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Difference between deoxyribose- and tetrahydrofuran-type abasic sites in the *in vivo* mutagenic responses in yeast

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ABSTRACT

We have analyzed the mutagenic specificity of an abasic site in DNA using the yeast oligonucleotide transformation assay. Oligonucleotides containing an abasic site or its analog were introduced into B7528 or its derivatives, and nucleotide incorporation opposite abasic sites was analyzed. Cytosine was most frequently incorporated opposite a natural abasic site (O) ('C-rule'), followed by thymine. Deletion of *REV1* decreased the transformation efficiency and the incorporation of cytosine nearly to a background level. In contrast, deletion of *RAD30* did not affect them. We compared the mutagenic specificity with that of a tetrahydrofuran abasic site (F), an abasic analog used widely. Its mutation spectrum was clearly different from that of O. Adenine, not cytosine, was most favorably incorporated. However, deletion of *REV1* decreased the transformation efficiency with F-containing oligonucleotide as in the case of O. These results suggest that the bypass mechanism of F is different from that of O, although the bypasses in both cases are dependent on *REV1*. We also found that the mutagenic specificity of F can be affected by not only the adjacent bases, but also a base located two positions away from F.

INTRODUCTION

Abasic sites are common DNA lesions formed by hydrolysis of N-glycosidic bonds of nucleotides in DNA, which releases the DNA base but leaves the phosphodiester backbone intact. Hydrolysis of the glycosidic bond can occur spontaneously, or enzymatically as an intermediate step in base excision repair (1,2). Abasic sites are known to act as strong blocks to the progression of DNA polymerases *in vitro*, but they can also be highly mutagenic *in vivo* (1,3). For example, abasic sites occur during base excision repair of dU in DNA. To remove dU from DNA, uracil is released by the action of uracil-DNA glycosylase, the product of the *UNG1* gene (4) in yeast. The resulting abasic site is cleaved by an AP-endonuclease, and then removal of the abnormal termini and repair DNA synthesis follow. In yeast, the major AP-endonuclease is the *APN1* gene product (5), but the more recently discovered APN2 AP-endonuclease can also play a role in some cases (6,7). Although abasic sites that escape the action of the AP endonucleases are expected to block DNA polymerases strongly, translesion synthesis (TLS) also occurs. The non-instructive nature of the abasic lesion can make the TLS highly mutagenic. For TLS of an abasic site, Rev1p, the product of the *REV1* gene, can insert dCMP opposite the abasic site (8,9). This terminal dCMP can then be extended by DNA polymerase ζ, composed of the *REV3* and *REV7* gene products (8,9). However, other pathways including other TLS polymerases, such as DNA polymerase η, the *RAD30* gene product, may also contribute to TLS (10). In addition, a role for Rev1p other than its dCMP transferase activity has been suggested during TLS of abasic sites (7,11) and 6-4 photoproducts (11,12).

The mutagenic properties of abasic sites have been investigated under a variety of experimental conditions. In *Escherichia coli*, predominant incorporation of A opposite an abasic site has been known as 'A-rule' (13–21). Recent studies in eukaryotes suggest that the A-rule may not be a common mechanism for preferential incorporations opposite abasic sites. An essentially random incorporation of nucleotides opposite natural abasic sites was observed in COS7 cells (22–24). In another study in COS cells, predominant incorporation of A was observed opposite a tetrahydrofuran-type abasic site (F) (25). In human lymphoblastoid cells, G was incorporated preferentially opposite natural abasic sites (26). In NIH3T3 cell, T, and not A, was mainly incorporated opposite an F with point mutations in the adjacent positions (27).

