DNA Sequence Analysis of Spontaneous Mutagenesis in *Saccharomyces cerevisiae*

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ABSTRACT

To help elucidate the mechanisms involved in spontaneous mutagenesis, DNA sequencing has been applied to characterize the types of mutation whose rates are increased or decreased in mutator or antimutator strains, respectively. Increased spontaneous mutation rates point to malfunctions in genes that normally act to reduce spontaneous mutation, whereas decreased rates are associated with defects in genes whose products are necessary for spontaneous mutagenesis. In this article, we survey and discuss the mutational specificities conferred by mutator and antimutator genes in the budding yeast *Saccharomyces cerevisiae*. The implications of selected aspects of the data are considered with respect to the mechanisms of spontaneous mutagenesis.

SPONTANEOUS mutations play a fundamental role in evolution and have been implicated in aging, carcinogenesis, and human genetic disease (Harmon 1981; Kirkwood 1989; Cooper and Krawczak 1990; Arber 1991; Drake 1991a; Loeb 1991, 1994; Wintersberger 1991; Caskey *et al.* 1992; Strauss 1992). They are thought to originate as a consequence of intracellular events, including the formation of DNA lesions, the occurrence of DNA synthesis errors during replication, repair and recombination, and the movement of transposable elements (Sargentini and Smith 1985; Smith and Sargentini 1985; Ramel 1989; Loeb and Cheng 1990; Drake 1991b; Kunkel 1992; Smith 1992; Amariglio and Rechavi 1993; Ames *et al.* 1993; Lindahl 1993).

DNA lesions can arise naturally through intracellular metabolism or the intrinsic instability of DNA. For example, there is evidence consistent with spontaneous alkylation, deamination, and loss of DNA bases, as well as their modification by reactive oxygen species (Loeb and Preston 1986; Ames *et al.* 1993; Lindahl 1993; Friedberg *et al.* 1995). Because a substantial fraction of endogenous DNA damage may involve the alteration or loss of nucleotide bases, many spontaneous mutations may be generated during replication past miscoding or noninstructional lesions. The accuracy of DNA synthesis on undamaged templates is attributed in large measure to DNA polymerase (pol)-mediated nucleotide selection and proofreading of replication errors, as well as to postreplicative mismatch correction of errors that escape proofreading (Loeb and Cheng 1990; Kunkel 1992; Goodman *et al.* 1993; Modrich 1994). The topography of the DNA molecule itself also is an important factor, however, and roles have been proposed for DNA secondary structures and misaligned template primers in the production of single and multiple base pair alterations (Ripley and Glickman 1983; Kunkel 1990). Insertions of transposable elements generally constitute larger sequence changes in DNA. Although transposon movement can be a relatively infrequent event, transposition rates may be increased by the presence of DNA damage, including that which occurs spontaneously (Bradshaw and McEntee 1989; Kunz *et al.* 1990, 1994a).

Two general strategies have been used in attempts to better understand the mechanisms responsible for spontaneous mutagenesis. The first is to characterize strains that have enhanced spontaneous mutation rates. The rationale for this route is that mutator phenotypes are expected to result from defects in genes whose products act to minimize genetic instability. Indeed, as expected, such studies have revealed that spontaneous mutations can arise through failure of DNA repair or processes that maintain the accuracy of DNA replication (Haynes and Kunz 1981; Sargentini and Smith 1985; Kramer *et al.* 1989a; Rebeck and Samson 1991; Michaels and Miller 1992; Smith 1992; Xiao and Samson 1993; Kunz *et al.* 1994a and references therein). The second approach is to isolate antimutator mutants with the aim to identify genes whose functions are required for spontaneous mutagenesis. A number of these mutants have been recovered, mainly in prokaryotic systems, and where characterized, they have been found to have alterations primarily in genes that encode DNA polymerases (Sargentini and Smith 1985; Morrison *et al.* 1989; Smith 1992; Drake 1993 and references therein; Fijalkowska *et al.* 1993).

Early studies of spontaneous mutagenesis in mutator and antimutator strains relied on genetic analysis of reversion, suppression, or forward mutation (for reviews, see Lawrence 1982; Sargentini and Smith 1985; Friedberg *et al.* 1995). Because of technological restrictions, the systems used did not identify the DNA sequence alterations involved, or they were unable to

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detect all types of mutation that occurred. These limitations were overcome by applying DNA sequencing techniques to examine forward mutations in prokaryotic and then eukaryotic organisms (Friedberg *et al.* 1995). The resulting data on the types of DNA sequence changes that occur, the rates at which they arise, their site specificity, and how these parameters are influenced by mutator and antimutator alleles have yielded insights into processes that act to maintain genetic stability and ensure accurate transmission of the hereditary material.

In this article, we review the modulation of spontaneous mutagenesis in mutator and antimutator strains of the budding yeast *Saccharomyces cerevisiae*, as revealed by DNA sequence analysis. First, we discuss briefly the selected aspects of systems used in the analysis of mutagenesis. Next, sequence changes arising spontaneously in a wild-type background are considered to establish the types of mutations that do occur and provide a basis for comparison. We then survey the effects of eliminating genes whose inactivation leads to a mutator or antimutator phenotype on the magnitude and specificity of spontaneous mutagenesis. Finally, we focus on a select number of findings with implications for the mechanisms of spontaneous mutagenesis in eukaryotic cells.

Assay systems: *In vivo* mutagenesis assay systems can be based on the characterization of forward or reverse mutations on a target gene (assay gene). Sequence analyses using forward systems have been reported for the *CAN1*, *LYS2*, *SUP4*-o, and *URA3* genes. Commonly used reversion systems are based on *ade2*, *his7*, *hom3*, or *lys2* alleles. Mutant strains are usually constructed by deleting part or all of the gene, disrupting the gene sequence by inserting additional DNA into the gene, or by introducing point mutations into the gene. Deletion of a significant portion or all of the gene is the preferable method because this usually results in complete loss of the gene's function. In contrast, disruptions and point mutations may result in partial retention or alteration of gene function, and different point mutations especially may produce different phenotypic effects. Assay genes can be located on a chromosome or a centromeric plasmid. This type of plasmid mimics chromosome behavior with respect to replication during S phase, copy number (predominantly single copy), and replicative stability (Campbell and Newlon 1991). Potential differences in chromatin structure and overall size, however, may influence metabolic processes that involve the target gene. This should be considered when interpreting data for plasmid-borne genes.

Forward systems are preferable in the study of mutagenesis because they can detect a wide variety of alterations at many sites within a gene. In contrast, reversion systems only detect specific changes and cannot be used to obtain mutational spectra or to map distributions of mutations. The advantage of a reversion system is that once the relevant change is known, sequencing is not usually required, thereby allowing rapid screening of a large number of samples. In this case, however, genotypic and phenotypic reversion to wild type are assumed to involve the same sequence change, but this may not necessarily be so. For example, intragenic suppression may be indistinguishable phenotypically from locus reversion. Reversion systems will not be considered further in this review.

