ULTRAVIOLET-INDUCED REVERSION OF *cyc1* ALLELES IN RADIATION-SENSITIVE STRAINS OF YEAST. III. *rev3* MUTANT STRAINS

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ABSTRACT

The role of the *REV3* gene function in UV-induced mutagensis in the yeast *Saccharomyces cerevisiae* has been examined by determining the reversion of 12 well-defined *cyc1* mutations in diploid strains homozygous for the *rev3-1* or *rev3-3* allele. The 12 *cyc1* alleles include one ochre, one amber, four initiation, two proline missense, and four frameshift mutations. We find that the *rev3* mutations reduce the frequency of UV-induced reversion of all of the *cyc1* alleles, though different classes of alleles respond to a different extent. These results imply that the *REV3* gene function is required for the production of a wide variety of mutational events, though probably not all, and show that each of the three *REV* loci have different mutational phenotypes. Such diverse phenotypes are not predicted by the unitary model for bacterial mutagenesis (CAILLET-FAUQUET, DEFAIS and RADMAN 1977; WITKIN 1976), suggesting that this is at best an incomplete description of eukaryotic mutagenesis.

GENETIC analysis of UV mutagenesis in *Saccharomyces cerevisiae* has led to the identification of more than ten gene loci that are active in the production of induced mutations (LEMONTT 1971, 1977; LAWRENCE and CHRISTENSEN 1976). Although such loci appear to act within a single process dependent on *RAD6* function (LAWRENCE and CHRISTENSEN 1976), a number of them, in contrast to comparable genes in *E. coli*, are concerned with the production of only some, and not all, induced mutations; that is, these genes have different mutational phenotypes.

The first example of such variation in phenotype was provided by the *rev2-1* mutation. This mutation, as well as mutations at the *REV1* and *REV3* loci, were isolated by LEMONTT (1971) from strains in which the frequencies of revertants of the ochre allele *arg4-17* induced by UV were much reduced. Although reversion frequencies of other auxotrophic alleles were also much reduced in *rev1* and *rev3* mutant strains (LEMONTT 1971), as well as forward mutations to auxotrophy (LEMONTT 1972), suggesting that these loci play a general role in UV mutagenesis, the *REV2* locus was shown by LEMONTT to have a much more restricted function. Subsequent observations demonstrate that this locus is concerned with the production of mutations that revert some, but not all, ochre mutations; it is not concerned with the production of mutations that revert a

diverse set of missense, amber or frameshift alleles (LAWRENCE and CHRISTENSEN 1978a).

In view of the limited mutational phenotype associated with the *REV2* locus, it might be argued that this gene plays only a very peripheral part in mutagenesis and that different mutational phenotypes would not be found for genes that are more directly concerned with this process. The discovery that the *REV1* gene function is also not required for the production of all mutational events (LAWRENCE and CHRISTENSEN 1976) suggests that this argument is not correct, however, and more recent work has shown that the *REV1* locus has a remarkably complex, and as yet incompletely understood, mutational phenotype (LAWRENCE and CHRISTENSEN 1978b).

In order to determine whether each of the yeast loci concerned with UV mutagenesis has a unique mutational phenotype, we have now examined this feature in *rev3* mutant strains, using the reversion of well-defined *cyc1* alleles to monitor the production of specific sets of base-pair addition, deletion or substitution events. The nature of the *cyc1* mutations, and in particular their modes of reversion, has been established by sequence analysis of iso-1-cytochrome *c*, for which the *CYC1* locus is the structural gene, isolated from revertant clones (see Table 1 for references) and in many instances these mutations lie within known nucleotide sequences.

We find that the *REV3* locus indeed possesses a unique mutational phenotype, unlike that of any of the other well-studied genes. The UV-induced reversion of all *cyc1* alleles examined is reduced in strains carrying the *rev3-1* or *rev3-3* mutations, though the extent of the reduction varies between different classes of allele, frameshift alleles reverting at an average frequency of about 11% of normal and most base-pair substitution alleles at a frequency of about 1 to 2% of normal. The base-pair substitution alleles *cyc1-115* and *cyc1-131*, which were found previously to be atypical of this class because they reverted by a process that was independent of the *REV1* gene function (LAWRENCE and CHRISTENSEN 1978b), were also atypical in these experiments, reverting at a frequency that was about 50% of normal. Such data suggest that the *REV3* gene function is required for the production of the great majority, but not all, types of mutation induced by UV.

