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DNA Sequence Analysis of Spontaneous Mutations in the *SUP4*-o Gene of *Saccharomyces cerevisiae*

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A collection of 196 spontaneous mutations in the *SUP4*-o gene of the yeast *Saccharomyces cerevisiae* was analyzed by DNA sequencing. The classes of mutation identified included all possible types of base-pair substitution, deletions of various lengths, complex alterations involving multiple changes, and insertions of transposable elements. Our findings demonstrate that at least several different mechanisms are responsible for spontaneous mutagenesis in *S. cerevisiae*.

Although spontaneous mutagenesis has been well studied in the yeast *Saccharomyces cerevisiae*, relatively little is known about the molecular nature of spontaneous mutational events or the factors that influence such events in this organism (for reviews, see references 6, 10, and 18). To improve our understanding of mutational mechanisms in *S. cerevisiae*, we recently developed a system in which mutations occurring in the tyrosine suppressor tRNA gene *SUP4*-o can be rapidly analyzed to determine the DNA sequence changes responsible (12). Here we describe our use of the *SUP4*-o system to analyze spontaneous mutations in *S. cerevisiae*.

In this system, *SUP4*-o is carried on the centromere plasmid YCpMP2. Autonomous yeast centromere-containing plasmids mimic the behavior of yeast chromosomes. They are maintained predominantly as single copies in haploid cells (a feature essential for mutant selection), exhibit typical chromatin organization, and replicate once per cell cycle in S phase (2). Forward mutations in the *SUP4*-o gene are detected by their elimination of suppressor activity (13). The haploid yeast strain MKP-o (12) carries ochre-suppressible markers which, in the absence of suppression, confer resistance to the arginine analog canavanine (*can1-100*), cause red pigmentation (*ade2-1*), or result in lysine auxotrophy (*lys2-1*). Cells harboring YCpMP2 are canavanine sensitive and form white, lysine-independent colonies. Loss of suppressor activity leads to the formation of canavanine-resistant, red or pink colonies unable to grow when replicated to lysine omission medium. Following mutant characterization in vivo, yeast DNA is isolated and transformed into *Escherichia coli* JF1754 (12) to retrieve the shuttle vector. Then the mutant *SUP4*-o genes are sequenced directly on linearized double-stranded YCpMP2 plasmid DNA by the dideoxynucleotide chain termination technique (17) by the procedure of Korneluk et al. (8).

To isolate independently occurring spontaneous mutants, cultures of MKP-o carrying YCpMP2 were grown to stationary phase (approximately 1.5×10^7 cells per ml) in medium selective for the plasmid. Under these conditions, 90% of the cells retain YCpMP2 (12). Cell suspensions were then plated on appropriate medium to select canavanine-resistant colonies. Among the total canavanine-resistant colonies detected, 2.6% (326 of 12,342) were classified as *SUP4*-o mutants (red or pink and Lys^-). On this basis, the mean *SUP4*-o mutation frequency was determined to be 2.4×10^{-6}, corresponding to a spontaneous mutation rate of 1.9×10^{-7} events per generation (calculated as described previously [12]). A collection of 196 spontaneous *SUP4*-o mutants, each isolated from a separate culture, was characterized by DNA sequencing.

Single base-pair substitutions were recovered at a frequency of 2.0×10^{-6} and accounted for 84% of the spontaneous mutants (Table 1). The rate of mutation was calculated to be 1.8×10^{-9} per base pair per generation. Both types of transition and all four transversions were identified, but there was a slight excess of transversions, and three times as many events occurred at G · C sites as at A · T sites (Table 2). Spontaneous base substitution mutagenesis at the chromosomal *SUP4*-o locus is also biased towards transversions and G · C sites (9), suggesting that the same mutational processes act on the plasmid and chromosomal copies of *SUP4*-o. In addition to single events, we also detected one nontandem, double-base-pair change involving A · T → G · C and G · C → A · T substitutions at positions +12 and +14, respectively. While the spontaneous base-pair substitutions were distributed throughout the *SUP4*-o gene, mutations occurred at three locations (+18, +83, and +88) much more frequently than at others (Fig. 1). No point mutations were detected 5′ or 3′ to the region encoding *SUP4*-o, and base-pair changes were found at only one position (+51) within the tRNA intron. Outside the intron, spontaneous

TABLE 1. Sequence alterations in spontaneous mutants

Alteration	No. detected (% of mutants examined)	Frequency ($\times 10^{-6}$)
Base-pair substitution		
Single change	164 (83.7)	2.01
Double change	1 (0.5)	0.012
Total	165 (84.2)	2.02
Deletion		
Single base pair	10 (5.1)	0.12
Multiple base pairs	6 (3.1)	0.074
Complex event	3 (1.5)	0.036
Total	19 (9.7)	0.23
Ty insertion	12 (6.1)	0.15

