THE ORIGIN OF SPONTANEOUS MUTATION IN *SACCHAROMYCES CEREVISIAE*

SIEW-KEEN QUAH, R. C. VON BORSTEL AND P. J. HASTINGS

Department of Genetics, The University of Alberta, Edmonton, Alberta, Canada T6G 2E9

Manuscript received July 2, 1980
Revised copy received October 20, 1980

ABSTRACT

Characterization of two antimutator loci in yeast shows that both are members of the same mutagenic repair system known to be responsible for almost all induced mutation (LAWRENCE and CHRISTENSEN 1976, 1979a,b; PRAKASH 1976). One of the these newly isolated antimutator mutations is an allele of *rev3* (LEMONTT 1971b). Two other alleles of *rev3* were tested and were also found to be antimutators. Double mutants carrying *rev3* and mutator mutations of *rad3*, *rad51* or *rad18* are like *rev3* single mutants with respect to spontaneous mutation rate, supporting the hypothesis (HASTINGS, QUAH and VON BORSTEL 1976) that many mutators in yeast act by channelling spontaneous lesions from accurate to mutagenic repair. However, the enhanced mutation rate seen in a radiation-resistant mutator mutant *mut1* is not dependent on *REV3*, but is dependent on another gene designated *ANT1*. An additive effect on the reduction in spontaneous mutation, seen in the *ant1 rev3* double-mutant strain, leads to the conclusion that at least 90% of spontaneous mutations seen in the wild type are caused by mutagenic repair of spontaneous lesions.

WHEN strains of yeast having enhanced mutation rates were isolated, it was found that a minimum of 10 genetic loci were responsible for the effect (HASTING, QUAH and VON BORSTEL 1976; ORD 1980). Most of these mutant strains are sensitive to mutagenic agents such as ultraviolet radiation (UV), gamma radiation and methyl methanesulfonate (MMS). This pleiotropic effect of mutator phenotype and sensitivity to mutagens is also observed among many of the repair-defective *rad* mutants. Of the 17 *rad* mutants examined, 10 are mutators (BRYCHCY 1974; BRYCHCY and VON BORSTEL 1977; HASTINGS, QUAH and VON BORSTEL 1976; VON BORSTEL, CAIN and STEINBERG 1971; S.-K. QUAH, unpublished results). Although none of the gene products of these mutator loci is known, it is unlikely that misreplication resulting from altered polymerases can account for such a large number of mutator loci in yeast.

It is evident from the study of induced mutagenesis in yeast that radiation-sensitive mutants show induced mutation frequencies that differ from those found in wild-type strains because the mutations cause changes in the proportions of lesions that are channelled into various repair processes. Some of these repair processes are mutagenic, others are not (LAWRENCE and CHRISTENSEN 1976; LEMONTT 1971a). That these same radiation-sensitive mutants affect the

rate of occurrence of spontaneous mutations strongly suggests that spontaneous lesions are repaired by systems having many steps in common with the systems repairing induced lesions. In other words, enhanced spontaneous mutation rates seen in mutator strains can be explained by the channelling of spontaneous lesions into mutagenic (error-prone) repair (HASTINGS, QUAH and VON BORSTEL 1976). This hypothesis leads to the prediction that antimutator strains that have lost mutagenic repair capacity could be isolated and that they will be deficient in repair. Strains defective in UV-induced mutagenesis have been isolated and analyzed (LAWRENCE and CHRISTENSEN 1976; LAWRENCE *et al.* 1974; LEMONTT 1971b; PRAKASH, HINKLE and PRAKASH 1979). Results from these studies suggest that error-prone repair occurs in yeast. We have attempted to show from the properties of antimutator mutants described in this report that at least one of the error-prone repair processes involved in repair of radiation-induced lesions is also available to spontaneous lesions and is the cause of the enhanced spontaneous mutation rate of some mutator strains.

The isolation of antimutator mutants that are involved in repair and affect the rate of spontaneous mutation allowed us to show that most spontaneous mutations in wild-type yeast also arise from mutagenic repair of spontaneous lesions.

MATERIALS AND METHODS

Yeast strains: Strains used in this study are presented in the text and in Table 1.

Media: The following media were used: YEPD medium (2% Difco peptone, 1% Difco yeast extract, 2% dextrose), YEPG medium (2% Difco peptone, 1% Difco yeast extract, 3% glycerol), FS or sporulation medium (2% potassium acetate, 0.1% glucose, 0.25% yeast extract, supplemented with the amino acids used in MC medium), synthetic complete or MC medium (0.67% Difco yeast nitrogen base without amino acids, 2% glucose, supplemented with amino acids as indicated in VON BORSTEL, CAIN and STEINBERG 1971). Growth-limiting medium is MC medium with lysine or histidine in a limiting concentration as indicated in the section on the box test for measuring spontaneous mutation rates. For the limiting lysine experiments, 5 μg/ml instead of 20 μg/ml was used. Solid MC medium was supplemented with 2% agar. Solid YEPD, YEPG and SF media were supplemented with 1.5% agar.

The mutational systems: The mutational systems required that all the strains used carry a minimum of two nonsense mutations, *lys1-1* and *ade2-1*, and a missense mutation, *his1-7*. Two other nonsense mutations, *arg4-17* and *trp5-48*, were often included but were not essential, except in a few cases where the *arg4-17* reversion rates were measured. Both *lys1-1* and *arg4-17* respond to nonsense suppressors that insert tyrosine at ochre codons. These forward mutations to suppressors (SS) can be distinguished from reversions at the nonsense site (locus) by virtue of their loss of red pigmentation due to suppression of the *ade2-1* mutation. These two mutational systems within a *lys1-1* reversions system were adopted by SCHULLER and VON BORSTEL (1974) in a 1,000-compartment fluctuation test (or box test) to measure spontaneous mutation rates. The third mutational system used involved the reversion of the missense mutation *his1-7* from histidine dependence to independence.

UV sensitivity test: Stationary phase cells grown on YEPD plates were harvested and suspended in 5 ml of M/15 KH_2PO_4, pH 4.5. Appropriate dilutions mere made and plated on YEPD. The plates were irradiated using a General Electric G30T8 germicidal lamp in a darkened room. A Laterjet UV meter was used for dosimetry.

Gamma radiation sensitivity test: Cells were prepared in the same way as described for UV radiation. A ^{60}Co Gamma-Cell 220, from Atomic Energy of Canada Ltd., was used.

TABLE 1

Strain numbers and genotypes

Diploid number	Parental haploid strains		Genotype
YO191	YO176–6D	a	*rev3–1 HIS+ HOM+*
	YO600–14C	α	
YO192	YO177–5C	a	*rev3–1 LYS+ ADE+*
	YO600–14C	α	
YO326	X1687–16C–10	a	*rev3–3 his5–2 HIS1+ HOM3+ leu2–10 met1–1*
	YO300–1B	α	
YO102	YO95–1B	a	*rev3–15(ant2) ARG+ trp?*
	YO300–1C	α	
YO414	E004–6D	a	*rad3–12*
	YO102–4B	α	*rev3–15 TRP+*
YO415	E004–6D	a	*rad3–12*
	YO192–3B	α	*rev3–1 TRP+*
YO418	E004–6D	a	*rad3–12*
	YO300–1C	α	
YO425	YO102–1C	a	*rev3–15 trp?*
	YO318–1A	α	
YO436	YO102–1C	a	*rev3–15 trp?*
	XV407–19B	α	*rad51(mut5–1)*
YO294	YO300–2C	a	
	rs4	α	*rad18–3* no auxotrophic marker
YO424	YO294–4A	a	*rad18–3 TRP+*
	YO102–4B	α	*rev3–15 TRP+*
YO417	YO294–4A	a	*rad18–3*
	YO192–3B	α	*rev3–1 TRP+*
YO189	YO800–1C	a	*mut1–1*
	YO102–1B	α	*rev3–15 ARG+ trp?*
YO43	XV803–4A	a	*ARG+ TRP+ HOM+ leu1–12*
	289	α	*mut1–1 ant1*
YO133	YO43–9A	a	*mut1–1 ant1 ARG+ TRP+ HOM+*
	YO300–1C	α	
YO75	YO43–8B	a	*ant1 ARG+ TRP+ HOM+ leu1–12*
	YO42–2A	α	*ARG+ TRP+ HOM+*

Unless noted otherwise, all strains carry *lys1–1, ade2–1, arg4–17, trp5–48, his1–7* and *hom3–10.*

Methyl methanesulfonate sensitivity test: Stationary-phase cells at a density of 5×10^6 in M/15 KH_2PO_4 buffer were spotted on YEPD medium supplemented with 0.035% MMS prepared as described by PRAKASH and PRAKASH (1977). The MMS was obtained from Terochem Laboratories Ltd., Edmonton.

