INHERITANCE OF SPONTANEOUS MUTABILITY IN YEAST[1,2]

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IT has become increasingly clear in recent years that spontaneous mutations are due principally to factors intrinsic to an organism rather than to external agents such as background radiation (VON BORSTEL 1969; MAGNI 1969). That is, mutability is itself a phenotypic character, subject to genetic control.

SPEYER, KARAM and LENNY (1966) demonstrated that a temperature-sensitive mutation in the gene specifying DNA polymerase in bacteriophage T4 increased the spontaneous mutation rate. They showed that this mutator caused transitions. Another DNA polymerase mutant from T4 has been shown to induce transversions (HALL and LEHMAN 1968). There is, however, no reason to believe that with the "normal," wild-type DNA polymerase gene no mistakes at all arise. On the contrary, it is reasonable to assume that mistakes caused by the DNA replication system are one source of spontaneous mutations. This assumption is supported by the discovery of antimutator activity among DNA polymerase mutants (DRAKE *et al.* 1969).

Genetic recombination has also been implicated as a source of spontaneous mutations. MAGNI and VON BORSTEL (1962) found a so-called "meiotic effect" for certain mutations in yeast. These mutations revert at a rate six- to twenty-fold higher during meiosis than during mitosis. MAGNI (1963) further showed that reversions during meiosis of such mutations, believed to be addition-deletion mutations, had a strong correlation with outside marker exchange. In addition, mutations of the base-substitution type did not show the meiotic effect (VON BORSTEL, BOND and STEINBERG 1964; MAGNI 1964, 1969). These studies demonstrated the role of recombination as an intrinsic factor causing spontaneous addition-deletion mutations in a meiotic organism.

In addition, the DNA repair system has been implicated as another source of spontaneous mutations. It is believed that radiation-sensitive strains are defective in their ability to repair lesions in DNA. If this is true, it suggests that the frequency of spontaneous mutations could be altered in those repair-deficient strains where the lesions occur naturally during DNA metabolism. That this is indeed the case has been shown for several species (BÖHME 1969), including yeast (ZAKHAROV, KOŽINA and FEDEROVA 1968; VON BORSTEL *et al.* 1968). In this paper

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we analyze the effects of several radiation-sensitive mutations on the frequencies and types of spontaneous mutations in the yeast *Saccharomyces cerevisiae.*

MATERIALS AND METHODS

Strains: The origin and derivation of yeast cultures is given in Tables 1 and 2. The original hybrid X1687 was synthesized by R. K. MORTIMER. The radiation-sensitive mutants were all derived from X1687-101B by sodium nitrite mutagenesis (RESNICK 1969). Spores from all crosses were tested for radiation sensitivity. In every case, whenever a radiation-sensitive spore was selected for analysis it proved to have mutator capacity.

Media: These autoclaved media are those used in most of the experiments; they have evolved from media used by HAWTHORNE and MORTIMER (1960). Complete medium (YEPD) is used for stock maintenance, and for assaying viability: 1% yeast extract, 2% bacto-peptone, 2% glucose, and 2% agar. Minimal medium plus vitamins (MV) is used for characterizing strains and analyzing mutants: 0.67% yeast nitrogen base without amino acids (Difco), 2% glucose, and 2% agar. Synthetic complete medium (SC) is used as a control medium for scoring nutritional phenotypes;