Mutational spectra derived from different assay systems should be compared with caution because of the inherent characteristics of the systems. Ideally an assay system should involve a target gene large enough to allow all possible types of mutation to occur and be recovered. While a larger target gene should be more representative of the genome, practical difficulties in sequencing could negate this advantage. Multiple sequencing sessions may be required to identify mutations in a large target. Even if a mutation is identified near the sequencing primer, the entire target has to be sequenced to verify that there are no additional alterations. Another desirable feature is a wide variety of sequence contexts, which helps maximize detection of sequence-specific effects. The proportion of intronic sequences in the assay system, however, should be low because many intronic mutations may not produce phenotypic changes. If nondetectable mutations are not randomly distributed with respect to class or location throughout the target gene, then mutational specificity will be biased and the mutational data will not be representative of the true spectrum. Thus, if possible, the ability of the system to detect all possible substitutions at all positions within the target should be characterized. If this requirement is not met, the interpretation of the spectral data should acknowledge this limitation. Irrespective of the size or sequence content of the assay target, a statistically significant number of mutants must be examined to construct a meaningful spectrum. Small data sets increase the probability that spectral features are caused by chance.

Spontaneous DNA sequence alterations: To date, mutations that occur spontaneously in wild-type cells have been characterized using the yeast centromere plasmid-borne *SUP4*-o allele, the *URA3* gene integrated at the chromosomal *HIS3* locus, and the endogenous *CAN1* gene (Giroux *et al.* 1988; Lee *et al.* 1988; Kunz *et al.* 1990; Kang *et al.* 1992; Roche *et al.* 1994; Tishkoff *et al.* 1997). The endogenous *LYS2* and *URA3* genes also have been used, but only to study insertions of the yeast transposable element Ty (Natsoulis *et al.* 1989). The most extensive analysis has involved *SUP4*-o. In total, eight different classes of mutation were detected at this locus (Table 1), nine if the double base pair substitution category is expanded to show individual tandem and nontandem double changes, and 13 if the complex changes are further subdivided. Single base pair substitution and deletion, multiple base pair deletion, duplication, and more complex replacements also were found at *URA3*, and single base pair substitution, dele-

tion and insertion, and complex changes were detected at *CAN1*. Despite the large difference in the numbers of mutations analyzed (*SUP4*-o: 582; *URA3*: 70; *CAN1*: 20), the single base pair substitutions and deletions constituted roughly similar fractions of the total events characterized and occurred at roughly comparable rates for the three genes. Although this points to common mutational mechanisms for genes on yeast centromere plasmids and chromosomes, the very small number of *ura3* and *can1* mutations analyzed mandates caution when making comparisons. In addition, the spontaneous mutation rate per base pair for *SUP4*-o is an order of magnitude higher than expected on the basis of comparison to mutation rates for other microorganisms, perhaps because *SUP4*-o encodes a tRNA or resides on a plasmid (Drake 1991a).

Spontaneous single base pair substitutions have been recovered at 65 of the 66 exon sites, and one of the two intron positions where such changes can be detected in *SUP4*-o with the genetic screen used. However, only 75% of the substitutions detectable in the exons (129 out of 174) and intron (three out of four) of the gene have been found to occur spontaneously so far, and some positions are mutated much more frequently than others. Both types of transition and all four transversions have been identified in *SUP4*-o (Table 2), but there is a slight excess of transversions (transversion:transition ratio = 1.44:1). Furthermore, events at G·C pairs outnumber those at A·T pairs by nearly 4:1 (367:97), a ratio that is almost threefold greater than that expected (1.5:1) on the basis of random occurrence of mutations at the G·C (41) and A·T (28) sites that can be detectably mutated. Collectively, the data suggest that the occurrence and repair/correction/editing of spontaneous DNA damage or replication errors in yeast is influenced by sequence context. In addition to single events, a tandem double and a tandem triple substitution, as well as two nontandem double substitutions, have been found in *SUP4*-o.

Some differences are apparent for substitution mutagenesis at the *URA3* and *CAN1* genes. The relative proportions of A·T → G·C transitions and A·T → T·A transversions at *URA3* differ, and transversions outnumbered transitions 3.3:1 compared with *SUP4*-o, but there were also fourfold more substitutions at G·C pairs, as observed for *SUP4*-o. No substitutions were detected at A·T pairs in *CAN1*, and the fractions of the three substitutions at G·C pairs differ from the corresponding values for *SUP4*-o, although the transversion:transition ratios (1.6) are similar for *SUP4*-o and *CAN1*. These differences may reflect experimental dissimilarities, the very small numbers of mutations analyzed for *URA3* and *CAN1*, or limitations caused by the redundancy of the genetic code on the types of substitutions that can be selected at individual sites in a protein-encoding gene.

Single and multiple base pair deletions (the latter ranging from eight to 807 base pairs in length) or insertions account for 18% of the *SUP4*-o mutations in the wild-type background. The majority (44 out of 49) of single base pair losses occurred in runs of two, three, or five base pairs with many of the deletions (40), as well as all (five) of the single base pair additions, taking place in a tract of five G·C pairs. The seven deletions and insertions at *URA3* and *CAN1* also occurred mainly in base pair runs. This pattern is consistent with a mutational mechanism involving misalignment of template and primer strands during replication through runs of repeated base pairs (Streisinger *et al.* 1966). The much greater number of deletions than insertions within the five-base pair run in *SUP4*-o implies either that the template strand "slips" and is stabilized more frequently than the primer strand or that the mutational intermediate resulting from primer strand slippage is corrected more efficiently. A number of the multiple base pair deletions retained single copies of short (two to six base pairs), repeated sequences originally present at the deletion termini and so may have been generated by nonhomologous recombination occurring between the repeats. All of the multiple base pair insertions involved integration of the yeast retrotransposon Ty. In at least one instance, the insertion may have been the remnant of a Ty element that excised from *SUP4*-o by homologous recombination between its flanking delta sequences. Interestingly, most (34 out of 39) of the Ty elements inserted between positions 37 and 38 of the tDNA sequence. The reason for this hot spot is not known, but it may depend in part on functional *RAD6* and *REV3* genes because deletion of either gene reduces Ty insertions at the hotspot by about one-third (Kang *et al.* 1992; Roche *et al.* 1994). The tendency for Ty elements to insert near tRNA genes (Boeke and Sandmeyer 1991) may also be involved, but it is unlikely to account for the hotspot by itself.

A duplication of seven base pairs and several more complex events were also detected. The complex events include a mutant with nontandem deletions of one and 32 base pairs, two mutants each having a single base pair substitution adjacent to a single base pair deletion, and five mutants having the sequence 5′-GATCTCA-3′ replaced with 5′-CCGGG-3′. The latter change was most likely caused by processing of a DNA secondary structure that was formed by pairing within a quasipalindromic sequence (Kohalmi *et al.* 1991). This possibility is strongly supported by finding in a separate study (Kohalmi *et al.* 1991) the other sequence replacement (5′-CCCGG-3′ converted to 5′-TGAGATC-3′) that could be templated by the same secondary structure.

The available data provide evidence that a large variety of DNA sequence alterations can occur spontaneously in yeast, pointing to the complexity of spontaneous mutagenesis in this organism. They also support hypotheses that implicate roles for DNA sequence–directed events in the generation of spontaneous mutations in eukaryotic cells.