Measuring spontaneous mutation rates (the lassie test): For a detailed description of this method, see VON BORSTEL (1978). This is a simple reversion test on solid MC medium with either lysine (10 or 20 μg/ml) or histidine (1 or 2 μg/ml) as a growth-limiting factor. Revertant colonies appeared on a thin lawn of auxotrophic cells and were counted after 10 days at 27°.

Measuring spontaneous mutation rates (the 10-tube fluctuation test): Five ml of liquid YEPD for each strain was placed in each of 10 test tubes. A 3-day-old whole colony from a YEPD plate was inoculated into each tube. These cultures were grown for 3 days on a shaker. The cells were then washed and counted. One ml from each culture was plated on 2 plates of MC—lysine

and 2 plates of MC — histidine. Revertants were counted after 5 days. This procedure was later simplified. Ten 5-day-old whole colonies grown on YEPD at a density of not more than 30 colonies per plate were separately suspended into 10 test tubes, each containing 2 ml of phosphate buffer. Cells were counted, and 1 ml from each tube was plated on one kind of omission medium. Ten colonies were plated for each reversion system. The number of revertants (per 10^8 cells) per culture were ranked and the median was used to compare the strains. This test is used qualitatively, mainly to show that reduced spontaneous mutation rates exhibited by the presumptive antimutator strains can be confirmed in a nonselective medium, in this case YEPD.

Measuring spontaneous mutation rates (the box test): This method of measuring spontaneous mutation rates has been described by SCHULLER and VON BORSTEL (1974), VON BORSTEL (1978) and VON BORSTEL, CAIN and STEINBERG (1971). It is essentially a 1,000-compartment lassie test, done in MC liquid medium. Each compartment contains a 1 ml culture. Depending upon the experiment, the concentrations of lysine used were 0.5, 1, 1.5 or 2 μg/ml, and histidine was at a concentration of either 0.2 or 0.3 μg/ml. Mutation rates were estimated from the proportion of compartments without revertants.

The isolation of ant1: Stationary phase cells from strain YO800–6C (*α mut1–1 lys1–1 ade2–1 his1–7 hom3–10*) were mutagenized by ethyl methanesulfonate (Eastman Kodak) following a modification of LINDEGREN's method (LINDEGREN *et al.* 1965) that was described by VON BORSTEL *et al* (1973). The mutant-screening protocol is essentially the same. Mutagenized cells were plated on YEPG (100 to 200 colonies per plate) and were incubated for 4 days. Each plate was then replica-plated onto a limiting lysine (10 μg/ml) and a YEPD second-master plate. Colonies that did not papillate as much as the *mut1–1* control strain on limiting lysine medium after 1 week were then subjected to a lassie test. About 10^6 cells from each clone were plated on a limiting lysine (10 μg/ml) plate. Revertants were counted after 10 days. Clones that showed counts lower than the *mut1–1* control were subjected to 2 other tests, *i.e.*: (1) the 10-tube fluctuation test with YEPD, and (2) the box test. Clone 289 isolated in this manner was found to show sensitivity to UV radiation. When outcrossed to the wild-type strain XV803–4A (**a** *lys1–1 ade2–1 his1–7 hom3–10*), tetratype tetrads were recovered suggesting that a second mutation, designated *ant1*, had been induced in clone 289.

The isolation of ant2 (= rev3–15): The *ant2* mutation was isolated in a haploid strain, XV846–10A (**a** *lys1–1 ade2–1 trp1–1 leu2–1 his1–7 hom3–10*), by VON BORSTEL and LYNCH (1978) in an experiment designed to determine the relative frequency of mutator and antimutator mutations induced by gamma radiation. Stationary-phase cells suspended in M/15 KH_2PO_4 phosphate buffer, pH4.5, were exposed to 10 Krad of ^{60}Co. Mutagenized cells were diluted and plated on YEPG. All plates were incubated at 30° for 3 days. Surviving colonies were subjected to the lassie test, using 2 μg/ml of histidine. Clone B5496, showing a sensitivity to UV and a reduced spontaneous reversion rate for *his1–7*, was confirmed as an antimutator by a 10-tube fluctuation test with YEPD and a box test. The B5496 mutation designated *ant2* was outcrossed to the wild-type strain XV185–6A (*α lys1–1 ade2–1 arg4–17 trp5–48 his1–7 hom3–10*). From this cross, the meiotic segregant YO95–1B, sensitive to UV, was used to expand the stock cultures carrying this antimutator mutation (see Table 1).

Construction of double mutants: The *rad3–12* and *rad6–1* mutations were isolated by COX and PARRY (1968). The *rev3–1* and *rev3–3* mutations were isolated by LEMONTT (1971b). The *rad18–3* (formerly *rs4* mutation was isolated by SNOW (1967). The *rad51* (formerly *mut5–1*) mutation (MORRISON and HASTINGS 1979) and the *mut1–1* mutation (VON BORSTEL *et al.* 1973) were isolated in our laboratory. Haploid strains used in these experiments were constructed by sporulating diploid strains whose genotypes relevant to this study are given in Table 1. Standard tetrad analysis procedures of yeast genetics were used throughout. Phenotypes of the spore cultures were determined by UV and gamma-ray sensitivity tests and by the lassie test. Single and double mutants were confirmed by backcrosses to tester strains. Wild-type strains referred to as *RAD+*, *REV+*, or *ANT+* in Table 1 are related to, but not necessarily isogenic with, XV185–14C and XV185–6A. We used more than one control strain from a cross or related crosses to overcome the lack of isogeny.

RESULTS

The antimutator mutation *ant2* was induced by gamma radiation and was found to cause reduced *his1–7* reversion. Further characterization showed that strains carrying this mutation are UV and MMS sensitive, but do not appear to be gamma-ray sensitive. Complementation, using UV survival, was found in diploids heterozygous for *ant2* and each of the following mutants: *ant1*, *rev1*, *rad6*, *rad9* and *rad18*, but, as shown in Figure 1, not *rev3*. Furthermore, 15 tetrads dissected from an *ant2/rev3* diploid gave no UV-resistant recombinant. UV-induced reversion of *his1–7*, *lys1–1* and *arg4–17* was found to be lower in *ant2* than in the wild-type strains (data not shown). We concluded that *ant2* is an allele of *rev3* and therefore shall be referred to as *rev3–15*. LEMONTT (1971b) isolated 14 *rev3* alleles by screening for resistance to UV-induced reversion.

The antimutator activity of strains carrying the new *rev3–15* allele was compared to those of *rev3–1* and *rev3–3* isolated by LEMONTT (1971b). It can be seen in Table 2 that all three *rev3* alleles significantly reduce *lys1–1*, *arg4–17* and *his1–7* reversion rates. Suppressor mutation rates appeared to be slightly reduced; a clearer reduction is observed in subsequent experiments.