The existence of diverse mutational phenotypes is not predicted by the unitary model for bacterial mutagenesis proposed by CAILLET-FAUQUET, DEFAIS and RADMAN (1977) and WITKIN (1976), suggesting that this is at best an incomplete description of eukaryotic mutagenesis. Our results imply that induced mutations are produced as a consequence of the joint action of a large number of gene products, partially independent sets being concerned with the production of mutations of different kinds or at different sites (LAWRENCE and CHRISTENSEN 1978a,b; present results) or with the formation of mutations from different premutational lesions (PRAKASH 1974, 1976; MCKEE and LAWRENCE, in preparation).

MATERIALS AND METHODS

Strains: The range of activity of the *REV3* gene function was examined by determining the

effect of the *rev3-1*, and in some instances the *rev3-3* mutant allele on the UV-induced reversion of 12 *cyc1* alleles, kindly supplied by FRED SHERMAN. These alleles include one ochre, one amber, four initiation, two proline missense and four frameshift mutations (see TABLE 1). The reversion of these alleles was studied in diploid strains homozygous for *arg4-17* and heteroallelic for *cyc1*, one of the alleles being *cyc1-1*, a nonrevertable deletion of the whole *CYC1* locus (PARKER and SHERMAN 1969; SHERMAN *et al.* 1975) and the other being one of 12 revertible *cyc1* alleles. Twelve sets of diploids were used in the *rev3-1* series of strains, one set for each *cyc1* allele, and five sets in the *rev3-3* series, each series being constructed in the manner used previously (LAWRENCE and CHRISTENSEN 1978a,b). In the *rev3-1* series, each set contained eight strains (*cyc1-6*, *cyc1-115*, *cyc1-131*, *cyc1-183*, *cyc1-239*, and *cyc1-331*), or four strains (*cyc1-13*, *cyc1-31*, *cyc1-51*, *cyc1-91*, *cyc1-133*, and *cyc1-179*), half the strains in each set being homozygous for *rev3-1*, and half heterozygous at this locus to act as wild-type controls. In the *rev3-3* series, each set contained four strains, two of which were homozygous and two heterozygous for this mutation. Each series of strains was relatively homogeneous with respect to genetic background both because the diploids all had one parent in common, carrying *cyc1-1*, *arg4-17* and a *rev3* mutation, and because most of the *cyc1* alleles came from an isogenic series. Meiotic segregants carrying *cyc1 alleles* were identified by spectroscopic examination of whole cells at —190° (SHERMAN and SLONIMSKI 1964), while the *rev3-1* and *rev3-3* alleles, kindly supplied by J. F. LEMONTT, were detected by their effect on the UV-induced reversion of *arg4-17* in the manner described previously (LAWRENCE and CHRISTENSEN 1978a,b).

Media and Methods: Strains were grown for three days at 30° in liquid YPD (1% Bacto yeast extract, 2% Bacto-peptone and 2% dextrose) and appropriate dilutions of washed cells plated onto SLY (0.17% Difco yeast base without ammonium sulfate and amino acids, 0.5% ammonium sulfate, 0.05% Bacto yeast extract, 1% DL-lactate and 1.5% Difco agar Noble, supplemented with all necessary nutrilites) for *cyc1* reversion, onto YPG (1% Bacto yeast extract, 2% Bacto peptone, 3% (V/V) glycerol, 1% Bacto agar) for corresponding estimates of viability, onto SD (0.17% Difco yeast base without ammonium sulfate and amino acids, 0.5% ammonium sulfate, 2% dextrose, 1.5% Difco agar Noble, supplemented with all nutrilites except arginine) for *arg4-17* revertants and onto fully supplemented SD for corresponding estimates of

TABLE 1

The type and position of nucleotide alterations in the various cyc1 *alleles studied*

cyc1 allele Type	Number	Mutant codon	Normal codon and position	Reference
Initiation mutants	13	AUPy or AUA	AUG – 1	
	51	CUG	AUG – 1	
	131	GUG	AUG – 1	STEWART *et al.* (1971)
	133	AGG	AUG – 1	
Ochre mutant	91 (≡9)	UAA	GAA 2	SHERMAN *et al.* (1974) SHERMAN and STEWART (1974)
Amber mutant	179	UAG	AAG 9	STEWART and SHERMAN (1972, 1973) SHERMAN *et al.* (1974)
Proline missense	6	CCU	GCU 12	PUTTERMAN *et al.* (1974)
mutants	115	CCPy	CUPy 14	STEWART and SHERMAN (personal communication)
Frameshift mutants	31	—UC?	UUC 3	SHERMAN and STEWART (1973)
	183	+A	AAA 10	STEWART and SHERMAN (1974)
	239	—G	AAG 4	SHERMAN and STEWART (1975)
	331	—A	GAA 2	

viability. The nutrilites in the *rev3–1* series were arginine, trytophan and adenine (20 mg/l) and in the *rev3–3* series, arginine, methionine (20 mg/l and leucine (30 mg/l). Ten or 20 plates were used for each dose and dilution for scoring *cyc1* revertants, five plates for *arg4–17* reversion and four plates for viability estimates. Further details with respect to experimental procedures can be obtained from earlier publications (LAWRENCE and CHRISTENSEN 1976, 1978a,b).