The previous reports on the mutagenic specificity of abasic sites in budding yeast, *Saccharomyces cerevisiae*, are also inconsistent. C was predominantly incorporated opposite an abasic site using a shuttle vector system (8). In another study with the *SUP4* gene on a plasmid, the frequency of

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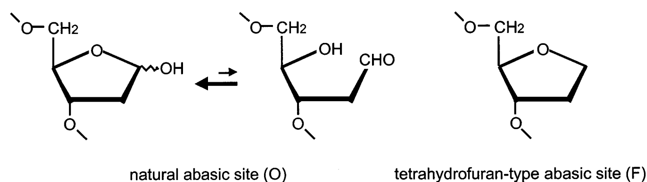

Figure 1. Structures of abasic sites used in this study.

spontaneous A:T → C:G events increased in the *apn1* mutant, deficient in the AP-endonuclease, suggesting the preferential insertion of G opposite spontaneously occurring abasic sites (28). Recently, the preferential incorporation of A was shown from the mutation spectrum in the deletion strain of *APN1* and *APN2* after methyl methansulfonate treatment (7). In two cases it has shown that Rev1p is necessary for the bypass through abasic sites (7,8,11). However the contribution of dCMP transferase activity is not clear.

In this study, we investigated mutagenic specificities of uracil (U), a natural abasic site (O) and an F (Fig. 1) using oligonucleotide transformation method. A *cyc1-31* mutation in a strain, B7528, can be reverted to wild-type phenotype Cyc+ by direct transformation with short oligonucleotides (29). We have applied this method to analyze mutagenic specificity of nucleotide analogs and a 6-4 photoproduct (12,30), using oligonucleotides with these lesions. Mutational specificity of the lesion can be easily determined by DNA sequencing analysis of the transformants under various genetic backgrounds. To improve the efficiency of transformation by F, a disruptant of *APN1* gene coding the major AP-endonuclease which can process F was prepared. Deletion strains of *REV1* and *RAD30* of B7528 were used for the investigation of the contribution of DNA polymerases performing a TLS.

MATERIALS AND METHODS

Media

Escherichia coli strains were grown in LB medium that was supplemented with 100 µg/ml ampicillin when required (31). Yeast strains were grown in YPD medium (1% yeast extract, 2% peptone and 2% glucose), YPG medium (1% yeast extract, 2% peptone and 3% glycerol) or minimal selective medium (32). For solid media, 1.5% agar was added.

Strains

The *E.coli* strain DH5α cells were used to amplify and manipulate all plasmids described in this paper. The *S.cerevisiae* strains used in this study are listed in Table 1. All yeast strains were derived from B7528, which was provided by Dr Fred Sherman of Rochester University. Sequence information was provided by the Stanford Yeast Genomic Database (http://genome-www.stanford.edu/Saccharomyces/). Transformations for gene disruption of yeast were performed by the lithium acetate method (33). Transformants were streaked and purified on appropriate minimal selective medium or YPD medium containing 200 mg/l G418.

A disruptant of *APN1* was constructed by introducing *hisG-URA3-hisG* (34) inserted *APN1* gene provided by Dr B. Demple of Harvard School of Public Health (5). *URA3* marker was subsequently eliminated on 5-fluoro-orotic acid medium. *Rev1* disruptants, *rev1* and *apn1 rev1*, were constructed using *hisG-URA3-hisG* cassette as described (12). The *rad30* disruption construct was generated using PCR-based gene disruption method with the *kanMX* module (35). PCR was performed using two primers, rad30DISLm (5′-TAGCGCAGGCCTGCTCATTTTTGAACGGCTTTGAT-AAAACAAGACAAAGCCGTACGCTGCAGGTCGAC-3′) and rad30DSRm (5′-AGGACGTTTTAGTTGCTGAAG-CCATATAATTGTCTATTTGGAATAGGATCGATGAAT-TCGAGCTCG-3′). The resulting PCR product was introduced into B7528. Disruption was verified by PCR using primer sets VERRAD30L (5′-TAGTCTTCTAGCGCAGGC-3′) and VERRAD30R (5′-ATCGCCTTCAAACTTCAGAG-3′), and VERrad30RK2 (5′-AAACGATCTAATTGATTAAGTCC-3′) and KAN1 (5′-CCTCGACATCTGCCC-3′). The plasmid for *apn2* disruption, pYORCYBLO19w (36), was kindly provided by Dr F. Malagon of Universidad de Sevilla. This plasmid has *apn2::kanMX* disruption. The *apn2::kanMX* fragment was released by digestion with *Hpa*I and *Xho*I and used to transform B7528 and *apn1*. Disruption was confirmed by PCR analysis using primer sets, APN2L (5′-ATGGAGA-AAAAGATGACAGGA-3′) and APN2R (5′-GAGGTTA-CTGACGATGACC-3′), and KAN1 and APN2R.