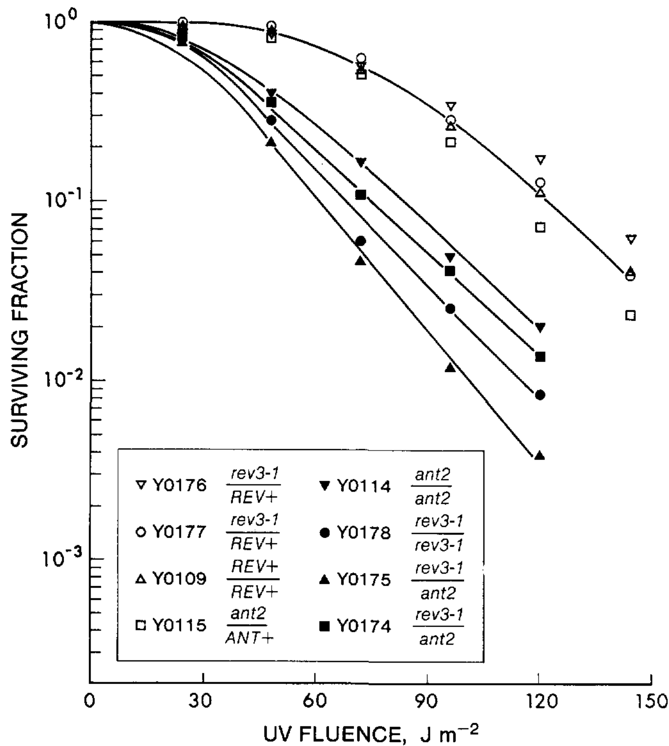

FIGURE 1.—UV survival curves of eight diploid strains showing that *ant2* does not complement *rev 3–1*.

TABLE 2

Antimutator activity of three rev3 *alleles*

Mutation	Strain	*lys1-1*						*his1-7*				*arg4-17*					
		Cells/comp. ($\times 10^{-6}$)	No. of comps.	No. of mutant compartments		Mutation rate ($\times 10^{8}$)		Cells/comp. ($\times 10^{-6}$)	No. of comps.	No. of mutant comps.	Mutation rate ($\times 10^{8}$)	Cells/comp. ($\times 10^{-6}$)	No. of comps.	No. of mutant compartments		Mutation rate ($\times 10^{8}$)	
				SS	Locus	SS	Locus							SS	Locus	SS	Locus
rev3-1	YO191-1C	3.66	898	48	3	0.75	0.05	1.84	987	46	1.29	4.72	986	89	2	1.00	0.02
	YO191-2D	4.07	1000	46	3	0.58	0.04	2.63	1000	167	3.47	—	—	—	—	—	—
	YO191-2C	4.23	996	52	4	0.63	0.05	1.57	999	24	0.75	—	—	—	—	—	—
	YO191-3C	2.81	999	47	0	0.86	NR*	2.74	1000	20	0.68	—	—	—	—	—	—
	YO192-3B	3.42	1000	93	4	1.43	0.06	2.44	998	117	2.56	5.41	995	120	4	1.19	0.04
	YO192-4D	4.63	999	109	7	1.25	0.08	2.60	1000	164	3.43	—	—	—	—	—	—
rev3-3	YO326-6C	2.34	1000	51	5	1.12	0.11	1.98	1000	131	3.54	4.55	895	160	2	2.17	0.02
	YO326-9B	2.15	1000	16	2	0.38	0.05	2.79	996	98	1.86	4.85	999	133	5	1.47	0.05
	YO326-10A	2.41	1000	34	4	0.72	0.08	2.22	998	98	2.25	4.59	995	141	3	1.66	0.03
rev3-15	YO102-1B	3.95	999	193	4	2.72	0.05	1.22	959	27	1.17	—	—	—	—	—	—
	YO102-1C	3.66	997	148	2	2.20	0.03	1.21	981	28	1.20	4.86	1003	139	6	1.54	0.06
	YO102-3C	4.75	1000	83	8	0.91	0.08	2.47	1000	100	2.12	4.21	996	164	2	2.14	0.02
	YO102-4B	4.23	1000	92	3	1.11	0.04	2.48	921	79	1.66	4.88	999	177	3	2.00	0.03
Wild type	YO191-1B	3.32	1000	64	28	1.00	0.43	1.92	997	249	7.49	—	—	—	—	—	—
	YO191-4B	3.58	997	120	12	1.79	0.17	1.87	997	160	4.63	—	—	—	—	—	—
	YO192-1B	4.14	1000	195	29	2.62	0.36	1.95	999	336	10.51	—	—	—	—	—	—
	YO192-4C	4.15	998	180	24	2.40	0.29	2.44	994	371	9.56	—	—	—	—	—	—
	YO326-2B	4.58	999	128	34	1.50	0.38	2.59	998	393	9.61	5.52	997	177	16	1.77	0.15
	YO326-3B	4.12	999	116	37	1.50	0.46	2.49	1000	373	9.37	2.71	900	26	11	0.54	0.23
	YO326-9A	4.39	1000	138	22	1.69	0.25	3.04	997	410	8.64	5.15	986	227	12	2.54	0.12
	YO326-10B	2.99	978	105	36	1.90	0.63	2.50	998	343	8.38	5.13	998	238	9	2.65	0.09
	YO102-1A	4.52	1000	117	32	1.38	0.36	1.10	996	193	9.80	—	—	—	—	—	—
	YO102-1D	3.82	1000	135	34	1.90	0.45	2.23	999	211	5.32	5.14	997	214	17	2.35	0.17
	YO102-3A	2.44	1000	62	10	1.31	0.21	—	—	—	—	—	—	—	—	—	—
	YO102-3B	3.84	997	133	15	1.82	0.20	1.92	1000	176	5.05	4.77	696	137	7	2.30	0.11

In order to test our hypothesis that radiation-sensitive mutator mutants derived their mutator phenotype from channeling of spontaneous lesions into mutagenic repair, we constructed strains doubly mutant at two genetic loci: a radiation-sensitive mutator mutation resulting in a block in error-free repair and a *rev3* mutation as a second block in mutagenic repair. The evidence for or against our hypothesis lies in the phenotype of such double mutants with respect to spontaneous mutation. The three radiation-sensitive mutator mutants chosen for this study represent three major epistasis groups as defined by UV- and gamma-radiation survival interaction in double mutants (BRENDEL and HAYNES 1973; COX and GAME 1974; GAME and COX 1972, 1973; GAME and MORTIMER 1974; KHAN, BRENDEL and HAYNES 1970; LAWRENCE and CHRISTENSEN 1976).

(1) Excision defective mutants in yeast (GAME and COX 1972; PRAKASH 1977a,b; PRAKASH and PRAKASH 1979; RESNICK and SETLOW 1972; REYNOLDS 1978; UNRAU, WHEATCROFT and COX 1971; WATERS and MOUSTACCHI 1974) are mainly UV sensitive and show enhanced UV-induced mutation frequencies (AVERBECK *et al.* 1970; ECKHARDT and HAYNES 1977; LAWRENCE and CHRISTENSEN 1976; LAWRENCE *et al.* 1974; MOUSTACCHI 1969; RESNICK 1969a; ZAKHAROV, KOZINA and FEDOROVA 1970). This is consistent with the idea that excision repair is largely error-free (LAWRENCE and CHRISTENSEN 1976). Studies of double mutants with regard to UV-induced mutagenesis showed that mutants defective in mutagenic repair are epistatic to mutants defective in excision repair (LAWRENCE and CHRISTENSEN 1976; LEMONTT 1971b). Only the *rad3* mutant shows the mutator phenotype; the other excisionless mutants tested (*rad1*, *rad2*, *rad4* and *rad10*) are normal (BRYCHCY and VON BORSTEL 1977).

(2) *rad51* (COX and GAME 1974; MORRISON and HASTINGS 1979) is one of many genes in yeast that are concerned mainly with the repair of ionizing radiation damage and only to a small extent with the repair of UV damage (GAME and COX 1973; GAME and MORTIMER 1974; HO 1975; RESNICK 1969b). The process is thought to be error-free for the repair of damage induced by UV and gamma radiation (LAWRENCE and CHRISTENSEN 1976; MCKEE and LAWRENCE 1979a), but mutagenic in the repair of damage induced by ethyl methanesulfonate (PRAKASH 1976). Five of seven X-ray-sensitive mutants belonging to the *RAD51* epistasis group are mutators (HASTINGS, QUAH and VON BORSTEL 1976; VON BORSTEL, CAIN and STEINBERG 1971; S.-K. QUAH, unpublished results).