TABLE 1

Sequence alterations identified in mutator and antimutator strains

Gene inactivated	Inactivation method[a]	Mutational target[b]	Number analyzed[c]	Rate $\times 10^{-7}$ (fold increase)[d]	Base pair substitution		Deletion		Insertion		Duplication	Complex change	Reference
					1 bp	2 bp	1 bp	>1 bp	1 bp	Ty			
Wild type													
		URA3	34	NR (NA)	88		6	3			3		Lee *et al.* 1988
		URA3	36	1.5 (NA)	83		8	3				6	von Borstel *et al.* 1993
		SUP4-o	354	2.8 (NA)	82	0.6	7	2	0.3	7	0.3	0.8	Kang *et al.* 1992
		SUP4-o	228	7.1 (NA)	76.3	0.9	11	2.2	1.7	5.7		2.2	Roche *et al.* 1992
		SUP4-o-Y	199	1.99 (NA)	87.5		7.5	0.5	2.5	1.5	0.5		Ramachandran 1996
		SUP4-o-I	191	1.91 (NA)	84.3		8.9	1.6	3.7		0.5	1	Ramachandran 1996
		CAN1(20°)	151 (Ty)	NR (NA)						13.9			Wilke *et al.* 1989
		URA3	83 (Ty)	NR (NA)						96			Natsoulis *et al.* 1989
		LYS2	59 (Ty)	NR (NA)						49			Natsoulis *et al.* 1989
		CAN1	24	2.8 (NA)	65		15		10			10	Tishkoff *et al.* 1997
Proofreading/polymerase													
POL3	PM	*SUP4*-o-Y	204	63.5 (32)	77		13.7		8.8			0.5	Ramachandran 1996
POL3	PM	*SUP4*-o-I	199	213.2 (112)	85.9		11.1		3				Ramachandran 1996
POL3	PM	*URA3* LR	31	24 (130)	64.5		22.6		12.9				Morrison and Sugino 1994
POL3	PM	*URA3* RL	36	20 (NR)	58.3		36.1		5.6				Morrison and Sugino 1994
POL2	PM	*URA3* LR	33	2.2 (12)	90.9		6.1		3				Morrison and Sugino 1994
POL2	PM	*URA3* RL	23	1.4 (NR)	87				13				Morrison and Sugino 1994
MUT7	PM	*URA3* (30°)	54	5.8 (3.9)	63		7.4	18.5	3.7			7.4	von Borstel *et al.* 1993
MUT7	PM	*URA3* (32°)	22	NR	68.2		13.6	18.2					von Borstel *et al.* 1993
MUT7	PM	*URA3* (34°)	26	NR	84.7		7.7	3.8				3.8	von Borstel *et al.* 1993
Mismatch correction													
PMS1	Δ	*SUP4*-o-Y	212	21.1 (10.6)	58.5		26.9		14.6				Ramachandran 1996
PMS1	Δ	*SUP4*-o-I	213	22.7 (11.9)	62.4		24.9		12.7				Ramachandran 1996
PMS1	Δ	*SUP4*-o	210	19.2 (6.9)	60		34.7	0.5	4.3			0.5	Yang 1995
MLH1	Δ	*SUP4*-o	67	12 (5)	43.2		49.3		7.5				B. A. Kunz (unpublished data)
MSH2	Δ 93%	*SUP4*-o	213	17.3 (6.2)	63.3		33.4		2.3	1			Yang 1995
MSH2	Δ	*CAN1*	20	40.0 (40)	15		80		5				Marsischky *et al.* 1996
MSH2	Δ	*CAN1*	20	29 (10)	15		70		15				Tishkoff *et al.* 1997
MSH3 MSH6	Δ	*CAN1*	22	36.5 (37)	31.9		63.6		4.5				Marsischky *et al.* 1996
MSH6	Δ 2.3kb	*CAN1*	21	18 (18)	86		14						Marsischky *et al.* 1996

(*continued*)

TABLE 1

Continued

Gene inactivated	Inactivation method[a]	Mutational target[b]	Number analyzed[c]	Rate $\times 10^{-7}$ (fold increase)[d]	Base pair substitution		Deletion		Insertion		Duplication	Complex change	Reference
					1 bp	2 bp	1 bp	>1 bp	1 bp	Ty			
Nucleotide excision repair													
RAD1	Δ 70%	*SUP4*-o	249	28.3 (4)	85.5	0.4	6.4	0.8		6.9			Kunz *et al.* 1990
RAD3-1	PM	*SUP4*-o	225	15.5 (3.2)	85.3	0.4	9.9	0.9	1.8	1.3		0.4	Yang *et al.* 1996
RAD3-102	PM	*SUP4*-o	101	42.9 (23)	82.2		8.9	3.0	3.9			2	Montelone *et al.* 1992
REV3 RAD1	Δ 70%	*SUP4*-o	202	6.24 (0.9)	72.8		9.4		2.5	12.8		2.5	Roche *et al.* 1994
RAD6-dependent repair													
RAD6	Δ 0.6 kb	*SUP4*-o	202	23.2 (4.9)	70.3		2	0.5		26.2	0.5	0.5	Kang *et al.* 1992
RAD6	Δ	*CAN1*	30 (Ty)	7.8 (NR)						53			Picologlou *et al.* 1990
RAD6	NR	*CAN1, URA3*	40 (Ty)	NR (NR)						26			Picologlou *et al.* 1990
RAD6	Δ	*CAN1*	60 (Ty)	NR (NR)						88			Liebman and Newman 1993
RAD18	Δ 1.6 kb	*SUP4*-o	212	14.3 (3)	93.4		1.9	0.9		2.8	0.5	0.5	Kunz *et al.* 1991
RAD27	Δ	*CAN1*	22	180 (63)		18.2		4.5			68.2	9.1	Tishkoff *et al.* 1997
REV3	Δ	*SUP4*-o	201	2.9 (0.4)	73.6		11.4	1	2.5	9		2.5	Roche *et al.* 1994
REV3 RAD6	Δ	*SUP4*-o	208	11.1 (1.6)	71.6		11.5			15.9		1	Roche *et al.* 1995a
REV3 RAD18	Δ	*SUP4*-o	209	5.7 (0.8)	91		6.6	0.5	0.9	0.5		0.5	Roche *et al.* 1995a
Recombinational repair													
RAD52	*TRP1*	*SUP4*-o	237	14.8 (2.1)	95.4	0.8	3.4		0.4				Kunz *et al.* 1989
REV3 RAD52	Δ, *TRP1*	*SUP4*-o	191	2.9 (0.4)	86.4		4.2	0.5	5.8	0.5		2.6	Roche *et al.* 1995a
Base excision repair													
APN1	Δ 724 bp	*SUP4*-o	199	21.7 (3.9)	83.5	0.5	5.5	1	2	7		0.5	Kunz *et al.* 1994a
APN1 MAG1	Δ, 700 bp	*SUP4*-o	67	3.9 (0.7)	98.5				1.5				Kunz *et al.* 1994a
UNG1	Δ 85%	*SUP4*-o	69	41 (10.8)	98.5	1.5							Impellizzeri *et al.* 1991
Nucleotide metabolism													
DCD1	Δ 0.39 kb	*SUP4*-o	207	9.5 (2)	88.4		9.2		0.5	0.5		1.4	Kohalmi *et al.* 1991

The values shown for each mutational class are percentages of the total mutations analyzed. The mutation rate for each class can be calculated by multiplying the overall rate by the percentage.

