ISOLATION AND CHARACTERIZATION OF AMBER SUPPRESSORS IN YEAST

SUSAN W. LIEBMAN, FRED SHERMAN AND JOHN W. STEWART

Department of Radiation Biology and Biophysics
University of Rochester School of Medicine and Dentistry
Rochester, New York 14642

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ABSTRACT

Nonsense suppressors were obtained in a haploid yeast strain containing eight nutritional mutations, that are assumed to be amber or ochre, and the *cyc1-179* amber mutation that has a UAG codon corresponding to position 9 in iso-1-cytochrome *c*. Previous studies established that the biosynthesis and function of iso-1-cytochrome *c* is compatible with replacements at position 9 of amino acids having widely different structures (STEWART and SHERMAN 1972). UV-induced revertants, selected on media requiring the reversion of one or two of the amber nutritional markers, were presumed to contain a suppressor if there was the unselected reversion of at least one other marker. The 1088 suppressors that were isolated could be divided into 78 phenotypic classes. Only 43 suppressors of three classes caused the production of more than 50% of the normal amount of iso-1-cytochrome *c* in the *cyc1-179* strain. Genetic analyses indicated that all of these highly efficient amber suppressors are allelic to one or another of the eight suppressors which cause the insertion of tyrosine at ochre (UAA) codons (GILMORE, STEWART and SHERMAN 1971). Furthermore, only tyrosine has been identified at position 9 in iso-1-cytochrome *c* in *cyc1-179* strains suppressed with these efficient amber suppressors.

NUMEROUS suppressors in the yeast *Saccharomyces cerevisiae* have been isolated and classified according to their ability to suppress particular alleles (for example see GILMORE 1967; HAWTHORNE and MORTIMER 1968; HAWTHORNE and LEUPOLD 1974). The codons suppressed by several of these suppressors have been unambiguously determined by their action on *cyc1* mutants with ochre (UAA) and amber (UAG) mutations that were identified by amino acid replacements in iso-1-cytochromes *c* from intragenic revertants (see SHERMAN and STEWART 1974). The amino acids inserted by these suppressors have been determined from protein analyses of iso-1-cytochromes *c* in these suppressed mutants. In addition, the efficiencies of suppression were estimated from the levels of iso-1-cytochromes *c* in intact cells and in cell extracts (GILMORE, STEWART and SHERMAN 1971; SHERMAN *et al.* 1973; LIEBMAN, STEWART and SHERMAN 1975a, b). It has been suggested that most of these suppressors arise by mutations of tRNA genes and that they are similar to the nonsense suppressors found in *E. coli*.

In one of the first extensive studies, GILMORE (1967) uncovered mutants of only eight distinct loci (the class I, set 1 suppressors) that efficiently suppressed all of a set of five markers and only these suppressors acted efficiently on the ochre mutant *cyc1–2*. Each suppressor caused the insertion of tyrosine at ochre sites, but did not act on amber mutants (GILMORE, STEWART and SHERMAN 1971). A lower-efficiency UAA suppressor, *SUQ5*-**o**, first investigated by COX (1965, 1971), was shown to cause the insertion of serine (LIEBMAN, STEWART and SHERMAN 1975a) and to become more efficient in the presence of the non-Mendelian genetic determinant ψ^+, which also increases the efficiencies of the tyrosine-inserting UAA but not UAG suppressors (COX 1971; LIEBMAN, STEWART and SHERMAN 1975b).

Two amber suppressors, *SUP6*-**a** and *SUP7*-**a**, which were chosen for their high efficiency of action on the amber allele *cyc1–76*, were shown to cause the insertion of tyrosine at amber sites. These suppressors are alleles of the class I, set 1, UAA-suppressors, *SUP6*-**o** and *SUP7*-**o**, but they do not act on ochre codons (SHERMAN *et al.* 1973).

In this paper, a comprehensive search for amber suppressors is described. 1088 suppressors were isolated and were divided into 78 phenotypic classes. Those that act with the highest efficiency on the amber marker *cyc1–179* are all alleles of one or another of the eight UAA suppressors that insert tyrosine. As expected, these UAG suppressors cause the insertion of tyrosine at the amber site. A preliminary account of some of these results has been reported (LIEBMAN, SHERMAN and STEWART 1973).

MATERIALS AND METHODS

Mutants of iso-1-cytochrome c: The *cyc1–76* mutant has an amber codon corresponding to amino acid residue position 71 in iso-1-cytochrome *c*, while the *cyc1–179* and *cyc1–138* mutants have amber codons corresponding to amino acid position 9. The origin and properties of these mutations, *cyc1–76*, *cyc1–179* and *cyc1–138*, which completely lack iso-1-cytochrome *c*, have been described in detail in previous publications (STEWART and SHERMAN 1972, 1973; SHERMAN *et al.* 1974). The *cyc1–179* and *cyc1–138* alleles appear to be exact repeats (SHERMAN and STEWART 1973).

Suppressors: In this paper, UAA suppressors refer to suppressors which act on UAA (ochre) mutants but not on UAG (amber) mutants. The UAA suppressors should not be confused with prokaryotic "ochre" suppressors, which act on both UAA and UAG mutants and which so far are found only in *E. coli* and *S. typhimurium* and not in yeast. Amber or UAG suppressors refer to suppressors which act on UAG mutants but not on UAA mutants.

We have found it convenient to designate the genotypes of suppressors with two types of nomenclature—a brief form and a complete but cumbersome form. In the brief form, UAA and UAG suppressors are simply indicated, respectively, by **o** and **a** following the locus designation. For example *SUP5*-**o** and *SUP5*-**a** refer, respectively to all UAA and UAG suppressors of the *SUP5* locus. This abbreviated nomenclature incorrectly implies that all UAA suppressors of a given gene are identical and likewise all UAG suppressors of a given gene are identical. A more complete genetic nomenclature should include the allele numbers to indicate suppressors of independent origin. While such designation may be unimportant in some studies, it is crucial in others. When such information is to be presented, the allele number will follow the **o** and **a** symbols. The same allele numbers have not been assigned to both UAG and UAA suppressors, since we are maintaining previously assigned allele numbers, and since some authors may

choose to omit the **o** and **a** symbols. Thus *SUP5-o1* and *SUP5-a2* designate, respectively, a particular UAA suppressor and a particular UAG suppressor.

Using the above nomenclature, the eight UAA suppressors which were shown to insert tyrosine (GILMORE, STEWART and SHERMAN 1971) are denoted as *SUP2-o1*, *SUP3-o1*, *SUP4-o1*, *SUP5-o1*, *SUP6-o1*, *SUP7-o1*, *SUP8-o1*, and *SUP11-o1*, or briefly as *SUP2-o*, *SUP3-o*, etc. The gene symbols *SUP2-a2*, *SUP3-a2*, *SUP4-a4*, *SUP5-a2*, *SUP6-a2*, *SUP7-a2*, *SUP8-a2* and *SUP11-a2* denote particular UAG suppressors that are allelic to these tyrosine-inserting UAA suppressors and that were isolated in a previous study (SHERMAN *et al.* 1973) and in this study. As described above, these UAG suppressors can be briefly denoted as *SUP2-a*, *SUP3-a*, etc. The UAA suppressor which causes the insertion of serine (LIEBMAN, STEWART and SHERMAN 1975a) is denoted as *SUQ5-o1*, or briefly as *SUQ5-o*. The non-Mendelian determinant $\psi+$ is required for the efficient expression of this suppressor. $\psi+$ also increases the efficiencies of the class I, set 1 UAA suppressors, and the presence of these suppressors in a $\psi+$ strain is usually lethal. On the contrary, $\psi+$ does not appear to act on UAG suppressors (COX 1965, 1971; LIEBMAN, STEWART and SHERMAN 1975b).