(3) The *RAD6* epistasis group consists of more than a dozen mutants (LAWRENCE 1976, 1979a,b), some of whose functions are implicated in mutagenic repair in induced mutagenesis; the *rad18* mutant is a member of this group (LAWRENCE and CHRISTENSEN 1976). Its mutator phenotype (VON BORSTEL, CAIN and STEINBERG 1971; and this report) tends to suggest that its gene product is concerned with error-free repair (LAWRENCE and CHRISTENSEN 1979a,b). UV-induced mutagenesis is more often normal than defective in *rad18* strains (LAWRENCE and CHRISTENSEN 1979a), and these strains showed somewhat elevated revertibility with nitrous acid (PRAKASH 1976).

Each of the mutator strains—*rad3*, *rad51* and *rad18* — was crossed to a *rev3* mutant. Spontaneous mutation rates of double mutants were compared to those of the single mutants and the wild-type control strain. The data are presented in Table 3. Table 4 gives the mean values and standard errors for each genotype. The results show that the *rev3* mutation is epistatic to all three radiation-sensitive mutations with respect to the rate of spontaneous mutation. The *lys1–1* reversion rates of all three types of double mutants are characteristic of those seen in *rev3* single mutants. The *his1–7* reversion rates are intermediate between those of *rev3* single mutants and wild-type strains. Spontaneous mutation rates to suppression seem to vary according to genotype. The double mutants involving either *rad3* or *rad51* show lower mutation rates than those of the *rev3* single mutants. The *rad18 rev3* double mutants, in contrast to *rad3 rev3* and *rad51 rev3*, show mutation rates to suppression more like those of the wild-type strains.

In view of the existence of a class of mutator mutants that are radiation resistant (HASTINGS, QUAH and VON BORSTEL 1976), such as *mut1* (GOTTLIEB and VON BORSTEL 1976; VON BORSTEL *et al.* 1973), it might be argued that there are other ways for a cell to regulate spontaneous mutation rates. In the absence of information as to whether the *mut1* mutation causes lesions or accumulates lesions because of defective repair, it is nevertheless likely that the *REV3* gene is required to process these lesions. We investigated this possibility by constructing *mut1 rev3* double mutants; Table 5 shows that mutator activity is still present in the double mutants. Unlike other mutator strains, the *mut1* mutation often reduced the *lys1–1* locus reversion rates below those of the wild type (GOTTLIEB and VON BORSTEL 1976; VON BORSTEL *et al.* 1973; S.-K. QUAH, unpublished results). Results presented in Table 5 tend to suggest that this antimutator activity of the *mut1* mutation might interact additively with the antimutator effect of the *rev3* mutation.

The observation that *mut1* mutator activity is independent of *REV3* gene function can be interpreted to mean that another mutagenic process repairs spontaneous lesions in *mut1* strains. To better understand *mut1*-mediated enhanced spontaneous mutagenesis, an antimutator mutation was induced in a *mut1* background. This second mutation, *ant1*, confers UV, but not gamma-ray, sensitivity upon the strain; it segregates independently from the *mut1* mutation. However, the mutation does not completely remove the mutator activity of *mut1* (Table 6). The double mutants still show mutator activity, but only at about 17% to 38%, depending on the marker, of the rates seen in *mut1* single mutants. Strains carrying only the *ant1* mutation were backcrossed to wild type; the results in Table 7 show that UV sensitivity segregated with reduced mutation rates for suppression and for reversion of *his1–7*. There may or may not be an effect on *lys1–1* locus reversion rates. We have found that *ant1* complements the following mutants for UV survival: *rev1*, *rev3–1*, *rev3–15*, *rad3*, *rad6*, *rad9*, *rad18* and *rad51*. In Figures 2, 3 and 4 we show the UV-survival interactions of *ant1* with *rev3–15* and representative mutants of the two known repair pathways, *rad6* and *rad3*. Since it has been shown that *rev3* and *rad6* have an epistatic interaction (LAWRENCE and CHRISTENSEN 1976), the difference in the interaction

TABLE 3

The effect of rev3 *in combination with* rad3, rad51 *and* rad18 *on spontaneous mutation rate*

Genotype	Strain	*lys1-1*						*his1-7*			
		Cells/comp. (× 10^{-6})	No. of comps.	No. of mutant compartments		Mutation rate (× 10^8)		Cells/comp. (× 10^{-6})	No. of comps.	No. of mutant comps.	Mutation rate (× 10^8)
				SS	Locus	SS	Locus				
rad3	YO414–1D	2.14	991	237	44	6.40	1.06	2.05	900	529	21.50
	YO414–4A	—	—	—	—	—	—	2.17	958	729	32.67
	YO414–4C	—	—	—	—	—	—	1.83	950	721	38.90
	YO415–2A	3.61	998	287	74	4.70	1.07	2.60	998	884	41.59
	YO415–6A	3.06	782	257	64	6.51	1.39	2.11	991	862	48.11
	YO418–5B	3.39	995	331	88	5.96	1.37	2.49	994	798	32.44
	YO418–6A	2.56	681	140	53	4.49	1.58	2.34	955	576	19.47
rad3 rev3	YO414–1B	2.68	987	45	7	0.87	0.13	1.95	948	145	4.25
	YO415–1A	2.25	984	52	5	1.21	0.11	1.77	993	200	6.37
	YO415–1C	2.73	986	35	5	0.66	0.09	1.61	887	117	4.34
	YO415–2C	3.23	997	48	1	0.76	0.02	2.83	998	104	1.94
	YO415–6B	3.42	986	46	6	0.70	0.09	1.86	995	148	4.26
rev3	YO414–1C	3.22	996	92	5	1.50	0.08	2.37	800	81	2.24
	YO414–4B	—	—	—	—	—	—	2.37	960	181	4.35
	YO414–4D	—	—	—	—	—	—	2.78	895	120	2.58
	YO415–2B	3.12	980	104	5	1.80	0.08	2.74	796	128	3.19
	YO415–6C	2.93	959	80	1	1.49	0.02	2.18	885	99	2.67
	YO425–1B	2.80	993	81	1	1.52	0.02	—	—	—	—
	YO425–1C	3.44	988	96	2	1.49	0.03	—	—	—	—
Wild type	YO414–2B	—	—	—	—	—	—	2.13	996	265	7.26
	YO415–1B	3.23	963	119	31	2.04	0.51	1.94	994	420	14.14
	YO415–1D	3.47	948	154	33	2.55	0.51	2.46	994	436	11.76
	YO415–2D	3.48	977	115	28	1.80	0.42	2.85	996	254	5.16
	YO415–6D	2.94	783	86	10	1.98	0.22	1.89	994	437	15.27
	YO418–8B	3.23	895	109	34	2.01	0.60	2.31	998	494	14.24
	YO418–8D	3.17	984	122	54	2.09	0.89	2.63	963	403	16.58
	YO425–1A	3.20	963	93	21	1.59	0.34	—	—	—	—
	YO425–1D	2.70	999	144	21	2.88	0.39	—	—	—	—

