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VARIATION OF MUTAGENIC ACTION ON NONSENSE MUTANTS AT DIFFERENT SITES IN THE ISO-1-CYTOCHROME *c* GENE OF YEAST

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ABSTRACT

Three ochre and two amber mutants in yeast have been definitively identified by the amino acid replacements in iso-1-cytochromes *c* from intragenic revertants. Except for rare and sometimes unusual changes, all of the replacements were single amino acids whose codons differed from UAA or UAG by one base. These assignments, which were based on the absence of tryptophan replacements in ochre revertants, could be corroborated from the studies of two groups of suppressors that were shown to act on either the ochre or amber mutants. All five nonsense mutants are located at different sites in the *cyc1* gene and all are at sites that can be occupied by amino acids having a wide range of structures. The relative frequencies of the amino acid replacements indicate that identical codons located at different sites may respond differently to a mutagenic agent. Notably glutamine replacements occurred almost exclusively in UV-induced revertants of only one ochre mutant *cyc1–9*, but not at all or at reduced proportions in the others. Similarly, lysine replacements occurred almost exclusively in the NA-induced revertants of only the ochre mutant *cyc1–72*, but not at all in the others. These and other results reveal that mutation of A·T base pairs by UV and nitrous acid are dependent upon the location of the codon within the gene as well as the location of the base pair within the codon. From these findings, it appears as if the type of base-pair changes induced by UV and nitrous acid are strongly influenced by adjacent nucleotide sequences.

IT has long been suspected that mutation at a site may depend not only on the nature of the affected base pair, but also on the relationship to neighboring base pairs. This concept first arose from finding nonrandom distributions of spontaneous and induced mutants at different sites within a gene. The occurrence of highly mutable sites, or so-called "hotspots" is best exemplified by the classical studies of the *r*II locus of bacteriophage T4 (BENZER 1961), but they are also observed in higher cells such as yeast (GUTZ 1961; SHERMAN, STEWART, JACKSON, GILMORE and PARKER 1974). Similarly the types and rates of induction and reversion of nonsense mutants of bacteriophages appear to be markedly influenced by the positions within the gene (CHAMPE and BENZER 1962; BRENNER, STRETTON and KAPLAN 1965; STRETTON and BRENNER 1967; VANDERBILT and TESSMAN 1970; KOCH 1971; SALT and RONEN 1971). While in some cases it is still difficult to interpret the high frequencies of mutations at certain

sites, there are instances where it is evident that mutation rates are influenced by the neighboring nucleotide sequences (BRENNER, STRETTON and KAPLAN 1965; STRETTON and BRENNER 1967; KOCH 1971; SALT and RONEN 1971). The mechanisms by which nearby nucleotide sequences can affect spontaneous and induced mutation are not understood. In this paper, we have summarized the amino acid replacements in iso-1-cytochromes *c* from intragenic revertants of nonsense mutants at different positions within the gene. The normal amino acid sequence of the protein and the results of the amino acid replacements can be used to evaluate the influences of adjacent nucleotide sequences. Also. restrictions imposed by unacceptable amino acids can be uncovered and both the types and the relative frequencies of the base-pair changes can be deduced. The amino acid replacements occurring spontaneously and induced by NA and UV in three ochre and two amber mutants are reported in this paper. These results clearly demonstrate that the mutational changes induced by UV and NA were dependent upon the position of the codon within the gene and the base pair within the codon, implying that the mode of action of these mutagens is strongly influenced by adjacent nucleotide sequences. Some of these amino acid replacements and their implications have been previously reported in preliminary (SHERMAN *et al.* 1969) and final (STEWART *et al.* 1972; STEWART and SHERMAN 1972) publications.

NONSENSE MUTANTS OF ISO-1-CYTOCHROME *C*

Two hundred and ten independently derived mutants of the structural gene for iso-1-cytochrome *c* have been isolated by the following three procedures: the spectroscopic scanning procedure, which consisted of examining yeast with a spectroscope in order to isolate mutants deficient in the α-band of cytochrome *c* (SHERMAN 1964); the benzidine staining procedure which primarily depends on the content of hemoproteins (SHERMAN *et al.* 1968); and more recently, the chlorolactate resistance method which depends on the lack of utilization of lactate, either due to the absence or to the nonfunction of iso-1-cytochrome *c* (SHERMAN, STEWART, JACKSON, GILMORE and PARKER 1974). Reverants of these *cyc1* mutants (referred to as *cy1* or cy_1 mutants in earlier publications) can be selected on lactate medium, and reversion due to intragenic events and extragenic suppressors can be conveniently distinguished by genetic and other tests (see SHERMAN and STEWART 1971; 1973; SHERMAN *et al.*, 1974). The mutational lesions in some of these *cyc1* mutants have been established by the amino acid replacements in iso-1-cytochromes *c* from intragenic revertants. The UAA ochre and UAG amber mutants can be recognized because they can revert by single base changes to any one of several amino acid codons, shown in Figure 1. These patterns of amino acid replacements have been used to identify three ochre and two amber mutants; all of these nonsense mutants are located at different sites in the *cyc1* gene and all are at sites that have no restrictions for any of the amino acid residue related to the nonsense codons by single base-pair differences. We believe that the amino acid replacements summarized below, along