TABLE 1

Genotype and origin of haploid strains

Strain	Genotype								Origin
1403								ura4-11	MAGNI (1969)
KC370	a uvs9-2	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		RESNICK (1969)
KC371	a uvs9-3	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		RESNICK (1969)
KC372	a uxs1-1	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		RESNICK (1969)
KC376	a xrs1-1	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		RESNICK (1969)
KC377	a xrs2-1	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		RESNICK (1969)
KC383	a xrs2-2	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		RESNICK (1969)
KC410-4D	a xrs3-1	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		RESNICK (1969)
KC614-6-16C	α	ade2	arg4-27	his5-2	lys1-1		leu1	thr4-1	RESNICK (1969)
X1687-12B	α	ade2-1	arg4-17	his5-2	lys1-1	trp5-48			GILMORE (1967)
X1687-16C	a	ade2-1	arg4-17	his5-2	lys1-1	trp5-48	leu1-12	met1	GILMORE (1967)
X1687-101B	a	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		RESNICK (1969)
XV103-4B	α	ade2-1	arg4-17	his5-2	lys1-1	trp5-48			XV103
XV103-4C	a xrs1-1	ade2-1	arg4-17	his5-2	lys1-1	trp5-48	leu1-12		XV103
XV103-7D	α xrs1-1	ade2-1	arg4-17	his5-2	lys1-1	trp5-48	leu1-12		XV103
XV104-15C	α			his5-2				ura4-11	XV104
XV104-20A	a xrs1-1			his5-2			leu1-12	ura4-11	XV104
XV108-3D	α	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		XV108
XV108-4A	α uxs1-1	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		XV108
XV108-6A	a uxs1-1	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		XV108
XV108-13B	a	ade2-1	arg4-17	his5-2	lys1-1		leu1-12		XV108
XV108-25C	α uxs1-1	ade2-1	arg4-17	his5-2	lys1-1	trp5-48			XV108
XV108-26C	a uxs1-1	ade2-1	arg4-17	his5-2	lys1-1	trp5-48			XV108
XV130-18A	α	ade2-1	arg4-17					ura4-11	XV130
XV130-21A	α			his5-2			leu1-12	ura4-11	XV130
XV130-17B	a uxs1-1	ade2-1		his5-2				ura4-11	XV130
XV131-5A	a uxs1-1	ade2-1		his5-2	lys1-1			ura4-11	XV131
XV136-21A	α xrs2-1	ade2-1	arg4-17	his5-2	lys1-1	trp5-48			XV136
XV136-21D	a	ade2-1	arg4-17	his5-2	lys1-1				XV136

TABLE 2

Origin of diploid strains

Strain	Origin	Strain	Origin
XV10	X1687-16C	XV117	XV103-4C
	X1687-12B		XV103-7D
XV103	KC376	XV118	KC376
	X1687-12B		X1687-12B
XV104	KC376	XV123	XV103-4C
	1403		XV103-7D
XV108	KC372	XV124	KC372
	X1687-12B		X1687-12B
XV109	XV103-4C	XV125	KC376
	XV103-7D		X1687-12B
XV110	XV108-26C	XV127	X1687-101B
	XV108-25C		XV103-4B
XV112	XV108-13B	XV130	KC372
	XV108-3D		1403
XV113	XV108-6A	XV131	KC372
	XV108-4A		1403
		XV136	KC377
			X1687-12B

it is MV supplemented as follows: adenine, arginine, lysine, histidine, tyrosine, phenylalanine, isoleucine, methionine, tryptophan, and uracil, each at 20 mg/l; serine, 375 mg/l; leucine, 30 mg/l; threonine, 350 mg/l; valine, 75 mg/l. In most of the experiments reported here, valine was not added to the medium, and occasionally neither were tyrosine, phenylalanine, and isoleucine. Omission media are used for studying revertants from auxotrophy to prototrophy and for ascertaining mutant character; it is SC lacking one of the supplements: —TRP, —ARG, —HIS, —LYS, —ADE, for example, indicate respectively SC minus tryptophan, SC minus arginine, SC minus histidine, SC minus lysine, and SC minus adenine. Growth-limiting medium (GL) is SC minus agar with one of the amino acids present in a limiting concentration to restrict the growth of the yeast cells at a titer below saturation. This permits recognition of revertants in our box experiments. Sporulation medium (F) is used for sporulating diploid strains: 2% potassium acetate, 0.1% glucose, 0.25% yeast extract, and 1.5% agar.

Measurement of revertant frequency: For studying revertants from auxotrophy to prototrophy, a synthetic complete medium was used with the relevant nutritional requirement present in limiting concentration to restrict the growth at a titer below saturation level. We call this "limiting medium." A suitable limiting titer with the strains used was 10^5–10^7 cells/ml, depending on the reversion rates of the particular mutant strains. The limiting concentrations of the nutrients were: for lysine 1.0 μg/ml, and for uracil 0.5 μg/ml. Control experiments not reported here showed that these limiting concentrations are not critical. In addition, the mutation rates are the same whether complete medium or minimal medium plus the strain requirements are used.