TABLE 3—Continued

Genotype	Strain	*lys1-1* Cells/comp. (× 10^{-6})	*lys1-1* No. of comps.	*lys1-1* No. of mutant compartments		*lys1-1* Mutation rate (× 10^8)		*his1-7* Cells/comp. (× 10^{-6})	*his1-7* No. of comps.	*his1-7* No. of mutant comps.	*his1-7* Mutation rate (× 10^8)
				SS	Locus	SS	Locus				
rad51	YO436–3B	—	—	—	—	—	—	1.61	1000	561	25.25
	YO436–4B	1.82	991	427	18	15.50	0.50	1.66	996	538	23.31
	YO436–11A	—	—	—	—	—	—	1.48	996	671	37.53
	YO436–2D	1.73	1000	408	40	15.16	1.18	1.44	1000	795	55.07
	YO436–5B	1.99	999	463	41	15.62	1.05	1.76	998	812	47.66
rad51 rev3	YO436–3C	0.93	997	12	3	0.65	0.16	1.53	1000	77	2.61
	YO436–4A	1.18	997	15	2	0.64	0.09	1.45	498	35	2.40
	YO436–11B	1.17	998	9	3	0.39	0.13	1.43	997	45	1.62
	YO436–7D	0.82	1000	7	1	0.43	0.12	0.87	993	71	4.25
rev3	YO436–3A	2.67	989	80	2	1.58	0.04	2.77	997	64	1.16
Wild type	YO436–3D	—	—	—	—	—	—	2.62	998	308	7.03
rad18	YO294–4A	2.12	1000	419	12	12.79	0.28	1.67	1000	610	28.15
	YO424–4D	2.26	1000	434	16	12.60	0.36	1.97	996	499	17.61
	YO424–3A	2.62	998	603	31	17.67	0.60	1.81	998	602	25.48
	YO417–2B	1.89	998	398	7	13.48	0.19	1.83	996	597	24.93
	YO424–5D	2.71	996	575	27	15.88	0.51	2.08	998	633	24.00
rad18 rev3	YO424–4B	2.16	1000	141	4	3.51	0.09	1.67	998	211	7.03
	YO424–3B	2.11	998	144	3	3.70	0.07	1.71	988	133	4.23
	YO417–2A	1.73	997	77	4	2.32	0.12	1.48	897	132	5.39
	YO424–5B	—	—	—	—	—	—	1.80	998	139	4.14
	YO424–6B	2.10	1000	156	1	4.04	0.02	1.89	934	95	2.82
	YO424–6D	2.63	803	77	0	1.92	NR	2.10	993	155	4.03
rev3	YO424–4C	3.62	1000	97	7	1.41	0.10	2.73	1000	133	2.48
	YO424–3C	3.20	995	104	7	1.72	0.11	2.24	962	86	2.09
	YO417–2D	3.61	925	42	2	0.64	0.03	2.51	999	92	1.91
	YO424–5A	3.49	997	112	4	1.71	0.06	2.65	999	129	2.56
Wild type	YO424–4A	3.25	1000	176	37	2.98	0.58	2.25	986	308	8.14
	YO424–3D	2.51	997	152	29	3.30	0.59	2.49	891	216	5.56
	YO417–2C	1.78	997	71	22	2.08	0.63	1.64	966	187	6.49
	YO424–5C	3.20	1000	161	23	2.74	0.36	2.51	999	288	6.71

* NR = No revertant.

TABLE 4

A summary of the mean mutation rates of single and double mutants with standard errors using data from Table 3

	Mutation rate ($\times 10^8$)								
	lys1-1								
	SS			Locus			*his1-7*		
Genotype	N	$\bar{x}$	SE	N	$\bar{x}$	SE	N	$\bar{x}$	SE
rad3	5	5.6	0.4	5	1.30	0.1	7	34	4.0
rad51	3	15.4	0.1	3	0.90	0.2	5	38	6.0
rad18	5	14.0	1.0	5	0.39	0.07	5	24	2.0
rad3 rev3	5	0.8	0.1	5	0.09	0.02	5	4.2	0.7
rad51 rev3	4	0.53	0.07	4	0.12	0.02	4	2.7	0.6
rad18 rev3	5	3.1	0.4	5	0.06	0.02	6	4.6	0.6
rev3	10	1.5	0.1	10	0.06	0.01	10	2.5	0.3
Wild type	12	2.3	0.2	12	0.50	0.05	12	10	1.0

N = number of strains; $\bar{x}$ = mean; SE = standard error.

of *ant1* with *rev3* and with *rad6* is inconsistent because *ant1* interacts epistatically with *rev3* but not with *rad6*.

Finally, to find out how much of the wild-type spontaneous mutation is *REV3* and *ANT1* dependent, we measured mutation rates of strains doubly mutant at these two loci. Results presented in Table 8 show that the mutation rates of *ant1 rev3* double mutants, depending on the markers tested, are like those of either *rev3* or *ant1* single mutants.

DISCUSSION

One interesting finding that has emerged from a genetic analysis of the origin of spontaneous mutation in yeast has been the uncovering of an antimutator mutation that is an allele of *rev3*. The *REV3* gene function has been shown to be involved in the mutagenic repair of UV-induced damage (LAWRENCE and CHRISTENSEN 1976, 1979b; LEMONTT 1971a,b). This mutagenic repair process in yeast appears to involve a large number of genes (LAWRENCE and CHRISTENSEN 1976, 1979a,b). The observation that spontaneous mutation rates are reduced in strains carrying *rev3* mutations suggests that this process is also responsible for a large proportion of spontaneous mutations seen in wild-type strains. The results of double-mutant studies showing that *rev3* mutation is epistatic to mutator mutations of *rad3*, *rad51* and *rad18* support the hypothesis (HASTINGS, QUAH and VON BORSTEL 1976) that enhanced spontaneous mutation rates seen in radiation-sensitive mutator strains are a result of mutagenic repair of spontaneous lesions, and that spontaneous lesions are recognized and repaired by the same enzymatic processes as those that repair induced lesions. The fact that a deficiency in nonmutagenic repair leads to higher spontaneous mutation rates can be taken as evidence that most spontaneous lesions are repaired nonmutagenically in a wild-type strain. Our results show that spontaneous mutations still occur, though at

TABLE 5

The mutator activity of mut1 *is independent of the* REV3 *gene product*

		lys1-1						*his1-7*			
		Cells/ comp. ($\times 10^{-6}$)	No. of comps.	No. of mutant compartments		Mutation rate ($\times 10^{8}$)		Cells/ comp. ($\times 10^{-6}$)	No. of comps.	No. of mutant comps.	Mutation rate ($\times 10^{8}$)
Genotype	Strain			SS	Locus	SS	Locus				
mut1	YO189–3C	0.83	996	764	2	88.25	0.12	2.29	929	740	34.64
	YO189–4B	0.68	896	612	3	84.30	0.25	2.21	948	690	29.15
mut1 rev3	YO189–3A	0.86	996	838	0	107.48	NR*	2.39	986	772	31.79
	YO189–4C	1.08	998	927	0	122.03	NR	2.22	995	771	33.45
rev3	YO189–3B	3.77	998	58	3	0.80	0.04	2.46	991	136	2.40
	YO189–4A	3.45	1000	71	7	1.07	0.10	2.52	971	142	3.14
Wild type	YO189–3D	2.92	1000	157	18	2.93	0.31	2.37	949	279	7.34
	YO189–4D	3.41	997	204	26	3.35	0.39	2.24	995	446	13.11

* NR = No revertant.

TABLE 6

The effect of ant1 *on* mut1

Genotype	Strain	Cells/ comp. ($\times 10^{-6}$)	No. of comps.	No. of mutant compartments		Mutation rate ($\times 10^{8}$)		Cells/ comp. ($\times 10^{-6}$)	No. of comps.	No. of mutant comps.	Mutation rate ($\times 10^{8}$)
				SS	Locus	SS	Locus				
mut1	XV800-6C	1.63	999	875	9	64.04	0.28	6.27	998	571	36.27
mut1 (UV-)	289	1.70	1000	293	2	10.18	0.06	0.94	997	304	19.09
mut1	YO43-9D	2.11	1000	988	5	105.03	0.12	1.56	987	732	41.98
	YO43-12A	2.06	994	894	15	55.67	0.37	1.12	1000	729	58.18
	YO133-1C	1.47	932	860	3	87.19	0.11	1.18	883	474	32.49
mut1 ant1	YO43-9A	2.30	992	502	16	15.33	0.35	1.49	997	517	24.08
	YO43-12D	2.06	997	463	5	15.16	0.12	1.26	1000	343	16.37
	YO133-1A	2.89	953	436	8	10.59	0.15	2.69	974	549	15.43
ant1	YO43-9B	2.29	990	14	17	0.31	0.38	1.36	998	118	4.64
	YO43-12B	2.64	991	27	11	0.52	0.21	1.55	997	167	5.93
	YO133-1D	4.06	840	27	25	0.40	0.37	2.03	935	218	6.53
Wild type	YO43-9C	1.89	998	48	17	1.30	0.45	1.12	999	244	11.78
	YO43-12C	2.38	995	74	16	1.62	0.34	1.40	1000	367	16.25
	YO133-1B	4.29	887	170	20	2.48	0.27	1.46	990	212	8.25

TABLE 7

Tetrad analysis showing segregation of UV-sensitivity with low spontaneous mutation rate

