Mutagenic effects of abasic and oxidized abasic lesions in *Saccharomyces cerevisiae*

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ABSTRACT

2-Deoxyribonolactone (L) and 2-deoxyribose (AP) are abasic sites that are produced by ionizing radiation, reactive oxygen species and a variety of DNA damaging agents. The biological processing of the AP site has been examined in the yeast *Saccharomyces cerevisiae*. However, nothing is known about how L is processed in this organism. We determined the bypass and mutagenic specificity of DNA containing an abasic site (AP and L) or the AP analog tetrahydrofuran (F) using an oligonucleotide transformation assay. The tetrahydrofuran analog and L were bypassed at 10-fold higher frequencies than the AP lesions. Bypass frequencies of lesions were greatly reduced in the absence of Rev1 or Pol₃z (rev3 mutant), but were only marginally reduced in the absence of Pol₁ (rad30 mutant). Deoxyctydine was the preferred nucleotide inserted opposite an AP site whereas dA and dC were inserted at equal frequencies opposite F and L sites. In the rev¹ and rev³ strains, dA was the predominant nucleotide inserted opposite these lesions. Overall, we conclude that both Rev1 and Pol₃z are required for the efficient bypass of abasic sites in yeast.

INTRODUCTION

An apurinic/apyrimidinic site (2-deoxyribose, AP) is one of the most frequently encountered DNA lesions in cells (1–3). Significant amounts of AP sites are spontaneously generated via hydrolytic depurination (4), and increased generation of AP sites occurs when cells are exposed to oxidative stress or to genotoxic agents such as ionizing radiation (4,5). Furthermore, AP sites are normal intermediates of base excision repair initiated by the glycosylases that respond to insults inflicted upon DNA by a wide variety of damaging agents (6,7). In addition to the AP sites, oxidized abasic lesions are also produced when cells are exposed to ionizing radiation or other sources of oxidative stress that generate significant amounts of reactive oxygen species (5,8). 2-Deoxyribonolactone (L) and the C4-oxidized 4-hydroxy-2-deoxyribose (C4-AP) are two of the more commonly observed oxidized abasic lesions (5,8). Recent studies suggest that these lesions are produced in significant amounts under certain conditions (9–12). Oxidized AP sites are also generated by various antitumor agents (13–15). For example, exposing DNA to the neocarzinostatin chromophore can lead to the generation of L sites (13,14) and interaction of DNA with bleomycin produces a significant amount of C4-AP sites (15,16).

In *in vitro* experiments, abasic lesions (AP, L and C4-AP) are processed efficiently by purified AP endonucleases, such as bacterial endonuclease IV and exonuclease III (6,7,17–19), which nick the DNA 5’ to the lesion. In contrast, the processing efficiency of oxidized abasic sites by the DNA lyase activities associated with many DNA glycosylases is quite variable (17,18,20–22). *Escherichia coli* DNA glycosylases such as endonuclease III, endonuclease VIII and formamidopyrimidine N-glycosylase as well as yeast endonuclease III homologs (Ntg1 and Ntg2) and oxoguanine N-glycosylase recognize an AP site efficiently and nick the DNA 5’ to the lesion (6). While *E.coli* endonuclease III also recognizes C4-AP, it cleaves DNA containing the lesion 100-fold less efficiently than does endonuclease IV or exonuclease III (17). Exposing endonuclease III to DNA containing L actually leads to the formation of an irreversible crosslinked complex (20,21).

In addition to *in vitro* biochemical analyses, the biological processing and consequences of AP sites have been well...
documented in *E. coli*, where these sites are both cytotoxic and mutagenic (1,4,6,7). Relatively little is known about the biological processing of oxidized AP sites. Early data indicated that these sites are also highly mutagenic and cytotoxic (23–26) and these earlier observations have been supported by more recent studies in *E. coli* (27–29). In *E. coli*, replication of an M13 single-stranded plasmid containing either AP, L or C4-AP sites requires the lesion bypass polymerases pol II, pol IV and pol V (27–29). These lesion bypass polymerases interact distinctly with each type of abasic lesion, generating a unique mutation spectrum for each. 2'-Deoxyadenosine (2'-dA), for example, is preferentially incorporated opposite an AP site, while dG is incorporated as well or better than dA opposite L (27,29,30). In addition, single nucleotide deletions are produced opposite AP and L sites in a sequence-dependent manner that involves a misinsertion–misalignment process (29). In contrast, a large proportion of the C4-AP bypass events results in 3 nt deletions (28).