[a] PM, point mutation Δ, deletion (the extent of a partial deletion is given in base pairs, kilobases, or as a percentage of the total coding sequence); *TRP1*, disruption with *TRP1*.

[b] The Y or I after *SUP4*-o indicates the two different orientations of the gene within the plasmid; the LR or RL after *URA3* indicates the two different orientations of the gene at the same location on chromosome III [see Morrison and Sugino (1994) for details]; mutations were selected at 20°, 30°, 32°, or 34°.

[c] Ty, screening of mutations for Ty insertions only.

[d] NR, not reported; NA, not applicable.

TABLE 2

Single base pair substitutions detected in mutator or antimutator strains

Gene	Mutational target	G·C→A·T	A·T→G·C	Total	G·C→T·A	G·C→C·G	A·T→C·G	A·T→T·A	Total	Reference
Wild type										
	URA3	16		16	16	32	4	32	84	Lee *et al.* 1988
	URA3	26.7	3.3	30	26.7	33.3	3.3	6.7	70	von Borstel *et al.* 1993
	SUP4-o	25.5	14.5	40	34.1	20.4	1.4	3.8	60	Kang *et al.* 1994
	SUP4-o	25.3	15.5	40.8	33.4	19.5	2.3	4.0	59.2	Roche *et al.* 1994
	SUP4-o	24.3	10.4	34.7	32.9	17.9	8.1	6.3	65.3	Ramachandran 1996
	SUP4-o	32.3	8.7	41	22.4	25.5	7.4	3.7	59	Ramachandran 1996
	CAN1	38.5		38.5	23	38.5			61.5	Tishkoff *et al.* 1997
Proofreading/polymerase										
POL3 (Y)	*SUP4-o*	15.4	10.3	25.7	20.5	1.3	6.4	46.1	74.3	Ramachandran 1996
POL3 (I)	*SUP4-o*	24.6	14	38.6	32.7	1.8	10.5	16.4	61.4	Ramachandran 1996
POL3	*URA3 LR*	20	45	65	5		20	10	35	Morrison and Sugino 1994
POL3	*URA3 RL*	19.0	9.5	18.5	43		9.5	19	61.5	Morrison and Sugino 1994
POL2	*URA3 LR*	23.3		23.3	10		3.3	63.4	76.7	Morrison and Sugino 1994
POL2	*URA3 RL*	10.0	5	15	25		20	40	85	Morrison and Sugino 1994
MUT7	*URA3* (30°)	5.9		5.9	11.8	41.2	11.8	29.3	94.1	von Borstel *et al.* 1993
MUT7	*URA3* (32°)	6.7		6.7	13.3	33.3		46.7	93.3	von Borstel *et al.* 1993
MUT7	*URA3* (34°)	13.6	4.5	18.1		40.9	18.2	22.8	81.9	von Borstel *et al.* 1993
Mismatch correction										
PMS1 (Y)	*SUP4-o*	28.2	7.3	35.5	44.4	12.9	4	3.2	64.5	Ramachandran 1996
PMS1 (I)	*SUP4-o*	27.0	7.5	34.6	42.1	15.8	3	4.5	65.4	Ramachandran 1996
PMS1	*SUP4-o*	24,6	7.9	32.5	46	15.1	4.8	1.6	67.5	Yang 1995
MLH1	*SUP4-o*	17.2	6.9	24.1	44.8	10.4	20.7		75.9	B.A. Kunz (unpublished data)
MSH2	*SUP4-o*	14.7	13.2	27.9	58.8	6.7	3.7	2.9	72.1	Yang 1995
MSH2	*CAN1*				67		33		100	Marsischky *et al.* 1996
MSH3MSH6	*CAN1*	14.2	28.6	42.8	57.2				57.2	Marsischky *et al.* 1996
MSH6	*CAN1*	55.6	11.1	66.7	27.8		5.5		33.3	Marsischky *et al.* 1996

(*continued*)

TABLE 2

Continued

Gene	Mutational target	G·C→A·T	A·T→G·C	Total	G·C→T·A	G·C→C·G	A·T→C·G	A·T→T·A	Total	Reference
Nucleotide excision repair										
RAD1	*SUP4-o*	24.7	6.6	31.3	34.1	26.6	3.3	4.7	68.7	Kunz *et al.* 1990
RAD3-1	*SUP4-o*	23.2	11	34.2	25.8	30.5	4.2	5.3	65.8	Yang *et al.* 1996
RAD3-102	*SUP4-o*	10.8	2.4	13.2	57.8	25.3	1.2	2.4	86.8	Montelone *et al.* 1992
REV3RAD1	*SUP4-o*	30.1	19.2	49.3	35.6	12.3	2.1	0.7	50.7	Roche *et al.* 1994
RAD6-dependent repair										
RAD6	*SUP4-o*	27.5	10.6	38.1	59.8	2.1			61.9	Kang *et al.* 1992
RAD18	*SUP4-o*	11.1	6	17.1	76.4	3.5		3	82.9	Kunz *et al.* 1991
REV3	*SUP4-o*	23.8	20.4	44.2	40.8	10.9	0.7	3.4	55.8	Roche *et al.* 1994
REV3RAD6	*SUP4-o*	14.7	0.7	15.4	81.2	2.7	0.7		84.6	Roche *et al.* 1995a
REV3RAD18	*SUP4-o*	6.4	5.8	12.2	84.6	2.1		1.1	87.8	Roche *et al.* 1995a
Recombinational repair										
RAD52	*SUP4-o*	26.5	5.8	32.3	23.5	37.6	2.2	4.4	67.7	Kunz *et al.* 1989
REV3RAD52	*SUP4-o*	26.1	11.5	37.6	44.8	7.3	1.8	8.5	62.4	Roche *et al.* 1995a
Base excision repair										
APN1	*SUP4-o*	11.5	9.7	21.2	18.8	6.7	20.6	32.7	78.8	Kunz *et al.* 1994a
APN1MAG1	*SUP4-o*	19.7	10.6	30.3	13.6	6.1	24.2	25.8	69.7	Kunz *et al.* 1991
UNG1	*SUP4-o*	94.2		94.2	2.9	2.9			5.8	Impellizzeri *et al.* 1991
Nucleotide metabolism										
DCD1	*SUP4-o*	13.1	13.7	26.8	5.5	58.5	5.5	3.8	73.2	Kohalmi *et al.* 1991

The values shown for each type of substitution are percentages of the total substitutions detected. The mutation rate for each type of substitution can be calculated by multiplying the relevant substitution rate determined from Table 1 by the percentage for each substitution. Other details are as described in the legend to Table 1.

Specificity of mutator and antimutator alleles

In the next sections, we describe briefly the mutational specificities of defects that confer mutator or antimutator phenotypes. The genes involved are cataloged according to the processes that they influence: proofreading, mismatch correction, DNA repair [nucleotide excision repair (NER), base excision repair, *RAD6*-dependent repair, and recombinational repair], and nucleotide metabolism. The data are summarized in detail in Tables 1 and 2, which also provide the relevant references.