RESULTS

The data given in Table 2, which are concerned with ther *rev3–1* series of strains, show that the UV-induced reversion of all *cyc1* alleles tested was reduced in diploids homozygous for this mutation, and very similar results were found in the *rev3–3* series (Table 3). These observations imply that the *REV3* gene function is employed in the production of most, and perhaps all, of the mutational alterations that can revert these *cyc1* alleles, a range of specificity that is in marked contrast to those of the *REV1* and *REV2* genes; despite the fact that mu-

TABLE 2

Influence of the rev3–1 *mutation on the UV-induced reversion of* arg4–17 *and various* cyc1 *alleles*

Type of allele		*rev3* phenotype	Induced revertants per 10^8 survivors 35 Jm^{-2}	70 Jm^{-2}
Ochre	*arg4–17*	*rev3–1**	43 ± 7 (40)	170 ± 28 (8)
		+	6020 ± 282 (95)	15269 ± 782 (42)
	cyc1–91	*rev3–1*	24 ± 10 (80)	56 ± 7 (37)
		+	2635 ± 97 (85)	8514 ± 743 (68)
Amber	*cyc1–179*	*rev3–1*	31 ± 9 (69)	98 ± 1 (14)
		+	1299 ± 196 (97)	4849 ± 28 (59)
Initiation	*cyc1–13*	*rev3–1*	3 ± 1 (92)	10 ± 1 (50)
		+	159 ± 4 (88)	378 ± 29 (71)
	cyc1–51	*rev3–1*	4 ± 4 (85)	3 ± 0 (23)
		+	118 ± 41 (100)	277 ± 33 (80)
	cyc1–131	*rev3–1*	41 ± 6 (81)	205 ± 42 (23)
		+	97 ± 6 (105)	367 ± 36 (73)
	cyc1–133	*rev3–1*	3 ± 0 (80)	19 ± 1 (30)
		+	272 ± 4 (86)	604 ± 109 (74)
Proline missense	*cyc1–6*	*rev3–1*	1 ± 1 (69)	3 ± 3 (18)
		+	119 ± 32 (93)	378 ± 99 (63)
	cyc1–115	*rev3–1*	498 ± 49 (74)	1457 ± 252 (24)
		+	1096 ± 81 (94)	3863 ± 446 (71)
Frameshift	*cyc1–31*	*rev3–1*	1 ± 1 (76)	1 ± 1 (16)
		+	67 ± 2 (80)	195 ± 20 (61)
	cyc1–183	*rev3–1*	4 ± 1 (82)	18 ± 8 (34)
		+	53 ± 4 (98)	191 ± 11 (71)
	cyc1–239	*rev3–1*	3 ± 1 (68)	23 ± 7 (15)
		+	19 ± 3 (100)	69 ± 7 (62)
	cyc1–331	*rev3–1*	2 ± 1 (75)	10 ± 3 (20)
		+	23 ± 2 (98)	86 ± 4 (66)

Percent survival in parentheses.
* Average of data from all strains.

TABLE 3

Influence of the rev3–3 *mutation on the UV-reversion of* arg4–17 *and various* cyc1 *alleles*

Type of allele		*rev3* phenotype	Induced revertants per 10^8 survivors 35 Jm^{-2}	70 Jm^{-2}
Ochre	*arg4–17*	*rev3–3**	70 ± 13 (24)	226 ± 63 (2)
		+	7753 ± 628 (89)	21538 ± 3484 (26)
	cyc1–91	*rev3–3*	105 ± 25 (83)	333 ± 133 (13)
		+	2641 ± 25 (94)	5849 ± 1100 (68)
Initiation	*cyc1–131*	*rev3–3*	63 ± 29 (63)	305 ± 172 (5)
		+	115 ± 7 (87)	380 ± 45 (58)
	cyc1–133	*rev3–3*	5 ± 2 (87)	11 ± 6 (27)
		+	305 ± 7 (89)	646 ± 109 (52)
Proline missense	*cyc1–115*	*rev3–3*	461 ± 50 (74)	1893 ± 12 (5)
		+	1151 ± 47 (100)	3487 ± 300 (52)
Frameshift	*cyc1–183*	*rev3–3*	13 ± 1 (48)	24 ± 3 (7)
		+	88 ± 16 (91)	236 ± 39 (63)

Percent survival in parentheses.
* Average of data from all strains.

tations at these three loci were isolated in an identical manner, it is clear that they have different phenotypes.