In yeast, the biological processing of defined AP sites has been examined using plasmid gap-filling assays (31,32), assays that require the conversion of a single-stranded plasmid to a duplex form (33) or transformation assays that target a lesion-containing oligonucleotide to a defined genomic region (34). In contrast to the ‘A-rule’ of AP bypass in *E. coli*, dC was incorporated preferentially opposite the AP site in each of the yeast assays. Genetically, AP bypass in yeast clearly requires the participation of the Pol ζ (a complex of the Rev3 and Rev7 proteins) and Rev1 polymerases, but Pol η (encoded by *RAD30*) has been implicated in the insertion of dCMP opposite an AP site under some circumstances (33). Although there is no information regarding the processing of oxidized AP sites in yeast, these sites are likely recognized by the yeast AP endonucleases and lyases in a similar manner as in *E. coli*. In the event that these oxidized lesions escape the action of Apn1, the major AP endonuclease in yeast, oxidized abasic sites are expected to block replicative DNA polymerases, and lesion bypass in the form of translesion synthesis or a template switching event would be needed for the cell to survive.

In the current study, we investigate the bypass and associated mutagenicity of 2-deoxyribonolactone, an oxidized AP site in yeast using single-strand oligonucleotide-mediated transformation, and directly compare its bypass with that of the natural AP site or the AP site analog, tetrahydrofuran (F). The genomic lys2ΔA746 –1 frameshift allele was used as the target for 30 nt oligomers (30mer) containing an extra nucleotide or defined abasic site located precisely at the position of the frameshift mutation. Integration of the oligonucleotide and subsequent bypass of the abasic site during DNA replication results in the addition of a nucleotide, thereby reverting the lys2ΔA746 frameshift allele and restoring a selectable Lys⁰ phenotype. Relative to an undamaged control 30mer, the frequency of Lys⁺ transformants obtained with a lesion-containing 30mer reflects the efficiency of lesion bypass. In addition to assessing bypass in wild-type strains, the contributions of the Rev1, Pol ζ and Pol η to the bypass of the different types of abasic sites were examined using appropriate mutant strains. Finally, the sequencing of individual Lys⁺ transformants from each background allowed the specificity of lesion bypass by the various TLS polymerases to be determined.

**MATERIALS AND METHODS**

**Oligonucleotides**

Oligonucleotide LysA30, which contain sequences that flank the lys2ΔA746 allele, and LysA30R, which has the same sequence as LysA30 but is of reversed polarity, were obtained from Qiagen. Oligonucleotides containing a unique tetrahydrofuran (LysA30-F) or uracil (LysA30-U) were also obtained from Qiagen. To obtain an oligonucleotide containing a 2-deoxyribose (LysA30-AP), LysA30-U was treated with an excess amount of uracil DNA glycosylase as described previously (35). LysA30-L contained a 2-deoxyribonolactone (L) and was prepared by UV irradiation (350 nm) of parental oligonucleotide containing the appropriate photochemical precursors (36,37). Figure 1 shows the structures of these various abasic sites. The sequences of the oligonucleotides used for yeast transformations and the lys2ΔA746 target site are listed in Table 1.

**Yeast strains**

*Saccharomyces cerevisiae* strains used in this study are listed in Table 2 and are all derived from *SJR922* (*MATa ade2-101 oc*).