Modification of suppressors by secondary mutations is denoted by appropriate symbols following the original gene designation. For example, the UAG suppressors obtained in this study by mutations of the UAA suppressors *SUP5-o1*, *SUP7-o1* and *SUQ5-o1* are denoted, respectively as *SUP5-o1-a1*, *SUP7-o1-a1* and *SUQ5-o1-a1*, or briefly as *SUP5-a*, *SUP7-a* and *SUQ5-a*. The symbol *SUP5-a2-a1* denotes a suppressor arising by a secondary mutation of *SUP5-a2* that lowered the efficiency of suppression and that is described in the accompanying paper (LIEBMAN and SHERMAN 1976).

Suppressible markers: The properties of the suppressible nutritional or resistance mutants are listed in Table 1. The suppressible auxotrophic markers, *met8-1*, *tyr7-1*, *trp1-1*, *ade3-26*, *ilv1-1*, *leu2-1*, *his5-2* and *lys1-1* were employed in the isolation of suppressors. These auxotrophic alleles were chosen either for their suitability in the selection of a wide variety of suppressors capable of acting on *cyc1-179* or for their known suppressibility by UAA suppressors. Ideally, these nutritional markers should require the insertion of a different spectrum of amino acids at the amber site and should require different efficiencies of suppression in order to be suppressed.

The *tyr7-1* and *trp1-1* alleles are presumed to be amber since they are acted on by suppressors of the *bona fide* amber mutations *cyc1-76* and *cyc1-179*, but are not suppressed by UAA suppressors (SHERMAN *et al.* 1973). The marker *met8-1* was suspected of being amber from its pattern of suppressibility (HAWTHORNE 1969a). HAWTHORNE (1969a,b) has described some suppressors

TABLE 1

Properties of nonsense mutants

Mutant	Phenotype	Suppressibility	Reference
met8-1	Requires methionine	UAG	HAWTHORNE (1969a)
*tyr7-1**	Requires tyrosine and phenylalanine	UAG	HAWTHORNE (1969a, b)
trp1-1	Requires tryptophan	UAG	HAWTHORNE (1969a, b)
ade3-26	Requires adenine and histidine	UAG	JONES (1972)
ilv1-1	Requires isoleucine	UAG	ROMAN and JACOB (1958)
leu2-1	Requires leucine	UAA	HAWTHORNE (1969a, b)
lys1-1	Requires lysine	UAA	GILMORE and MORTIMER (1966)
lys2-1	Requires lysine	UAA	HAWTHORNE (1969a, b)
his5-2	Requires histidine	UAA	GILMORE and MORTIMER (1966)
can1-100	Resistant to canavanine	UAA	MORTIMER (unpublished)
tyr7-1,**o**	Requires tyrosine and phenylalanine	UAA	MAGNI (unpublished)

* or *aro7-1*.

which act on both *tyr7-1* and *trp1-1* while others act on *tyr7-1* but not on *trp1-1*. Furthermore, most suppressors which act on *trp1-1* and *tyr7-1* also act on *met8-1*, but the converse is not true (MORTIMER, personal communication). These characteristics suggested that the alleles *met8-1*, *tyr7-1* and *trp1-1* differed either in the amino acid replacements that were acceptable at the mutant sites or they differed in the efficiencies required to suppress them.

The *ade3-26* allele was suspected of being amber since it was acted on by a suppressor of the presumed amber mutant, *tyr7-1*, but was not suppressed by *SUP2-o*, a class I, set 1, UAA suppressor (JONES 1972). JONES (1972) has hypothesized that only polar mutations can be recovered at the *ade3* locus. If so, any amino acid inserted at the *ade3-26* mutant site should be acceptable. As will be shown below, it now appears that *met8-1* and *ade3-26* are indeed amber mutations since they can each be suppressed by suppressors which act on the *bona fide* amber allele *cyc1-179* but are not suppressed by UAA suppressors.

The *leu2-1* allele appear to be an ochre mutant, since it can be suppressed by the tyrosine-inserting UAA suppressors (HAWTHORNE 1969b; GILMORE, STEWART and SHERMAN 1971), whereas it is not acted on by tyrosine-inserting UAG suppressors (SHERMAN *et al.* 1973). This marker was employed because HAWTHORNE and LEUPOLD (1974) found it to be suppressed by omnipotent suppressors which appear to act on certain UAA, UAG and UGA mutants.

The presumed UAA mutations, *his5-2* and *lys1-1*, were used to determine whether any of the *cyc1-179* suppressors could act on UAA. The *his5-2* and *lys1-1* markers are suppressed by the tyrosine-inserting and serine-inserting UAA suppressors (GILMORE 1967; LIEBMAN, STEWART and SHERMAN 1975a) but are not acted on by tyrosine-inserting amber suppressors (SHERMAN *et al.* 1973). Also, *his5-2* and *lys1-1* are not acted on by the omnipotent suppressors (HAWTHORNE and LEUPOLD 1974).

The *ilv1-1* mutant included in this study is apparently different from the *ilv1-1* mutant described by HAWTHORNE and LEUPOLD (1974). We found that our *ilv1-1* mutant, which was obtained as i_a (ROMAN and JACOB 1958), was not suppressed by suppressors which acted on the ochre alleles *his5-2*, *lys1-1* or *leu2-1*. Furthermore, the results described subsequently indicate that ROMAN's *ilv1-1* is a true amber mutation since it is suppressed by suppressors which act on *cyc1-179*.

Genetics: Suppressor segregation—Conventional techniques of crossing, sporulation and dissection were employed in the construction of appropriate haploid strains. The segregation of the *SUP* genes was scored by suppression of one or more of the appropriate markers by spotting cell suspensions as described by SHERMAN *et al.*, (1974). The segregation and efficiency of suppression of the *cyc1* genes was scored by estimating the level of cytochrome *c* in whole cells at low temperature (—190°) using a spectroscope (SHERMAN and SLONIMSKI 1964).

Identification of cycl *alleles*—X-ray-induced mitotic recombination, as described by SHERMAN *et al.* (1974), was used to distinguish between the different *cyc1* alleles segregating in a cross. The unknown *cyc1* segregants were crossed to *cyc1* tester strains and suspensions of the diploid crosses were spotted on lactate medium and were X-irradiated. However, amber *cyc1* alleles were not readily distinguished with this procedure if efficient amber suppressors were present, since recombinant colonies often could not be distinguished above the background growth due to suppression of the *cyc1* alleles. Therefore, special diploid tester strains were constructed to be homozygous for the mating-type genes, α/α or **a**/**a**, and for the appropriate *cyc1* mutations, *cyc1-76* or *cyc1-138*. When these testers were crossed to suppressor-bearing segregants whose *cyc1* amber alleles were to be determined, the background growth of the resultant triploids on lactate medium was not excessive and recombinant colonies could be distinguished. Diminution of suppression efficiency in the triploids probably results from the reduced dosage of the suppressor gene.