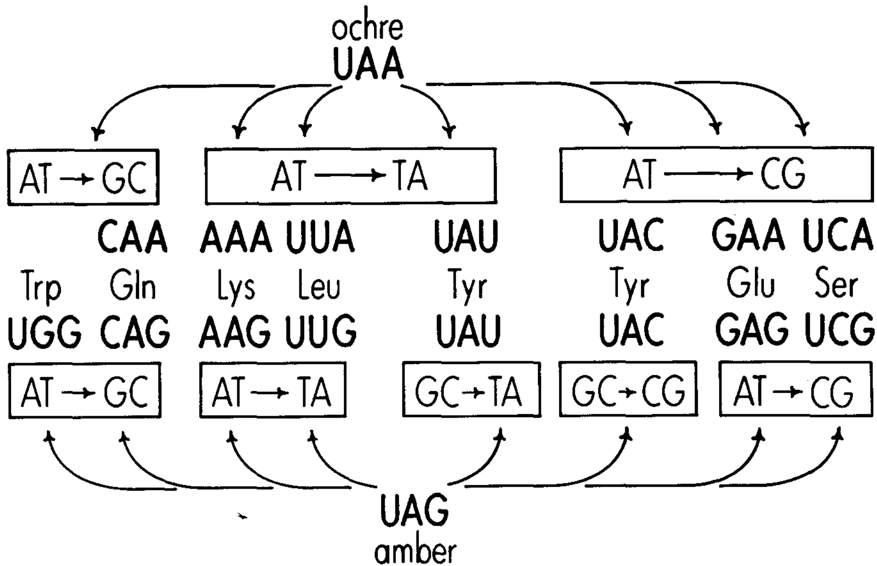

FIGURE 1.—The mutational events that lead to amino acid replacements in revertants of ochre and amber mutants by single base-pair changes. The mRNA codons are presented either below or above the amino acids they specify. The DNA base-pair changes that are associated with the amino acid replacements are circumscribed by boxes. Neither the transitions that interconvert these nonsense mutants, nor the transition that generates UGA from UAA, cause amino acid replacements.

with the studies of the nonsense suppressors, presented in the next section, definitely established the amber and ochre assignments in these five mutants.

The most extensive number of replacements comes from reverants of the nonsense mutant *cyc1–9*, which contains a UAA codon at the site corresponding to the glutamic acid residue at position 2 in normal iso-1-cytochrome *c*. This *cyc1–9* mutant, which was detected by the benzidine staining procedure (SHERMAN *et al.* 1968), contains no detectable iso-1-cytochrome *c*. The primary structure of iso-1-cytochromes *c* from over 150 intragenic revertants have been examined. These include the 45 proteins from the initial study of spontaneous revertants and revertants induced with UV, NA, X-rays, ethyl methanesulfonate, diethyl sulfate, methyl methanesulfonate, 1-nitrosoimidazolidone-2, and *N*-methyl-*N'*- nitro-*N*-nitrosoguandine (STEWART *et al.* 1973), as well as 14 additional proteins from revertants induced with X-rays and α-particles (SHERMAN and STEWART in preparation; see SHERMAN and STEWART 1973), and 25 additional proteins from spontaneous revertants and UV- and NA-induced revertants that are reported for the first time in this paper. The single amino acid replacements in 81 of these revertant proteins are presented in Table 1. Two other revertant proteins, induced by X-irradiation and α- particles, contained, respectively, Met-*Ile*-Glu-Phe- and (Met)Thr-*Leu*-*Leu*- replacement of the normal (Met)Thr-Glu-Phe- sequence; these are most simply explained by two concomitant base-pair substitutions. The remaining revertant, induced by NA,

TABLE 1

Origin and properties of the five cyc1 *nonsense mutants and the amino acid replacements in iso-1-cytochromes* c *from intragenic revertants*

Mutant	Lesion	Inducing mutagen	Selection method	Residue position	Normal residue	Replacements in revertant iso-1-cytochromes c*									Suppressed by:†	
						Trp	Gln	Lys	Leu	Tyr	Glu	Ser	Others	Total	*SUP2–1* etc.	*SUP7–2*
cyc1–2	UAA	NA	spectroscope	21	Gln	0	8	4	8	12	0	1	0	33	+	0
cyc1–9	UAA	UV	benzidine	2	Glu	0	42	8	11	15	1	2	3	84	±	0
cyc1–72	UAA	UV	chlorolactate	66	Glu	0	21	20	7	6	7	0	1	62	+	0
cyc1–76	UAG	UV	chlorolactate	71	Glu	4	1	1	4	1	0	1	0	12	0	+
cyc1–179	UAG	UV	chlorolactate	9	Lys	17	6	0	15	8	1	1	4	52	0	+

* Amino acid replacements in iso-1-cytochromes *c* from intragenic revertants that occurred spontaneously and that were induced by various mutagens (see text), including NA and UV (see Table 2). From: *cyc1–2*, STEWART and SHERMAN (1968; in preparation); *cyc1–9* STEWART *et al.* (1972), SHERMAN and STEWART (in preparation); *cyc1–72*, STEWART and SHERMAN (in preparation); *cyc1–76*, STEWART and SHERMAN (1973); *cyc1–179*, STEWART and SHERMAN (1972), SHERMAN and STEWART (in preparation).