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**Table 1. DNA sequences of the lys2ΔA746 target site and the oligonucleotides used for transformation**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>lys2ΔA746</td>
<td>3'-CCCTTTGTGTTCTAAAGTTTAA–CTGCTCGAGTTC-5'</td>
</tr>
<tr>
<td>LysA30</td>
<td>5'-CAAGATTCTAAATTGACGACCTCAAGCAT-3'</td>
</tr>
<tr>
<td>LysA30-F</td>
<td>5'-CAAGATTCTAAATTGACGACCTCAAGCAT-3'</td>
</tr>
<tr>
<td>LysA30-AP</td>
<td>5'-CAAGATTCTAAATTGACGACCTCAAGCAT-3'</td>
</tr>
<tr>
<td>LysA30-L</td>
<td>5'-CAAGATTCTAAATTGACGACCTCAAGCAT-3'</td>
</tr>
<tr>
<td>LysA30R</td>
<td>3'-CAAGATTCTAAATTGACGACCTCAAGCAT-5'</td>
</tr>
</tbody>
</table>

A portion of the non-coding strand of the lys2ΔA746 allele is given, which is complementary to the coding strand sequence of the transforming oligonucleotides (LysA30-X). The dash in the lys2ΔA746 sequence corresponds to position of the frameshift mutation. The additional nucleotide present in each oligonucleotide is indicated in bold type. F, tetrahydrofuran; S, 2-deoxyribose; L, 2-deoxyribonolactone.

**Table 2. Yeast strains used in this study**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>SJR922</em></td>
<td><em>MATa ade2-101 oc his3Δ200 ura3Δ Nco lys2ΔA746</em></td>
<td>(38)</td>
</tr>
<tr>
<td><em>SJR1777</em></td>
<td><em>MATa ade2-101 oc his3Δ200 ura3Δ Nco lys2ΔA746</em></td>
<td>(38)</td>
</tr>
<tr>
<td><em>SJR922</em></td>
<td><em>MATa ade2-101 oc his3Δ200 ura3Δ Nco lys2ΔA746</em></td>
<td>This study</td>
</tr>
<tr>
<td><em>SJR922</em></td>
<td><em>MATa ade2-101 oc his3Δ200 ura3Δ Nco lys2ΔA746</em></td>
<td>This study</td>
</tr>
<tr>
<td><em>SJR922</em></td>
<td><em>MATa ade2-101 oc his3Δ200 ura3Δ Nco lys2ΔA746</em></td>
<td>This study</td>
</tr>
</tbody>
</table>
his3Δ200 ura3ΔNco lys2ΔA746) (38). APN1 was deleted by transforming cells with BamHI/EcoRI-digested pSCP19A, which contains the apn1Δ:HIS3 allele (39). Deletions of the complete open reading frames of RAD30, REV1 or REV3 were obtained following transformation with an appropriate PCR fragment and selection for genicin-resistant transformants (40). PCR fragments were derived using genomic DNA from the appropriate null strain (OPEN BIOSYSTEMS) as template.

**RESULTS**

**Correction efficiency of the lys2ΔA746 allele using single-stranded oligonucleotides**

The efficiency of allele correction in yeast following transformation with short single-stranded oligonucleotides is dependent on both the length and concentration of the oligonucleotide (42,43). To determine the background reversion and transformation efficiencies at the LYS2 locus, wild-type (WT) and apn1Δ strains containing the lys2ΔA746 allele (deletion of an adenine from the coding strand) were transformed with the 30 nt oligomers LysA30R and LysA30 (see Table 1 for sequences and Table 2 for yeast strains used in this study). LysA30 has the same sequence and polarity as the LYS2 coding strand and contains the nucleotide that is deleted in the lys2ΔA746 allele. Its incorporation into genomic DNA restores a Lys+ phenotype. The LysA30R 30mer has the same sequence but reverse polarity as LysA30. Any transformants obtained using this oligonucleotide reflect non-specific background. When 300 pmol of the oligonucleotides were used, the number of revertants obtained with LysA30R was <1% of the number obtained with LysA30 (Table 3). It should be noted that the frequency of revertants obtained after electroporation with LysA30R was no different from the background reversion frequency, indicating that the process of transformation is not mutagenic in this assay. In addition, the presence/absence of Apn1 did not affect the transformation efficiency of the undamaged LysA30 oligonucleotide. These results are similar to those obtained using the cyc1-31 frameshift allele as a target in oligonucleotide-mediated transformation (34,42,43) and establish the suitability of the lys2ΔA746 allele for examining the bypass of various AP sites in yeast.