Identification of suppressor loci—A random spore test, depicted schematically in Figure 1, was devised to test whether any of the UAG suppressors isolated here were alleles of previously identified UAA suppressors. The test consists of crossing a UAA suppressor-bearing strain by a UAG suppressor-bearing strain, followed by selecting segregants which do not contain UAA suppressors, and then by determining the fraction of these that carry the UAG suppressor. If the suppressors are very closely linked, all segregants lacking UAA suppressors should carry the UAG suppressor; if they are not linked, approximately one-half of the segregants lacking UAA

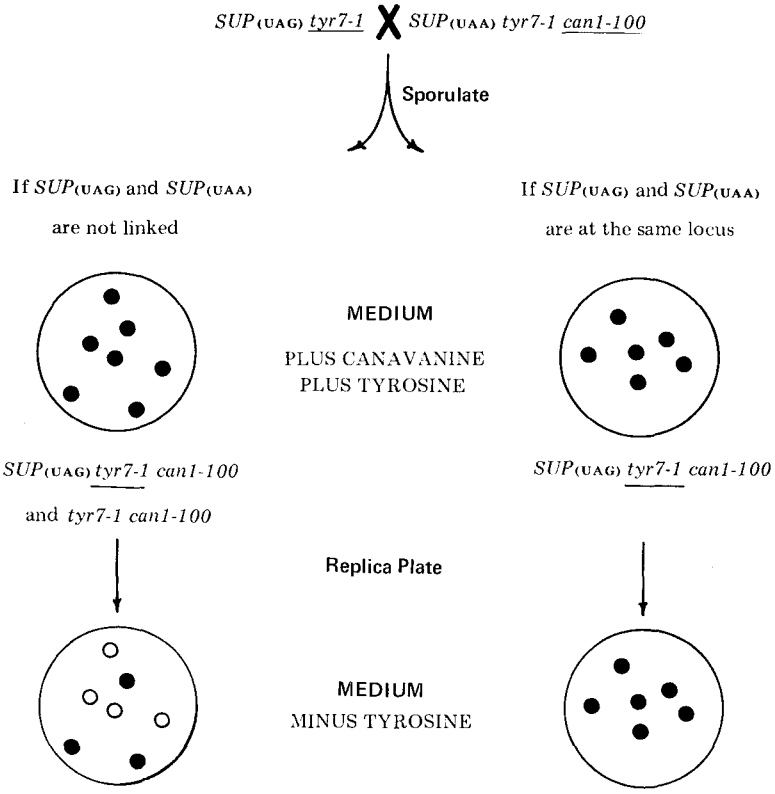

FIGURE 1.—Schematic diagram of random spore analyses to determine if UAG and UAA suppressors are at the same locus. The suppressed alleles are indicated by *underlines*. Filled circles indicate the presence of a colony, while open circles indicate the absence of a colony. The presence of phenylalanine in medium, which is required for growth by *try7–1*, is not shown in the diagram. For further explanation see the text.

suppressors should also lack the UAG suppressor. Simultaneous selection against diploids and UAA suppressor segregant was achieved by having the ochre allele *can1–100* (MORTIMER, unpublished) heterozygous in the cross. Only haploid strains lacking UAA suppressors and carrying the *can1–100* allele should grow on medium containing canavanine, since resistance to canavanine, conferred by *can1–100*, is recessive and is suppressed by UAA suppressors. To detect amber suppressors in the segregants that grew on canavanine medium, the UAG allele *tyr7–1* was made homozygous in the cross; colonies that grew on canavanine medium containing tyrosine and phenylalanine were replica plated onto medium lacking tyrosine, where only UAG-suppressor segregants could grow. Thus, tester strains were constructed to contain one or another of the UAA suppressors, the ochre allele *can1–100* and the amber allele *tyr7–1*. Each of the UAG suppressors coupled with the *tyr7–1* allele was crossed to each tester strain. The diploids were sporulated and the sporulation mixture was streaked on medium containing canavanine, tyrosine and phenylalanine. Colonies that grew were replica plated onto plates that lacked tyrosine. Missing colonies on the medium lacking tyrosine indicated that the suppressors were not allelic. If all the colonies which grew on the canavanine medium also grew on the medium lacking tyrosine, the suppressors were assumed to be allelic.

Preparation of iso-1-cytochromes c: Large batches of the suppressed *cyc1–179* strains were grown under depressed conditions by slowly pumping medium into the fermentor, using the

procedure described by PRAKASH, STEWART and SHERMAN (1974). In some cases low-temperature spectroscopy of the fermentor-grown cells indicated that they contained less than 15% of the wild-type level of cytochrome *c* even though greater than 50% of the wild-type level of iso-1-cytochrome *c* was present in the original inoculum of the suppressed *cyc1–179* mutant. Such batches were either discarded, or were pooled and analyzed separately from batches containing a high level of cytochrome *c*. This phenomenon, which reflects an instability of the suppressors, is described in detail in the accompanying paper (LIEBMAN and SHERMAN 1976).

The methods used to purify the iso-1-cytochromes *c* have been described previously (SHERMAN *et al.* 1968; LIEBMAN, STEWART and SHERMAN 1975a). Cells were autolyzed by stirring in ethyl acetate at 4°. Cytochrome *c* was then absorbed on a course cation-exchange resin, Amberlite CG50(Na^+), from which it was eluted. The iso-1-cytochrome *c* and iso-2-cytochrome *c* were then separated by chromatography on a fine cation exchange resin and the iso-1-cytochrome *c* was further purified on a Sephadex G75 column.

Identification of the structural changes in iso-1-cytochrome c: The methods used for peptide mapping have been described in detail by STEWART *et al.* (1971). Tryptic and chymotryptic digests of samples of 0.1 μmol of cytochrome *c* were prepared by reaction of 1% w/v cytochrome *c* and 0.1% w/v enzyme in 0.25% w/v ammonium bicarbonate for 2.75 hr at 37°. The digests were lyophilized and were subjected to electrophoresis on Whatman 3MM paper wet with pyridine/acetic acid/water (5:0.18:95) pH 6.5, for 1.67 hr at 20 V/cm, and then to descending chromatography in *n*-butanol/pyridine/acetic acid/water (15:10:3:12) for 16 hr. The peptide maps were developed by sequential dipping through the collidine/ninhydrin reagent, the Ehrlich reagent for tryptophan, and the Pauly reagent for histidine and tyrosine, in that order:

RESULTS

Isolation of suppressors: Suppressors were isolated in strain SL210–3A which contains the amber mutant *cyc1–179* as well as the suppressible auxotrophic markers *met8–1*, *tyr7–1*, *trp1–1*, *ade3–26*, *ilv1–1*, *leu2–1*, *his5–2* and *lys1–1*. These auxotrophic alleles were chosen either for their suitability in the selection of a wide variety of suppressors capable of acting on *cyc1–179* or for their known suppressibility by UAA suppressors. It is believed that these nutritional markers may require the insertion of a different spectrum of amino acids at the amber site and may require different efficiencies of suppression in order to be suppressed (see MATERIALS AND METHODS).