† GILMORE, STEWART and SHERMAN (1971); SHERMAN *et al.* 1973; SHERMAN *et al.* (1974).

so far has not been identified but it also appears to be the result of more than one base-pair change. Peptide mapping, amino acid analysis and sequencing of several selected samples have established that the 81 single amino acid replacements are located at position 2, and all of these replacements comprise the set of the amino acids that have codons differing from UAA by one base. In contrast, there was no case of a tryptophan replacement in any of these 84 revertants proteins or in 72 other revertant proteins from another study not tabulated here (LAWRENCE, STEWART, SHERMAN and CHRISTENSEN 1974). The structural diversity of the amino acid residues at position 2, and the deletion of this region in other types of revertants (STEWART *et al.* 1971; SHERMAN and STEWART 1973), leaves little doubt that tryptophan at position 2 would result in a functional iso-1-cytochrome *c*. We therefore conclude that the lesion in *cyc1–9* is ochre.

The same pattern of amino acid replacements was the basis for the conclusion that *cyc1–2* and *cyc1–72* are ochre mutants. The *cyc1–2* mutant was detected by the spectroscopic scanning procedure in the first systematic attempt to isolate cytochrome *c*-deficient mutants (SHERMAN 1964) and was the key mutant for establishing that the *cyc1* locus determines the primary structure of iso-1-cytochrome *c* (SHERMAN *et al.* 1966). Initial peptide mapping and amino acid analysis of total proteins and of heme peptides demonstrated that the two revertants, *CYC1–2-A* (SHERMAN *et al.* 1966) and *CYC1–2-D* (see SHERMAN *et al.* 1970) had alterations confined to positions 19 through 27. On the basis of these altered peptide maps and of the normal sequence reported by NARITA *et al.* (1963) and NARITA and TITANI (1969) for cytochrome *c* from *Saccharomyces oviformis*, it was incorrectly suggested that the ochre lesion in *cyc1–2* corresponds to residue position 20 (STEWART and SHERMAN 1968; see SHERMAN *et al.* 1970). Reinvestigation of the partial sequence of normal iso-1-cytochrome *c* revealed that the normal residues at positions 20 and 21 are, respectively, leucine and glutamine and not glutamic acid and leucine as originally reported for *Saccharomyces oviformis* (LEDERER, SIMON and VERDIÈRE 1972; STEWART and SHERMAN, in preparation). In addition, sequencing of altered heme peptides from several *cyc1–2* revertants established unambiguously that the replacements occur at position 21 (STEWART and SHERMAN, in preparation). Presented in Table 1 are the amino acid replacements in iso-1-cytochromes *c* from 33 *cyc1* revertants that were obtained spontaneously or induced by UV, NA, ethyl methanesulfonate and 1-nitrosoimidazolidone-2 (STEWART *et al.*, in preparation). Thus *cyc1–2* gives rise to revertants having all the amino acid replacements that are expected for an ochre mutant except glutamic acid.

The *cyc1–72* mutant was detected by the chlorolactate procedure (SHERMAN *et al.*, 1974). Iso-1-cytochrome *c* in 29 revertants of *cyc1–72*, obtained spontaneously or induced with UV, NA, diethyl sulfate and 1 nitrosoimidazolidone-2, were either normal or had a glutamic acid residue replaced by either leucine, glutamine, or lysine. Because none of the 29 revertants had tryptophan replacing glutamic acid, *cyc1–72* was identified as ochre. The replaced residue was shown to be glutamic acid 66 by sequencing several altered peptides that were

released by chemical cleavage at the tryptophan 64 residue (STEWART and SHERMAN, in preparation). An additional 33 revertant proteins from *cyc1–72* have been more recently examined. All but one of the 62 revertants appear to have arisen by a single base-pair change; the exceptional protein has not yet been completely characterized, but it is multiply altered. All of the expected amino acid replacements of an ochre codon were observed except serine.

It was established that the defect in *cyc1–179* is due to a UAG amber codon corresponding to residue 9 by examining altered iso-1-cytochromes from 42 intragenic revertants which were obtained spontaneously or induced with UV, NA, diethyl sulfate, and X-rays (STEWART and SHERMAN 1972). An additional ten intragenic revertants obtained with α-particles were examined (SHERMAN and STEWART, in preparation; see SHERMAN and STEWART 1973). Partial sequencing of five proteins, peptide maps, and amino acid compositions established that 48 out of the 52 revertant proteins contained the single amino acid replacements presented in Table 1. The four remaining revertants, which were derived by treatments with NA, X-rays and α-particles, are most simply accounted for by concomitant substitutions of 2 or 3 base pairs (STEWART and SHERMAN 1972) or by the deletion of 6 base pairs (see SHERMAN and STEWART 1973). The single amino acid replacements include all of the amino acids whose codons differ from UAG by one base, except for lysine which is found at this position in normal iso-1-cytochrome *c*.