A deletion strain of *UNG1* was constructed from pMK201 that consists of a 9.1 kb insert containing the *UNG1* gene as described (4) except that *hisG-URA3-hisG* was used as a selection marker (34). The plasmid pMK201 was provided by Dr Kim Gerik of Washington University School of Medicine.

Oligonucleotides

The oligonucleotides used directly for transformation in this study are listed in Table 2. Wild-type oligonucleotide (oligo-W) and 26mer dUMP-containing oligonucleotides (oligo-U) were purchased from Genset (Kyoto, Japan). To produce natural abasic site-containing oligonucleotide (oligo-O), oligo-U was treated with uracil-DNA glycosylase (Gibco BRL, Tokyo, Japan). Oligo-U (500 pmol) was incubated for

Table 1. Strains used in this study

Strain	Genotype	Source
B7528	*MAT*a *cyc1-31 cyc7-67 lys5-10 ura3-52*	Moerschell *et al.* (29)
COY7	*MAT*a *cyc1-31 cyc7-67 lys5-10 ura3-52 apn1::hisG*	This study
COY14	*MAT*a *cyc1-31 cyc7-67 lys5-10 ura3-52 rad30::kanMX*	This study
COY35	*MAT*a *cyc1-31 cyc7-67 lys5-10 ura3-52 rev1::hisG-URA3-hisG*	Otsuka *et al.* (12)
COY43	*MAT*a *cyc1-31 cyc7-67 lys5-10 ura3-52 apn1::hisG rev1::hisG-URA3-hisG*	This study
COY41	*MAT*a *cyc1-31 cyc7-67 lys5-10 ura3-52 apn2::kanMX*	This study

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Table 2. DNA sequences of the *CYC1* target site and a list of 26mer oligonucleotides used for yeast transformation in the present study

CYC1	(Met)Thr -Glu -Phe -Lys -Ala -Gly -Ser -Ala -Lys -Lys -Gly--------
	---ATAATGACTGAATTCAAGGCCGGTTCTGCTAAGAAAGGT------
B7528 (*cyc1-31*)	(Met)Thr -Glu -Stop
	---ATAATGACTGAATA-AAGGCCGGTTCTGCTAAGAAAGGT------
Oligo-W	5′-ATAA^{1}TGACTGAATTC^{12}AAGGCCGGTTC^{23}-3′
Oligo-U	5′-ATAATGACTGAATT**U**AAGGCCGGTTC-3′
Oligo-O	5′-ATAATGACTGAATT**O**AAGGCCGGTTC-3′
Oligo-F	5′-ATAATGACTGAATT**F**AAGGCCGGTTC-3′
Oligo-AF	5′-ATAATGACTGAACA**F**AAGGCCGGTTC-3′
Oligo-CF	5′-ATAATGACTGAACC**F**AAGGCCGGTTC-3′
Oligo-GF	5′-ATAATGACTGAACG**F**AAGGCCGGTTC-3′
Oligo-TF	5′-ATAATGACTGAACT**F**AAGGCCGGTTC-3′

The first and second lines show DNA sequences of wild-type *CYC1* and *cyc1-31* mutant, respectively. Nucleotides in the oligonucleotides are numbered by numbering the A residue in the ATG initiation codon as 1. The position of abasic analog is indicated by a bold letter. U, deoxyuridine; O, natural abasic site; F, tetrahydrofuran.

1 h at 37°C with 1 U of uracil-DNA glycosylase in 20 mM Tris–HCl (pH 8.0), 1 mM EDTA, 0.01 M DTT, 0.1 mg/l BSA. F-containing oligonucleotide (oligo-F) was synthesized using dSpacer amidite monomer purchased from Glen Research Corp. (Sterling, VA, USA).