Limiting medium was inoculated with a cell concentration of 5×10^3 cells/ml. A Brewer Automatic Pipetting Machine, model No. 40 (Baltimore Biological Laboratory) was used to deliver the medium in 1 ml aliquots to 10–12 compartmented culture boxes (the culture boxes, each having 100 compartments, were developed by F. J. DE SERRES, unpublished, and made on special order by Falcon Plastics). The medium was stirred continuously with a magnetic stirrer during delivery. The calibration of the syringe was checked before and after each experiment by delivering 20 squirts into a 25 ml graduate. After being filled, each box was sealed with masking

tape and incubated at room temperature (24–25°C). The boxes were handled carefully to ensure that the contents were not agitated.

The yeast cells settle to the bottom of each compartment, and revertant colonies form there. Up to 20 revertants per compartment can be counted accurately. Note, however, that this information was not used in computing the mutation rates by the method used below.

After the growth of the inoculated cells has reached a plateau on the second day, the revertant colonies begin to appear. These were counted, and the number was recorded on the lid of each box by marking the date and the number per compartment. The counting was repeated daily for at least 12 days.

The frequency of revertants in the original inoculum was determined by plating on solid omission medium at the time the boxes were prepared. At the end of an experiment, the cell titer was determined by counting the cells microscopically in a hemocytometer. For every box of 100 compartments, cells from two compartments (containing no revertants) were counted.

Revertant analysis: In experiments involving the suppressible lysine mutant, revertant colonies were picked and further analyzed by inoculation onto Petri plates containing lysine omission medium. If there was more than one colony in a compartment, only the largest one was picked. These were incubated for 5 days. They were then replica-plated onto complete, synthetic complete, minimal, and omission media for each of the requirements of the particular strain. Thus we determined whether the reversions were at the *lys*1 locus or could be attributed to a super-suppressor. If the revertant grows only on the lysine omission medium, it is considered to be a reversion at the locus. This method gives only a rough approximation of the reversion rate at the mutant locus since some of the revertants may be attributable to suppressors that do not affect the other loci. Nevertheless, the single-requirement revertants still provide a reasonable estimate of locus mutations, comprising fewer than 5% suppressors (MAGNI, unpublished).

Computation of mutation rates: Let N be the number of compartments in an experiment, and N_0 the number of compartments without revertants. From the zeroth term of a Poisson distribution we have

$$e^{-m} = N_0/N, \tag{1}$$

where m equals the average number of mutational events (not mutants) per compartment. Most of these mutational events are due to new mutations arising during the growth of the cells in the limiting medium, but some are due to mutants present in the inoculum. We correct for this "background" by

$$m_g = m - m_b, \tag{2}$$

where m_b is the average number of mutants per compartment in the inoculum (as determined by direct plating), and m_g is the corrected average number, i.e., the mutational events occurring during the growth in the compartments. This can be converted to the mutational events per cell per generation, M, by

$$M = m_g/2C \tag{3}$$

where C is the number of cells per compartment after growth has ceased in the limiting medium. The factor of two in the denominator is necessary because the number of cell generations in the history of a culture is approximately twice the final number of cells. Actually, the proper value for this numerical factor depends upon the point(s) in the cell cycle at which growth is terminated in the limiting medium and also upon the distribution of mutation production over the cell cycle. Since it enters only as a scale factor in all mutation rate calculations, relative mutation rates are unaffected by the value used.

This method for determining mutation rates is due to LURIA and DELBRÜCK (1943). The principal advantage of the method is that the results are not affected by many types of selection. Since we only score the presence or absence of a mutational event in a culture, it is clearly irrelevant whether the mutants grow faster or slower than non-mutants.

In those experiments where the mutants are further analyzed into categories the mutation rate may be partitioned by

$$M_i = f_i M, \tag{4}$$

where M_i is the mutation rate (per cell per generation) for the ith category and f_i is the fraction of the mutants tested which were found to be in the ith category.

RESULTS

Kinetics: The method for measuring mutation rates is based upon the following considerations. A mutant requiring, for instance, lysine is inoculated into a medium containing some lysine but not enough to allow growth to occur to saturation. If at any time during growth of the culture a mutation to lysine independence occurs, the mutant cells will continue to grow after the lysine is exhausted. A large number (about a thousand) of small cultures (about 1 ml each) are started, and we measure the fraction of cultures in which one or more mutations occur.