A less extensive study involving 12 revertants that were obtained spontaneously or that were induced by UV, NA and diethyl sulfate also unambiguously identified the lesion as *cyc1–76* as a UAG amber mutation (STEWART and SHERMAN 1973). The single amino acids, presented in Table 1, were determined to replace glutamic acid 71 in these 12 revertant proteins by subjecting them to amino acid analysis, peptide mapping and sequencing fragments produced by cyanogen bromide digests. The replacements in the revertant proteins comprise all but one of the amino acids that would be expected to arise from a UAG mutant by single base-pair changes; the one residue not found as a replacement, glutamic acid, is at this position in the normal protein. It should be emphasized that tryptophan, the critical replacement which differentiates amber and ochre mutations, constitutes approximately one-third of the replacement in both *cyc1–179* and *cyc1–76* revertants. The site of replacements in revertants of the five nonsense mutants are presented in Figure 2 along with the normal sequence of iso-1-cytochrome *c*.

NONSENSE SUPPRESSORS

Thus it appears as if ochre and amber mutants can be distinguished on the basis of tryptophan replacements in intragenic revertants. However, it is still possible that tryptophan replacements at certain sites result in unfunctional iso-1-cytochromes *c* and therefore the assignments of ochre codons by this criterion alone are not indisputable. While it is clear that tryptophan residues are very likely to be acceptable at the *cyc1–9* site, it is not as evident that this is true for the *cyc1–2 and cyc1–72* sites. Supporting evidence for the amber and

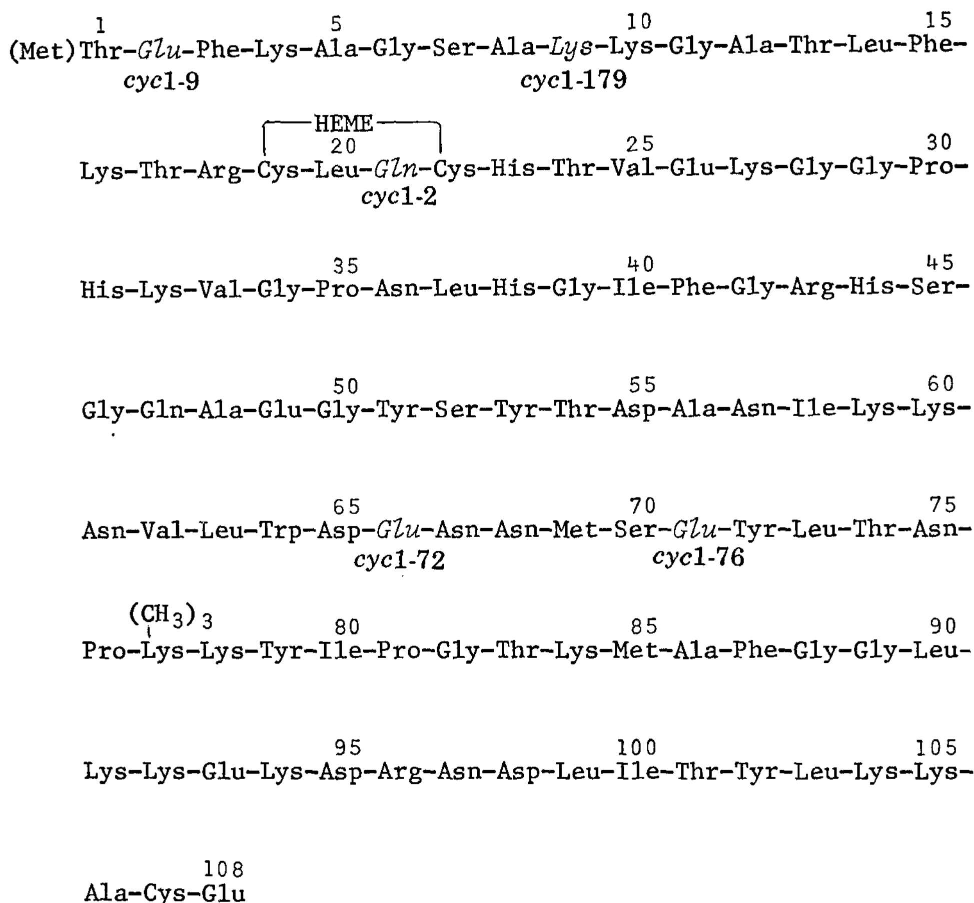

FIGURE 2.—The amino acid sequence of iso-1-cytochrome *c* (NARITA and TITANI 1969; LEDERER, SIMON and VERIERE 1972; STEWART and SHERMAN in preparation). The residues that are replaced in revertant iso-1-cytochrome *c* from the five nonsense mutants are shown in italics, directly above the corresponding *cyc1* mutant designations. Also shown are the sites of heme attachments at positions 19 and 22, the ε-N-trimethyllysine at position 77 (DELANGE, GLAZER and SMITH 1970), and the amino terminal residue of methionine that is excised from the normal protein (STEWART *et al.* 1971).

ochre assignments comes from studies of the action of nonsense suppressors on the *cyc1* mutants.