Oligonucleotide transformation and sequencing analysis of the transformants

The transformation was performed by electroporation method (12). In a typical experiment, 300 pmol of an oligonucleotide was added to 40 µl of competent cells. Colonies of transformants (Cyc^{+} strain) grew out of the uniform lawn of the untransformed cells (Cyc^{-} strain) on YPD plates after 5 days incubation. Transformants that can grow on non-fermentable carbon sources were purified on YPG plates. DNA sequencing analysis of the transformants was made as described previously (12).

RESULTS

Efficiency of transformation with oligonucleotide containing abasic sites

The strain B7528 and its derivatives used in this study have Cyc^{-} phenotype because of the *cyc1-31* mutation, a point mutation composed of one base change and one deletion as shown in Table 2. Oligonucleotides containing the wild-type sequence of *CYC1* (oligo-W) can restore the wild-type reading frame to give Cyc^{+} transformants if the oligonucleotide is integrated into the correct chromosomal position. We have synthesized oligonucleotides containing an abasic site or dU at position 12. These modified oligonucleotides can still restore the wild-type reading frame if a nucleotide is incorporated opposite the modified site by TLS. Incorporation of any nucleotide at this position can restore Cyc^{+} phenotype (12,29,30,37). Assuming that oligonucleotides are incorporated at the same efficiency, the ratio of transformation efficiency with oligonucleotide containing a lesion to that with control oligonucleotide (oligo-W) indicates the frequency of read-through past the lesion that escapes repair (Fig. 2). The nucleotide incorporated opposite the lesion can be determined by DNA sequencing analysis.

B7528 and its derivatives were transformed with oligo-W, oligo-U, oligo-O or oligo-F. Transforming efficiencies are shown in Table 3. The efficiencies of oligonucleotide containing U and O were 6 and 7% of oligo-W in B7528, respectively. The transforming efficiencies of U were similar to those of O. This seems to result from the efficient removal of uracil by cellular uracil-DNA glycosylase as discussed below. In the case of oligo-F, the transforming efficiency in B7528 was 0.33% of oligo-W. The low transforming activities of modified oligonucleotides can be affected by the efficient removal of a lesion by a repair system, which causes the loss of the wild-type reading frame. To improve transformation efficiencies of the modified oligonucleotides, we disrupted either the *APN1* or *APN2* gene, which encode the major and minor AP-endonucleases. Indeed, the transforming efficiencies of oligo-F in *apn1* were clearly increased, but not in the *apn2* disruptant. This suggests that Apn1p, but not Apn2p, would cleave the 5′ end of F effectively *in vivo*.

To investigate contributions of TLS polymerases, *rev1* and *rad30* disruptants were used. The deletion of *REV1* in B7528 reduces the transforming efficiency with oligonucleotides containing abasic analogs. This effect of *rev1* deletion is also seen in the comparison of *apn1* and *apn1 rev1* strains. These results indicate that *REV1* contributes to the bypass of O and F abasic sites. In contrast, the efficiencies of oligo-U and oligo-O in *rad30* deletion strain were almost the same as that in B7528.

Nucleotide incorporation frequencies opposite O

The DNA sequence of the region where the oligonucleotides were introduced was determined. Nucleotides incorporated opposite the position occupied by U and O in the transforming oligonucleotides are shown in Table 4. C was most frequently incorporated in B7528 and an *apn1* mutant, and then A and T were incorporated at a much lower level. The same mutagenic specificity was observed in a *rad30* strain. On the other hand, C was only poorly incorporated in *rev1* and *apn1 rev1* mutants. These results suggest that the deoxycytidyl transferase activity of Rev1p inserts C opposite O. G was most frequently incorporated for U and O in *rev1* defective mutants. The yeast Polη, a *RAD30* product, efficiently inserts G opposite a natural abasic site *in vitro* (38). Incorporation of G