A distinct advantage of using the lys2ΔA746 rather than the cyc1-31 allele in this type of analysis is that the position of the transforming oligonucleotide need not be constrained to the precise position or immediate vicinity of the frameshift mutation to be corrected. As shown in other studies with this allele, a compensatory +1 frameshift can occur anywhere within an ~150 bp ‘reversion window’ defined by stop codons in alternative reading frames (38,41). Although not exploited in the current studies, this feature of the lys2ΔA746 system will allow a detailed examination of sequence context effects on lesion bypass during the replication of chromosomal DNA. Finally, selection with the LYS2-based system is very clean. There is little, if any, background growth of mutants on the selective medium, and extragenic suppression has never been observed.

**Bypass efficiencies of defined abasic sites**

Previous oligonucleotide-mediated transformation experiments with the cyc1-31 allele (34) demonstrated that AP sites are bypassed at similar frequencies in WT and apn1Δ strains (7.2 and 8.6%, respectively). In contrast, the bypass

<table>
<thead>
<tr>
<th>Strain</th>
<th>No oligonucleotide</th>
<th>LysA30-R (300 pm)</th>
<th>LysA30 (300 pm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>58 ± 9</td>
<td>57 ± 8</td>
<td>8186 ± 1388</td>
</tr>
<tr>
<td>(apn1)</td>
<td>62 ± 11</td>
<td>55 ±10</td>
<td>8880 ± 1470</td>
</tr>
</tbody>
</table>
frequency of F increased over 10-fold in an apn1 mutant relative to a WT strain in these studies (6.2 and 0.33%, respectively). Preliminary experiments with the lys2ΔA746 allele as a target for an AP- or F-containing 30mer (LysA30-AP or LysA30-F, respectively) indicated that the efficiency of transformation with either oligonucleotide was at or below the background obtained with LysA30R control 30mer, suggesting that both lesions were efficiently repaired in this system (data not shown). Because the predominant repair of these lesions is expected to occur through the action of the Apn1 AP endonuclease (44), all subsequent experiments were conducted in an apn1Δ background.

In contrast to previous observations with the cyc1-31 allele where F and AP bypass occurred at comparable frequencies (~8%; (34)), F was bypassed in the lys2ΔA746 system almost 10-fold more efficiently than an AP site in an apn1 mutant (31.8 and 3.4%, respectively; Table 4). It is interesting to note that the oxidized L lesion was also efficiently bypassed (23.4%). We considered the possibility that the inefficient bypass of the AP relative to the L and F lesions in the apn1 mutant might reflect repair by enzymes other than Apn1. In addition to Apn1, it is known that an AP site can be recognized by the minor AP endonuclease, Apn2 (45), as well as by the yeast homologs of E.coli endonuclease III, Ntg1 and Ntg2 (46). The number of transformants obtained with LysA30-AP was similar, however, for the apn1 single, the apn1 apn2 double and the apn1 ntg1 ntg2 triple mutants (data not shown). These data suggest that the significantly lower transformation efficiencies exhibited by oligonucleotides containing AP is not a repair-related issue, but rather reflects simply inefficient bypass in this particular context.

The bypass frequency of F did not decrease significantly in the apn1 rad30 mutant, but there was a significant, 65% reduction in the apn1 rev1 and apn1 rev3 mutants (Table 4). Bypass of the oxidized L occurred in a qualitatively similar manner. It was unaffected by loss of Polη (apn1 rad30 mutant), but was reduced >85% in the apn1 rev1 and apn1 rev3 mutants. In spite of the low transformation efficiencies with 30mer containing the AP lesion, a similar trend was evident. The bypass efficiency of the AP site did not decrease significantly in the apn1 rad30 double mutant, but there was a significant, 62 and 50% reduction in the apn1 rev3 and apn1 rev1 mutants, respectively (Table 4). These data suggest that Polη (Rad30) plays only a minor, if any, role in the bypass of these lesions.

