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Genetics

# Site and strand specificity of UVB mutagenesis in the *SUP4*-o gene of yeast

[cyclobutane dimer/(6-4) photoproduct/germicidal UV/strand preference]

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ABSTRACT **DNA sequencing was used to characterize 208 mutations induced in the *SUP4*-o tRNA gene of the yeast *Saccharomyces cerevisiae* by UVB (285–320 nm) radiation. The results were compared to those for an analysis of 211 *SUP4*-o mutations induced by 254-nm UVC light. In each case, >90% of the mutations were single base-pair changes but G·C → A·T transitions predominated and accounted for more of the mutations induced by UVB than UVC. Double substitutions, single base-pair deletions, and more complex events were also recovered. However, UVB induced 3-fold more tandem substitutions than UVC and nontandem double events were detected only after irradiation with UVC. Virtually all induced substitutions occurred at sites where the pyrimidine of the base pair was part of a dipyrimidine sequence. Although the site specificities were consistent with roles for cyclobutane dimers and pyrimidine-pyrimidone(6-4) lesions in mutation induction, preliminary photoreactivation data implicated cyclobutane dimers as the major form of premutational DNA damage for both agents. Intriguingly, there was a preference for both UVB- and UVC-induced mutations to occur at sites where the dipyrimidine was on the transcribed strand.**

Exposure to sunlight has been linked with the development of human skin cancer and experimental evidence indicates that among the wavelengths that penetrate the ozone layer, those responsible for carcinogenesis are within the UVB (280–320 nm) region of the solar spectrum (1–3). Although the precise mechanism is unknown, DNA damage, as expressed by genetic mutation, is very likely to be involved in the induction of cancer by sunlight. Indeed, the human disorder xeroderma pigmentosum involves defective repair of UV-induced DNA lesions and sensitizes affected individuals to solar photocarcinogenesis (4). Furthermore, sunlight has been shown to mutagenize Chinese hamster cells (5) and UVB wavelengths are mutagenic to bacterial, yeast, and mammalian cells (6–8).

It is generally considered that cyclobutane pyrimidine dimers and pyrimidine-pyrimidone(6-4) photoproducts [(6-4) photoproducts] are the most important premutational DNA lesions induced by UVC (200–280 nm) radiation (9, 10). Although UVB wavelengths also produce these two types of photoproduct (6, 10–12), other lesions such as DNA strand breaks and thymine glycols are induced by UVB treatment (13, 14). The precise roles of these various lesions in UVB mutagenesis, and thus the mechanism(s) involved, have yet to be established. There is evidence implicating cyclobutane dimers and (6-4) photoproducts in the mutagenicity of UVB radiation (12, 15), but there also are indications that the nondimer lesions may contribute significantly (6, 15). To add to the complexity of this situation, other factors may influence the production of UVB-induced mutations. For example, UVC-induced cyclobutane dimers are selectively repaired in the transcribed strand of transcriptionally active *dhfr*, *lacI*, or *URA3* genes in human and rodent, bacterial, or yeast cells, respectively (16–18), and the same may be true for (6-4) photoproducts (19). Such differential repair predicts a bias for UVC mutagenesis at sites where the dipyrimidine sequence is on the nontranscribed strand and this has been found recently for the *hprt* gene in Chinese hamster cells (20). Consequently, mutation induction by UVB might also exhibit a strand preference.

Characterization of the DNA sequence alterations produced by UVB irradiation could yield important clues about the lesions and mechanism(s) involved in UVB mutagenesis and help determine whether it is influenced by processes such as differential repair. To assay mutational specificity, we previously developed a system for the DNA sequence analysis of forward mutations in the suppressor tRNA gene *SUP4*-o of the yeast *Saccharomyces cerevisiae* (21). Here we have used this system to examine mutation induction by polychromatic UVB light and have compared the results to those for UVC (254 nm) mutagenesis. Our results suggest that cyclobutane dimers are responsible for the majority of UVB- and UVC-induced *SUP4*-o mutations and indicate a preference for mutagenesis at sites where a dipyrimidine sequence is on the transcribed strand.

## MATERIALS AND METHODS

**Strain, Plasmid, and Media.** The haploid, repair-proficient yeast strain MKP-op (*MATα*, *can1-100*, *ade2-1*, *lys2-1*, *ura3-52*, *leu2-3,112*, *his3-Δ200*, *trp1-Δ901*, YCpMP2) was used. *SUP4*-o, an ochre suppressor allele of a yeast tyrosine tRNA gene, is carried on YCpMP2 (21) a centromere-containing shuttle vector. Such plasmids mimic chromosome behavior and are maintained predominantly as single copies in haploid yeast cells (22). Media for growth of yeast and mutant selection have been described (21).