The kinetics of appearance of mutants is shown in Figure 1. The first mutants appear after 3–4 days of incubation. This is about the time necessary for a single cell to make a colony under the conditions used (i.e. room temperature and anaerobiosis). By 6–8 days most of the mutants have appeared. This is what one would expect for mutations that occur at the end of growth. After this time there is only a slow increase in the number of mutants. We presume that this is due

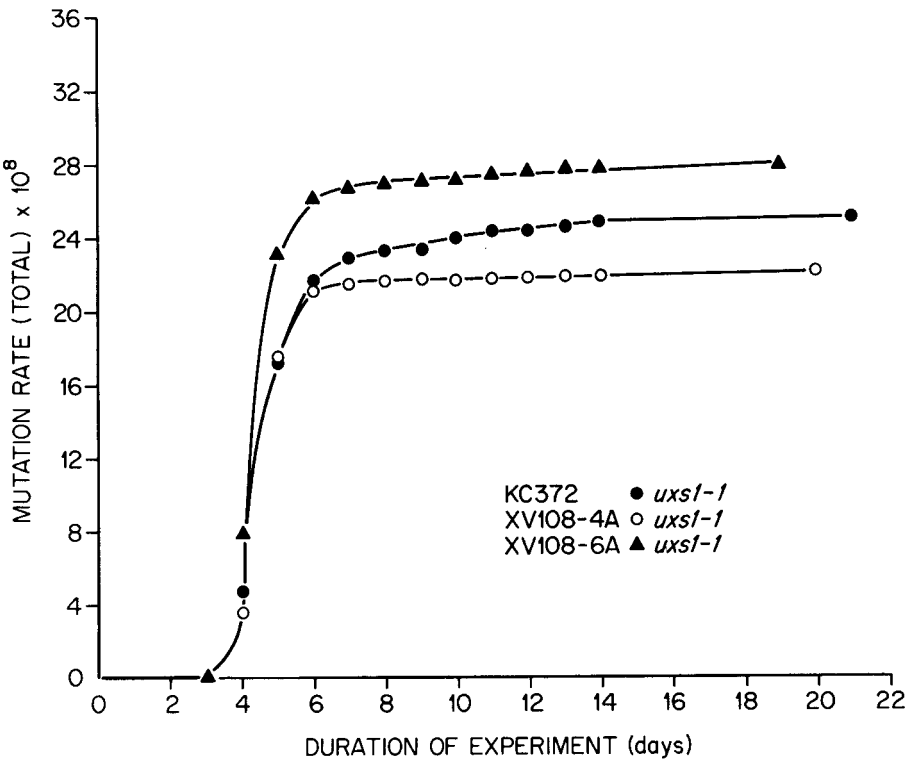

FIGURE 1.—Kinetics of mutant appearance for haploid strains bearing the radiation-sensitive allele *uxs*1–1.

to a small amount of cell division resulting from cell death and reutilization of nutrients. This slow, late rise in mutant frequency is rather variable; in some cases the slope is higher than that shown in Figure 1, and in other cases there is no rise at all.

The growth of some of our strains, particularly the ones carrying the *xrs*1-1 allele, is somewhat slower; in such strains the appearance of mutants is somewhat delayed and the initial slope is less. To allow for this, we have arbitrarily selected the rates calculated for days 8 and 10 as the mutation rates for experiments with diploid and haploid strains, respectively.

Effect of radiosensitivity alleles: The rates of reversion to lysine independence were determined for a number of haploid strains carrying seven radiation-sensitive alleles at five loci. The mutation rate as calculated on the 10th day of incubation is given under the heading "Mutation rates (Total)" in Table 3. The effects of the different alleles vary. The radiosensitivity alleles *uxs*1-1 and *xrs*1-1 confer a greatly increased (five- to sevenfold) spontaneous mutation rate. A moderate increase (twofold) is shown by the allele *xrs*3-1. The remainder of the strains showed no increase in mutation rate; indeed, it seems that the mutation rate is even decreased by some of the radiosensitivity mutations. It should be clear from these data that strains with the same radiosensitivity phenotype may