There are numerous extragenic suppressors that have the ability to restore the function of some, but not all mutant alleles occurring at probably every locus in yeast (HAWTHORNE and MORTIMER 1963; MORTIMER and GILMORE 1968). One group of these "super-suppressors" map at any one of eight district loci, and belong to class I, set 1, since they efficiently suppress the five mutants *trp5–48*, *arg4–17*, *his5–2*, *lys1–1* and *ade2–1* (GILMORE 1967). Each of the eight suppressors *SUP2–1*, *SUP3–1*, *SUP4–1*, *SUP5–1*, *SUP6–1*, *Sup7–1*, *SUP8–1* and *SUP11–1*, caused the production of 5% to 18% of the normal amount of iso-1-

cytochrome *c* when they are individually coupled to the *cyc1–2* mutant. All eight of the suppressed proteins contain a residue of tyrosine at the position which corresponds to the site of the ochre codon (GILMORE, STEWART and SHERMAN 1971). Similarly, *SUP7–1* was shown to cause the insertion of tyrosine at position 2 in iso-1-cytochrome *c* from the suppressed *cyc1–9* strain but the level was only approximately 1% of the normal amount. Additional class I, set 1 suppressors appeared to act on *cyc1–9*, but like *SUP7–1*, only inefficiently. In contrast, as described below, none of the several class I, set 1 suppressors that were tested caused the formation of iso-1-cytochrome *c* with the *bona fide* amber mutants *cyc1–76* and *cyc1–179*.

Recently systematic searches were made for suppressors that would efficiently act on the *cyc1–76* and *cyc1–179* alleles (SHERMAN *et al.*, 1973; LIEBMAN, SHERMAN and STEWART 1973). The *cyc1–76* and *cyc1–179* mutants were coupled to a variety of markers, including suppressible markers that were suspected of being amber, as well as some of the nutritional ochre markers previously described. In the two studies cited above, the total cytochrome *c* content was estimated in approximately 1,500 revertant strains that were believed to contain amber suppressors. Approximately 50 of these suppressors caused the production of over 30% of the normal amount of iso-1-cytochrome *c*, and many of these were subjected to genetic analyses. So far all of the efficient amber suppressors that have undergone genetic tests have been found to be allelic to one or another of the eight ochre-specific suppressors described above and all of the suppressed iso-1-cytochromes *c* that were subjected to analyses had tyrosine residues at the amber sites.

All 210 *cyc1* strains were tested with four of the suppressors (*SUP2–1*, *SUP6–1*, *SUP8–1*, *SUP11–1*) that efficiently suppress the ochre mutant *cyc1–2* and with one of the suppressors (*SUP7–2*) that efficiently suppress the two amber mutants *cyc1–76* and *cyc1–179* (SHERMAN *et al.* 1974). The results of the action of these suppressors on the five nonsense mutants, summarized in Table 1, indicate a high degree of specificity, which is in complete agreement with the assignments based on the absence or presence of tryptophan replacements. It has been suggested from these results that the highest efficiency suppressors are altered forms of tyrosine t-RNA which act on either amber or ochre codons but, unlike *E. coli* ochre suppressors, not on both nonsense codons.

AMINO ACID REPLACEMENTS

In light of the definitive identification of the nonsense codon in these five *cyc1* mutants, it is now possible to consider the amino acid replacements in their intragenic revertants and accurately describe the corresponding base-pair changes. While numerous mutagens have been used in the reversion studies, the highest number of revertants we have chosen to examine are those that arose spontaneously and those that were induced by UV and NA, and we will discuss only these data. It should be pointed out that the spontaneous revertants were derived from different subclones and therefore were of independent origin. Also, care was taken to ensure that the induced revertants were in all likelihood the

result of the treatments and were not simply preexisting spontaneous mutants. In most experiments the induced frequencies were over a hundredfold greater than the untreated spontaneous frequencies, and in no case was an induced mutant studied unless there was at least a tenfold increase. Most treatments consisted of either 1600 ergs mm^{-2} of UV light or 15 minutes with 50 mM nitrous acid.

The tabulation of the amino acid replacements in spontaneous and UV- and NA-induced revertants from different *cyc1* strains is presented in Table 2. It should be mentioned that not all of the *cyc1* strains were directly derived from the same parent strain. Unlike the other four *cyc1* mutants, the *cyc1–2* mutant was derived from the normal strain D273–10B. The original mutant B-295 (SHERMAN 1964), and three different segregants, each carrying the same *cyc1–2* gene, were used to obtain the 23 revertants, and because of the low numbers, all of their replacements are tabulated together. The strains B-577 (*cyc1–72*), B-581 (*cyc1–76*) and B-699 (*cyc1–179*) were directly isolated from the normal strain D311-3A by the chlorolactate method, and these three strains are therefore closely related. However, it should be remembered that the *cyc1–9* mutant was detected by the benzidine staining procedure which requires the use of ρ^- strains. For this reason the original *cyc1–9* ρ^- mutant, although isolated from D311–3A, was crossed to normal strains in order to obtain the *cyc1–9* ρ^+ segregants JP109–3A and JP109–6A.

The relative frequencies of amino acid replacements in revertants from these original strains strongly indicated that some of the *cyc1* mutants were responding differently to the NA and UV treatments. Notably, all eleven of the UV-induced revertants from the *cyc1–9* strains, JP109–3A and JP106–6A, contained glutamine replacements, while at least two types of replacements were found in the UV-induced revertants of the other nonsense mutants. Also, NA induced a preponderance of lysine replacements in the *cyc1–72* strain, B-577, while this replacement was completely absent in all of the NA-induced revertants from the other *cyc1* strains. Other differences in the pattern of amino acid replacements include the lack of glutamine replacements in UV-induced revertants from *cyc1–2* and *cyc1–76*, while this replacement is prevalent in UV-induced revertants of all other mutants.