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TABLE 2. Spontaneous base-pair substitutions

Change	No. detected (% of total substitutions)
Transitions	
A · T → G · C	27 (16.3)
G · C → A · T	39 (23.5)
Total	66 (39.8)
Transversions	
A · T → T · A	10 (6.0)
A · T → C · G	4 (2.4)
G · C → C · G	33 (19.9)
G · C → T · A	53 (31.9)
Total	100 (60.2)

TABLE 3. Characterization of spontaneous deletions

Mutant(s)	No. of deleted base pairs	Location of deletion
10, 27-1, 52-1, 60, 62, 217, 228, Co-2	1	+79 → +83
62-1	1	+84 → +86
132	1	+18 or +19
189	1	+15
160, Co-31-1	7	+58 → +64[a]
6-1	8	+89 → +96
134	9	+89 → +97
10	32	+88 → +119[b]
126	135	+13 → −122
143	248[c]	−1 → −248
230	625	+70 → −555
114	807[c]	+83 → −724

[a] Present in a mutant also having a 5-base-pair insertion in the deleted region.
[b] Present in a mutant also having a single-base-pair deletion in +79 → +83.
[c] Values are only estimates because sequence repeats are present at the deletion termini.

base-pair substitutions were recovered at 56 of the 75 available sites in the gene. We also characterized induced base-pair changes at intron positions +40 and +42 and at seven additional sites (+4, +8, +20, +64, +72, +74, and +81) not recovered here (Kunz et al., unpublished observations). In studies involving the chromosomal *SUP4*-o locus (1, 9), spontaneous point mutations have been identified at another intron site (+43) and at two other positions (+13 and +70) not yet detected in *SUP4*-o on the plasmid. Thus, to date, base-pair substitutions that inactivate suppression have been located at four sites within the intron and at 65 of the remaining 75 sites in the *SUP4*-o gene.

Deletions, ranging from the loss of a single base pair to the elimination of up to 807 base pairs, were also detected (Table 3). These events accounted for 9.7% of the total mutations identified, corresponding to a frequency of 2.3×10^{-7}. In nine of the mutants, single G · C pairs were deleted from the tracts of G · C pairs extending from +79 to +83 or +84 to +86. Slipped mispairing of nascent and template strands during replication in runs of repeated base pairs (19) could have been responsible for these events and for the loss of 8 and 9 base pairs in mutants 6-1 and 134, respectively (Table 3). Two mutants (114 and 143) retained single copies of short, repeated sequences (4 and 2 base pairs, respectively) originally present at the deletion termini and so may have been generated by nonhomologous recombination occurring between the repeats (11). Three other mutants featured more complex alterations. In one (mutant 10), a deletion of 32 base pairs from +88 through +119 was accompanied by the removal of a single G · C pair from the +79 to +83 region. The other two mutants (Co-31-1 and 160) both had the sequence 5′-GATCTCA-3′ at +58 to +64 replaced by 5′-CCGGG-3′. It has been proposed that quasi-palindromic sequences might play a role in the production of this type of complex event by pairing out of register to stabilize mutational intermediates and template concurrent multiple changes (5, 14). Intriguingly, a DNA secondary structure which could be processed to yield the replacement we detected at +58 to +64 could be formed by pairing within an inverted repeat present in the region involved (Fig. 2).

Twelve mutants were found to contain nonplasmid DNA extending 5′ from position +17 (one mutant), +37 (10 mutants), or +43 (one mutant). Sequencing of the first 100 to 200 nucleotides of the insertions showed that they had conserved features of the 330-base-pair delta sequences that flank transposable Ty elements in *S. cerevisiae* (15, 16). Eight inserts began with the sequence 5′-TGTTGGAATA-3′. The remaining four insertions began with 5′-TG-3′ and had a *Hin*fI recognition site at position 34. Computer-facilitated comparisons of the sequenced ends of each of the