Although the induced reversion frequencies of all the *cyc1* alleles tested were reduced in *rev3–1* or *rev3–3* strains and although in all cases, except the *cyc1–131* set in the *rev3–3* series, these reductions were statistically significant, the extent of the reduction was nevertheless variable among the different *cyc1* alleles. The results from strains carrying the initiation mutation *cyc1–131* and the proline missense allele *cyc1–115* are particularly noticeable in this respect, showing that these alleles revert at a frequency that is about 50% of normal in *rev3* strains, rather than about 1% to 2% of normal as found with the remaining base-pair substitution alleles. Very similar results were obtained in both the *rev3–1* and *rev3–3* series of strains, showing that this is not the consequence of any particular *rev3* allele or genetic background. Moreover, the frequency of *arg4–17* reversion in the *rev3* strains carrying *cyc1–115* and *cyc1–131* was no different from that in the other *rev3* strains, so that there is no evidence for the existence of any kind of *rev3* suppressor in these particular diploids. Previous results (LAWRENCE and CHRISTENSEN 1976), based on two determinations with a single strain, suggested that the UV-induced reversion frequency of *cyc1–131* was reduced by a greater factor than two in *rev3–1* strains. The reason for this is not known, but since the present results are based on four *rev3–1* and two *rev3–3* strains, the data given in Tables 2 and 3 are the more reliable. We conclude that a rather small effect of the *rev3* mutations on reversion is a characteristic and unusual feature of the *cyc1–115* and *cyc1–131* alleles. Such results reinforce previous observations that the mutational events which revert these alleles are the consequence of an unusual process (LAWRENCE and CHRISTENSEN 1978). Both alleles

can revert in several ways, but it is not yet known whether only some of these occur in *rev3* strains or whether the frequency of all of them is reduced by a factor of two.

Apart from these two base-pair substitution alleles, the frame shift mutations also seem to form a separate, if rather heterogeneous, class. On average, the frameshift alleles revert at a frequency about one tenth of normal in *rev3* strains, though the results for *cyc1–239* and in particular *cyc1–31* show rather large deviations from this average; in *rev3* strains, the *cyc1–31* allele reverts at a frequency that is less than 1% of normal, a frequency that is more characteristic of base-pair substitution alleles (apart from *cyc1–115* and *cyc1–131*) than of the frameshift mutations. Such behavior appears to parallel previous observations with the *rev1* mutant strains; *cyc1–31*, like the base pair substitution alleles other than *cyc1–115* and *cycl1–131*, reverted by a *REV1*-dependent process, while the other frameshift alleles did not.

The characteristic reversion frequencies, induced by UV, of the various *cyc1* alleles studied, and also the characteristic effects of the *rev3* mutations on these frequencies, are properties associated with specific sets of base-pair alterations within the *cyc1* locus itself. Virtually all of the revertants, the frequencies of which are listed in Tables 2 and 3, are the consequence of intragenic mutations; very few suppressors of either the translational or nontranslational variety were scored in these experiments. Nonsense suppressors are very rarely recovered on lactate medium (PRAKASH and SHERMAN 1973), and while nontranslational, or metabolic, suppressors that allow growth on lactate in the absence of normal levels of cytochromoe *c* can be found in all *cyc1* strains, most are recessive and are not expressed in diploid cells. Any that occur are readily detected by their very slow growth and particularly by spectroscopic examination of intact cells according to the method of SHERMAN and SLONIMSKI (1964). Their absence from the revertant colonies scored was verified by such spectroscopic examination of a sample of six mutants from each of two *rev3*, and two *REV3*$^+$, strains in the *rev3–1* series. Similarly, as shown previously (LEMONTT 1971; LAWRENCE and CHRISTENSEN 1978b), most of the revertants of *arg4–17* are of the locus type, in both *REV*$^+$ and *rev3* strains.

As expected from the results of LEMONTT (1971), the data in Tables 2 and 3 also show that the strains carrying *rev3* mutations are more sensitive with respect to survival than wild type, though the difference is not great, particularly on the nonsynthetic glycerol medium (YPG) used in estimating *cyc1* reversion. The difference is greater on synthetic dextrose medium (SD), used in estimating *arg4–17* reversion, though the sensitizing effect of *SD* is much less than that found in experiments with *rev2* strains (LAWRENCE and CHRISTENSEN 1978a).