Proofreading: DNA pols δ, ε, and γ are the proofreading-proficient polymerases identified in yeast (Campbell 1993; Morrison and Sugino 1993). Exonucleolytic proofreading–deficient (Exo$^-$) mutants of pols δ and ε exhibit a mutator phenotype with a mutation rate increase of up to 130-fold for pol δ Exo$^-$ over wild type. The pol ε Exo$^-$ mutator confers a much less pronounced mutator effect that is consistent with mammalian DNA pol ε being more accurate than pol δ during *in vitro* replication (Thomas *et al.* 1991). The magnitude of the pol ε Exo$^-$ mutator effect (~10-fold increase in mutation rate) also suggests that the higher fidelity of pol ε results more from presynthetic error control mechanisms than from proofreading. Analysis of mutations generated in *SUP4*-o and *URA3* by the pol δ Exo$^-$ mutator, as well as mutations in *URA3* by the pol ε Exo$^-$ mutator, revealed that increases in single base pair events (substitutions, deletions, and insertions) accounted for virtually the entire increase in the mutation rates. (No sequence data were presented for spontaneous mutagenesis at *URA3* in the wild-type background, so the magnitude of the rate increases for the individual classes of mutation could not be determined. Single base pair mutations, however, were the only types of event recovered at *URA3* in the mutator strains.) Among the single base pair substitutions detected in *SUP4*-o, the G·C → C·G and A·T → T·A transversions exhibited the smallest and greatest rate increases, respectively. C/C mismatches are corrected postreplicatively much less efficiently in *Saccharomyces cerevisiae* than are G/G mismatches (Bishop *et al.* 1989; Kramer *et al.* 1989b). Thus, the data suggest that pol δ produces relatively few C/C mispairs when replicating *SUP4*-o. This interpretation is supported for pol δ and is extended to pol ε by the failure to detect any G·C → C·G transversions among 91 single base pair substitutions induced within a chromosomal copy of the *URA3* gene by a pol δ Exo$^-$ or pol ε Exo$^-$ mutator. The *SUP4*-o data also suggest that pol δ proofreads A/A and/or T/T mismatches more efficiently than other base mismatches, a possibility that is consistent with the relative inefficiency with which T/T mismatches in *SUP4*-o are corrected (Ramachandran 1996; Yang *et al.* 1996). Interestingly, the magnitude and specificity of the pol δ Exo$^-$ mutator was influenced by the orientation of the *SUP4*-o gene with respect to the origin of replication (Ramachandran 1996). The implications of this observation are discussed below. Although an attempt was made to examine the effect of *URA3* orientation on the specificities of the pol δ Exo$^-$ or pol ε Exo$^-$ mutators (Morrison and Sugino 1994), too few substitutions were characterized (20–30 per *URA3* orientation) to arrive at any statistically meaningful conclusion. Nonetheless, it is worth noting that some differences in the relative fractions of substitutions upon inversion of *URA3* in the pol δ Exo$^-$ background are similar to certain orientation-dependent differences detected for *SUP4*-o.

The *mut7-1* allele confers a temperature-sensitive defect in pol δ that results in a mutator phenotype. It is not known, however, whether the mutation influences the polymerizing or proofreading activities of the polymerase. Several observations argue against a proofreading defect. Elimination of proofreading by pol δ is not lethal, whereas *mut7-1* confers temperature-sensitive lethality. Furthermore, it is clear that the mutator effect is much smaller than that observed for the proofreading deficiency, although viability at 30° indicates that the full effect of the *mut7-1* allele is not apparent at this temperature. In addition, multiple base pair deletions, complex changes, and G·C → C·G transversions were recovered at *URA3* in the *mut7-1* background, but not at *URA3* for the pol δ Exo$^-$ mutator. One must be cautious in interpreting the latter findings because the data necessary to determine the magnitude of the *mut7-1* mutator effect at 32° and 34° were not presented, and at 30°, as many as 25% of the mutations analyzed could have been spontaneous in origin.

Mismatch correction: The specificities of mutators caused by inactivation of the *PMS1*, *MSH2*, and *MLH1* or *MSH3* plus *MSH6* mismatch repair genes have been characterized. The mutation rates were increased in all mismatch correction-deficient strains relative to the wild type, reflecting the importance of mismatch correction in maintaining genetic stability. At *SUP4*-o, for which specificity data for the wild-type background were provided, only the rates of single base pair events were increased in *pms1*, *msh2*, and *mlh1* strains, with deletions/insertions showing the greatest increases. Recently, *MSH2* was reported to be required for correction of a 26-base pair insertion mismatch formed in cells undergoing meiosis (Kirkpatrick and Petes 1997). No multiple base pair insertions/deletions were recovered in the *msh2* strains, however, suggesting either that such mismatches form much less frequently in mitotic cells or that the role of *MSH2* in large loop resolution may be meiosis specific. Interestingly, the *SUP4*-o specificity data indicate that the *mlh1* mutator features a larger proportion of single base deletions/insertions than the spectra for *pms1* and *msh2*. It has been suggested that the Pms1p, Msh2p, and Mlh1p proteins might function in the same pathway, possibly with interaction between Pms1p and Mlh1p (Prolla *et al.* 1994). If so, one might

expect the mutational specificities of all three mutators to be identical. There may be, however, different substrate-dependent mismatch repair complexes leading to differences in spectra, depending on the mismatch repair gene affected (Marsischky *et al.* 1996). Although inactivation of *MSH2* resulted in a much greater proportion of deletions/insertions at *CAN1* than observed for *SUP4*-o, relatively few mutations were sequenced, so the significance of this finding remains to be established. The *msh6* mutator produced mainly base pair substitutions in *CAN1*, with a much smaller fraction of single base pair deletions than observed for any other mismatch correction defect. Simultaneous inactivation of *MSH3* and *MSH6*, however, resulted in a spectrum of *can1* mutations closer to that for the *msh2* mutator. With the exception of the *MSH6* strain, more transversions than transitions were recovered in all of the mismatch correction-deficient mutants. Changing the orientation of *SUP4*-o within its plasmid replicon did not alter the spectrum of mutations obtained in a *pms1* strain, suggesting that for naturally occurring mismatches, gene orientation influences neither the direction nor efficiency of mismatch repair (Ramachandran 1996).

Nucleotide excision repair: *RAD1* encodes an endonuclease that is thought to function in a complex with Rad10p, incising the DNA backbone 5′ to the lesion (Friedberg 1988; Friedberg *et al.* 1995). The mutation rate of the *rad1* mutant was moderately higher than the wild-type strain, with increments in the rates of single base pair substitution and deletion and Ty insertion accounting for the entire rate increase. The rates of all six types of base pair substitutions were increased in the *rad1* background. Most of the single base pair deletions occurred in runs of two or more base pairs, consistent with the mutational intermediate being a nucleotide loop that arose through strand slippage during replication. It was suggested that the increase in the rate of single base pair events might reflect a decrease in mismatch correction (Kunz *et al.* 1990). *RAD1*, however, is not required for correction of heteroduplex plasmids that carry base mismatches (Kang and Kunz 1992). Furthermore, the increased rates of substitutions and deletions in the *rad1* background are dependent on *REV3*, which encodes a DNA polymerase (ξ) that is believed to function in translesion synthesis (Nelson *et al.* 1996b). This implicates translesion synthesis in the generation of spontaneous mutations that arise in the *rad1* mutant. Although there is evidence that Rad1p functions in the mismatch repair of a 26-base pair nucleotide loop formed during meiosis (Kirkpatrick and Petes 1997), only three multiple base pair deletions/insertions involving just two or three base pairs were detected among 249 *SUP4*-o mutations that were analyzed for the *rad1* strain.