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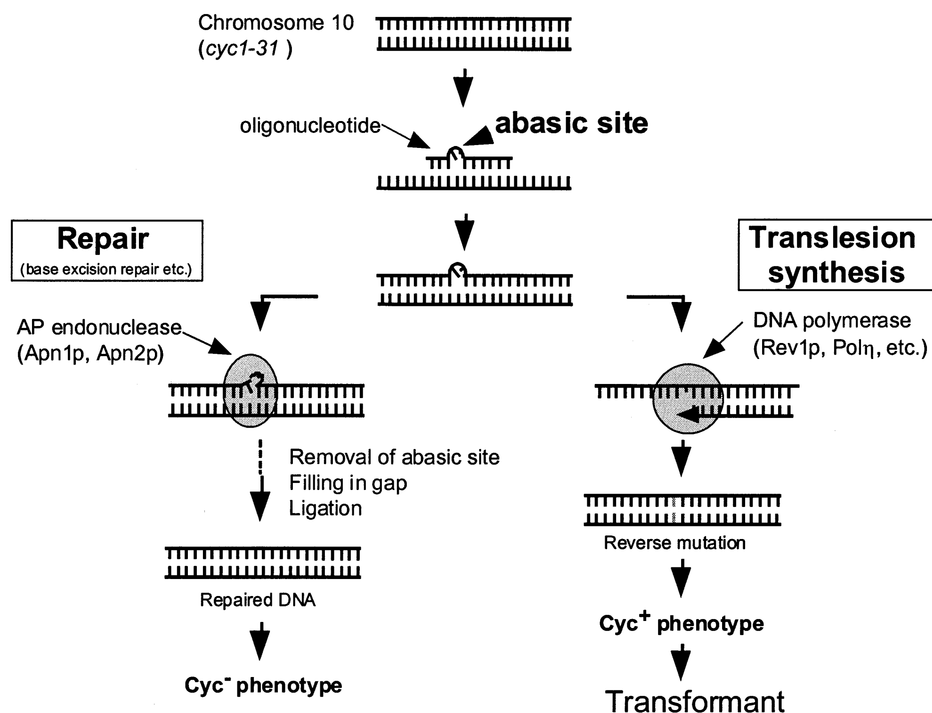

Figure 2. A putative mechanism of transformation with an oligonucleotide containing an abasic site. The *S.cerevisiae CYC1* system was used for analyzing mutagenic specificity of abasic sites. The *cyc1-31* mutant does not produce active iso-1-cytochrome *c* because of the frameshift/nonsense mutation on *CYC1* gene. The mutant gene can be altered by transformation with synthetic oligonucleotides containing an abasic analog if a nucleotide is incorporated opposite the lesion by TLS.

Table 3. Transforming activities of oligonucleotides containing abasic analogs in B7528 and its derivatives

Relevant genotype	Oligonucleotide	Transformation efficiency Transformants/μg	% of control
Wild-type (B7528)	Oligo-W	1599 ± 1172	100
	Oligo-U	88 ± 48	6.0 ± 1.8
	Oligo-O	117 ± 68	7.2 ± 1.1
	Oligo-F	3.9 ± 2.1	0.33 ± 0.20
	No oligo	0.3 ± 0.8	0.04 ± 0.09
apn1	Oligo-W	3164 ± 1292	100
	Oligo-U	313 ± 133	8.1 ± 2.2
	Oligo-O	339 ± 139	8.6 ± 2.4
	Oligo-F	217 ± 190	6.2 ± 4.9
	No oligo	1.3 ± 1.6	0.2 ± 0.3
rad30	Oligo-W	1510 ± 183	100
	Oligo-U	110 ± 17	7.3 ± 1.0
	Oligo-O[a]	105	7.4
	Oligo-F	4.3 ± 1.8	0.28 ± 0.10
	No oligo	0	0
rev1	Oligo-W	581 ± 375	100
	Oligo-U	1.7 ± 1.7	0.28 ± 0.31
	Oligo-O	3.4 ± 2.9	0.88 ± 1.2
	Oligo-F	0.20 ± 0.30	0.02 ± 0.02
	No oligo	0	0
apn1 rev1	Oligo-W	2579 ± 569	100
	Oligo-U	4.0 ± 1.4	0.14 ± 0.05
	Oligo-O	4.5 ± 3.1	0.18 ± 0.13
	Oligo-F	2.6 ± 2.5	0.13 ± 0.14
	No oligo	0	0
apn2	Oligo-W	1348 ± 872	100
	Oligo-U	91 ± 58	8.3 ± 3.1
	Oligo-O[a]	113	17.4
	Oligo-F	3.7 ± 2.9	0.30 ± 0.23
	No oligo	0.1 ± 0.2	0.06 ± 0.13

Efficiencies shown are the average of three to six independent experiments unless otherwise indicated.
[a]The average of two independent experiments.