**Detection of *SUP4*-o Mutants.** Forward mutations in *SUP4*-o were detected as described (21) by scoring for reduced suppression of the three ochre markers *can1-100*, *ade2-1*, and *lys2-1*. Selection for diminished suppression of all three markers detects at least a 30% decrease in the production of functional *SUP4*-o tRNA (23) and is unlikely to bias mutant recovery significantly (24).

**UV Experiments.** Irradiation and subsequent steps were carried out under yellow light to avoid photoreactivation. The UVB source was two 15-W midrange bulbs (Ultraviolet Products, San Gabriel, CA) having >95% of their total output between 270 and 400 nm with the peak output at 325 nm (manufacturer's specifications). Suspensions of stationary-phase cells in sterile $H_2O$ ($1.5 \times 10^7$ cells per ml as determined by a Coulter Counter) were stirred and irradiated in plastic Petri dishes with the lids on. The lid filtered out all wave-

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lengths below 285 nm [determined with a Milton Roy (Rochester, NY) 3000 spectrophotometer]. Using the spectrophotometric data and the bulb specifications, 31% of the transmitted radiation was calculated to lie between 285 and 320 nm with 69% between 320 and 400 nm. When all wavelengths below 316 nm were eliminated with a Mylar D clear plastic filter (DuPont), the fluence used here did not induce *SUP4*-o mutations (data not shown). Thus, for our purposes, we consider the lamp to be primarily a UVB source. The incident dose rate through the lid was adjusted to 22 $J \cdot m^{-2} \cdot sec^{-1}$ with a UVX radiometer fitted with a UVX-31 midrange sensor (Ultraviolet Products). The UVC source was a Sylvania G30T8 bulb with >98% of its output at 254 nm (manufacturer's specifications) and the incident dose rate was set to 2.4 $J \cdot m^{-2} sec^{-1}$ with a UVX radiometer fitted with a UVX-25 shortrange sensor. For UVB, aliquots of the irradiated cell suspension, diluted when necessary, were plated to measure survival and select mutants. For UVC, aliquots of the cell suspension were diluted when necessary and spread on appropriate medium, and the plates were irradiated with the lids off. After incubation of the plates in the dark at 30°C for 6 days, viability and the *SUP4*-o mutation frequency were determined.

**Other Procedures.** DNA isolation, bacterial transformation, and DNA sequencing were performed as described (21, 25, 26).

## RESULTS

**Induction and Characterization of *SUP4*-o Mutants.** Doses of UVB (9768 $J/m^2$) and UVC (60 $J/m^2$) that decreased cell survival to 10%, enhanced the *SUP4*-o mutation frequency by 70- and 145-fold, respectively, the maximum levels of induction we observed (data not shown). To minimize the contribution of spontaneous events, we used these doses to collect mutations for DNA sequence analysis. In total, 299 *SUP4*-o mutants were characterized and the data for 91 UVC-induced mutants were pooled with the results for 120 mutants (also induced by 60 $J/m^2$) examined earlier (26). For both UVB- and UVC-induced mutagenesis, single base-pair changes predominated (Table 1). Tandem double substitutions, single base-pair deletions, and more complex events consisting of combinations of substitutions and deletions were also recovered after each treatment (Tables 1 and 2). However, significantly more tandem double changes were detected after UVB than UVC irradiation ($P < 0.05$). Nontandem double substitutions were found only after treatment with UVC. Analysis of mutations induced in the *Escherichia coli supF* gene by 313-nm UVB radiation (27) gave different results, likely reflecting dissimilarities in the two experimental systems (mutations in *supF* were scored in bacteria after passage through monkey cells of *supF*-bearing plasmids that had been irradiated *in vitro* with three different but very large doses of monochromatic 313-nm light).

**Types and Distributions of Substitutions.** Substitutions that occurred as single events, or as part of multiple mutations, are collated in Table 3 (the relative fractions are the same when only single events are tabulated). All six possible types of base-pair substitution were induced by UVB and UVC, with transitions outnumbering transversions by 15-fold for UVB and by 7-fold for UVC. For both agents, G·C → A·T events predominated but their relative fraction among the total changes was substantially greater for UVB than for UVC (87% vs. 70%) ($P < 0.001$). The increase in G·C → A·T transitions for UVB occurred at the expense of substitutions at A·T pairs relative to UVC and resulted in a ratio of G·C → A·T to A·T → G·C transitions of 13:1 for UVB compared with 4:1 for UVC.