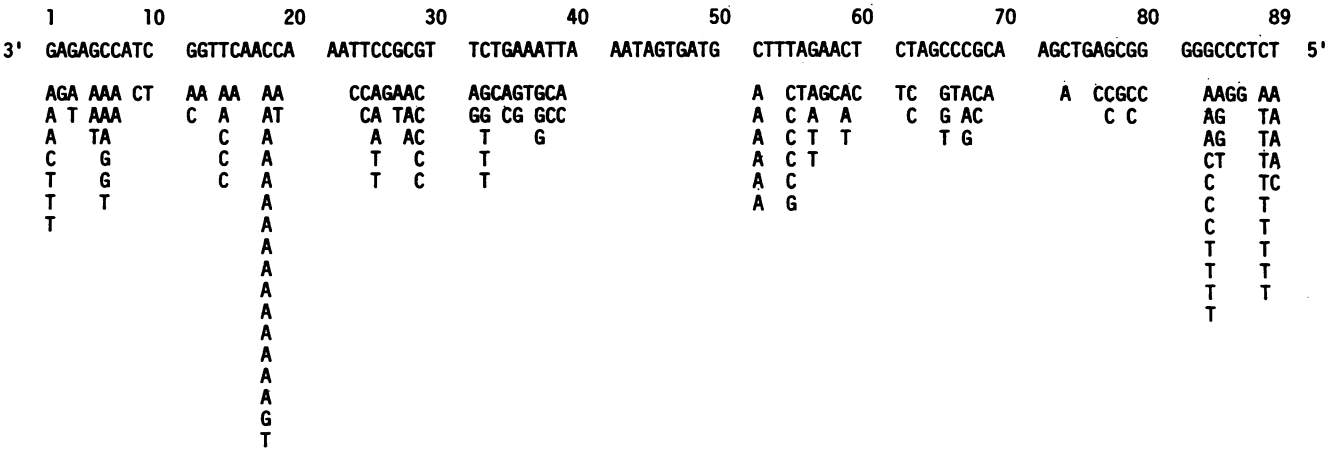

FIG. 1. Distribution of spontaneous base substitutions in *SUP4*-o. For simplicity, only the region of the transcribed strand encoding the tyrosine tRNA is shown. The anticodon is located at +36 to +38, and the 14-base-pair intron is inferred to extend from +40 through +53, as for the *sup4*$^+$ allele (7). The YCpMP2 yeast chromosomal DNA fragment carrying *SUP4*-o extends from −87 to +156.

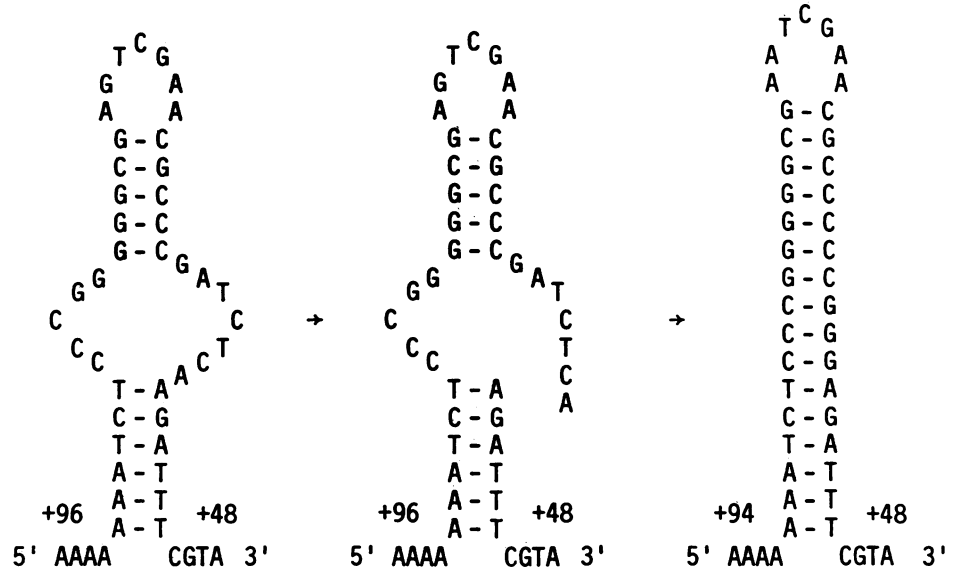

FIG. 2. Inverted repeat that potentially can direct the replacement of the sequence 5'-GATCTCA-3' with 5'-CCGGG-3', as diagrammed. The replacement perfects the structure of the palindrome. Only the transcribed strand is shown.

12 inserts with established sequences for delta elements revealed striking homologies (up to 100%). Agarose gel electrophoresis of the 12 mutant plasmids following digestion with *Bam*HI indicated that 11 were larger than YCpMP2 by 6 kilobase pairs, the approximate size of a Ty element (15). Thus, we concluded that each of the 11 contained an intact transposon. The 12th plasmid was just slightly larger than YCpMP2, consistent with the presence of a solo delta sequence. Therefore, the insertion in this plasmid may be the remnant of a transposon that had excised by homologous recombination between its flanking deltas (15, 16). Insertion of Ty elements accounted for 6.1% of the total spontaneous mutants characterized. From this value, the frequency of Ty transposition into *SUP4*-o on the plasmid was calculated to be 1.5×10^{-7}. However, this may be an overestimate because of the apparent hot spot for transposition at +37. Although the factors that promote transposition of Ty are not well understood, insertion generally has been found to occur in A+T-rich sequences (3, 4, 20). Consistent with this observation, Ty integrated at *SUP4*-o sites in regions of high A · T pair density.