TABLE 3

Rate of reversion to lysine independence for haploid strains

Strain	Radiosensitivity alleles*	Mutation rates ($\times 10^8$)			Number of revertants	
		Total	Locus	S.S.	Locus	S.S.
KC614-6-16C	—	4.8	0.7	4.1	31	197
X1687-12B	—	4.3	0.5	3.8	16	117
X1687-16C	—	3.6	0.4	3.2	14	113
X1687-101B	—	5.5	0.5	5.0	17	173
XV108-3D	—	4.6	0.3	4.3	15	193
XV136-21D	—	4.5	0.8	3.7	32	139
(Average)	—	4.6	0.5	4.1	..	...
KC372	*uxs*1-1	24.0	0.3	23.7	6	507
XV108-4A	*uxs*1-1	21.7	0.1	21.6	3	490
XV108-6A	*uxs*1-1	27.2	0.2	27.0	5	638
(Average)	*uxs*1-1	24.3	0.2	24.1	..	...
KC376	*xrs*1-1	30.6	1.2	29.4	18	459
XV103-4C	*xrs*1-1	30.9	1.3	29.6	18	489
XV103-7D	*xrs*1-1	36.1	0.9	35.2	14	535
(Average)	*xrs*1-1	32.5	1.1	31.4	..	...
XV136-21A	*xrs*2-1	3.2	0.5	2.7	18	106
XV136-21C	*xrs*2-1	3.0	0.5	2.5	24	126
(Average)	*xrs*2-1	3.1	0.5	2.6	..	...
KC383	*xrs*2-2	3.4	0.7	2.7	20	82
KC410-4D	*xrs*3-1	10.2	0.3	9.9	11	347
KC370	*uvs*9-2	2.0	0.3	1.7	11	52
KC371	*uvs*9-3	4.7	0.5	4.2	24	207

* The following alleles have been renamed: *uvs*9-2 = *rad*2-16; *uvs*9-3 = *rad*2-17; *uxs*1-1 = *rad*18-2 (GAME and COX, 1971).

show vastly different spontaneous mutation rates. But some of the strains containing radiation sensitivity genes are indeed mutators.

Nature of the revertants: The reversion to lysine independence in these strains can occur by two different mechanisms. There can be a reversion at the *lys*1 locus itself (which may or may not be a true back mutation), or there can be a forward mutation at any one of a large number of suppressor loci. These suppressors, called supersuppressors, will in general suppress other nonsense mutants included in the genetic makeup of the strains we have used. Revertants from the above experiments were picked and tested to see whether they had lost nutritional requirements other than that for lysine. In almost every case, a revertant which had lost another requirement had lost the requirements for adenine, arginine, histidine, and tryptophan simultaneously. That is, the *lys*1-1 suppressors were almost exclusively Class I suppressors according to the scheme of GILMORE (1967) and HAWTHORNE and MORTIMER (1968). The results of this analysis are shown in Table 3 under the headings "Mutation rates (Locus, S.S.)," where S.S. signifies super-suppressor.

We note first that only about 10% of the reversions in control strains are at the *lys*1 locus. This means that the locus mutation rate is calculated from small numbers, and is hence subject to a rather large experimental error. The increase in mutation rate due to *uxs*1-1 is seen to be due solely to an increase in the super-suppressor rate. In fact the mutation rate at the locus appears to have dropped somewhat. We cannot exclude, however, that this drop is not an artifact due to the extremely unfavorable ratio of super-suppressor to locus mutants. In the experiments with *uxs*1-1 more than half of the compartments (i.e. cultures) had at least one mutant colony. Only one colony from each compartment was picked. If there was any slight selection in favor of picking a super-suppressor revertant, or if some fraction of the locus mutants were contaminated with super-suppressor revertants from the same culture, some of the locus mutations would be missed. For this reason we are not certain whether *uxs*1-1 affects the spontaneous mutation rate at the locus; in any event it does not increase it.

The X ray-sensitive mutation *xrs*1-1 also confers a greatly increased super-suppressor reversion rate. With this sensitivity allele there is also an increase in the reversion rate at the *lys*1 locus, although this increase appears to be proportionately less than that for the super-suppressors. Additional experiments not reported here were carried out with the super-suppressible alleles *his*5-2 and *arg*4-17, and with the same results. However, the same possibility for artifacts exists here as with the *uxs*1-1 experiments, so we cannot be certain that there is a large differential increase caused by *xrs*1-1.

The data for the remaining radiosensitivity mutants are sparser. Nevertheless, the changes, both increases and decreases noted above, for the total reversion rate are reflected in the super-suppressor rates.