Revertants were obtained from SL210–3A either spontaneously or after UV treatments of 30 or 50 Jm^{-2} and were selected on synthetic media requiring the reversion of one or two nutritional markers. The revertants were picked and subcloned, their requirements were determined by replica plating on omission media, and their cytochrome *c* content was estimated by low-temperature spectroscopy. Those strains which exhibited the unselected reversion of at least one marker in addition to the reversion of a selected marker were presumed to contain a suppressor. The revertants which lost the requirement of only one marker either could be intragenic revertants or could contain suppressors that act on only single markers; this distinction was not made, and such possible suppressors were excluded.

The number of revertants and suppressors isolated with the various types of media are listed in Table 2. At least one additional marker was concomitantly reverted in all of the revertants isolated on media requiring the reversion of two markers. Surprisingly, only 13.4% of the revertants isolated on a medium lacking

TABLE 2

Numbers of revertants

Selected on media lacking:	Number of revertants	Number of suppressors*	% suppressors
Methionine	134	116	86.6
Tyrosine	250	50	20.0
Adenine	79	41	51.9
Methionine and tyrosine	133	133	100
Methionine, isoleucine and valine	40	40	100
Methionine and tryptophan	150	150	100
Methionine and leucine	145	145	100
Methionine and adenine	140	140	100
Adenine, isoleucine and valine	48	48	100
Adenine and tryptophan	50	50	100
Adenine and leucine	10	10	100
Tyrosine, isoleucine and valine	50	50	100
Tyrosine and tryptophan	50	50	100
Tyrosine and adenine	50	50	100
Tyrosine and leucine	15	15	100
Totals	1344	1088	

* Suppressors acting on at least one marker which was not selected by reversion; suppressors acting on single markers were not distinguished from intragenic revertants.

only methionine appeared to be intragenic revertants. Perhaps this is because the *met8–1* mutant can be suppressed by a large number of diverse suppressors.

Classification of suppressors: The 1088 suppressor-bearing revertants have been grouped in 78 classes on the basis of phenotypic expression of the markers in SL210–3A. For *cyc1–179*, the degree of suppression was scored by estimating the cytochrome *c* level in whole cells as as described in Table 3. For the nutritional markers, levels of growth on omission data were scored after 2 and 7 days of incubations as indicated in Table 3. Each of the suppressor strains was also scored for ability to grow on complete glucose and glycerol media.

Many of the suppressor-bearing strains did not grow or grew poorly on glycerol medium and were partially or completely deficient in cytochromes $a{\cdot}a_3$, *b* and c_1. The number of such suppressors in each class is listed in Table 3. Since low mutagenic treatments were used, these multiple effects might well be due to the pleiotropic effects of single genes. In some pedigrees poor growth on glycerol did segregate with the amber suppressors. In other cases, however, the amber suppressors could be separated from the cytochrome deficiencies and poor growth on glycerol medium as previously reported by SHERMAN *et al.* (1973).

Only six of the suppressors of UAG acted on the ochre alleles *his5–2* and *lys1–1* and only one of these, a class 35 mutant, strongly suppressed one of the ochre alleles. None of these suppressors acted efficiently on *cyc1–179*. Genetic analyses have not been performed on these six strains to determine whether they contain multiple suppressor mutations or a single suppressor capable of acting on UAA and UAG codons.

TABLE 3

Phenotypic classes of suppressors

Class	Suppressible alleles									No. of revert.	No. of glycerol negative
	cyc1-179	*met8-1*	*tyr7-1*	*trp1-1*	*ade3-26*	*ilv1-1*	*leu2-1*	*his5-2*	*lys1-1*		
1	+	+	+	+	+	+	−	−	−	36	5
2	+	+	+	+	+	±	−	−	−	5	5
3	+	+	+	+	+	−	−	−	−	2	1
4	±	+	+	+	+	+	−	−	−	76	6
5	±	+	+	+	+	±	−	−	−	4	3
6	±	+	+	±	+	+	−	−	−	9	4
7	±	+	+	±	+	±	−	−	−	4	3
8	±	+	+	+	+	−	−	−	−	4	4
9	±	±	±	±	±	−	−	−	−	1	0
10	±	+	±	±	−	−	−	−	−	2	2
11	±	−	±	−	−	−	−	−	−	1	0
12	±	−	−	−	+	−	−	−	−	27	23
13	−	+	+	+	+	+	−	−	−	432	16
14	−	+	+	+	+	±	−	−	−	75	25
15	−	+	+	+	±	+	−	−	−	4	0
16	−	+	+	±	+	+	−	−	−	143	2
17	−	+	±	+	+	+	−	−	−	2	0
18	−	+	+	+	±	±	−	−	−	14	1
19	−	±	+	+	±	+	−	−	−	1	0
20	−	+	+	±	+	±	−	−	−	4	4
21	−	±	+	+	±	±	−	−	−	1	0
22	−	+	+	±	±	±	−	−	−	1	0
23	−	+	+	+	+	−	−	−	−	7	7
24	−	+	+	+	±	−	−	−	−	3	0
25	−	+	+	±	+	−	−	−	−	1	0
26	−	±	±	±	+	−	−	−	−	1	1
27	−	+	+	−	+	+	−	−	−	5	1
28	−	+	±	±	−	−	−	−	−	2	0
29	−	±	+	−	±	−	−	−	−	4	4
30	−	+	±	−	−	−	−	−	−	1	0
31	−	+	−	±	−	−	−	−	−	3	0
32	−	+	−	−	±	−	−	−	−	1	0
33	−	−	+	−	−	±	−	−	−	2	0
34	−	−	+	−	±	−	−	−	−	5	4
35	±	+	+	+	±	±	+	±	+	1	1
36	±	+	+	+	+	+	±	−	−	1	0
37	±	+	+	+	+	−	+	−	−	1	1
38	±	+	+	+	±	−	+	−	−	1	1
39	±	+	±	±	±	−	±	−	−	1	1
40	±	+	+	+	−	±	+	−	−	1	0
41	±	+	+	+	−	−	+	−	−	6	5
42	±	+	+	±	−	−	+	−	−	2	1
43	±	+	−	±	−	−	±	−	−	3	3
44	±	+	−	−	±	−	±	−	−	3	2
45	±	±	−	−	−	−	±	−	−	1	0
46	−	+	+	+	+	+	+	±	±	2	0
47	−	+	+	±	±	±	+	±	−	2	0
48	−	+	+	+	±	±	+	−	±	1	0