The *RAD3* gene encodes an ATPase/helicase that is not only necessary for NER, but that is also involved in transcription (Friedberg 1988: Friedberg *et al.* 1995). *RAD3* is essential because of its latter role and, consequently, only mutators caused by point mutations in *RAD3* have been characterized. *rad3-1* and *rad3-102* increased the *SUP4*-o mutation rate by 3.2- and 23-fold, respectively. The substantial difference in the magnitude of the mutation rate increases most likely reflects the different effects of the two-point mutations on Rad3p activity. For example, *rad3-1* renders cells very sensitive to UV radiation, whereas *rad3-102* slightly decreases UV resistance. In both cases, the mutation rate enhancements were caused primarily by increases in the rates of single base pair events, and the relative fractions of base pair substitutions, deletions, and insertions were similar. The transversion:transition ratio, however, was 3.8-fold greater in *rad3-102* (7.2) than in *rad3-1* (1.9), and the distributions of substitutions within *SUP4*-o were quite different (Montelone *et al.* 1992; Yang *et al.* 1996). Interestingly, the transversion:transition ratio for the latter strain was closer to that observed for the *rad1* mutant (2.2), which also is highly UV sensitive. Despite some similarities in the spectra for *rad3-1* and *rad1*, there are indications that the mutational mechanisms associated with the two mutators may not be identical. Ty insertions were increased only in the *rad1* strain, and *rad3-1* but not *rad1* has been found to increase the efficiency of base mismatch correction (Yang *et al.* 1996). If the enhanced efficiency of mismatch repair offsets the *rad3-1* mutator effect to some extent, then the Rad3 protein might make a more substantial contribution to genetic stability than that suggested by the magnitude of the *rad3-1*-mediated increase in the *SUP4*-o mutation rate alone. The status of mismatch repair in the *rad3-102* mutant has not been assessed so far.

***RAD6*-dependent repair:** Members of the *RAD6* epistasis group for UV sensitivity are thought to function in postreplication repair or damage tolerance via translesion synthesis (Haynes and Kunz 1981; Friedberg 1988; Friedberg *et al.* 1995; Nelson *et al.* 1996a,b). The influence of inactivating the *RAD6*, *RAD18*, and *REV3* genes on the specificity of spontaneous mutagenesis has been determined using single- and double-mutant strains.

The *RAD6* gene product is an ubiquitin-conjugating enzyme that is capable of ubiquitinating histones H2A, H2B, and H3 *in vitro* (Jentsch *et al.* 1987; Haas *et al.* 1991). The *rad6* mutator increased the *SUP4*-o mutation rate by about fivefold, specifically promoting the two-base pair transitions, G·C → T·A transversions, and Ty insertions. The substitutions were not caused by a failure to correct mismatches that could have given rise to the detected base changes. Not only were the Ty insertions increased in number, but they also occurred at considerably more locations throughout *SUP4*-o than observed for the wild-type strain, suggesting that protein ubiquitination influences both the rate and target site specificity of Ty retrotransposition. Promotion of the four different

types of mutation by the *rad6* mutator was *REV3* dependent, but the *REV3* requirement was not uniform for each of the three mutational classes, with *REV3* function necessary for 60–98% of the changes, depending on the class of mutation.

RAD18 encodes a protein that contains DNA- and nucleotide-binding motifs and that has an ATPase activity (Bailly *et al.* 1997). The *rad18* mutator is unique among eukaryotic mutators identified to date in that it displays a highly specific phenotype. Only the rate of G·C → T·A transversions is increased, by about eightfold. Again, this effect is not caused by a mismatch correction deficiency. Intriguingly, extracts of *rad18* cells are defective in nicking supercoiled plasmid DNA containing methylene blue plus light-induced DNA lesions (T. Y.-K. Chow and B. A. Kunz, unpublished results). These lesions include 8-hydroxyguanine (Kasai and Nishimura 1984), which can give rise to G·C → T·A transversions during DNA replication (Shibutani *et al.* 1991; Wood *et al.* 1992; Moriya 1993). Rad18p and Rad6p form a heterodimeric complex with ubiquitin-conjugating, DNA-binding, and ATP hydrolytic activities (Bailly *et al.* 1997). The mutational specificity data suggest that this complex might be required to limit the production of G·C → T·A transversions by DNA damage, and the function of *RAD18* in spontaneous mutagenesis might be to direct the complex to the relevant lesions. Thus, it is not surprising that the production of G·C → T·A transversions by the *rad18* mutator also is *REV3* dependent, and that the degree of *REV3* dependency is similar to that observed for *rad6*-mediated G·C → T·A transversion.

RAD27 apparently functions in a novel mutation avoidance pathway that minimizes the occurrence of duplications of sequences flanked by short direct repeats. The *rad27* mutator greatly increased the rate of these events and promoted complex sequence changes. In contrast to all yeast mutators characterized to date, the rate of single base pair events did not appear to be increased. Because only a small number of mutations were analyzed, however, it remains possible that the rate of single base pair substitutions, deletions, or insertions is enhanced by *rad27*, but to a much lesser extent than the rates for the events detected.

As mentioned above, the *REV3* gene product is a DNA polymerase that is likely to be required for translesion synthesis (Nelson *et al.* 1996b). Defects in this gene confer an antimutator phenotype (Cassier *et al.* 1980; Quah *et al.* 1980) whose specificity has been characterized. The rates of all classes of spontaneous mutation detected in an isogenic wild-type strain were reduced in the *rev3* background, although the major portion of the overall mutation rate decrease was associated primarily with single base pair substitutions, deletions, and insertions. This observation, plus the fact that the rate reductions for other events were small or few mutations were recovered, makes it difficult to say whether the data indicate a very broad specificity for the antimutator. It has been suggested that *RAD6* might influence other members of the *RAD6* epistasis group via ubiquitination of their gene products (Sung *et al.* 1990). The manifestation of the *rev3* antimutator phenotype in the *rad6* background, however, implies that ubiquitination of Rev3p by Rad6p is not necessary for the role of Rev3p in spontaneous mutagenesis.

Inactivation of the DNA damage-inducible gene *DDR48* also has been reported to confer an antimutator phenotype (Treger and McEntee 1990). Subsequent analyses of locus reversion and suppression of *lys2-1*, forward mutation at *CAN1* and *SUP4*-o, and the specificity of spontaneous mutation at *SUP4*-o in *ddr48* strains, however, did not confirm the antimutator phenotype (Roche *et al.* 1995b).