The distributions of the substitutions are given in Fig. 1. No mutations were detected 5′ or 3′ to the coding sequence. A total of 47 sites were mutated but only 27 of these were common to both distributions. Nevertheless, at least 90% of the substitutions in each distribution occurred at sites mutated by both agents, and the distributions appeared to be very similar. Hot spots for G·C → A·T transitions were detected at sites 51 and 86, and transitions were also induced frequently at other G·C pairs mutated by UVB and UVC. Although the distributions overlapped considerably, there were differences. In particular, A·T pairs in which at least two UVC-induced mutations occurred (sites 23, 24, 31, 36–39, and 58) were comparatively poor targets for UVB mutagenesis. By using the Monte Carlo estimate of the *P* value of the hypergeometric test (29) to statistically compare the two distributions, we determined that the probability of random sampling error being the cause of any differences was <0.5%.

**Site Specificity.** Assuming that the UVC-induced *SUP4*-o mutations were likely targeted by dipyrimidine lesions, the similarities in the distributions of the substitutions led us to

Table 1. Characterization of *SUP4*-o mutations

| Sequence alteration | UVB No. (%) | UVB Frequency × $10^{-6}$ | UVC No. (%) | UVC Frequency × $10^{-6}$ |
|---|---|---|---|---|
| Substitution | | | | |
| Single | 189 (90.9) | 127 | 197 (93.3) | 271 |
| Tandem | 16 (7.6) | 11 | 6 (2.8) | 8 |
| Non-tandem | — | | 4 (1.9) | 6 |
| Deletion −1 | 2 (1.0) | 1 | 2 (1.0) | 2 |
| Other | 1 (0.5) | 1 | 2 (1.0) | 2 |
| Total | 208 | 140 | 211 | 290 |

Table 2. Multiple mutations and deletions

| Sites | Changes | No. detected UVB | No. detected UVC |
|---|---|---|---|
| 18, 19 | C → T, C → T | 2 | 1 |
| 19, 20 | C → T, A → T | 1 | 1 |
| 25, 26 | C → T, C → T | 2 | — |
| 31, 32 | T → C, C → T | 1 | — |
| 36, 38 | A → C, T → C | — | 1 |
| 38, 42 | T → A, A → T | — | 1 |
| 50, 51 | G → T, C → T | — | 1 |
| 52 → 54 | −1 | 1 | — |
| 65, 66 | C → T, C → T | 2 | 1 |
| 65 → 67, 74 | −1, T → G | — | 1 |
| 79 → 83 | −1 | 1 | 2 |
| 80, 81 | G → A, G → A | 1 | 1 |
| 84, 87, 89 | C → G, −1, T → A | 1 | — |
| 84, 85 | C → T, C → T | 3 | 1 |
| 84 → 86 | −1, C → G | — | 1 |
| 84, 86 | C → T, C → T | — | 2 |
| 85, 86 | C → T, C → T | 3 | — |
| 87, 88 | T → C, C → A | 1 | — |

Changes are given for the transcribed strand (see Fig. 1).

Table 3. Base-pair substitutions

| Substitution | No. detected (%) UVB | No. detected (%) UVC |
|---|---|---|
| G·C → A·T | 194 (87.0) | 154 (70.3) |
| A·T → G·C | 15 (6.7) | 39 (17.8) |
| Total | 209 (93.7) | 193 (88.1) |
| G·C → T·A | 5 (2.2) | 5 (2.3) |
| G·C → C·G | 4 (1.8) | 5 (2.3) |
| A·T → C·G | 1 (0.4) | 3 (1.4) |
| A·T → T·A | 4 (1.8) | 13 (5.9) |
| Total | 14 (6.3) | 26 (11.9) |