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Table 4. Efficiencies of nucleotide incorporations opposite the lesions

Oligonucleotide	Strain	Base-specific read-through % (*N*)[a]				
		A	C	G	T	Other mutations
Oligo-U	B7528	1.2 (4)	3.0 (10)	0.6 (2)	1.2 (4)	0.0 (0)
	apn1	1.7 (6)	4.9 (17)	0.0 (0)	1.5 (5)	0.0 (0)
	rad30	0.0 (0)	6.5 (16)	0.4 (1)	0.4 (1)	0.0 (0)
	rev1	0.0 (1)	0.0 (0)	0.3 (19)	0.0 (0)	0.0 (0)
	apn1 rev1	0.1 (7)	0.0 (0)	0.1 (8)	0.0 (0)	0.0 (0)
	apn2	0.0 (0)	6.1 (17)	0.7 (2)	0.7 (2)	0.7 (2)
Oligo-O	B7528	0.3 (1)	4.5 (13)	0.3 (1)	2.1 (6)	0.0 (0)
	apn1	0.4 (1)	5.4 (15)	0.4 (1)	2.2 (6)	0.4 (1)
	rad30	0.4 (1)	5.0 (13)	0.0 (0)	1.9 (5)	0.0 (0)
	rev1	0.1 (3)	0.4 (12)	0.4 (13)	0.0 (1)	0.0 (0)
	apn1 rev1	0.0 (1)	0.0 (0)	0.2 (14)	0.0 (0)	0.0 (0)
	apn2	1.2 (1)	6.3 (5)	2.4 (2)	6.3 (5)	1.2 (1)
Oligo-F	B7528	0.1 (11)	0.1 (6)	0.0 (0)	0.1 (6)	0.1 (6)
	apn1	2.9 (12)	1.2 (5)	1.9 (8)	0.0 (0)	0.2 (1)
	rad30	0.1 (7)	0.1 (4)	0.0 (0)	0.0 (1)	0.1 (6)
	rev1	0.0 (1)	0.0 (0)	0.0 (1)	0.0 (0)	0.0 (2)
	apn1 rev1	0.1 (18)	0.0 (2)	0.0 (1)	0.0 (0)	0.0 (0)
	apn2	0.1 (8)	0.0 (3)	0.0 (2)	0.0 (1)	0.1 (6)

[a]Base-specific read-through (%), *E*, indicates how frequently each base was incorporated opposite lesions. *N* is the number of transformants in which each base was incorporated opposite the lesion. $E = N / N_T \times E_T$ where N_T is the number of total transformants examined for each lesion in each strain, and E_T is total read-through (%) for each lesion in each strain shown in Table 2. For example, B7526 strain cells were transformed with Oligo-U, and DNA sequence opposite the position occupied by U in the oligonucleotide was determined for 20 transformants (N_T). Among them, four had A opposite the position of U. This number was normalized by total transformants examined (N_T) to give 0.2. The base-specific read-through efficiency (*E*), 1.2%, was obtained by multiplying the value by total read-through efficiency for B7528 transformed with Oligo-U (E_T), 6% (Table 2). All other values were obtained in a similar way using *N* and transforming efficiencies shown in Table 2.

may indicate the contribution of Polη to bypass O in the absence of Rev1p. The transforming efficiencies and the mutation spectra of U were similar to those of O. This shows that U on the introduced oligonucleotide is almost completely removed in the yeast cell to create O. We confirmed that this is due to the action of uracil-DNA glycosylase coded by the *UNG1* gene. Actually, the transformation efficiencies of a *UNG1*-deleted derivative of B7528 with oligo-U were as high as 80% of those with oligo-W oligonucleotide. A was incorporated opposite the position occupied by U in all nine transformants obtained from the *ung1* disruptant.