Tetrad	UV	*lys1–1* Cells/comp. ($\times 10^{-6}$)	*lys1–1* No. of comps.	*lys1–1* No. of mutant compartments SS	*lys1–1* No. of mutant compartments Locus	*lys1–1* Mutation rate ($\times 10^{8}$) SS	*lys1–1* Mutation rate ($\times 10^{8}$) Locus	*his1–7* Cells/comp. ($\times 10^{-6}$)	*his1–7* No. of comps.	*his1–7* No. of mutant comps.	*his1–7* Mutation rate ($\times 10^{8}$)
YO75–2A	S	2.02	1000	23	11	0.58	0.27	2.43	983	156	3.55
–2B	S	2.05	998	21	10	0.52	0.25	2.76	990	256	5.43
–2C	R	1.76	999	44	20	1.28	0.57	2.35	997	440	12.41
–2D	R	1.88	998	66	16	1.82	0.43	2.18	974	299	8.40
YO75–3A	R	4.05	997	125	23	1.65	0.29	2.29	977	528	16.98
–3B	R	4.07	998	202	43	2.78	0.54	2.96	926	415	10.02
–3C	S	5.11	997	51	21	0.51	0.21	3.04	973	171	3.16
–3D	S	4.55	996	40	25	0.45	0.28	2.44	953	295	7.24
YO75–4A	S	3.23	995	42	17	0.67	0.27	3.23	771	195	4.51
–4B	S	3.08	995	16	14	0.26	0.23	2.60	990	308	7.10
–4C	R	2.29	999	116	20	2.70	0.44	2.48	990	472	13.04
–4D	R	2.04	999	57	27	1.44	0.67	2.02	1000	602	22.75

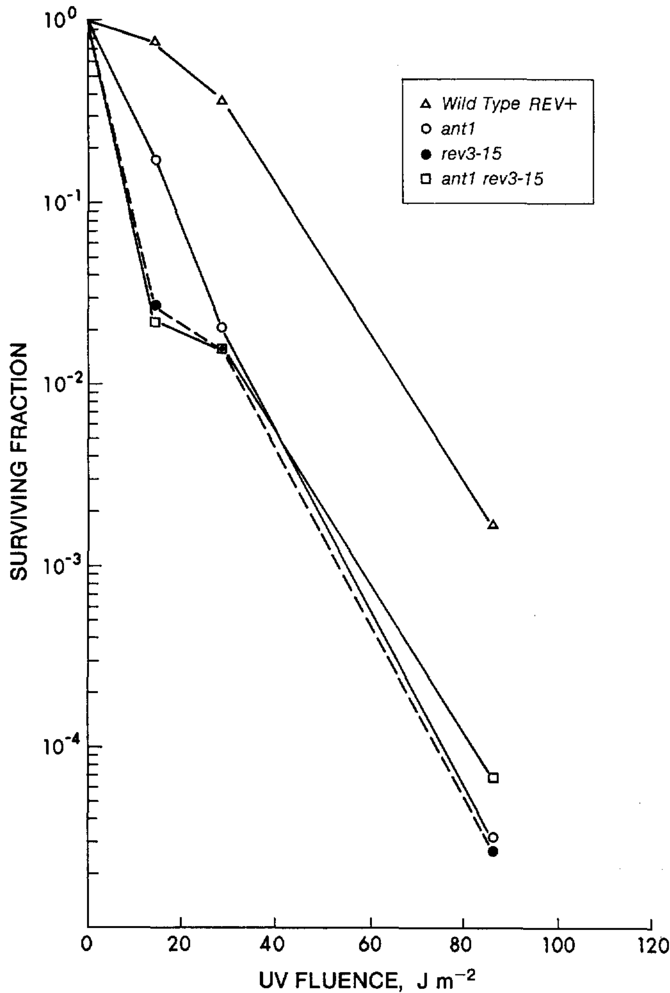

FIGURE 2.—UV survival curves for wild-type (YO119-3B), *ant1* (YO119-3D), *rev3-15* (YO119-3C) and *ant1 rev3-15* (YO119-3A) haploid strains. Three other *ant1 rev3-15* double-mutant strains gave survival curves similar to the *rev3-15* single-mutant strain.

a reduced rate, in *rev3* strains. It is not known whether these mutations are due to leakiness of the *rev3* mutation, to unrepaired misreplication errors or to a minor mutagenic pathway, as yet unidentified, operating in these strains.

It appears, as shown in Tables 2 and 3, that the *rad3 rev3* and *rad51 rev3* double mutants specifically reduce suppressor mutation rates to levels lower than those of the *rev3* single mutants. A possible explanation is that the repair processes controlled by the *RAD3* and *RAD51* gene loci are slightly mutagenic. It is conceivable that excision repair of a spontaneous lesion could result in an intermediate substrate that is potentially more mutagenic. On the other hand, it is difficult to measure the spontaneous mutation rate accurately enough at such

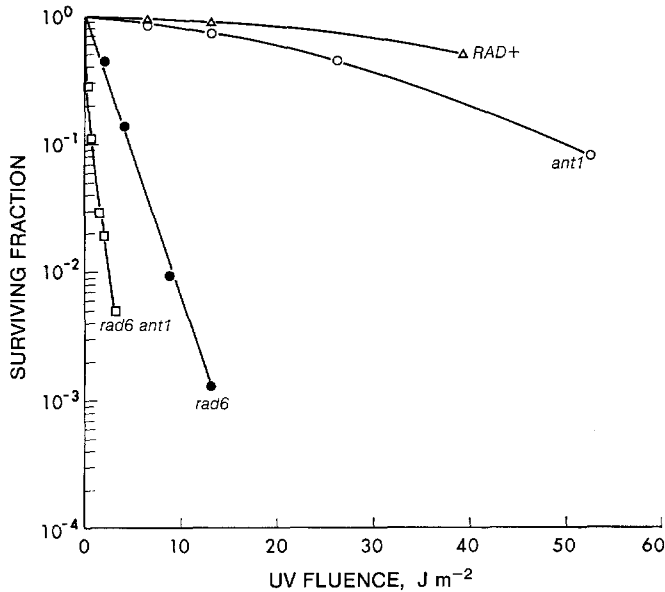

FIGURE 3.—UV survival curves for wild-type (YO106–2C), *ant1* (YO106–2B), *rad6–1* (YO106–2D) and *rad6 ant1* (YO106–2A) haploid strains. Two other experiments using different sets of tetratype tetrads gave similar results.

low rates to exclude totally any modifier effects. However, if the lower mutation rate is due to segregation of a modifier gene, the results shown in Table 2 give no evidence for tight linkage. Unlike *rad3 rev3* and *rad51 rev3* double mutants, *rad18 rev3* double mutants show suppressor mutation rates similar to those of the wild-type strains. This implies that a proportion of the spontaneous mutation seen in *rad18* single mutant strains is not caused by the *REV3* gene product.

When we examined the influence of the *rev3* mutation on a *mut1* mutator strain that is insensitive to the lethal effect of radiation or MMS, we found that the *REV3* gene function is not required for the enhanced mutation seen in *mut1* mutant strains. This implies that other spontaneous lesions or configurations exist that are repaired by processes independent of those defined by the *RAD3*, *RAD51*, *RAD18* and *REV3* loci. The isolation of an antimutator strain, *ant1*, which is also a repair-deficient mutant, is compatible with the conclusion that another mutagenic process besides that controlled by the *REV3* gene exists. The incomplete action of the *ant1* mutation in the reduction of mutator activity in the *mut1* strain could be attributed to leakiness of the *ant1* mutation or to other causes that have yet to be uncovered.