In summary, we analyzed a collection of 196 mutations arising spontaneously in the *SUP4*-o gene of *S. cerevisiae*. Several classes of mutation were detected, pointing to the complexity of spontaneous mutagenesis in *S. cerevisiae*. Furthermore, certain of these results support the hypothesis (5) that DNA sequence-directed events may generate spontaneous mutations in eucaryotic cells.

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LITERATURE CITED

1. **Allison, D. S., S. H. Goh, and B. D. Hall.** 1983. The promoter sequence of a yeast tRNA^{tyr} gene. Cell **34**:655–664.
2. **Clarke, L., and J. Carbon.** 1985. The structure and function of yeast centromeres. Annu. Rev. Genet. **19**:29–56.
3. **Fink, G. R., P. Farabaugh, G. Roeder, and D. Chaleff.** 1980. Transposable elements in yeast. Cold Spring Harbor Symp. Quant. Biol. **45**:575–580.
4. **Gafner, J., and P. Philippsen.** 1980. The yeast transposon Ty1 generates duplications of target DNA on insertion. Nature (London) **286**:414–418.
5. **Golding, G. B., and B. W. Glickman.** 1985. Sequence-directed mutagenesis: evidence from a phylogenetic history of human α-interferon genes. Proc. Natl. Acad. Sci. USA **82**:8577–8581.
6. **Haynes, R. H., and B. A. Kunz.** 1981. DNA repair and mutagenesis in yeast, p. 371–414. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Knapp, G., J. S. Beckwith, P. F. Johnson, S. A. Fuhrman, and J. Abelson.** 1978. Transcription and processing of intervening sequences in yeast tRNA genes. Cell **14**:221–236.
8. **Korneluk, R. G., F. Quan, and R. A. Gravel.** 1985. Rapid and reliable dideoxy sequencing of double-stranded DNA. Gene **40**:317–323.
9. **Kurjan, J., B. D. Hall, S. Gillam, and M. Smith.** 1980. Mutations at the yeast *SUP4* tRNA^{tyr} locus: DNA sequence changes in mutants lacking suppressor activity. Cell **20**:701–709.
10. **Lawrence, C. W.** 1982. Mutagenesis in *Saccharomyces cerevisiae*. Adv. Genet. **21**:173–254.
11. **Nalbantoglu, J., D. Hartley, G. Phear, G. Tear, and M. Meuth.** 1986. Spontaneous deletion formation at the *aprt* locus of hamster cells: the presence of short sequence homologies and dyad symmetries at deletion termini. EMBO J. **5**:1199–1204.
12. **Pierce, M. K., C. N. Giroux, and B. A. Kunz.** 1987. Development of a yeast system to assay mutational specificity. Mutat. Res. **182**:65–74.
13. **Rasse-Messenguy, F., and G. R. Fink.** 1973. Temperature-sensitive nonsense suppressors in yeast. Genetics **75**:459–464.
14. **Ripley, L. S., and B. W. Glickman.** 1983. Unique self-comple-

mentarity of palindromic sequences provides DNA structural intermediates for mutation. Cold Spring Harbor Symp. Quant. Biol. **47**:851–861.

15. **Roeder, G. S., and G. R. Fink.** 1983. Transposable elements in yeast, p. 299–328. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
16. **Rothstein, R., C. Helms, and N. Rosenberg.** 1987. Concerted deletions and inversions caused by mitotic recombination between delta sequences in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:1198–1207.
17. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
18. **Sargentini, N. J., and K. C. Smith.** 1985. Spontaneous mutagenesis: the roles of DNA repair, replication and recombination. Mutat. Res. **154**:1–27.
19. **Streisinger, G., Y. Okada, J. Emrich, J. Newton, A. Tsugita, E. Terzaghi, and M. Inouye.** 1966. Frameshift mutations and the genetic code. Cold Spring Harbor Symp. Quant. Biol. **31**:157–179.
20. **Williamson, V. M., D. Cox, E. T. Young, D. W. Russell, and M. Smith.** 1983. Characterization of transposable element-associated mutations that alter yeast alcohol dehydrogenase II expression. Mol. Cell. Biol. **3**:20–31.