and thus is permanently destacked (14). This configuration may occasionally prevent polymerase from recognizing the 5′ site as one appropriate for nucleotide insertion, leading to a targeted single base deletion. Alternatively, the correct insertion of adenine at the 3′ site, followed by nascent strand slippage, may produce these events. Accurate replication may depend on the formation of T · A base pairs, since nuclear magnetic resonance studies demonstrate that the 3′ T in the *trans-syn* isomer (14) and both nucleotides in the *cis-syn* isomer (5, 14) are capable of forming such hydrogen-bonded structures. By extension, substitution mutations may arise from the formation of T · T and to a lesser extent T · G mispairs; in *E. coli*, T→A transversions and T→C transitions, in a ratio of 4:1 to 5:1, are induced by both isomers, suggesting that saturation of the 5-6 double bond itself within the context of these structures encourages such events (7). The mutations observed are very different from those expected from an abasic site (8) and appear to result from the misinstructional rather than the noninstructional properties of the template (7).

These results also demonstrate, perhaps not surprisingly, that the frequency with which DNA polymerase bypasses a mutagenic lesion and the frequencies with which different errors are made (unlike the mutation spectrum) do depend strongly on the particular proteins responsible for replication and the particular cellular conditions in which it takes place. Previous observations (1, 2, 7–9) showing that various UV photoproducts possess unique and characteristic mutational properties in *E. coli*, each unlike those for abasic sites, imply that the structure of the mutagen-modified template itself is also an important determinant of these properties. Taking these two pieces of information together, it is likely that bypass and error frequencies, at least for T-T cyclobutane dimers, result from a specific interaction between a particular set of replication proteins and a particular template structure.

If the conclusions described above are confirmed and prove to be fairly general, there would appear to be little prospect of predicting the mutagenic properties of lesions from the structure of the mutagen-modified DNA template. The ability to make such predictions would have the attractive feature of greatly simplifying mutagen testing. However, bypass frequency and the error frequency of bypass, which are probably the more important determinants of mutagenicity in most circumstances, appear to be the least predictable properties. It is nevertheless still possible that the results for *S. cerevisiae* will prove to be a good predictor for other eukaryotes. Further, the mutation spectrum, the most predictable feature, can be an important property when mutations of a particular type are of concern, as might be the case with certain oncogenes.

A second aim of these experiments was to determine whether UV-inducible gene products are required for bypass replication and mutagenesis in *S. cerevisiae*, as they clearly are in *E. coli*. Single-stranded vectors are useful for this

purpose because they exclusively detect such an event, and the homogeneity of the population of photoproduct-carrying vector molecules results in high resolution. As shown by the data given in Tables 1 and 2, there is no evidence for an SOS-like phenomenon in *S. cerevisiae*. UV irradiation of yeast cells merely reduces their capacity to be transformed, uniformly for control and photoproduct-containing vectors alike. These observations are consistent with others showing that UV irradiation has little effect on the regulation of yeast gene products known to be essential for induced mutagenesis (6, 13). Therefore, *S. cerevisiae* and *E. coli* may use quite different means to regulate bypass replication and mutagenesis.

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