TABLE 3—Continued

Class	Suppressible alleles									No. of revert.	No. of glycerol negative
	cyc1–179	*met8–1*	*tyr7–1*	*trp1–1*	*ade3–26*	*ilv1–1*	*leu2–1*	*his5–2*	*lys1–1*		
49	—	+	+	+	+	+	±	—	—	2	0
50	—	+	+	+	±	±	+	—	—	3	0
51	—	+	+	+	±	±	±	—	—	1	0
52	—	+	+	±	±	±	+	—	—	8	0
53	—	+	±	±	±	±	+	—	—	1	0
54	—	+	+	+	±	—	+	—	—	8	6
55	—	+	+	±	±	—	+	—	—	4	2
56	—	+	±	±	+	—	±	—	—	1	0
57	—	±	+	+	±	—	±	—	—	1	0
58	—	+	±	±	±	—	±	—	—	1	1
59	—	+	+	+	—	±	+	—	—	21	1
60	—	+	+	±	—	±	+	—	—	18	0
61	—	+	±	±	—	±	+	—	—	6	0
62	—	+	+	—	—	±	+	—	—	2	0
63	—	+	±	—	±	—	±	—	—	1	1
64	—	—	±	±	±	—	±	—	—	1	0
65	—	+	+	+	—	—	+	—	—	48	8
66	—	+	+	+	—	—	±	—	—	6	2
67	—	+	+	±	—	—	+	—	—	15	0
68	—	+	±	+	—	—	+	—	—	2	0
69	—	±	+	+	—	—	+	—	—	2	2
70	—	±	±	+	—	—	+	—	—	1	1
71	—	+	±	±	—	—	+	—	—	5	0
72	—	+	±	±	—	—	±	—	—	6	0
73	—	+	—	+	—	—	+	—	—	1	0
74	—	+	—	±	—	—	+	—	—	1	0
75	—	+	±	—	—	—	+	—	—	3	0
76	—	+	—	—	—	—	+	—	—	1	0
77	—	+	—	—	—	—	±	—	—	8	0
78	—	—	—	—	±	—	±	—	—	1	0
										1088	Total

The levels of suppression of the nutritional markers are indicated by: +, good growth by 2 days; ±, slight growth by 7 days; and —, no sign of growth by 7 days. The levels of suppression of the *cyc1–179* mutant are indicated by the amount of cytochrome *c* compared to the normal value: +, approximately 75% of the normal value; ±, approximately 5% to 30% of the normal value; and —, the low level characteristic of mutants completely lacking iso-1-cytochrome *c* to approximately 5% of the normal value.

Many of the suppressors of one or more of the following alleles also act on *cyc1–179*: *met8–1*, *tyr7–1*, *trp1–1*, *ade3–26*, *ilv1–1* and *leu2–1*. Genetic analyses and assays for iso-1-cytochrome *c*, described later, established that suppressors of the nutritional mutations and of the *bona fide* amber alleles *cyc1–179* and *cyc1–76* almost always segregated together. This suggests that *met8–1*, *tyr7–1*, *trp1–1*, *ade3–26*, *ilv1–1* and *leu2–1* are all amber alleles. However, it should be recalled (see MATERIALS AND METHODS) that *leu2–1* is apparently an ochre allele that may be suppressed by certain suppressors of amber mutants. Dominance and growth characteristics (see below) have revealed a general distinction between the suppressors of amber mutants that act on *leu2–1* and those that do not.

Out of the 1088 suppressor strains that were isolated, only 192 appeared to have increased amounts of cytochrome *c*, ranging from a barely detectable elevation to nearly the normal level. The majority of the suppressor strains contained the low level of cytochrome *c* that is characteristic of *cyc1* strains (including the original *cyc1–179* strain) which completely or almost completely lack iso-1-cytochrome *c* but which contain the normal low-amount of iso-2-cytochrome *c*. Thus it appears as if most of the suppressors either do not act on *cyc1–179* or act with a low degree of efficiency. Most of the work which follows is concerned with the 192 suppressors that act on *cyc1–179*.

Dominance or recessiveness of suppressors: To further characterize the suppressors which act on *cyc1–179*, 170 of the 192 suppressor strains with elevated levels of cytochrome *c* were crossed to SL183–21C (*α cyc1–76 met8–1 tyr7–1 trp1–1 ade3–26 ilv1–1 leu2–1 lys2–1 his5–2*). The resulting diploids were heterozygous for the respective suppressors and were homozygous for the suppressible nutritional markers *met8–1*, *tyr7–1*, *trp1–1*, *ade3–26*, *ilv1–1*, *leu2–1* and *his5–2*. In addition the diploids were heteroallelic for the amber alleles *cyc1–179* and *cyc1–76*. The dominance or recessiveness of the suppressors was determined by comparing suppressor action in the original haploid and the corresponding diploid strain described above. This was accomplished firstly by comparing the growth of these strains on the various types of omission media which support growth only when a nutritional allele is suppressed. Secondly, the cytochrome *c* content of the haploid and diploid strains was compared by low-temperature spectroscopy.

Each of the suppressors which acted on amber mutations but did not act on any of the ochre mutations were dominant or semi-dominant for all suppressed alleles. Generally, the degree of dominance of a given suppressor's action was the same no matter which suppressed nutritional allele was scored. However, the actions of all suppressors in the same class were not identical. What these differences in dominance within a class represent is unknown, but at least for the highly efficient suppressors (classes 1, 2 or 3) the genetic mapping described subsequently establishes that the dominance distinctions do not correspond to different suppressor loci.

The dominance studies suggest that some of the class distinctions made on the basis of suppression of *ilv1–1* may not be significant. This is because in some cases a suppressor that apparently does not act on *ilv1–1* in the haploid does suppress this marker in the diploid. The reversal of the expected haploid and diploid growth rates on the omission media could usually be related to poor growth of the haploid strains. In the case of the very efficient suppressors of *cyc1–179*, which are segregated into classes 1, 2 and 3 solely by differences in suppression of *ilv1–1*, genetic studies have established that classes 1, 2 and 3 include suppressors from the same loci.

A few of the 170 suppressors were entirely recessive, as judged from growth on omission media and cytochrome *c* levels. All of the recessive suppressors acted on the *leu2–1* ochre allele in addition to amber markers. Indeed, with the sole exception of the class 35 suppressor, which acts on the ochre alleles *his5–2* and

lys1–1 in addition to *leu2–1*, all of the suppressors which acted on *leu2–1* were recessive. These results suggest that recessive suppressors which act on both ochre and amber codons may be fundamentally different from the dominant or semi-dominant suppressors which act specifically on either ochre (see MORTIMER and GILMORE 1968) or amber codons. Therefore we also examined the dominance or recessiveness of a few other suppressors from among classes 13 through 34 and 46 through 78 that do not act on *cyc1–179*. Ten out of 11 amber-specific suppressors were dominant, while the exceptional suppressor from class 26 was recessive. Twenty-three out of 29 suppressors which act on *leu2–1* in addition to amber alleles were recessive, while six were semi-dominant. Two other suppressors which acted on *his5–2* or *lys1–1* as well as *leu2–1* were semi-dominant. Thus virtually all suppressors that act on only amber mutations are dominant or semi-dominant. while most suppressors that do not act on *his5–2* or *lys1–1* but do suppress the ochre allele *leu2–1* as well as amber mutations are recessive.