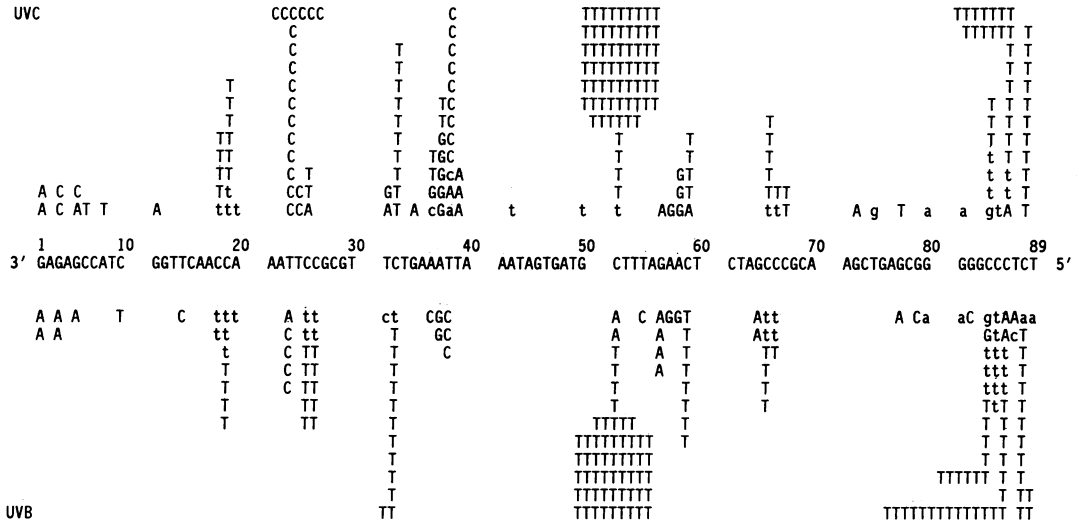


FIG. 1. Distribution of substitutions in *SUP4*-o. Only the region of the transcribed strand encoding the tRNA is shown. The anticodon is at 36–38 and the 14-base-pair intron is inferred to extend from 40 to 53 as for the *sup4*+ allele (28). Lowercase letters indicate changes in multiple mutants (for more detail, see Table 2). A UVC-induced G·C → C·G transversion within 84–86 is not shown because identification of its exact position was prevented by concurrent loss of a G·C pair from the same region.

compare the site specificities of UVB and UVC mutagenesis. There are 23 locations in *SUP4*-o where two dipyrimidine sequences overlap (i.e., Y-Y*-Y, where the asterisk indicates the mutated base). We analyzed only changes that could be associated with the 5′ or 3′ base in specific dipyrimidine sequences (i.e., 5′ R-Y*-Y 3′ or 5′ Y-Y*-R 3′) (Y = pyrimidine; R = purine). Several interesting features emerged (Table 4). First, UVB induced proportionally fewer substitutions at TT sequences than UVC, consistent with the observed decrease in mutagenesis at A·T pairs. Second, CT sequences were infrequently mutated by UVB and UVC, although this might reflect the relatively small number of available sites. Third, the fraction of UVB-induced substitutions at TC and CC sequences (87%) was significantly greater than that for UVC (69%) ($P < 0.01$). Fourth, the majority of mutations tabulated for UVB and UVC occurred at the 3′ base and for both agents this bias was more pronounced for transitions than transversions. If 83% of the UVB-induced substitutions at CT*C, CC*T, and TC*T sequences also occurred at the 3′ base of a dipyrimidine, then 88 of the 100 substitutions at overlapping sites could have been at TC or CC sequences (Table 5). Although UVC mutagenesis also appeared to prefer TC and CC sites, the proportion of events at these sequences (69%) was very close to the fraction of sites available (64%). However, 53 of the 65 UVC-induced substitutions at overlapping sites could have been at TC and CC sequences (Table 5). When this value is included in the analysis of site specificity, the fraction of UVC-induced substitutions at TC plus CC sites (73%) is significantly larger than expected on the basis of random occurrence ($P < 0.02$).

**Strand Specificity.** UVC-induced DNA damage is preferentially repaired on the transcribed strand of transcriptionally active genes in bacterial, yeast, rodent, and human cells (16–18). Thus, we anticipated that the majority of *SUP4*-o mutations would be at sites where the dipyrimidine sequences are on the nontranscribed strand. Surprisingly, we found the exact opposite preference. For both UVC and UVB, ≈90% of the induced substitutions occurred at sites where the pyrimidines were part a dipyrimidine sequence on the transcribed strand (Table 6). This did not reflect a bias in the distribution of dipyrimidines since each of the four different types was apportioned evenly between the two strands. When the hot spots at positions 51 and 86 (accounting for ≈42% of the substitutions for UVB and UVC) were excluded, there were still 5-fold and 3-fold more UVB- and UVC-induced substitutions, respectively, at sites where the dipyrimidine is on the transcribed strand.