The fact that the *rev3* mutation is epistatic to *ant1* with respect to UV survival can be taken to mean that *ant1* belongs to the *RAD6* epistatic group; however, *ant1* is not defective in UV-induced mutagenesis as determined by our test systems (data not shown). Furthermore, we have found that *ant1 rad6* double mutants are more sensitive to the lethal effects of UV radiation than is either single mutant, a result inconsistent with the finding that *rad6* is epistatic to *rev3*

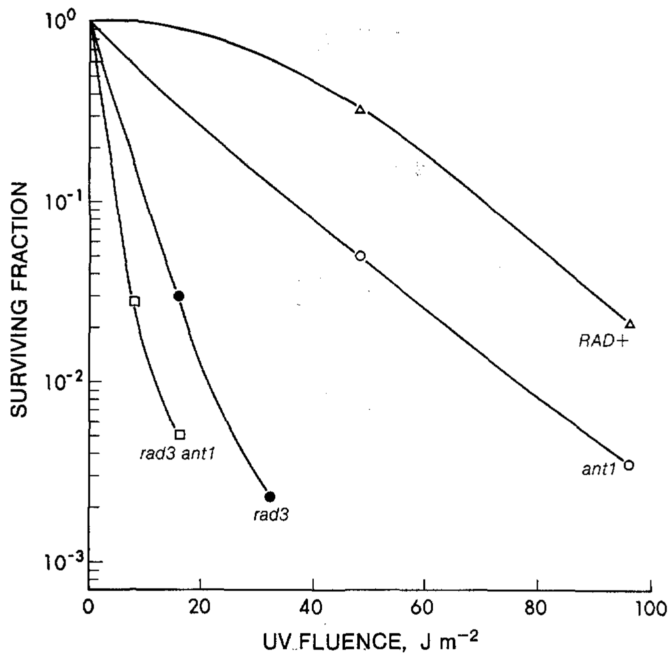

FIGURE 4.—UV survival curves for wild-type (YO169-1A), *ant1* (YO169-1B), *ant1 rad3* (YO169-1C) and *rad3* (YO169-1D) haploid strains. Two other experiments using different sets of tetratype tetrads gave similar results.

(LAWRENCE and CHRISTENSEN 1976). Although *rev3* is epistatic to *ant1* with regard to UV survival, they show distinct mutation phenotypes; the *rev3* mutant exhibits a strong effect on *lys1-1* and *his1-7* reversions; whereas, *ant1* is more effective on suppressor mutations. The *rev3 ant1* double mutant shows a spontaneous mutation phenotype characteristic of *ant1* for suppressor mutations and of *rev3* for *lys1-1* and *his1-7* reversions. Together, their functions appear to account for about 90% of the spontaneous mutation of these test alleles in the wild type. The independent behavior of these two mutations with respect to the spontaneous mutation rate is consistent with the idea that their gene functions are employed to produce different mutational events. Such a conclusion has been reached by LAWRENCE and CHRISTENSEN (1976, 1978a,b, 1979b) on UV-induced mutagenesis. Examples of variation in mutation phenotypes were provided in their studies of UV-induced reversion of *cyc1* alleles in strains defective in induced mutagenesis. They showed that the *RAD6* function appears to be necessary for the production of all kinds of mutations at all sites; in contrast, the *REV3* gene function is required to revert most, but not all, mutational events. MCKEE and LAWRENCE (1979a,b) proposed that the *RAD6* system was composed of several sets of overlapping functions, in which partially different sets of genes are required by different types of mutations, mutations at different positions in the genome, and the formation of mutations by different mutagens. The findings with *ant1* and *rev3* support this view, extending it to spontaneous mutation.

TABLE 8

Mutation rate of ant1 rev3-15 *double mutant*

		lys1-1						*his1-7*			
				No. of mutant compartments		Mutation rate ($\times 10^8$)					
Genotype	Strain	Cells/comp. ($\times 10^{-6}$)	No. of comps.	SS	Locus	SS	Locus	Cells/comp. ($\times 10^{-6}$)	No. of comps.	No. of mutant comps.	Mutation rate ($\times 10^8$)
ant1 rev3	YO119-2A	—	—	—	—	—	—	2.79	835	43	0.95
	YO119-2C	—	—	—	—	—	—	2.53	953	94	2.05
	YO119-3A	3.64	986	19	4	0.27	0.06	2.72	918	41	0.84
	YO119-4A	4.00	954	47	10	0.63	0.13	2.81	949	63	1.22
ant1	YO119-3D	4.90	967	50	18	0.54	0.19	3.03	909	326	7.34
	YO119-4B	3.95	975	44	26	0.58	0.34	2.63	881	205	5.03
rev3	YO119-3C	3.80	982	73	8	1.02	0.11	2.30	988	103	2.39
	YO119-4C	3.22	991	99	0	1.64	NR*	2.46	962	110	2.47
Wild type	YO119-2B	—	—	—	—	—	—	2.72	994	552	14.49
	YO119-2D	—	—	—	—	—	—	2.10	981	395	12.27
	YO119-3B	2.71	968	180	35	3.79	0.68	1.79	973	169	5.32
	YO119-4D	3.53	993	83	42	1.24	0.61	2.23	951	456	14.67

* NR = No revertant.

Since *rev3* mutants are antimutators, one would expect from the mutational phenotype based on UV-induced mutagenesis that *rad6* mutants would also be antimutators. This apparently is not so, for *rad6* mutations have been shown to enhance, rather than reduce, the spontaneous mutation rate (HASTINGS, QUAH and VON BORSTEL 1976; LAWRENCE and CHRISTENSEN 1979a). This inconsistency might lie in the complexity of the *RAD6*-dependent functions that have been discussed by LAWRENCE and CHRISTENSEN (1979a). They proposed that the *RAD6*-dependent functions can be described as a highly branched process dependent on the cooperative action of more than a dozen loci. In addition to the mutagenic repair functions, there are at least two different error-free functions: (1) a *RAD18*-dependent process that is responsible for UV and trimethoprim resistance, and (2) a *RAD9*- and *RAD15*-dependent process that is responsible for the error-free repair of gamma-ray damage. Metabolic suppressor mutations were found to suppress only the former process. Since *rad6* and *rad18* mutants are both mutators, it can be interpreted that the *RAD18*-dependent process also repairs spontaneous lesions and, when this error-free process is not available, these lesions are repaired mutagenically by the *REV3* gene product. This idea is consistent with the fact that *rad18 rev3* double mutants are not mutators. The lack of an antimutator effect seen in *rad6* single mutants may occur because of a compensatory increase in mutagenic repair by the *REV3* gene function. If this were true, the influence of a *rad6* mutation on spontaneous mutation rates would be more obvious in a *rev3* background. The phenotypes of *rad6 rev3* double mutants are yet to be determined.

We do not know the origin or nature of the spontaneous lesions that lead to spontaneous mutation in yeast. At present, we visualize the functions that are lost in these antimutator mutants as functions connected with the ability of repair polymerases to cross lesions in a variety of situations. An important question for further work is the origin of the low level of spontaneous mutation still present in *ant1 rev3* double-mutant strains.

We wish to acknowledge the excellent technical assistance of ELIZABETH GRANT, AUDREY LYNCH, ANNA S. H. CHUI and ELIZABETH RICHARD. This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

AVERBECK, D., W. LASKOWSKI, F. ECKARDT and E. LEYMANN-BRAUNS, 1970 Four radiation sensitive mutants of Saccharomyces: Survival after UV- and X-ray-irradiation as well as UV-induced reversion rates from isoleucine-valine dependence to independence. Molec. Gen. Genet. **107**: 117–127.

BRENDEL, M. and R. H. HAYNES, 1973 Interactions among genes controlling sensitivity to radiation and alkylation in yeast. Molec. Gen. Genet. **125**: 197–216.

BRYCHCY, T., 1974 Spontaneous mutability of UV-sensitive yeast. M.Sc. Thesis, Department of Genetics, University of Alberta, Edmonton, Alberta, Canada.

BRYCHCY, T. and R. C. VON BORSTEL, 1977 Spontaneous mutability in UV-sensitive excision-defective strains of Saccharomyces. Mutation Res. **45**: 185–194.

COX, B. S. and J. C. GAME, 1974 Repair systems in Saccharomyces. Mutation Res. **26**: 257–264.

COX, B. S. and J. M. PARRY, 1968 The isolation, genetics and survival chracteristics of ultraviolet light-sensitive mutants in yeast. Mutation Res. **6**: 37–55.