DISCUSSION

The most distinctive feature that has emerged from a genetic analysis of the process of induced mutagenesis in yeast, of the kind described in this report and earlier papers, has been the observation that each well-studied gene involved in

this process has a characteristic and different mutational phenotype, implying that each gene function is involved in the production of characteristic and at least partially different sets of mutational alterations. Different phenotypes of this kind are often found even when a single mutagen like UV is employed (LEMONTT 1971; LAWRENCE and CHRISTENSEN 1976, 1978a,b) and the diversity of phenotypes is even more striking when different mutagens are used (PRAKASH 1976; MCKEE and LAWRENCE, in preparation).

The results in Tables 2 and 3 show that the *REV3* gene has a mutational phenotype quite different from the other genes concerned with induced mutagenesis (see Table 4 for a summary of these phenotypes). Unlike the *REV1* and *REV2* activities, the *REV3* gene function is employed in the production of at least some of the mutational events required to revert each of the 12 *cyc1* alleles studied. These events include a wide range of transitions, transversions, base-pair additions and deletions. In contrast the *REV1* gene function is not required for base-pair addition or deletion, with the possible exception of those changes required to revert *cyc1–31*, and is also not used in the production of the mutations that revert the initiation mutant, *cyc1–131*, and the proline missense mutant, *cyc1–115* (LAWRENCE and CHRISTENSEN 1978b). Yet a different pattern is observed with the *REV2* gene, whose function enhances the UV-induced reversion frequency of some, but not all, ochre alleles (LAWRENCE and CHRISTENSEN 1978a). Mutations at the three *REV* loci, isolated in an identical manner (LE-

TABLE 4

Summary of results showing the influence of rev1, rev2, rev3 *and* rad6 *mutations on the reversion of various* cyc1 *alleles*

Type and number of *cyc1* allele		*rev1*	*rev2*	*rev3*	*rad6*
Ochre	17 = 2	—	+		
	72	—	+		
	91 = 9	—	+/—	—	—
Amber	76	—	+		
	84	—	+		
	179	—	+	—	
Initiation	13	—		—	
	51	—		—	
	131	+	+	+/—	—
	133	—	+	—	
Proline	6	—		—	
missense	115	+	+	+/—	—
Frameshift	31	—		—	
	183	+	+	—	—
	239	+	+	—	
	331	+		—	

+ indicates wild-type reversion frequency.
+/— indicates that reversion frequency is 25% to 50% of wild-type value.
— indicates that reversion frequencies are very low; in *rev1* strains about 5% of wild-type value, in *rev3* strains about 2%, and in *rad6* strains no detectable reversion above spontaneous levels.

MONTT 1971), therefore possess distinctive and different phenotypes with respect to their influence on UV mutagenesis. Finally, the phenotype of the *rev3* mutations is also different from that of *rad6–1*; reversion of all markers tested, including *cyc1–115* and *cyc1–131*, is greatly and equally reduced in *rad6–1* strains (LAWRENCE *et al.* 1974; LAWRENCE and CHRISTENSEN 1976; and unpublished data), unlike the variable effect of the *rev3* mutations on the reversion of different types of alleles.

The observation that the *rev3* mutations variably reduce the induced-reversion frequencies of different classes of *cyc1* alleles implies that at least some mutational pathways remain operational in strains carrying these radiation-sensitive alleles; although the overall rate of reversion is lower, such cells retain the ability to produce some mutations that revert the atypical base-pair substitution alleles, *cyc1–115* and *cyc1–131*, and to a lesser extent those that revert the frameshift alleles other than *cyc1–31*. The induced reversion of the typical base-pair substitution alleles, on the other hand, is reduced to such a great extent in *rev3* strains that the few revertants formed could well be due to a very low level of *REV3* activity, produced either by very slight leakiness of these mutations or low-level suppression of some kind; it cannot be excluded, however, that minor mutational pathways exist in those cases also. Since each of the *cyc1* alleles listed in Table 1 can revert in several ways, it is possible that the mutational pathways that remain operational in *rev3* mutant strains are concerned with the production of only one or a few of these kinds of revertants, rather than with the formation of some fraction of each type. A definitive answer to this question cannot be given until protein from revertant classes has been analyzed. There are, however, suggestive correlations between the frequencies of different revertant classes in wild-type strains and the factor by which reversion frequencies are decreased by the *rev3* mutations. For example, the *cyc1–131* allele, which contains the valine codon GTG in place of the normal ATG initiation codon, reverts either by G·C to A·T transition to restore this codon at the normal −1 site or by an unknown alteration that leads to the relocation of the initiation codon at the −2 site (STEWART *et al.* 1971). These two types of revertants occur with approximately equal frequency, but a third type, the mutational relocation of the initiation codon at the lysine 4 site, is rare; such revertants produce only 50% of the normal amount of iso-1-cytochrome *c* (STEWART *et al.* 1971), a difference that is readily detectable by spectroscopic examination, and none was found in a sample of 24 revertants in the present experiments or of 48 in previous work (LAWRENCE and CHRISTENSEN, 1978b). It is therefore possible that only one of the two major classes of revertants occurs in *rev3* mutant strains. Similarly, the *cyc1–115* allele, which contains a proline codon CCPy in place of the leucine 14 codon, reverts by G·C to A·T transition at the first position of the triplet, at the second position of the triplet, or at both positions simultaneously, at roughly equal frequencies (STEWART and SHERMAN, personal communication). Again, it is possible that only one or two of these classes of revertants occur in *rev3* strains. Finally, each of the frameshift alleles, *cyc1–31*, *cyc1–183*, *cyc1–239*, and *cyc1–331*, is capable of reverting in several ways and at several sites (SHERMAN and