## DISCUSSION

The types and distributions of *SUP4*-o mutations induced by polychromatic UVB radiation were strikingly similar to those for UVC mutagenesis, suggesting the involvement of the same lesion(s) and/or mechanism(s). Where also noted in studies of UVC mutagenesis, certain of the features common to both spectra, including the predominance of G·C → A·T transitions and the preference for substitutions at the 3′ base of dipyrimidine sequences, have been interpreted to indicate that cyclobutane dimers and (6-4) photoproducts may target UVC-induced mutations (9, 30, 31). Thus, the marked sim-

Table 4. Site specificity of UV-induced base-pair substitutions

| Site 5′ → 3′ | Sites available† 5′ | Sites available† 3′ | UVB Sites detected 5′ | UVB Sites detected 3′ | UVB Change and base mutated: Transition 5′ | UVB Change and base mutated: Transition 3′ | UVB Change and base mutated: Transversion 5′ | UVB Change and base mutated: Transversion 3′ | UVC Sites detected 5′ | UVC Sites detected 3′ | UVC Change and base mutated: Transition 5′ | UVC Change and base mutated: Transition 3′ | UVC Change and base mutated: Transversion 5′ | UVC Change and base mutated: Transversion 3′ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TT | 4 | 5 | 1 | 4 | 1 | 10 | 1 | 1 | 1 | 5 | — | 35 | 4 | 5 |
| CT | 4 | 1 | 2 | — | 3 | — | — | — | 2 | — | 3 | — | — | — |
| Total | 8 | 6 | 3 | 4 | 4 | 10 | 1 | 1 | 3 | 5 | 3 | 35 | 4 | 5 |
| TC | 4 | 9 | — | 5 | — | 66 | 1 | 2 | 1 | 5 | — | 71 | 1 | 3 |
| CC | 6 | 6 | 2 | 3 | 14 | 21 | 1 | 2 | 3 | 4 | 11 | 18 | — | 1 |
| Total | 10 | 15 | 2 | 8 | 14 | 87 | 2 | 4 | 4 | 9 | 11 | 89 | 1 | 4 |

†Only sites where base-pair substitutions can be detected in *SUP4*-o are tabulated.

Table 5. Substitutions at overlapping dipyrimidines

| Sequence 5′ → 3′ | Site | Change | No. detected UVB | UVC |
|---|---|---|---|---|
| TT*T | 36 | A | — | 2 |
| | | C | — | 1 |
| | | G | 1 | 1 |
| CT*T | 14, 24, 31, 57 | A | — | 1 |
| | | C | 3 | 3 |
| | | G | — | 1 |
| CT*C | 87 | A | 1 | — |
| | | C | 1 | — |
| CC*T | 25 | A | — | 1 |
| | | T | 7 | 2 |
| TC*T | 3, 32, 56, 88 | A | 1 | — |
| | | G | — | 2 |
| | | T | 34 | 22 |
| TC*C | 86 | A | 2 | 1 |
| | | G | — | 1 |
| | | T | 38 | 22 |
| CC*C | 66, 80–82, 85 | G | 1 | — |
| | | T | 11 | 5 |

Sites are given for the mutated bases (*). The changes are the substitutions that occurred.

ilarities that we observed between UVB and UVC mutagenesis argue that these lesions may also be the major types of premutational damage induced by polychromatic UVB radiation in *SUP4*-o.

The UVC dose used here is expected to induce (6-4) photoproducts at TT and CT sequences only rarely (32). If this is also true for UVB, then for both agents the transitions and transversions detected at TT and CT dipyrimidines were most likely due to cyclobutane dimers. Still, the majority of the UVB-induced *SUP4*-o mutations that could be unambiguously assigned to specific dipyrimidines were G·C → A·T transitions at the 3′ base of TC and CC sequences. Where similar results have been obtained for UVC mutagenesis (9, 30, 31), they have been considered to favor (6-4) photoproducts over cyclobutane dimers as premutational lesions at these sequences for several reasons: (*i*) (6-4) photoproducts form most often at TC and CC sequences, whereas cyclobutane dimers form most frequently at TT sequences and least often at CC sequences (31); (*ii*) structural determinations of the TC (6-4) photoproduct reveal that only the 3′ base should not pair correctly while both bases of the TT cyclobutane dimer exhibit weakened hydrogen bonding with the 5′ base showing more distortion (33, 34); and (*iii*) DNA polymerases preferentially insert adenine opposite UVC lesions in DNA (35). However, we have determined that photoreactivation, which is specific for cyclobutane dimers, can reduce the frequency of both UVB and UVC mutagenesis at *SUP4*-o by ≈80% (unpublished data). Experimental evidence does not support the possibilities that photoreactivation of cyclobutane dimers in UVC-irradiated yeast enhances repair of other mutagenic photoproducts and/or eliminates a signal for the induction of mutational processes that would act on other lesions (36). Therefore, we suggest that the majority of UVB- and UVC-induced mutations recovered at TC and CC sites in this study were targeted by cyclobutane dimers.