We conclude that our mutator genes affect the super-suppressor loci. Furthermore, in at least one case, they affect the suppressor loci and the suppressible locus differentially. In the discussion we shall argue that these two facts mean that we have a mutator gene which causes addition-deletion mutations.

TABLE 4

Rate of reversion to lysine independence for diploid strains

Strain	Radiosensitivity alleles	Mutation rates ($\times 10^8$)			Number of revertants	
		Total	Locus	S.S.	Locus	S.S.
XV10	—	5.7	0.2	5.4	4	93
XV112	—	5.8	0.8	5.0	21	125
XV127	—	4.9	0.1	4.8	3	118
(Average)	—	5.5	0.4	5.1	..	...
XV108	*uxs*1-1/+	6.7	0.6	6.1	12	113
XV124	*uxs*1-1/+	3.4	0.5	2.9	8	51
(Average)	*uxs*1-1/+	5.0	0.6	4.5	..	...
XV110	*uxs*1-1/*uxs*1-1	17.8	0.2	17.6	3	233
XV113	*uxs*1-1/*uxs*1-1	19.7	0.6	19.1	9	305
(Average)	*uxs*1-1/*uxs*1-1	18.7	0.4	18.3	..	...
XV118	*xrs*1-1/+	3.5	0.4	3.2	10	89
XV125	*xrs*1-1/+	4.3	0.1	4.2	2	79
(Average)	*xrs*1-1/+	3.9	0.2	3.7	..	...
XV117	*xrs*1-1/*xrs*1-1	27.2	0.8	26.4	15	477
XV123	*xrs*1-1/*xrs*1-1	39.2	0.6	38.6	4	277
(Average)	*xrs*1-1/*xrs*1-1	33.2	0.7	32.5	..	...

Dominance relations: For the two mutators showing the greatest effect, heterozygotic and homozygotic diploid strains were constructed. The rates for reversion to lysine independence were measured for these strains as well as for diploid control strains (Table 4). In both cases the reversion rates for heterozygotes were substantially the same as the control rates, whereas the reversion rate for the homozygotes was very much larger (three- to sixfold). Thus the mutator activities of both *uxs*1-1 and *xrs*1-1 are recessive.

It is also interesting to compare the homozygous diploids with corresponding haploids. Naively, one would expect a mutation rate for a diploid strain to be twice that of a haploid strain when expressed as mutations/cell/generation, because the diploid contains twice as many genomes/cell. A comparison of the results in Table 3 with those in Table 4 shows that this is not the case. In other words, the number of genomes is not the limiting factor in the cellular mutation rate in these strains.

Cytoplasmic petites: It was accidentally observed that the cytoplasmic petite character has mutator activity. The results are presented in Table 5. The total

TABLE 5

Rate of reversion to lysine independence in cytoplasmic petite [rho⁻] strains

Strain	Radiosensitivity alleles	Mutation rates ($\times 10^8$)			Number of revertants	
		Total	Locus	S.S.	Locus	S.S.
X1687-101B[rho⁻]	—	7.1	2.1	5.0	99	232
KC377 [rho⁻]	*xrs*2-1	5.1	1.8	3.3	108	195

mutation rate is scarcely different from the control rates of Table 3. The mutation rates for the *lys*1-1 locus, however, are several times higher.

Reversion of a frameshift mutant: According to MAGNI (1969), *ura*4-11 is a frameshift mutant. This mutation was induced and characterized in yeast strains which are only distantly related to the ones carrying our mutators. In crossing these strains to construct the proper genotypes for our experiments, we discovered that there were factors segregating that influenced the uracil revertant frequency. These factors do not influence radiation sensitivity. We report here (Table 6) only the results with those strains that we believe are free of these extraneous factors.

It can be seen from Table 6 that both of our strong mutators *uxs*1-1 and *xrs*1-1 enhance the rate of reversion of *ura*4-11. The factor of enhancement is in fact considerably greater than that for the *lys*1-1 suppressor (Table 3). For another putative frameshift mutant, *thr*3-10 (MAGNI 1969), we have found a greatly increased reversion index in strains carrying *xrs*1-1.