While it is clear from the revertants of these initial strains that differences exist, it was still possible, although unlikely, that these differences reflected a variation in genetic background and not the particular *cyc1* allele. Therefore studies were undertaken to see if the patterns of amino acid replacements could be repeated with strains from different genetic pedigrees. In particular, we wished to examine the exclusive glutamine replacements induced by UV in *cyc1–9* strains and the predominant lysine replacements induced by NA in the *cyc1–72* strain. Figure 3 outlines the manipulations that were performed to reduce the genetic differences between strains carrying the *cyc1–9* and *cyc1–72* genes. The original *cyc1–72* mutant, B-577, was crossed to one of the *cyc1–9* segregants, JP109–6A, and one *cyc1–72* segregant, D734–1D, was chosen for the backcross, D-738. Four segregants of a single tetrad from D-738 were used for the reversion studies. The distribution of amino acid replacements presented in Table 2 substantiates the

TABLE 2

Amino acid replacements in iso-1-cytochromes c from spontaneous and UV- and NA-induced revertants

Gene	Strain	Mutagen	Amino acid replacements							
			Trp	Gln	Lys	Leu	Tyr	Glu	Ser	Total
cyc1-2	Various	None	0	0	1	1	2	0	1	5
		UV	0	0	0	7	4	0	0	11
		NA	0	5	0	0	2	0	0	7
cyc1-9	JP109-3A	UV	0	3	0	0	0	0	0	3
	JP109-6A	None	0	2	0	0	2	1	1	6
		UV	0	8	0	0	0	0	0	8
		NA	0	4	0	1	0	0	0	5
	D738-10C	None	0	1	0	0	2	0	0	3
		UV	0	5	0	1	0	0	0	6
		NA	0	1	0	1	1	0	1	4
	D738-10D	None	0	0	0	1	1	0	0	2
		UV	0	5	0	1	0	0	0	6
		NA	0	2	0	1	1	0	0	4
	Total	None	0	3	0	1	5	1	1	11
		UV	0	21	0	2	0	0	0	23
		NA	0	7	0	3	2	0	1	13
cyc1-72	B-577	None	0	2	0	0	1	5	0	9*
		UV	0	4	0	4	3	0	0	11
		NA	0	1	8	0	0	0	0	9
	D738-10A	None	0	3	0	0	0	0	0	3
		UV	0	3	0	1	0	0	0	4
		NA	0	1	5	0	0	0	0	6
	D738-10B	None	0	2	0	0	0	1	0	3
		UV	0	2	0	2	0	0	0	4
		NA	0	2	4	0	0	0	0	6
	Total	None	0	7	0	0	1	6	0	15*
		UV	0	9	0	7	3	0	0	19
		NA	0	4	17	0	0	0	0	21
cyc1-76	B-581	None	1	0	0	0	1	0	1	3
		UV	1	0	0	2	0	0	0	3
		NA	2	0	0	1	0	0	0	3
cyc1-179	B-699	None	2	0	0	0	4	0	0	6
		UV	1	5	0	4	0	0	0	10
		NA	4	1	0	5†	0	0	0	10

* Includes one case of an unknown replacement, which required more than one base change.
† Includes one case of a double base-pair change which resulted in -Leu-Glu- at positions 9 and 10.