STEWART 1973, 1975; STEWART and SHERMAN 1974). It is not yet known whether all, or only some, of those various types of revertants occur in *rev3* mutant strains.

Genetic analysis of induced mutagenesis in yeast therefore shows that this process has the following properties. First, the mutagenic effectiveness of all mutagens tested, including UV, gamma rays (LAWRENCE *et al.* 1970, 1974) and a variety of chemical mutagens (PRAKASH 1974), depends on the *RAD6* function, and this function appears to be necessary for the formation of mutations at all sites and of all kinds (LAWRENCE *et al.* 1974; unpublished data). This implies that there is a single process, or set of related processes, that produce mutations; that this process is probably related to the repair or bypass of DNA damage; and that the great majority of mutagens are effective by virtue of "misrepair" rather than "misreplication." Yeast contrasts with *E. coli* in this respect since mutagens such as ethyl methanesulfonate and nitrosoguanidine are thought to act *via* misreplication in the bacterium (KONDO *et al.* 1970). Second, this process depends on the cooperative action of a large number of genes; more than a dozen loci have been identified so far (LAWRENCE and CHRISTENSEN 1976; LEMONTT 1971, 1977) and there is no reason to believe that this list is complete. Third, partially independent sets of gene functions are employed to produce mutations of different kinds or at different sites (LAWRENCE and CHRISTENSEN 1976, 1978a,b; present results) and to process different premutational lesions (PRAKASH 1976; McKEE and LAWRENCE, in preparation). In a formal sense the *RAD6* dependent mutagenic "pathway" is therefore best described as a highly branched process. Fourth, mutagenesis does not appear to be related to recovery in any causally simple manner. Although most of the mutation-deficient mutants are also more easily killed by radiation or other inactivating agents than are wild-type strains (LEMONTT 1971; LAWRENCE and CHRISTENSEN 1976), in keeping with the hypothesis that induced mutagenesis in yeast is a consequence of a mutagenic recovery process involving either the repair or bypass of DNA damage, the extent of the mutational deficiency in different strains is not commensurate with the degree of sensitivity. When grown on YPG medium, strains carrying mutations at one of the three *REV* loci are about equally sensitive with respect to UV killing, but have very different mutational deficiencies (see Table 4). On synthetic dextrose medium, *rev2* strains are more sensitive than those carrying *rev1* or *rev3* mutations, though they have the smallest mutagenic deficiency. A poor correlation between mutability and survival is also found with other mutants in the *rad6* pathway, while some of the *umr* mutants are no more sensitive to UV than is wild type (LEMONTT 1977). Mutational deficiency is therefore only loosely correlated with sensitivity.

In some instances, the poor correlation may be due to the simultaneous involvement of a gene in both mutagenic and nonmutagenic recovery, and the extreme sensitivity of *rad6* strains compared to the otherwise fairly similar *rev3* mutants is probably caused by this situation. The mutagenic and nonmutagenic activities of the *RAD6* locus can be separated both by mutations within and outside this gene; *rad6–1* strains carrying a nontranslational suppressor approach

wild-type strains in UV resistance, but are unable to mutate (LAWRENCE and CHRISTENSEN, unpublished data), while strains carrying the new *rad6–4* allele are almost as sensitive as *rad6–1* strains, but are capable of mutating (DOUTHWRIGHT-FASSE, personal communication). The *RAD9* gene appears to be active in either mutagenic or nonmutagenic recovery, depending on the type of DNA damage. Strains carrying a *rad9* mutation are incapable of mutating with chemical mutagens (PRAKASH 1974, 1976), are highly deficient in UV mutagenesis (LAWRENCE and CHRISTENSEN 1976), but mutate normally with gamma rays (McKEE and LAWRENCE 1978a). Such strains are nevertheless extremely sensitive to gamma rays, equally so in fact as those carrying *rad6–1* (McKEE and LAWRENCE, unpublished data) and only moderately sensitive to UV or chemical mutagens.