Recombinational repair: The *rad52* gene has been implicated in the recombinational repair of DNA double-strand breaks and in postreplication repair of UV-induced DNA damage in NER-deficient cells (Friedberg 1988; Friedberg *et al.* 1995). Disruption of *RAD52* results in a small increase in the *SUP4*-o mutation rate, with the mutator primarily affecting substitutions at G·C pairs. Unlike the situation for the *rad1*, *rad6*, and *rad18* mutators, *REV3* is essential for the *rad52* mutator effect. These results suggest that *RAD52* is involved in the repair of spontaneous DNA damage that, if left unrepaired, can give rise to spontaneous mutation only via *REV3*-dependent processing. This may be true for other members of the *RAD52* epistasis group (Haynes and Kunz 1981) because the Rad51p and Rad52p proteins interact *in vivo* (Milne and Weaver 1993), and because enhanced reversion of the *lys1-1* and *his1-7* alleles by the *rad51* mutator also exhibits complete *REV3* dependence (Quah *et al.* 1980). The nature of the DNA damage involved is not known. It is not clear, however, how processing a DNA double-strand break might generate primarily transitions and transversions at G·C pairs. Perhaps *RAD52* is required for the recombinational repair of daughter-strand gaps that are formed opposite certain types of spontaneous lesions. Inactivation of *RAD52* might then leave only *REV3*-dependent translesion synthesis to fill the gaps.

Base excision repair: *APN1* encodes the major apurinic/apyrimidinic (AP) endonuclease involved in base excision repair in *S. cerevisiae* (Popoff *et al.* 1990). Consistent with endogenous AP sites being mutagenic lesions, deletion of *APN1* resulted in a mutator, and characterization of *SUP4*-o mutations revealed increased rates mainly for single base pair substitution and Ty insertion. Among the substitutions, the rate of total transversions involving replacement by a G·C pair was increased 10-fold compared to a threefold rate increase for total transversions to an A·T pair. In particular, the rate of A·T → C·G events was 59-fold greater in the *apn1* strain. Elimination of the *N*3-methyladenine glycosylase (*MAG*) in the *apn1* mutant decreased the A·T → C·G

transversion rate by 83% relative to the *apn1* strain and reduced the rate of total substitutions to that for the wild-type parent. These results suggest that replication past unrepaired AP sites resulting from spontaneous DNA alkylation is largely responsible for the substitution specificity of the *apn1* mutator. The data also point to activation of Ty retrotransposition by spontaneously occurring DNA damage.

UNG1 encodes the yeast uracil-DNA-glycosylase (Impellizzeri *et al.* 1991). Inactivation of *UNG1* increased the *SUP4*-o mutation rate by 10-fold, with an increase in the rate of G·C → A·T transitions accounting for 93% of the mutator effect. This result is consistent with failure to remove uracil resulting from spontaneous deamination of cytosine.

Nucleotide metabolism: There is considerable evidence that regulation of intracellular DNA precursor levels is an important factor in minimizing spontaneous genetic instability (Kunz *et al.* 1994b). Accordingly, a defect in the gene (*DCD1*) encoding deoxycytidylate deaminase greatly increased the intracellular dCTP: dTTP ratio and conferred a modest mutator effect. Only the rate of single base pair events, mainly substitution, was increased. As might be expected on the basis of the elevated dCTP pool, ~80% of the substitutions could have involved replacement with C compared to 37% for the wild-type strain. Given the DNA precursor imbalance, a decrease in the proportion of G·C → A·T transitions and a corresponding increase in the fraction of A·T → G·C transitions might have been expected. Although the proportion of G·C → A·T events decreased by 50%, the fraction of A·T → G·C events did not change significantly, and there was an increase in the proportion of G·C → C·G and A·T → C·G transversions. Rather than misincorporation during replicative DNA synthesis, these substitutions are likely to be caused by misincorporation of dCTP during repair of apurinic sites because the *dcd1* mutator effect is eliminated by inactivation of *APN1* (B. A. Kunz, unpublished results).

Insights from DNA sequence analysis: Characterization of the specificities of yeast mutator and antimutator alleles has revealed that defects in various genes elicit a range of distinct spontaneous mutational spectra. Furthermore, dissimilarities in spectra have been observed for alleles of the same gene (*rad3-1 vs. rad3-102*) or for genes thought to be involved in the same repair process (*rad1* and *rad3*, *rad6*, and *rad18*). Such findings point to unexpected subtleties in the control of genetic stability in *S. cerevisiae* and presumably in other eukaryotic organisms. Next, we highlight several observations that bear on the mechanistic aspects of spontaneous mutation.

The A rule *vs.* endogenous abasic sites in eukaryotic cells: The mutational specificity of AP sites was first explored in transfection experiments in which single-stranded bacteriophage DNA was introduced into *E. coli*, with the specific changes established by analysis of the DNA recovered from the cells (Schaaper *et al.* 1983; Kunkel 1984). The mutations found were mainly transversions that apparently resulted from the insertion of dAMP residues opposite the AP sites during replication. Analysis of the template specificity of abasic sites during *in vitro* DNA synthesis by a variety of DNA polymerases indicated a preference for the insertion of dAMP (Sagher and Strauss 1983; Randall *et al.* 1987; Takeshita *et al.* 1987). These and other results gave rise to the so-called "A rule" (noninstructional insertion of adenine by DNA polymerase), which has since been applied to explain the mutational specificity of a variety of DNA lesions that lack obvious coding capacity (Strauss 1991). Analysis of the *apn1-Δ1* mutator effect indicates that unrepaired endogenous AP sites have significant mutagenic potential in eukaryotes but, surprisingly, with a mutational specificity different from that expected on the basis of the A rule (Kunz *et al.* 1994a). In particular, a large increase in the rate of transversion to G·C base pairs was observed (Table 2), with most of these changes attributed to the loss of DNA purines. Interestingly, the magnitude of the *apn1-Δ1* mutator effect is dramatically reduced by a deletion in *DCD1* (B. A. Kunz, unpublished results), which results in a decrease in the intracellular dGTP pool (Kohalmi *et al.* 1991). Consequently, AP sites in yeast appear to trigger the insertion of dGMP during translesion DNA synthesis. This preference did not arise from a selection bias in the mutational assay system used because, in *SUP4*-o, the insertion of dAMP opposite purines can be detected at more sites than insertion of dGMP (63 *vs.* 52 sites, respectively) (Kohalmi and Kunz 1992). Experiments involving the transfection of AP site-containing DNA into eukaryotic cells have also suggested that the mutational specificity of these lesions might be different in nucleated cells (Gentil *et al.* 1990; Cabral Neto *et al.* 1992; Kamiya *et al.* 1992). More recently, the product of the yeast *REV1* gene has been shown to insert cytosine opposite a site-directed AP lesion *in vitro* (Nelson *et al.* 1996a). Whether the Rev1 protein is required for mutagenesis at endogenous AP sites, however, remains to be determined. Nonetheless, the foregoing observations argue that the A rule cannot be readily extrapolated to eukaryotic cells.