**Mutagenic specificity of defined abasic sites**

Using a short oligonucleotide that targets the cyc1-31 allele, Otsuka et al. (34) found that dC was the most frequent nucleotide incorporated opposite an AP site, whereas dA was slightly favored over dC for incorporation opposite F (34). Although the primary goal in our studies was to determine the frequencies with which each nucleotide is incorporated opposite the oxidized L, we also examined insertion specificities during the bypass of AP and F lesions. It should be noted that only those Lys” transformants containing an insertion at the position of the abasic site in the transforming oligonucleotide were included in these analyses. As mentioned previously, no ‘true’ reversions of the lys2ΔA746 allele have ever been observed spontaneously, so any mutations that precisely restore the deleted nucleotide must reflect translesion synthesis past the corresponding abasic site. The nucleotide insertion distribution for each strain/lesion was based on the sequences of 20–30 bypass events.

With all three lesions, the majority of the insertion events were dC or dA, with an insertion of dG or dT being observed only sporadically (Table 5). As expected based on earlier studies, dC was preferentially incorporated opposite an AP site (68%), followed by dA (32%) (Table 5). Although a similar insertion bias for dC opposite the AP site was observed in the apn1 rad30 mutant, dA was preferentially incorporated in the apn1 rev1 and apn1 rev3 mutants (64 and 82%, respectively). In contrast to the clear bias for dC insertion during AP bypass in the apn1 mutant, dA and dC were incorporated opposite F and L sites at roughly comparable levels (40–50% for each; Table 5). As for the AP lesion, loss of Polη (apn1 rad30 mutant) had little effect on the

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### Table 4. Bypass frequencies for various abasic sites

<table>
<thead>
<tr>
<th>Strain</th>
<th>F (%)</th>
<th>Lactone (%)</th>
<th>AP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>apn1</td>
<td>0.318 ± 0.038 (100)</td>
<td>0.234 ± 0.14 (100)</td>
<td>0.034 ± 0.008 (100)</td>
</tr>
<tr>
<td>apn1 rad30</td>
<td>0.274 ± 0.033 (86.2)</td>
<td>0.272 ± 0.076 (117.4)</td>
<td>0.024 ± 0.009 (70.6)</td>
</tr>
<tr>
<td>apn1 rev1</td>
<td>0.114 ± 0.042 (35.8)</td>
<td>0.033 ± 0.029 (14.1)</td>
<td>0.017 ± 0.009 (50)</td>
</tr>
<tr>
<td>apn1 rev3</td>
<td>0.116 ± 0.019 (36.5)</td>
<td>0.034 ± 0.011 (14.5)</td>
<td>0.013 ± 0.005c (38.2)</td>
</tr>
</tbody>
</table>

Bypass frequency = (No. of lesion)/(No. of control).

No. of lesions = number of transformants obtained with lesion-containing oligonucleotide minus number of transformants obtained with lysA30R.

No. of controls = number of transformants obtained with oligonucleotide LysA30 minus number of transformants obtained with lysA30R; n = number of transformation experiments.

*a n = 5.

*b n = 3.

*c Bypass frequency was determined by DNA sequencing.