The existence of complex mutant phenotypes with respect to induced mutagenesis of the kind discussed above, and also the poor correlation between mutational deficiency and survival, is not predicted by the model for mutagenesis recently proposed for *E. coli* (CAILLET-FAUQUET, DEFAIS and RADMAN (1977), suggesting that it provides at best an incomplete description of yeast mutagenesis and may even be inappropriate for eukaryotes. According to this model, UV-induced mutations arise as a consequence of the suppression of the editing function of the DNA polymerase, allowing the previously stalled replication complex to move past noninstructive lesions, although at the cost of often inserting incorrect bases at these sites. With the exception of misreplication events during normal DNA synthesis, all types of mutations at all sites in the genome are, therefore, produced in a single step by a process that is directly related to recovery, and radiation sensitive mutations that efficiently prevent this recovery should also abolish all UV-induced mutagenesis. The *E. coli* mutations *recA* and *lexA* have this phenotype, and also the yeast *rad6–1* allele, but the majority of the yeast mutations do not. It is unlikely, therefore, that the functions of the corresponding wild-type alleles play an essential part in a recovery process of this sort, and if they play an inessential one, enhancing the fidelity of base selection within the replication complex for example, other reasons must be found to explain the moderate radiation sensitivity of strains carrying *rev* or similar mutations. A similar objection applies to post-replicational roles, such as mismatched base-pair correction, and there is no evidence that the *REV* genes play such a role (LAWRENCE and CHRISTENSEN 1978b). It is, therefore, unlikely that the RADMAN model, by itself, provides an adequate description of yeast mutagenesis, the *rad9* data discussed above being particularly difficult to accommodate within this scheme, though it may account for the production of some classes of mutations.

The existence of different mutational phenotypes among yeast, but not *E. coli*, loci suggests at first sight that the mechanism of mutagenesis in prokaryotes may be different from that in eukaryotes. This conclusion need not be correct, however, since the *E. coli recA* and *lexA* mutations, unlike the yeast *rev* alleles, were not selected on the basis of the mutational deficiency that they impart; mutations comparable to the *rev* alleles have only recently been isolated in *E. coli* and

their mutational phenotype has yet to be examined in detail (KATO and SHINOURA 1977). The data from yeast and bacteria are much more similar if attention is directed only at mutations selected for radiation sensitivity and in particular if the reversion of only a few alleles is monitored in such radiation-sensitive strains.

Although the data given in Tables 2 and 3 show that the *REV3* gene function is employed in the production of a much greater variety of mutational alterations than is the *REV1* gene function, a more detailed comparison of these results with those obtained previously (LAWRENCE and CHRISTENSEN 1978b) reveals a number of parallels that presumably reflect some intrinsic properties of the *cyc1* alleles and their reversion, though these are not yet understood. The base-pair substitution alleles, *cyc1–115* and *cyc1–131*, behave quite differently from the others in this class in both *rev1* and *rev3* mutant strains, for example, and the results for the frameshift allele, *cyc1–31*, are much more similar to the data for nonsense alleles than to those for the other frameshifts in both cases.

The atypical behavior of *cyc1–115* and *cyc1–131* does not appear to be explainable in terms of the primary structure of DNA, since they cannot be differentiated from other base-pair substitution alleles in terms of base change, site or surrounding nucleotide sequence (LAWRENCE and CHRISTENSEN 1978a). More probably, their unique reversion process is the result of other features, such as base modification, the location of nucleosomes or the presence of DNA-binding molecules. The atypical behavior of the incompletely understood frameshift allele, *cyc1–31*, may be due to the need for both base-pair substitution and base-pair addition, though there is no evidence to support this hypothesis. Whatever the explanation, it is clear that mutation in Saccharomyces is an unexpectedly complex process that is influenced by properties of chromatin other than nucleotide sequence.

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LITERATURE CITED

CAILLET-FAUQUET, P., M. DEFAIS and M. RADMAN, 1977 Molecular mechanisms of induced mutagenesis. Replication *in vivo* of bacteriophage ϕ174 single stranded, ultraviolet light irradiated DNA in intact and irradiated host cells. J. Mol. Biol. **117**: 95–112.

KATO, T., and Y. SHINOURA, 1977 Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. Mol. Gen. Genet. **156**: 121–131.

KONDO, S., H. ICHIKAWA, I. IWO and T. KATO, 1970 Base change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities. Genetics **66**: 187–217.