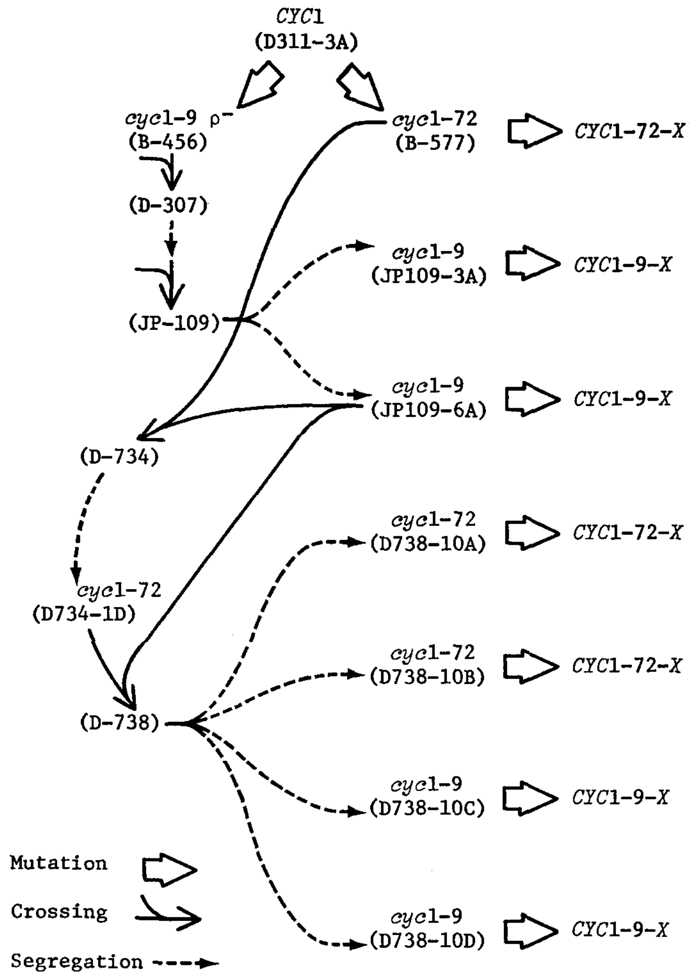

FIGURE 3.—An outline of the genetic operations that were undertaken in order to obtain the several *cyc1-9* and *cyc1-72* strains used for isolating intragenic revertants. The strain numbers are shown in parentheses; intragenic revertants are denoted by *CYC1-9-X* and *CYC1-72-X*. The original mutant, *cyc1-9* ρ^-, underwent two successive crosses, and the two meiotic segregants JP109-3A and JP109-6A, were chosen for the initial reversion studies of *cyc1-9*. The original isolate of *cyc1-72* (B-577) was used directly for reversion studies. In order to minimize genetic differences between strains carrying the *cyc1-9* and *cyc1-72* alleles, the original *cyc1-72* mutant, B-577, was crossed to the *cyc1-9* segregant, JP109-6A; from this cross a *cyc1-72* meiotic segregant, D734-1D, was chosen for a backcross to JP109-6A; from this cross, D-738, two *cyc1-9* and two *cyc1-72* meiotic segregants of a single tetrad were chosen for the additional reversion studies. The amino acid replacements in iso-1-cytochromes *c* from revertants of these seven *cyc1-9* and *cyc1-72* mutant strains are presented in Table 2.

observations with the initial strains and indicates that there are no apparent differences between strains having the same *cyc1* gene. All of the UV- and NA-induced replacements in the revertants of the three ochre mutants are tabulated together in Table 3, along with the corresponding base-pair changes.

TABLE 3

Amino acid replacements in iso-1-cytochromes c from UV- and NA-induced revertants of the three ochre mutants and the corresponding base-pair changes (summarized from Table 2)

Amino acid replacement	Base in UAA altered	Base-pair change	UV			NA		
			cyc1-2	*cyc1-9*	*cyc1-72*	*cyc1-2*	*cyc1-9*	*cyc1-72*
Glutamine	First	AT → GC	0	21	9	5	7	4
Lysine	First	AT → TA	0	0	0	0	0	17
Glutamic acid	First	AT → CG	0	0	0	0	0	0
Leucine	Second	AT → TA	7	2	7	0	3	0
Serine	Second	AT → CG	0	0	0	0	1	0
Tyrosine	Third	AT → TA }	4	0	3	2	2	0
Tyrosine	Third	AT → CG }						
		Total	11	23	19	7	13	21

DISCUSSION

The determination of amino acid replacements in revertants of nonsense mutants is an ideal method for investigating the influences of adjacent nucleotides on mutation. In contrast to studies where base-pair changes are deduced only by indirect genetic tests with a high degree of uncertainty, amino acid replacements can accurately establish the type of base-pair changes associated with the mutations. Also, the pathways for reversion of nonsense codons are varied, and the same type of change occurring at different base pairs within the codon can be distinguished (see Figure 1). Thus amino acid replacements reveal the relationships of the mutated base pair to the adjacent base pairs within the codon as well as to the base pairs of neighboring codons.

As shown in Figure 1, the transition and both transversions of AT base pairs in ochre codons can result in amino acid replacements. These changes, as well as transversion of the GC base-pairs, potentially can occur in revertants of amber mutants. Of all of the single base-pair substitutions, only the GC → AT transition is not observed, since this change leads to the formation of the UAA ochre codon from the UAG amber codon. It is not unreasonable to anticipate the detection of all of the changes expected for reversions, since nonsense mutations can occur at numerous sites, including sites that are relatively insensitive to amino acid changes.

In this paper we have tabulated the amino acid replacements that led to the identification of three ochre and two amber mutants, and we have summarized the results of studies with suppressors that corroborated these assignments. These five nonsense mutants all occur at different sites that can be occupied by amino acids having a wide range of structures. While numerous mutagens have been used for inducing revertants, in this paper we have tabulated and discussed only the 159 amino acid replacements that arose spontaneously or that were induced by UV or NA. The relative frequencies of the different amino acid replacements revealed that some of the *cyc1* mutants were responding differently to the mutagenic action of NA and UV. Amino acid replacements in revertants from

interbred strains strongly indicated that these differences are the property of the specific *cyc1* allele and not the property of other undefined genes. These variations in reversion of nonsense mutants point out the complexity of the molecular processes involved in UV and NA mutagenesis.

The mutagenic specificity of NA has been recently investigated in yeast by quantitatively measuring the reversion frequencies of various *cyc1* mutants that have defined lesions and known pathways for reversion (PRAKASH and SHERMAN 1973). One of the tester strains, *cyc1–131*, contains an altered initiation codon, GUG, which can revert back to normal by a GC → AT transition (STEWART *et al.* 1971). NA caused reversion in this strain approximately 100-fold greater than in any of the other testers, including the *cyc1–9* ochre and the *cyc1–179* amber strains. It appears from these results that NA induces primarily GC → AT transitions, at least at one site. Also the GC → AT transition that produced *cyc1–2* by NA mutagenesis is meagerly suggestive for this mode of action. This GC → AT transition is the only type of base-pair change not manifested in revertants of ochre and amber mutants (see above).