Nucleotide incorporation frequencies opposite F

Efficient read-through opposite F only takes place in an *apn1* strain (Table 3 and 4). A was preferentially incorporated opposite F in oligo-F. G was the next, then C. The spectrum was clearly different from that of O in each strain. Deficiency of Rev1p suppressed the incorporation of all of A, G and C in an *apn1 rev1* strain. This suggests a function of Rev1p other than deoxycytidyl transferase. However, the transferase may still be active, because the ratio of incorporation of C to A was lower in *apn1 rev1* than in *apn1*. It is also notable that the preferential incorporation of A was clearly observed in *APN1* proficient strains, B7528, *rad30* and *apn2*, although the efficiencies were very low.

Effect of adjacent bases on mutagenicity of F

Bypass efficiency of F by human Polκ and human Polι is influenced by the sequence context 5′ to the abasic site *in vitro* (39–41). We used four kinds of oligonucleotides (oligo-AF, oligo-CF, oligo-GF, oligo-TF) in order to examine whether the sequence context influences bypass of an abasic site in a yeast cell. The transformation efficiency of each oligonucleotide in *apn1* strain was 150–330 transformants/μg of oligonucleotide, similar to that of oligo-F. A was most favorably incorporated opposite F in oligo-AF, oligo-CF and oligo-GF, as in oligo-F (Table 5), but the efficiencies were different. C was most frequently incorporated when oligo-TF was introduced. The mutagenic specificity was influenced by a nucleotide 5′ of F. This is consistent with the previous report (40). The mutagenic specificity of F in oligo-TF is different from that in Oligo-F. Nucleotides 5′ and 3′ of F are the same in the two oligonucleotides, and only the nucleotides 2 nt away from the lesion differ, namely C in oligo-TF and T in oligo-F.

DISCUSSION

In the present study, we determined the *in vivo* mutagenic specificity of three types of DNA lesions in yeast: U, removal of which forms O inside cells, O and F. Surprisingly the mutagenic specificities of F were different from U or O in yeast cells, while U and O are almost the same in their mutagenic properties. C was most frequently incorporated opposite O or U. On the other hand, A was favorably incorporated opposite F, although read-through of both abasic sites required Rev1p function.

F has often been used as a structural analog of the cyclic hemiacetal form of O for the mutagenic studies of abasic sites (7,19,21,25,27). We compared F with O by side-by-side experiments using the *in vivo* assay system, and the results showed clear differences in the mutagenic specificity of two abasic sites. Shibutani *et al.* (21) reported the template activity of O, F and a deoxyribitol (a reduced form of O) in primer extension with Klenow fragment of the *E.coli* DNA polymerase I or calf thymus DNA polymerase α. The frequency of nucleotide insertion opposite all the three types of abasic site was in the order of A > G > C > T. The frequency of TLS past abasic sites was highest in F, O and then

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Table 5. Nucleotide incorporations opposite an F adjacent to a different nucleotide in *apn1* mutant strain

Oligonucleotide	No. of transformants with the nucleotide opposite F (%)				
	A	C	G	T	Other mutations
Oligo-AF	15 (68)	6 (27)	0 (0)	0 (0)	1 (4.5)
Oligo-CF	13 (59)	8 (36)	1 (4.5)	0 (0)	0 (0)
Oligo-GF	11 (52)	8 (38)	1 (4.8)	0 (0)	1 (4.8)
Oligo-TF	8 (36)	11 (50)	2 (9.1)	1 (4.5)	0 (0)
Oligo-F	12 (46)	5 (19)	8 (31)	0 (0)	1 (3.8)

deoxyribitol. The results indicate that O and F are similar in miscoding specificity, but different in the ability to block DNA synthesis. If this is also the case in the yeast replication machinery, the difference in the mutational specificity may indicate differences in the DNA polymerases involved. Because F blocks replication less than O, it might be possible that the replicative polymerase can more frequently continue polymerization with the aid of Rev1p as described below. A recent report by Avkin *et al.* (42) is interesting from this view. They found that a replicative polymerase is involved in translesion of F in human cells and inserts A opposite F.