Genetic basis of suppression: Analyses of the revertants that presumably contained suppressors were undertaken to establish the genetic basis of the revertant phenotypes. Genetic analyses were undertaken with the following 25 revertants that were isolated from SL210–3A (**a** *cyc1–179 met8–1 tyr7–1 trp1–1 ade3–26 ilv1–1 leu2–1 his5–2 lys1–1*) and that had elevated levels of cytochrome *c*: eight from class 1; one from class 2; one from class 3; three from class 4; two from class 5; two from class 10; four from class 41; one from class 42; two from class 43; and one from class 44. These strains were crossed to either SL183–21C (*α cyc1–76 met8–1 tyr7–1 trp1–1 ade3–26 ilv1–1 leu2–1 his5–2 lys2–1*), SL152–2B (*α cyc1–179 tyr7–1 leu2–1 lys2–1*) or D597–3D (*α cyc1–1 tyr7–1 trp1–1*), which contain one or more of the original suppressible alleles. The segregation of various nutritional markers was determined by scoring meiotic progeny for growth on appropriate types of omission media. Generally, all of the homozygous nutritional markers tested which were not suppressed in the original revertant were also not suppressed in any of the segregants. Also, the suppression of all the homozygous markers which were suppressed in the original revertant segregated together in all the meiotic progeny. Thus it is clear that the revertant characteristics all result from a suppressor mutation rather than the chance association of several independent reversions at the auxotrophic loci.

In 23 of the 26 pedigrees, there was a clear 2:2 segregation for the suppressed nutritional alleles, indicating that the suppressor was a single Mendelian character. In two pedigrees, however, of the crosses SL-337 and SL-338, involving, respectively, one of the eight suppressors from class 1 that was tested and the only tested class 3 suppressor, there was no indication of suppressors in any of the segregants even though three or four viable spores were generally recovered from each ascus. The instability of these suppressors will be discussed later. In a pedigree involving a class 10 suppressor, segregation of the suppressor indicated that two genes were required to produce the suppressor phenotype.

Of special interest in the 23 pedigrees containing single suppressors was the determination of whether the suppressors in the segregants acted on the amber mutations *cyc1–179* and *cyc1–76*. Low-temperature spectroscopy indicated that

increased levels of cytochrome *c* segregated with the suppressor in all but two pedigrees, those involving a class 5 and a class 44 suppressor; in these two revertants, apparently, the increased cytochrome *c* content was not due to the suppressor.

The suppressors isolated in all the other genetically analyzed revertants appeared to act on one or both of the *cyc1* amber alleles. In several of the pedigrees involving class 1, 4 and 5 suppressors, the suppressed *cyc1–76* segregants had higher cytochrome *c* levels than the suppressed *cyc1–179* segregants. Apparently the *cyc1–76* mutation can be suppressed more efficiently than the *cyc1–179* allele by a variety of amber suppressors. The coincident segregation of a suppressor and increased levels of cytochrome *c* suggests, but does not prove that the suppressor is acting on the *cyc1* mutations. It is also possible that pleotropic effects of the suppressor increase the iso-2- cytochrome *c* level. In this study, final proof that a suppressor acts on an amber *cyc1* mutation has been provided by isolating iso-1-cytochrome *c* from the suppressed strains.

The above-described genetic studies provided a means with which to verify the validity of the suppressor classifications made on the basis of the phenotypes of the original revertants. Most of the suppressor characteristics did persist through meiosis, a fact which supports the classification scheme. However, as mentioned above, the increased level of cytochrome *c* in two of the revertants was not consistently observed in the meiotic segregants; so these suppressors were probably classified incorrectly. In three different pedigrees, some segregants contained suppressed alleles that were not suppressed originally; in four other pedigrees, the number of suppressed alleles was reduced in some segregants. These discrepancies may be due to modifiers which affect the efficiency of the suppressors. While such modifiers probably confuse some classifications made on the basis of the original phenotypes, most meiotic segregants were identical to the original suppressor mutants, and if differences occurred, they were usually slight.

Many of the suppressor strains did not grow, or grew poorly on glycerol complete medium and did not grow well on glucose complete medium. In many pedigrees, the poor growth associated with the original revertants did not segregate with the suppressors; in other pedigrees the poor growth and suppressor characteristics were clearly associated. One pattern that emerges from these data is that most of the recessive suppressors which act *leu2–1* as well as on UAG cause poor growth on complete media. Indeed the class 41 suppressors segregating in two pedigrees are nearly recessive lethals.

In several pedigrees involving class 1, 2 or 3 suppressors which acted efficiently on *cyc1–179*, poor growth segregated with the suppressors. In other pedigrees, these same suppressors had lost the ability to efficiently suppress *cyc1–179*, while they continued to suppress the auxotrophic amber alleles. In these pedigrees, suppression was no longer associated with poor growth. These observations, reported in detail in the accompanying paper (LIEBMAN and SHERMAN 1976), suggested that the efficient amber suppressors could become low-efficiency suppressors, and that poor growth was caused by the efficient amber-suppression.

Identification of suppressor loci: Since two highly efficient amber (UAG) suppressors had previously been shown to be alleles of class I, set 1 UAA suppressors (SHERMAN *et al.* 1973), it appeared that some of the 43 suppressors isolated in this study, which acted efficiently on *cyc1–179* (classes 1, 2 and 3), might be alleles of the tyrosine-inserting UAA suppressors. Another possibility was that some of these suppressors were alleles of the serine-inserting UAA suppressor *SUQ5*-**o**, since as will be described below, a high-efficiency UAG suppressor allele, *SUQ5*-**o***1*-**a***1* has been isolated from this suppressor. A random spore test, described in MATERIALS AND METHODS and depicted schematically in Figure 1, was devised to test these possibilities. The test consists of crossing the unknown UAG suppressors with the UAA suppressor-bearing tester-strains, followed by selecting segregants which do not contain UAA suppressors, and then by determining the fraction of these that carry the UAG suppressor. If the suppressors are very closely linked, all segregants lacking UAA suppressors should carry the UAG suppressor; if they are not linked, approximately one-half of the segregants lacking UAA suppressors should also lack the UAG suppressor.

The major problem encountered in this test was that some of the diploids did not sporulate. When this occurred, UAG suppressor segregants from various pedigrees were mated to the UAA suppressor tester-strains. In addition, a variety of tester strains were tried in an effort to improve sporulation.

All but two of the 43 originally highly efficient amber (UAG) suppressors have been identified as alleles of one or another of the eight class I, set 1 suppressors which cause the insertion of tyrosine at ochre codons, while none of the 43 suppressors were identified as alleles of the *SUQ5*-**o** suppressor. The remaining two suppressors could not be fully analyzed since they did not sporulate with one or more of the testers, and we were unable to obtain other strains containing these two suppressors that could be used in the random spore tests. In two pedigrees, SL-338 and SL-337, analyzed for this purpose, the amber suppressors were not present in any of the progeny even though spore viability was high, indicating that the suppressors reverted. Several other crosses involving these suppressors each resulted in extremely poor spore viability. The unstable property of these two suppressors was not sufficient reason to suggest that they are not alleles of one or another of the eight UAA suppressors. Various degrees of instability and spore inviability, approaching the situation cited above, were also found with the amber suppressors that were established to be allelic to the UAA suppressors (see LIEBMAN and SHERMAN 1975), and these two unidentified suppressors appear to fall at one end of a continuum.