ECKARDT, F. and R. H. HAYNES, 1977 Induction of pure and sectored mutant clones in excision-proficient and deficient strains of yeast. Mutation Res. **43**: 327–338.

GAME, J. C. and B. S. COX, 1972 Epistatic interactions between four *rad* loci in yeast. Mutation Res. **16**: 353–362. ——, 1973 Synergistic interactions between *rad* mutations in yeast. Mutation Res. **20**: 35–44.

GAME, J. C. and R. K. MORTIMER, 1974 A genetic study of X-ray-sensitive mutants in yeast. Mutation Res. **24**: 281–292.

GOTTLIEB, D. J. C. and R. C. VON BORSTEL, 1976 Mutation in *Saccharomyces cerevisiae*: *mut1–1*, *mut1–2* and *mut2–1*. Genetics **83**: 655–666.

HASTINGS, P. J., S.-K. QUAH and R. C. VON BORSTEL, 1976 Spontaneous mutation by mutagenic repair of spontaneous lesions in DNA. Nature (London) **264**: 719–722.

HO, K., 1975 Induction of DNA double-strand breaks by X-rays in a radiation-sensitive strain of yeast *Saccharomyces cerevisiae*. Mutation Res. **30**: 327–334.

KHAN, N. A., M. BRENDEL and R. H. HAYNES, 1970 Supersensitive double mutants in yeast. Molec. Gen. Genet. **107**: 376–378.

LAWRENCE, C. W. and R. B. CHRISTENSEN, 1976 UV mutagenesis in radiation-sensitive strains of yeast. Genetics **82**: 207–232. ——, 1978a Ultraviolet-induced reversion of *cyc1* alleles in radition-sensitive strains of yeast. I. *rev1* mutant strains. J. Molec. Biol. **122**: 1–21. ——, 1978b Ultraviolet-induced reversion of *cyc1* alleles in radiation-sensitive strains of yeast. II. *rev2* mutant strains. Genetics **90**: 213–226. ——, 1979a Metabolic suppressors of trimethoprim and ultraviolet light sensitivities of *Saccharomyces cerevisiae* mutants. J. Bact. **139**: 866–876. ——, 1979b Ultraviolet-induced reversion of *cyc1* alleles in radiation-sensitive strains of yeast. III. *rev3* mutant strains. Genetics **92**: 397–408.

LAWRENCE, C. W., J. W. STEWART, F. SHERMAN and R. B. CHRISTENSEN, 1974 Specificity and frequency of ultraviolet-induced reversion of an iso-1-cytochrome *c* ochre mutant in radiation-sensitive strains of yeast. J. Molec. Biol. **85**: 137–162.

LEMONTT, J. F., 1971a Pathways of ultraviolet mutability in *Saccharomyces cerevisiae*. I. Some properties of double mutants involving *uvs9* and *rev*. Mutation Res. **13**: 311–317. ——, 1971b Mutants of yeast defective in mutation induced by ultraviolet light. Genetics **68**: 21–33.

LINDEGREN, G., Y. L. HWANG, Y. OSHIMA and C. C. LINDEGREN, 1965 Genetical mutants induced by ethyl methanesulfonate in Saccharomyces. Can. J. Genet. Cytol. **7**: 491–499.

MCKEE, R. H. and C. W. LAWRENCE, 1979a Genetic analysis of gamma-ray mutagenesis in yeast. I. Reversion in radiation-sensitive strains. Genetics **93**: 361–373. ——, 1979b Genetic analysis of gamma-ray mutagenesis in yeast. II. Allelic-specific control of mutagenesis. Genetics **93**: 375–381.

MORRISON, D. P. and P. J. HASTINGS, 1979 Characterization of the mutator mutation *mut5–1*. Molec. Gen. Genet. **175**: 57–65.

MOUSTACCHI, E., 1969 Cytoplasmic and nuclear genetic events induced by UV light in strains of *Saccharomyces cerevisiae* with different UV sensitivities. Mutation Res. **7**: 171–185.

ORD, R. W., 1980 Two mutators of *S. cerevisiae*: *mut7* and *mut8*. M.Sc. Thesis, Department of Genetics, University of Alberta, Edmonton, Alberta, Canada.

PRAKASH, L., 1976 Effect of genes controlling radiation sensitivity on chemically induced mutations in *Saccharomyces cerevisiae*. Genetics **83**: 285–301. ——, 1977a Repair of pyrimidine dimers in radiation-sensitive mutants *rad3*, *rad4*, *rad6* and *rad9* of *Saccharomyces cerevisiae*. Mutation Res. **45**: 13–20. ——, 1977b Defective thymine dimer excision in radiation-sensitive mutants *rad10* and *rad16* of *Saccharomyces cerevisiae*. Molec. Gen. Genet. **152**: 125–128.

PRAKASH, L., D. HINKLE and S. PRAKASH, 1979 Decreased UV mutagenesis in *cdc8*, a DNA replication mutant of *Saccharomyces cerevisiae*. Molec. Gen. Genet. **172**: 249–258.

PRAKASH, L. and S. PRAKASH, 1977 Isolation and characterization of MMS-sensitive mutants of *Saccharomyces cerevisiae*. Genetics **86**: 33–55. ——, 1979 Three additional genes involved in pyrimidine removal in *Saccharoyces cerevisiae*: *RAD7, RAD14* and *MMS19*. Molec. Gen. Genet. **176**: 351–359.

RESNICK, M. A., 1969a Induction of mutations in *Saccharomyces cerevisiae* by UV. Mutation Res. **7**: 315–332. ——, 1969b Genetic control of radiation sensitivity in *Saccharomyces cerevisiae*. Genetics **62**: 519–531.

RESNICK, M. A. and P. MARTIN, 1976 The repair of double-strand breaks in the nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. Molec. Gen. Genet. **143**: 119–129.

RESNICK, M. A. and J. K. SETLOW, 1972 Repair of pyrimidine dimer damage induced in yeast by ultraviolet light. J. Bact. **109**: 979–986.

REYNOLDS, R. J., 1978 Removal of pyrimidine dimers from *Saccharomyces cerevisiae* nuclear DNA under nongrowth conditions as detected by a sensitive enzymatic assay. Mutation Res. **50**: 43–56.

SCHULLER, R. and R. C. VON BORSTEL, 1974 Spontaneous mutability in yeast. I. Stability of lysine reversion rates to variation of adenine concentration. Mutation Res. **24**: 17–23.

SNOW, R., 1967 Mutants of yeast sensitive to ultraviolet light. J. Bact. **94**: 571–575.

UNRAU, P., R. WHEATCROFT and B. S. COX, 1971 The excision of pyrimidine dimers from DNA of ultraviolet irradiated yeast. Molec. Gen. Genet. **113**: 359–362.

VON BORSTEL, R. C., 1978 Measuring spontaneous mutation rates in yeast. pp. 1–24. In: *Methods in Cell Biology*, Vol. 20. Edited by D. M. PRESCOTT. Academic Press, Inc., New York, San Francisco, London.

VON BORSTEL, R. C., K. T. CAIN and C. M. STEINBERG, 1971 Inheritance of spontaneous mutability in yeast. Genetics **69**: 17–27.

VON BORSTEL, R. C. and A. LYNCH, 1978 Mutators and antimutators in *Saccharomyces*. Proc. XIV Intl. Cong. Genetics, Moscow, 1978, Part II, p. 210.

VON BORSTEL, R. C., S.-K. QUAH, C. M. STEINBERG, F. FLURY and D. J. C. GOTTLIEB, 1973 Mutants of yeast with enhanced spontaneous mutation rates. Genetics Suppl. **73**: 141–151.

WATERS, R. and E. MOUSTACCHI, 1974 The disappearance of ultraviolet-induced pyrimidine dimers from the nuclear DNA of exponential and stationary phase cells of *Saccharomyces cerevisiae* following various post-irradiation treatments. Biochim. Biophys. Acta. **353**: 407–419.

ZAKHAROV, I. A., T. N. KOZINA and I. V. FEDOROVA, 1970 Effects de mutation vers la sensibilite au rayonnement ultraviolet chez la levure. Mutation Res. **9**: 31–39.

Corresponding editor: J. W. DRAKE