Another interesting difference between F, and O or U is the effect of *APN1* endonuclease on the transforming activity. The transforming efficiencies of oligo-O and -U in the *apn1* strain increased by only 1.2 and 1.4-fold compared with those in the wild-type, respectively, while those of oligo-F increased by 19-fold. This might be related to the fact that a natural abasic site can be repaired by not only AP endonucleases, but also AP lyases, which cannot act on F. However, the true mechanism is unknown.

We tested whether TLS polymerases, Polη and Rev1p, were required for replication past O and F. The read-through of O and F definitely depended on Rev1p, even their mutagenic properties were different from each other. Rev1p possesses a deoxycytidyl transferase activity to insert C opposite O (9) and F (7). The spectrum of nucleotide incorporation opposite F showed predominant incorporation of A in all tested strains. These results suggest that Rev1p function, other than its deoxycytidyl transferase activity, may be required for TLS past F. In other words, Rev1p has a second function as proposed before (7,11). The Rev1p second function was also observed in the mutagenesis by 6-4 photoproducts (11,12). In addition to the incorporation of C and A, we also found a significant incorporation of T opposite O. This incorporation was not observed in *rev1* deletion mutants. This incorporation may again relate to Rev1p function. A further experiment using *rev1* mutants lacking deoxycytidyl transferase activity is required for the clear demonstration of this role.

Deletion of *RAD30* did not influence the efficiency of transformation and the mutagenic spectrum in any of U, O or F. Contribution of Polη on the bypass of the abasic sites may be low in yeast, although the polymerase may play some role which may become apparent in the bypass in the absence of Rev1p. This result agrees with the *in vitro* study on Polη by Haracska *et al* (10). These results indicate that a replicative polymerase interacting with Rev1p may insert A opposite O and F.

The experiment comparing oligonucleotides that differ in a base 5′ of F gave an unexpected result (Table 5). Surprisingly, oligo-TF and oligo-F showed clearly different mutagenic specificity. This is, to our knowledge, the first report that a base not adjacent to the lesion can affect its mutagenic property.

There are discrepancies in the reported mutagenic specificity of abasic sites in yeast. Favorable incorporation of C opposite O is found using a shuttle vector containing O (8), whereas two other reports indicated different specificity for abasic sites produced in genomic DNA intracellularly. An incorporation of G (28) and A (7) opposite abasic sites was reported. It is supposed that the inconsistency of mutagenic specificity of abasic sites might depend on whether the lesion is on a plasmid or in the genome (7). However, our results indicate that C is inserted most frequently opposite O (62% of the transformants with oligo-O), even when the translesion took place in the genome. Another possibility is that the mutagenic specificity of an abasic site already present in the DNA before transformation is different from that produced inside cells due to the interaction with the glycosylases. Our present results appear to be against this as far as uracil-DNA glycosylase is involved, because O and U showed similar mutagenic specificity.

We can find some differences between O and U, although they show similar mutagenic potential. The incorporation of A opposite the lesion in oligo-U in B7528 and *apn1* was higher than in oligo-O. This might be due to the U escaped from the action of uracil-DNA glycosylase, but it is difficult to explain the absence of the incorporation of A opposite the lesion in oligo-U in other strains. A low-level incorporation of C in *rev1* strains was observed for oligo-O, but not for oligo-U. Other glycosylases or base excision repair systems might interact with TLS systems more strongly than uracil-DNA glycosylase. Another difference between U and O was found in the *rev1* strain. O still produced many C insertions, whereas U did not (Table 4). Table 4 also shows the clear difference between F and O (U) in the *apn1 rev1* strain. However, further study is required to confirm these differences under very low transforming activities.

In conclusion, our data indicate that a very small structural difference in the DNA lesions or their environment can influence the mutation spectrum greatly. The specificity can be changed by the presence (O) or absence (F) of an OH residue at position –1 of deoxyribose in abasic sites, or a base 2 nt away from the lesion.

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