### Table 5. Nucleotide insertions opposite abasic sites

<table>
<thead>
<tr>
<th>Strains</th>
<th>% Nucleotide inserted (no.)</th>
<th>F (dA</th>
<th>dG</th>
<th>dC</th>
<th>dT</th>
<th>F (dA</th>
<th>dG</th>
<th>dC</th>
<th>dT</th>
<th>L (dA</th>
<th>dG</th>
<th>dC</th>
<th>dT</th>
</tr>
</thead>
<tbody>
<tr>
<td>apn1</td>
<td>32.0 (8)</td>
<td>68.0 (17)</td>
<td>51.7 (15)</td>
<td>3.4 (1)</td>
<td>41.4 (12)</td>
<td>3.4 (1)</td>
<td>56.7 (17)</td>
<td>—</td>
<td>43.3 (13)</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>apn1 rad30</td>
<td>19.1 (4)</td>
<td>80.9 (17)</td>
<td>65.0 (13)</td>
<td>5.0 (1)</td>
<td>25.0 (5)</td>
<td>5.0 (1)</td>
<td>47.4 (9)</td>
<td>5.3 (1)</td>
<td>47.4 (9)</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>apn1 rev1</td>
<td>63.6 (14)</td>
<td>9.1 (2)</td>
<td>18.2 (4)</td>
<td>9.1 (2)</td>
<td>93.8 (15)</td>
<td>—</td>
<td>6.2 (1)</td>
<td>—</td>
<td>90.9 (20)</td>
<td>5.3 (1)</td>
<td>47.4 (9)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>apn1 rev3</td>
<td>81.8 (18)</td>
<td>13.6 (3)</td>
<td>4.5 (1)</td>
<td>—</td>
<td>100 (31)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>81.0 (17)</td>
<td>14.3 (3)</td>
<td>4.8 (1)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

AP, deoxyribose; F, tetrahydrofuran; L, 2-deoxyribonolactone; no., number of transformants.
nucleotide insertion preference opposite the F and L lesion. However, loss of Rev1 or Rev3 resulted in a much higher dA:dC insertion ratio opposite the F and L lesions (Table 5). Taken together, these results indicate that dA and dC are the preferred nucleotides for insertion opposite a variety of abasic sites and provide further support for preferential insertion of dC by the combined action of Rev1 and Polζ.

**DISCUSSION**

The biological processing of AP sites is well documented in both yeast and *E. coli* 1,4,6,7,39,44. In *E. coli*, dA is most frequently incorporated opposite an AP site (the ‘A’ rule) whereas in yeast, dC is clearly preferred in most assays (the ‘C’ rule) 1,4,32. The one reported exception to the C-rule (47) has been explained in alternative ways (31,33) and is unlikely to violate the rule for AP site bypass in yeast. In agreement with the mutation specificity of AP sites in bacteria, polymerases Pol I, II and IV preferentially incorporate dA opposite an AP site (27–29). Yeast contains three TLS polymerases that could be important in bypassing AP sites: Rev1, Polζ (Rev3–Rev7 complex) and Polη (Rad30). Consistent with the C-rule, Rev1 has been shown to have deoxycytidyl transferase activity (48). In *in vitro*, Rev1 inserts dCMP opposite an AP site, and the terminal dCMP can then be extended by Polζ (49).

Using a short lesion-containing oligonucleotide to introduce an AP site into the yeast CYC1 locus, Otsuka et al. (34) showed that this lesion was bypassed in a manner dependent on Rev1 (an involvement of Polζ was not examined) but not on Rad30 (34). As expected, dC was preferentially inserted opposite the lesion, and recent data have confirmed that the catalytic activity of Rev1 is indeed required for this bypass (48). In the current study, we have also used oligonucleotide-mediated transformation to demonstrate that AP sites are similarly bypassed at low frequency when introduced at the *LYS2* locus, that dC is the preferred base inserted opposite the AP site and that the bypass is dependent on both Rev1 and Polζ (Rev3). In addition, our data are consistent with only a minor role for Rad30 in the bypass of genomic AP sites. While these transformation data are consistent with those obtained when examining AP bypass within a gapped plasmid (31,32), it has been reported that all three yeast TLS polymerases are involved in the bypass of an AP site engineered into a completely single-strand plasmid (33). One possible explanation for these conflicting results is that the local sequences that flank the AP site influence the polymerase specificity. Because there is a general agreement between the *lys2ΔA746* and *cyc1-31* genomic bypass studies and the gap-filling assay, all of which involve different sequence contexts, it is unlikely that sequence context is the determining parameter. A second possible explanation is that the major difference resides in the use of a completely single-stranded plasmid as opposed to duplex (or partially duplex) DNA as the template for *in vivo* bypass. With regard to the oligonucleotide-mediated transformation studies reported here and elsewhere, lesion bypass occurs in the context of chromosomal DNA so that the lesion is expected to interact with the full repertoire of replication complexes. We suggest that the accessory proteins within the replication complex stalled at an AP site interact much more favorably with Rev1 and Rev3 than with Rad30.