LAWRENCE, C. W., J. W. STEWART, F. SHERMAN and F. L. X. THOMAS, 1970 Mutagenesis in ultraviolet-sensitive mutants of yeast. Genetics **64**: S36–S37.

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. Mol. Biol. **85**: 137–162.

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 radiation-sensitive strains of yeast II. *rev2* mutant strains. Genetics **90**: 213–226. ——, 1978b Ultraviolet-induced reversion of *cyc1* alleles in radiation-sensitive strains of yeast I. *rev1* mutant strains. J. Mol. Biol. **122**: 1–21.

LEMONTT, J. F., 1971 Mutants of yeast defective in mutation induced by ultraviolet light. Genetics **68**: 21–23. ——, 1972 Induction of forward mutations in mutationally defective yeast. Molec. Gen. Genetics **119**: 27–42. ——, 1977 Pathways of ultraviolet mutability in *Saccharomyces cerevisiae*. III. Genetic analysis and properties of mutants resistant to ultraviolet-induced forward mutation. Mutation Res. **43**: 179–204.

PARKER, J. H. and F. SHERMAN, 1969 Fine structure mapping and mutational studies of genes controlling yeast cytochrome *c*. Genetics **63**: 9–22.

PRAKASH, L., 1974 Lack of chemically induced mutation in repair-deficient mutants of yeast. Genetics **78**: 1101–1118. ——, 1976 Effect of genes controlling radiation sensitivity on chemically induced mutation in *Saccharomyces cerevisiae*. Genetics **83**: 285–301.

PRAKASH, L., and F. SHERMAN, 1973 Mutagenic specificity: reversion of iso-1-cytochrome *c* mutants of yeast. J. Mol Biol. **79**: 65–82.

PUTTERMAN, G. J., E. MARGOLIASH and F. SHERMAN, 1974 Identification of missense mutants by amino acid replacements in Iso-1-cytochrome *c* from yeast. J. Biol. Chem. **249**: 4006–4015.

SHERMAN, F., and P. P. SLONIMSKI, 1964 Respiration-deficient mutants of yeast. II. Biochemistry. Biochem. Biophys. Acta **90**: 1–15.

SHERMAN, F., and J. W. STEWART, 1973 Mutations at the end of the iso-1-cytochrome *c* gene of yeast. pp. 55–86. In: *The Biochemistry of Gene Expression in Higher Organisms*. Edited by J. K. POLLAK and J. W. LEE. Australian and New Zealand Book Co. Pty. Ltd., Sydney. ——, 1974 Variation of mutagenic action on nonsense mutants at different sites in the iso-1-cytochrome *c* gene of yeast. Genetics **78**: 97–113. ——, 1975 The use of iso-1-cytochrome *c* mutants of yeast for elucidating the nucleotide sequences that govern initiation of translation. pp. 175–191. In: *Proceedings of the Tenth FEBS Meetings*.

SHERMAN, F., J. W. STEWART, M. JACKSON, R. A. GILMORE, and J. H. PARKER, 1974 Mutants of yeast defective in iso-1-cytochrome *c*. Genetics **77**: 255–284.

SHERMAN, F., M. JACKSON, S. W. LIEBMANN, A. M. SCHWEINGRUBER and J. W. STEWART, 1975 A deletion map of *cyc1* mutants and its correspondence to mutationally altered iso-1-cytochrome *c* of yeast. Genetics **81**: 51–73.

STEWART, J. W., F. SHERMAN, N. A. SHIPMAN and M. JACKSON, 1971 Identification and mutational relocation of the AUG codon initiating translation of of iso-1-cytochrome *c* in yeast. J. Biol. Chem. **246**: 7429–7445.

STEWART, J. W. and F. SHERMAN, 1972 Demonstration of UAG as a nonsense codon in bakers' yeast by amino acid replacements in iso-1-cytochrome *c*. J. Mol. Biol. **68**: 429–443. ——, 1973 Confirmation of UAG as a nonsense codon in bakers' yeast by amino acid replacements of glutamic acid 71 in iso-1-cytochrome *c*. J. Mol. Biol. **78**: 169–184. ——, 1974 Yeast frameshift mutations identified by sequence changes in iso--1-cytochrome *c*. pp. 102–127. In: *Molecular and Environmental Aspects of Mutagenesis*. Edited by L. PRAKASH, F. SHERMAN, M. W. MILLER, C. W. LAWRENCE and H. W. TABER. C. C. Thomas Pub., Inc., Springfield, Illinois.

WITKIN, E. M., 1976 Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bact. Rev. **40**: 869–907.

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