DISCUSSION

We have demonstrated that the *uxs*1-1 strain is associated with a mutator, and that it specifically acts on super-suppressor genes and not on a super-suppressible locus. An argument has been presented by MAGNI, VON BORSTEL and STEINBERG (1966) that enhancement in mutation rates of super-suppressor genes during meiosis without concurrent enhancement of mutation rate of the super-suppressible locus indicates that the former is the result of mutation through the addition or deletion of a base or bases in the DNA, since super-suppressible mutations have been shown to be of the base-substitution type. This does not exclude the possibility that super-suppressors may mutate by base-substitutions. However, if the super-suppressible mutant does not also revert at a higher rate, then the enhancement of the mutation rate of the super-suppressor is most likely due to some other type of mutational event, e.g., either an addition or a deletion of a

TABLE 6

Rate of reversion to uracil independence

Strain	Radiosensitivity allele	Mutation rate ($\times 10^8$)	Number of revertants
XV104-15C	—	0.06	17
XV104-15C	—	0.03	15
XV130-18A	—	0.03	6
XV130-21A	—	0.01	2
(Average)	—	0.03	..
XV130-17B	*uxs*1-1	0.30	25
XV131-5A	*uxs*1-1	0.41	25
(Average)	*uxs*1-1	0.36	..
XV104-20A	*xrs*1-1	0.96	92
XV104-20A	*xrs*1-1	0.84	80
(Average)	*xrs*1-1	0.90	..

base. It is self-evident that nonsense mutants could revert to sense or missense either by transition or by transversions; in any case, a variety of base substitutions are possible which could restore a gene product to a functional state. In addition, the mutator associated with *uxs*1-1 enhances the reversion rate of *ura*4-11, a frameshift mutant as defined by the meiotic effect. Hence, it is our interpretation that the mutator associated with the radiation-sensitive mutant *uxs*1-1 is an addition-deletion mutator.

Although the supporting data are much sparser, and the mutator effect is somewhat weaker, essentially the same conclusions can be drawn for the X ray-sensitive mutant strain *xrs*3-1.

The X ray-sensitive mutant strain *xrs*1-1 behaves the same, except for one important point. It enhances reversion at the super-suppressible locus as well. Thus it seems likely that the mutator associated with *xrs*1-1 can stimulate both base-substitution and addition-deletion mutations.

Both of the mutators that we have characterized in this manner, and are associated with *uxs*1-1 and *xrs*1-1, are recessive; that is, the mutator activity is not detectable in the heterozygote. This suggest that the mutator activity results from the loss of a function rather than from the alteration of a function; it is rather likely that the functional alteration of an enzyme would result in partial dominance.

Several investigators have suggested that certain mutator genes may be mutants of genes that encode enzymes which repair damage incurred from exposure to radiation. It is reasonable to assume that these or similar mechanisms are involved in repairing potential mutations arising spontaneously. A bit of caution in this regard is suggested by our results with the cytoplasmic petities [rho$^-$]. Two independently arisen [rho$^-$] strains showed mutator activity—in these cases, base-substitution mutator activity. The [rho$^-$] mutator must act in an indirect manner. There is no evidence that the mutators in the radiation-sensitive strains act otherwise.

SUMMARY

Spontaneous mutation rates were measured in several radiation-sensitive and "wild-type" strains of yeast. The method used for measuring the rates is by a 1000-compartment fluctuation test where growth is eventually stopped by limitation of one of the genetically controlled requirements; revertant colonies form in liquid medium in the bottom of each compartment. The kinetics of mutant appearance was determined; it was seen that after their appearing over a three- or four-day interval, mutants appear only infrequently in older cultures. It was found that three out of seven radiation-sensitive alleles at five loci are associated with enhanced mutation rates. Two of these radiation-sensitive mutants (*uxs*1-1 and *xrs*3-1) confer enhanced mutation rates in Class I super-suppressors but not at the suppressible (nonsense) locus; the other radiation-sensitive mutant (*xrs*1-1) enhances the rate of mutation at both the nonsense locus and the suppressors, but much more in the latter. The radiation-sensitive mutant *uvs*9-2 depresses the mutation rate for the suppressors. The mutators associated with

*uxs*1-1 and *xrs*1-1 are both recessive. Also these two mutators enhance mutation rates for the frameshift mutant *ura*4-11. We argue that the mutators act by affecting the addition or deletion of bases in DNA. Two separately derived cytoplasmic petites [rho$^-$] are base-substitution mutators.

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