This notion is supported by our observations that the ability to bypass F and L were also predominantly dependent upon Rev1 and Rev3 and only marginally on Rad30 (Table 4).

Despite the fact that F is only recognized by AP endonucleases and is not processed by AP lyases *in vitro* (50), the tetrahydrofuran analog has been used as a model AP site for examining the mechanism of lesion bypass in *E. coli* and yeast (27,29,34). With respect to lethality, mutagenicity and interactions with lesion bypass polymerases, F appears to be a good model for an AP site in SOS-induced *E. coli* cells, although it should be noted that dC instead of dA was preferentially incorporated opposite F (27,29). In uninduced *E. coli* cells, nucleotide insertion specificity was similar for both AP sites and F, but the polymerases involved differed. Pol II and pol IV played major roles in the bypass of AP sites, whereas F bypass required the participation of pol V (29).

In yeast, the biological responses toward F and AP sites are clearly different. Despite the observation that AP and F were bypassed at similar frequencies at the *CYC1* locus in an *apn1* mutant, dC was preferentially inserted opposite an AP site, whereas dA was incorporated at a much higher frequency than dC opposite F (34). In our experiments targeting the *lys2ΔA746* allele, Apn1-defective yeast bypassed F almost 10-fold more efficiently than an AP site. Relative to the bypass observed with the *cyc1-31* allele (34), F was bypassed 4-fold more frequently in the *lys2ΔA746* assay, and the AP site 2-fold less frequently. In agreement with the *cyc1-31* data, there was a striking bias for dC to be inserted opposite an AP site during correction of the *lys2ΔA746* allele, but dA and dC were inserted at equal frequencies opposite F (Table 5). These data clearly demonstrate that F is a poor model for an AP site with respect to both bypass efficiency and mutagenic specificity in yeast.

Recent reports have provided significant information regarding enzyme recognition, lethality and mutagenicity of the oxidized L lesions in *E. coli*. Using a plasmid replication assay, bypass of L requires the participation of the lesion bypass polymerases pol II, pol IV and pol V (27–29). Both AP sites and oxidized abasic sites are 100% lethal *in E. coli* lacking all three lesion bypass polymerases (27). In the current study, we demonstrated that, like AP sites, the bypass of L requires Rev1 and Polζ, and that its bypass is mutagenic. It is interesting to note that an L lesion was bypassed ~10-fold more efficiently than AP lesions in *apn1* and *apn1 rad30* mutants. These data suggest that if *apn1* cells are exposed to ionizing radiation or stress conditions that generate significant amounts of reactive oxygen species, a significant percentage of the induced mutations could potentially be the result of bypass at an L rather than at an AP site. The ability of yeast to bypass L clearly required the participation of Rev1 and Rev3, but not Rad30. In summary, the experiments reported here provide additional support for the C-rule of AP bypass in yeast and indicate that tetrahydrofuran (F) is not an appropriate substitute for examining the efficiency and specificity of AP bypass *in vitro*. In addition, these experiments reveal both similarities between the bypass of AP and oxidized abasic sites in terms of the TLS polymerases required, but differences in terms of nucleotide insertion preference. Under conditions where significant amount of L lesions are produced, L may contribute significantly to the overall mutagenesis induced by abasic sites. Finally, it should be noted that the development
of the lys2A7A46 allele as a target for introducing lesion-containing oligonucleotides into a chromosomal context for subsequent bypass will allow a detailed analysis of the influence of flanking sequences on bypass efficiency and specificity.

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