Roles of pol δ in DNA replication: DNA pols α, δ, and ε are implicated in the replication of eukaryotic cellular DNA (Bambara and Jessee 1991; Wang 1991; So and Downey 1992; Campbell 1993; Morrison and Sugino 1993; Hübscher and Spadari 1994; Stillman 1994). Polymerase α most likely functions in the initiation of leading strand and Okazaki fragment synthesis, but the precise roles of pols δ and ε are still under debate. The mutational specificity data for yeast DNA pol δ that lacks 3′ → 5′ exonuclease activity (Tables 1 and 2) might be interpreted to suggest that pol δ replicates only one of the two DNA strands at a yeast replication fork, leaving open the possibility that pol ε rep-

licates the other strand. This interpretation of the specificity data for pol δ is based on the following rationale. The type and location of errors that result from replication by DNA polymerases depend in part on the sequence of the template DNA (Kunkel 1992; Izuta *et al.* 1995). Thus, the pattern of mutations that are produced when a DNA polymerase replicates one strand of a gene might differ from the mutation pattern for replication of the other strand by the same polymerase. If only a single replication fork traverses the gene in question, the sequences of the templates within the gene will depend on the orientation of that gene with respect to the origin from which the replication fork initiates. Should a DNA polymerase replicate, for example, only the leading strand template, then for one orientation, the polymerase would produce a mutational spectrum dictated in part by the sequence of the transcribed strand. For the other orientation, the sequence of that template strand would be changed so that a different spectrum, directed in part by the sequence of the nontranscribed strand, might result. If the DNA polymerase replicates both strands to the same extent, then there might be little difference in the mutational spectrum that is produced upon gene inversion. In either orientation, the polymerase would encounter the sequences of both the transcribed and nontranscribed strands.

A single DNA replication fork does traverse the plasmid-borne *SUP4*-o gene (Ramachandran 1996). Consequently, the orientation of this gene within the plasmid dictates whether an individual strand is part of the leading or lagging strand template. Inversion of *SUP4*-o to change the sequence context of these templates alters the magnitude and specificity of the mutator due to elimination of proofreading by DNA pol δ (Tables 1 and 2), but it does not influence mismatch correction in this gene (Ramachandran 1996). Thus, the specificity data are consistent with pol δ encountering a different sequence context upon inversion of *SUP4*-o, therefore replicating just one template strand at the DNA replication fork. Two other interpretations of these data, however, must be considered. First, the leading and lagging strand replication complexes most likely involve at least some different accessory factors. Dissimilarities (aside from polymerases) in the constitutions of the complexes might lead to different fidelities of DNA replication, and the failure of pol δ to proofread might amplify these differences in accuracy. Consequently, even if pol δ replicated both DNA templates, the pattern of errors produced for the two orientations of *SUP4*-o might still differ, although it is difficult to say whether this would be true for the overall mutation rate. Second, it is conceivable that replication *in vivo* by eukaryotic DNA polymerases is dissociated from proofreading by the same enzymes. In this case, the specificity data might suggest that it is the proofreading rather than polymerizing activity of pol δ that operates on only one of the two template strands. If so, then one would have to question why pol δ corrects errors on only one of the two strands it replicates and why a different $3' \rightarrow 5'$ exonuclease corrects the other strand. Regardless of such conundrums, the differences in mutational specificity upon inversion of *SUP4*-o also suggest that for any gene, the spectrum and distribution of spontaneous mutations may depend in part on the position and orientation of the gene with respect to the nearest active origin of replication.

Specificity of mismatch repair: The yeast mismatch correction genes *MLH1*, *MSH2*, and *PMS1* have been shown to participate in a system that recognizes single base pairs and deletion/insertion mispairs (Kramer *et al.* 1989b; Yang 1995; Marsischky *et al.* 1996). Comparison of the mutational specificity data for a wild-type strain and isogenic mismatch correction mutants reveals that the rate of single base pair deletion/insertion in *SUP4*-o is increased much more than the rate of single base pair substitution (Table 1). This observation indicates that deletion mispairs are recognized more efficiently than substitution mispairs by the mismatch correction system, consistent with the situation in *E. coli* (Schaaper and Dunn 1987, 1991). The rate increase is the same for transitions and transversions (Table 2), however, indicating that there is little difference in the efficiencies with which naturally occurring transitions and transversion mispairs are recognized in yeast. In contrast, *E. coli* data point to much more efficient recognition of transition than transversion mispairs (Schaaper and Dunn 1987, 1991), suggesting that there may be fundamental differences in base mispair recognition by the general mismatch correction systems in prokaryotes and eukaryotes. Collectively, these observations also indicate that differences in the relative proportions of spontaneous single base pair substitutions and deletions/insertions may not only reflect different frequencies of mutational intermediate formation, but may also reflect differences in the repair of specific mismatches by *MLH1*/*MSH2*/*PMS1*-dependent mismatch correction.

Contribution of translesion synthesis to spontaneous mutagenesis: Evidence has been presented that bacteriophage T4 and *E. coli* antimutators are very specific and, therefore, may influence only particular pathways for error discrimination (Drake 1993; Fijalkowska *et al.* 1993). On the other hand, the *rev3* antimutator has relatively broad specificity that affects at least all base pair substitutions as well as single base pair deletions/insertions (Tables 1 and 2). Indeed, the magnitude of the antimutator effect suggests that ≥60% of the spontaneous single base pair substitutions and deletions might be caused by translesion synthesis. If so, the Rev3 DNA polymerase might be involved in processing many different lesions, and, therefore, a substantial fraction of spontaneous mutagenesis in yeast might reflect mechanisms for tolerance rather than error-prone repair of DNA damage. The fact that the *rev3* antimutator counteracts

several *rad* mutators, although to different extents (Roche *et al.* 1994, 1995a), implies that the relevant DNA lesions are normally substrates for error-free repair. Furthermore, certain of the data suggest that if left unrepaired by error-free mechanisms, some endogenous DNA lesions may give rise to mutations almost exclusively via *REV3*-dependent processing (*e.g.*, see data for *RAD52*).

Inducibility of Ty transposition by spontaneous DNA damage: Insertion of the Ty retrotransposon into *SUP4*-o was increased in strains having *apn1*, *rad1*, or *rad6* deletions (Table 2). *APN1* is required for the repair of abasic sites and free radical-induced strand breaks, and defects in *RAD1* (NER) and *RAD6* (postreplication repair?) sensitize cells to a variety of DNA-damaging agents. Thus, spontaneously occurring DNA lesions might mobilize Ty elements (Kunz *et al.* 1990, 1994a). Consistent with this hypothesis, treatment with UV or 4-nitroquinoline-1-oxide, mutagens that produce DNA damage subject to NER, has been reported to activate Ty transposition (Bradshaw and McEntee 1989). Spontaneous transposition rates, however, are not increased by mutator alleles of *RAD3* or *RAD18*, which greatly sensitize cells to UV (Kunz *et al.* 1991; Yang *et al.* 1996). In addition, the enhancement of Ty transposition by inactivation of *RAD6*, which encodes a ubiquitin-conjugating enzyme (Jentsch *et al.* 1987), might not necessarily be related to defective DNA repair. Picologlou *et al.* (1990) suggested that either failure to ubiquitinate Ty proteins or altered chromatin structure in *rad6* strains, resulting in increased access of transposition complexes to DNA, might be responsible. Such effects also might account for the broadened target site specificity for Ty insertion into *SUP4*-o in the *rad6* background. On the other hand, Ty target site specificity also is expanded by deleting *REV3* (Roche *et al.* 1994). Although this again points to a role for spontaneous DNA damage in Ty transposition, it is clear that the situation is not straightforward.

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