The number of amber suppressors found to be allelic to each of the eight UAA suppressors by the random spore analysis are listed in Table 4. The classifications

TABLE 4

Number of amber suppressors assigned to each locus

Suppressor locus	*SUP2*	*SUP3*	*SUP4*	*SUP5*	*SUP6*	*SUP7*	*SUP8*	*SUP11*	Total
Number of suppressors	9	7	5	6	1	2	4	7	41

TABLE 5

Identification of the loci of high efficiency suppressors by tetrad analyses

Haploid parents	No. asci analyzed	Viable spores per ascus				Segregation of amber suppressor : ochre suppressor*													
						4-spored asci					3-spored asci				2-spored asci			1-spored asci	
		4	3	2	1	4:0	3:1	2:2	1:3	0:4	3:0	2:1	1:2	0:3	2:0	1:1	0:2	1:0	0:1
L-113 × SL270-3C (*SUP2*-o)	8	4	3	0	1	0	0	4	0	0	0	2	1	0				0	1
SL292-5B × SL252-1B (*SUP3*-o)	8	4	3	1	0	0	1†	3†	0	0	0	3	0	0	1	0	0		
L-177 × SL251-10B (*SUP4*-o)	11	10	1	0	0	0	0	10	0	0	0	0	1	0					
SL309-2C × SL171-13B (*SUP5*-o)	13	12	1	0	0	0	0	12	0	0	0	1	0	0					
SL311-4C × SL127-1D (*SUP7*-o)	11	10	1	0	0	0	0	10	0	0	0	1	0	0					
L-270 × SL273-3C (*SUP8*-o)	7	7	0	0	0	0	0	7	0	0									
L-206 × SL259-10D (*SUP11*-o)	7	7	0	0	0	0	0	7	0	0									

* Scored by the suppression of homozygous amber and ochre markers.

† The only homozygous ochre marker present in the SL292-5B × SL252-1B cross was *his5-2*. The *his5-2* marker could not be scored for suppression in segregants containing the unsuppressed *ade3-26* marker, because this marker causes a requirement for histidine in addition to adenine. However, since both requirements of the *ade3-26* amber marker are suppressible, it was possible to determine that no segregant contained both amber and ochre suppressors. Thus, presence of the amber suppressor was scored directly and presence of the ochre suppressor was inferred.

of several suppressors have been verified by tetrad analyses of diploid strains which, according to the random spore results, should have contained allelic UAG and UAA suppressors. Suppression of the homozygous amber and ochre markers was used to score for the presence of each suppressor. If the suppressors were allelic, each segregant should contain either a UAA or UAG suppressor but never both or neither. Indeed, as shown in Table 5, this was the case in every pedigree examined. Thus we believe that the random spore tests accurately identified the loci of the amber suppressors.

Suppressors acting efficiently on *cyc1–179* can mutate so that there is inefficient or no suppression of the *cyc1–179* marker while there is still suppression of at least some of the nutritional markers (LIEBMAN and SHERMAN 1976). The inefficient suppression was shown to be due to the result of mutations either of the suppressor gene or of extraneous genes. Thus it seemed possible that some of the amber suppressors which did not act or acted poorly on *cyc1–179* (classes 4–34) were alleles of the highly efficient suppressors, and that some distinctions in suppressor phenotypes were due to secondary mutations. To test this possibility, 61 low-efficiency suppressors, from among classes 4, 5, 6, 7, 8, 9, 13, 14, 16, 18, 20, 23 and 24, were examined for allelism with *SUP2–***o** and *SUP5–***o** which are two of the class I, set 1 UAA suppressors. By using the random spore method, it was shown that none of these suppressors mapped at the *SUP2* or *SUP5* loci. In the same manner, it was established that 25 of these low-efficiency amber suppressors, from classes 4, 5, 6, 7, 8, 13, 14, 16, 20, and 23, were not allelic to any of the eight tyrosine-inserting UAA suppressors or the serine-inserting UAA suppressor *SUQ5–***o**.

Altered iso-1-cytochromes c *in the suppressed amber strains*: The two amber suppressors, *SUP6–***a2** and *SUP7–***a2**, were previously shown to cause the insertion of residues of tyrosine at positions corresponding to the sites of amber codons (SHERMAN *et al.* 1973). The six strains that are listed in Table 6, each containing the *cyc1–179* marker and one of the other six efficient amber suppressors, were chosen for preparations of iso-1-cytochromes *c*. In several cases, the cells harvested from the fermentor contained only a low level of cytochrome *c* even though *cyc1–179* was efficiently suppressed in the original inoculum. These cells retained a low-efficiency variant of the suppressor, since the auxotrophic amber

TABLE 6

Iso-1-cytochrome c *from suppressed amber strains*

Strain no.	Genotype	Approx. iso-1-cytochrome *c* level in fermentor-grown cells (% of normal amount)	Amino acid replacing lysine 9
SL290–2C	*cyc1–179 SUP2-***a2**	50%	tyrosine
SL315–3C	*cyc1–179 SUP4-***a4**	50%	tyrosine
SL350–2A	*cyc1–179 SUP5-***a2**	50%	tyrosine
SL308–4D	*cyc1–179 SUP11-***a2**	50%	tyrosine
L-210	*cyc1–179 SUP3-***a2**	50%	(impure)
SL332–1C	*cyc1–179 SUP8-***a2**	<10%	(impure)

markers were still suppressed. This phenomenon, which reflects an instability of the efficient suppressors, is described in detail in the accompanying paper (LIEBMAN and SHERMAN 1976).

Chromatographic analyses of cytochrome *c* from all the strains listed in Table 6 revealed fractions corresponding in position to iso-1-cytochrome *c* and iso-2-cytochrome *c*. In contrast, no iso-1-cytochrome *c* could be detected from the unsuppressed *cyc1–179* strain (STEWART and SHERMAN 1972).

Iso-1-cytochromes *c* of sufficient purity for structural analysis could be obtained from only four of the strains that contained either suppressors *SUP2–a2*, *SUP4–a4*, *SUP5–a2* or *SUP11–a2*. Peptide maps of tryptic and chymotryptic digests of these suppressed iso-1-cytochromes *c* differed from the peptide maps of wild-type iso-1-cytochrome *c* and were identical to the peptide maps of iso-1-chromes *c* that were obtained from the intragenic revertants of *cyc1–179*, in which a tyrosine residue replaced the lysine residue at position 9. The sites of enzymatic cleavage near residues 9, along with the positive response for tyrosine using the Pauly reagent, are such that these peptide maps establish the existence of only a tyrosine replacement at position 9 (STEWART and SHERMAN 1972). Thus, suppression of *cyc1–179* by *SUP2–a2*, *SUP4–a4*, *SUP5–a2* or *SUP11–a2* results in iso-1-cytochrome *c* containing a tyrosine residue at the position of the amber codon.

The iso-1-cytochrome *c* isolated from the efficiently suppressed strain L-210 (*cyc1–179 SUP3–a2*) was contaminated with proteolytic enzymes, which frustrated attempts to clearly identify the amino acid residue at position 9. The inefficiently suppressed cells of strain SL332–1C (*cyc1–179 SUP8–a2*) yielded only a small amount of cytochrome *c* that chromatographed at the position of iso-1-cytochrome *c*. While peptide maps could not be obtained with sufficient clarity to deduce the alteration in iso-1-cytochrome *c*, the results nevertheless confirmed that the cytochrome *c* was indeed iso-1-cytochrome *c*.