Table 6. Strand specificity of UV mutagenesis

| Dipyrimidine location | No. of substitutions at dipyrimidines (%) UVB | UVC |
|---|---|---|
| Transcribed strand | 203 (91.0) | 187 (86.2) |
| Nontranscribed strand | 20 (9.0) | 30 (13.8) |

Substitutions can be detected at 7 TC, 7 CC, 6 TT, and 6 CT sites on the transcribed strand and at 7 TC, 5 CC, 9 TT, and 6 CT sites on the nontranscribed strand (including overlaps in both cases). Two UVC-induced substitutions were not at dipyrimidine sequences and were not included in this analysis.

Given the foregoing, it is interesting that UVB induced 70% fewer substitutions at TT sequences and that nontandem substitutions were recovered after UVC, but not after UVB, irradiation. The simplest explanation for the paucity of mutations at TT sequences would be a decrease in the relative proportion of TT dimers. Although the ratio of CC to TT dimers in DNA irradiated *in vitro* has been found to be 2-fold greater at 300 nm than at 254 nm (11), it remains to be determined whether a similar effect large enough to account for our results occurs *in vivo*. The precise mechanism responsible for nontandem substitutions is unknown but if reduced fidelity of DNA replication is necessary for translesion synthesis (37), they might arise as replication errors at a damaged and a subsequent nondamaged site. For our results, this would imply that replicational fidelity decreased for the bypass of UVC, but not UVB, photoproducts, which seems doubtful. Alternatively, nontandem events could be due to concurrent substitutions at closely opposed photoproducts. If so, our data would suggest that UVB produced fewer closely opposed premutational lesions than UVC, a reasonable possibility since UVB induced less *SUP4*-o mutations than UVC on a per lethal hit basis.

Selective repair of cyclobutane dimers on the transcribed strand of transcriptionally active genes has been demonstrated (16–18). This led us to expect that most *SUP4*-o mutations would be at sites where the dipyrimidine is on the nontranscribed strand, as found for UVC-induced *hprt* mutations in excision repair-proficient Chinese hamster cells (20). Instead, ≈90% of the UVB- and UVC-induced substitutions in *SUP4*-o occurred at sites where the dipyrimidine was on the transcribed strand. A similar bias was also found for the *hprt* gene, but only in an excision repair-deficient Chinese hamster cell line (20). Since these cells did not remove pyrimidine dimers from either strand of the *hprt* gene, it was proposed that error rate differences in leading and lagging strand translesion synthesis accounted for the strand bias in this cell line. If so, the error rate must have been greater for the transcribed strand. It seems unlikely that such an error rate difference could have been responsible for the strand preference in *SUP4*-o. The yeast strain we used is excision repair proficient, the operation of excision repair on yeast plasmids appears to accurately reflect its action on yeast genomic chromatin (38), and UVC damage is preferentially repaired in the transcribed strand of a transcriptionally active, plasmid-borne gene in yeast (18). Thus, by analogy with the *hprt* data (20), selective repair of UV damage on the transcribed strand of *SUP4*-o should have precluded any bias attributable to a greater error rate during translesion synthesis on this strand.

Unlike the genes that have been used to study strand-specific repair of UVC-induced photoproducts (16–18, 20), *SUP4*-o is transcribed by eukaryotic RNA polymerase III. Promoters for transcription by this enzyme are internal and transcription factor complexes that assemble on *SUP4*-o *in vitro* cover the entire gene (39) with at least some components interacting directly with the nontranscribed strand (40). Furthermore, RNA polymerase III reads through such complexes without disrupting them (41) so that the transcription factors may remain associated with the nontranscribed strand for extended periods. Long-term binding of these factors to *SUP4*-o might inhibit UV photoproduct formation on the nontranscribed strand and/or serve to attract excision repair enzymes to pyrimidine dimers on this strand. These possibilities are supported by the demonstrations that: (*i*) binding of transcription factor IIIA to a 5S rRNA gene *in vitro* markedly reduces induction of UVC lesions within the bound

region (42); and (*ii*) photolyase stimulates excision repair in yeast in the dark, presumably by binding at cyclobutane dimers (43).

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