Excluding this undetectable GC → AT base pair change, it is evident that other changes vary with the location of the base pair. Except for the *cyc1–72* mutants, approximately one-half of NA-induced reversions of amber and ochre mutants occur by AT → GC transitions, while the remaining half occur by various types of transversion, with AT → TA transversions of the middle base pair (leucine replacements) being most common (Table 3). In contrast, NA induces predominantly lysine replacements in *cyc1–72* strains, which occur by an AT → TA transversion of the first base pair in the ochre triplet. NA did not induce lysine replacements in any of the other ochre or amber mutants, nor did it induce any leucine or tyrosine replacements in *cyc1–72*, which occur by AT → TA transversion of, respectively, the second and third base pair in the codon. Thus the action of NA is dependent not only on the position of the codon in the gene but also on the position of the base pair in the codon.

In studies with the bacteriophage T4, NA induced mutations that were believed to be primarily AT → GC and GC → AT transitions (FREESE 1959; BAUTZ-FREESE and FREESE 1961; CHAMPE and BENZER 1962). These conclusions relied on the specificities of base analogs to cause only GC → AT and AT → GC transitions and of hydroxylamine to cause only the GC → AT transition. In a study on the interconversion of nonsense codons, it was also concluded that NA induced primarily transitions in the single-stranded bacteriophage S13 (VANDERBILT and TESSMAN 1970). The apparent absence of transversions in the bacteriophage studies of NA mutagenesis is in striking contrast to the abundance of transversions in yeast. While it is possible that this difference is real, the specific action of base analogs, upon which the bacteriophage conclusions depend, are not unquestionable (KREIG 1963). Analysis of mutant proteins from tobacco mosaic virus directly established that NA induces mainly A→G and C→U transitions (see SADGOPAL 1968). While the deamination of bases may account for the specific action of NA on the RNA virus, this picture certainly does not explain

its mutagenic action on yeast nor probably on bacteriophages (VANDERBILT and TESSMAN 1970).

Examination of primary structures of iso-1-cytochromes *c* from intragenic revertants revealed that UV caused a variety of types of forward mutations in 17 *cyc1* mutants (see SHERMAN and STEWART 1973; STEWART and SHERMAN 1974). The *cyc1* mutants of these studies were restricted to the 10% of the amino terminal region of the protein which is virtually expendable. All transitions and transversions potentially can give rise to observable mutants, either by mutation of the AUG initiation codon or by producing UAA and UAG mutations at var. rious sites. The distribution of base-pair substitutions indicated that UV did not induce selective types of changes.

In addition, the reversion frequencies of well-characterized *cyc1* mutants indicated that UV does not induce selective changes which could be explained by its action on a single type of base pair (PRAKASH and SHERMAN 1973). Nevertheless the rate of UV-induced reversion of *cyc1–9* is extremely high in comparison to other *cyc1* strains, including the *cyc1–2* ochre (PARKER and SHERMAN 1969), the *cyc1–179* amber (PRAKISH and SHERMAN 1973), other ochre mutants (LAWRENCE, unpublished results), as well as other *cyc1* mutants that may contain revertible AT base pairs (PRAKASH and SHERMAN 1973). The unusually high UV mutability of *cyc1–9* is even more surprising in view of the fact that the revertants contained almost exclusively replacements of glutamine. In contrast, this replacement was absent in the UV-induced revertants of *cyc1–2* ochre and the *cyc1–76* amber strains and occurred in approximately one-half of the UV-induced revertants of the other nonsense mutants. It is clear that the *cyc1–9* mutant contains a particular nucleotide sequence which is especially prone to UV mutation in a specific manner. In fact, it appears as if all three of the ochre mutants may be giving rise to a different distribution of amino acid replacements in UV-induced revertants.

So far we have been unable to explain the variations of the UV- and NA-induced changes from simple differences of base pairs surrounding the nonsense codons. However, because of the degeneracy of the genetic code, not all of the adjacent base pairs are known, except for the region of the *cyc1–9* nonsense codon that has been sequenced by frameshift mutations (STEWART and SHERMAN 1974).

While one could suggest that the rate and type of induced changes are solely dependent on the ability of certain sequences to be highly reactive to mutagens, studies with UV-sensitive strains indicate that this may not be the case, and that the type of repair of the damaged DNA may be a determining factor. A different distribution of amino acids, comprising serine, glutamic acid, leucine, as well as glutamine, was observed in revertants of a *cyc1–9* strain when it was coupled with the *rad6* gene, which increases UV inactivation and decreases UV reversion (LAWRENCE, STEWART, SHERMAN and CHRISTENSEN 1974). It appears that the type and rate of UV mutagenesis is dependent on error-prone repair enzymes, whose actions are strongly influenced by nucleotide sequences.

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ADDENDUM: Recently it was found that the frequencies of nitroquinoline oxide-induced revertants corroborated the assignments of amber and ochre mutants (PRAKASH, L. and F. SHERMAN, 1974 Differentiation between amber and ochre mutants of yeast by reversion with 4-nitroguinoline-1-oxide. Genetics **77**: 245–254).

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