Mutation of UAA suppressors to UAG suppressors: A study was undertaken firstly to determine whether UAG suppressors would have the same properties if they arose from the wild type or from UAA suppressors and secondly to isolate and examine the properties of a UAG suppressor derived from the serine-inserting UAA suppressor *SUQ5–o1*. To facilitate the selection of mutations of the UAA suppressors *SUP5–o1* and *SUP7–o1* to UAG suppressors, ψ^- strains were constructed with the following alleles: the UAG alleles *cyc1–179*, *tyr7–1* and *trp1–1*; the UAA alleles *his5–2*, *lys1–1* and *can1–100*; and either the *SUP5–o1* or *SUP7–o1* suppressor. Following UV-irradiation of these strains, revertants were selected on a synthetic medium which contained canavanine, histidine, and lysine but which lacked tyrosine and tryptophan. The presence of canavanine selected for the expression of the *can1–100* allele and thus against the UAA suppressor. At the same time, the absence of the amino acids required by the *tyr7–1* and *trp1–1* markers resulted in selection for a UAG suppressor. Because of these stringent requirements, very few colonies appeared on the selective medium. Four out of 8 revertants examined had lost the UAA suppressor since *his5–2* and *lys1–1* were no longer suppressed. Furthermore, each of these 4 revertants had

concurrently gained a UAG suppressor which was similar to those derived from the wild-type alleles of one of the 8 tyrosine-inserting suppressors, since in addition to the selected reversion of *tyr7–1* and *trp1–1*, low-temperature spectroscopy indicated that *cyc1–179* was also efficiently suppressed. To verify that the new amber suppressors were alleles of *SUP5–o* and *SUP7–o*, diploids were constructed with each of the original UAA suppressors as well as with the corresponding presumed-allelic UAG suppressor, called *SUP5–o1–a1* or *SUP7–o1–a1*. Indeed 11 out of 11 and 7 out of 7 of the tetrads analyzed from these crosses, with *SUP5–o1–a1* and *SUP7–o1–a1* respectively, were parental ditype for the suppressors.

The UAA suppressor *SUQ5–o1* was mutated to a UAG suppressor by a procedure similar to that described above. We constructed a ψ^+ *SUQ5–o1* strain containing the UAG alleles *cyc1–179*, *met8–1*, *tyr7–1* and *trp1–1* and the UAA alleles *lys1–1*, *leu2–1* and *can1–100*. Selection for a loss of the UAA and an addition of a UAG suppressor was accomplished by plating on synthetic medium containing lysine, leucine, canavanine and tryptophan and lacking methionine and tyrosine. Out of 23 UV revertants examined, one had lost *SUQ5–o1* UAA suppressing activity since *lys1–1* and *leu2–1* were no longer suppressed. This strain had concurrently gained a UAG suppressor, since the UAG alleles *trp1–1* and *cyc1–179* were both suppressed in addition to the selected suppression of *tyr7–1* and *met8–1*. This new UAG suppressor, called *SUQ5–o1–a1*, was verified to be an allele of *SUQ5*, since 18 out of 18 tetrads from pedigrees containing *SUQ5–o1* and *SUQ5–o1–a1* were parental ditype for the suppressors. Further genetic analyses of the new, *SUQ5–o1–a1*, UAG-suppressor indicated that its properties were identical to the highly efficient UAG suppressors that insert tyrosine. *SUQ5–o1–a1* acts on the auxotrophic UAG alleles *met8–1*, *tyr7–1*, *trp1–1*, *ade3–26* and *ilv1–1*. Furthermore low-temperature spectroscopy indicated that *SUQ5–o1–a1* efficiently suppresses *cyc1–179* and *cyc1–76*.

DISCUSSION

Nonsense suppressors were obtained in a strain carrying eight suppressible auxotrophic markers, by selecting for revertants on media having 15 different combinations of nutritional deficiencies. Suppressors of UAG were presumed to be present in 1088 such revertants which were divided into 78 phenotypic classes by their patterns of suppression of *cyc1–179* and of five presumed amber and three presumed ochre nutritional markers. Amber-specific suppressors constitute four-fifths of the suppressors isolated, while the remaining one-fifth of the suppressors act on UAG and on UAA.

While only two amber-specific suppressor classes were previously uncovered in haploid strains (HAWTHORNE and MORTIMER 1968), as many as 34 phenotypic amber-specific suppressor classes were revealed in this study. Some of these classes may not represent differences that are necessarily attributable to a single suppressor, since genetic analysis of many suppressors was not undertaken and it is unknown whether or not the same phenotype would be observed in the sexual

progeny after crossing. Evidence described in the accompanying paper (LIEBMAN and SHERMAN 1976) and elsewhere shows that the pattern of suppression, or the class number in Table 3, of certain suppressors can be modified by other mutations. Furthermore, the apparent suppression of *cyc1–179* was found in a few strains to be due to increased levels of iso-2-cytochrome *c*, rather than to suppression of the UAG triplet in the mRNA of iso-1-cytochrome *c*. Also, false-negative scores in Table 3 were obtained with strains unable to grow on complete glycerol medium and which grew poorly on complete glucose medium. This caused, for example, independently isolated *SUP4–a* suppressors to be assigned to three different classes. However, the large number of phenotypic distinctions observed suggests that there are numerous valid classes. Indeed, of the 25 suppressors analyzed, most of the suppressor characteristics did persist through meiosis. Furthermore, genetic analyses have verified that at least two classes make up the previously reported class IX suppressors described by HAWTHORNE and MORTIMER (1968) that efficiently suppress *met8–1*, *tyr7–1* and *trp1–1*. This was established since suppressors in classes 4, 5, 6, 7, 8, 13 and 14 are not alleles of any of the class 1, 2 or 3 suppressors, although suppressors in all these classes would fall into class IX of HAWTHORNE and MORTIMER (1968) since they all act efficiently on *met8–1*, *tyr7–1* and *trp1–1*.

The efficiencies of the suppressors isolated in the present study have been estimated by their action on the amber mutant *cyc1–179*, using spectroscopic determinations of the cytochrome *c* levels in intact cells. The *cyc1–179* allele is particularly suitable for such measurements since functional proteins are formed with apparently most or all amino acids at its nonsense site (STEWART and SHERMAN 1972). Furthermore, it is evident that a more precise measure of efficiency of suppression can be obtained with *cyc1* mutants than with nutritional mutants since the levels of suppressed iso-1-cytochromes *c* can be determined directly instead of being inferred from growth rates. Indeed the only criterion that permitted the distinction of suppressors in classes 1, 2 and 3 from classes 4, 5, 8, 13 and 14 was efficiency of suppression of *cyc1–179*.

All but one of the UAG-specific suppressors tested, which ranged from very efficient to very inefficient suppressors of *cyc1–179*, were dominant or semi-dominant. Those which acted on *cyc1–179* most efficiently were associated with diminished cell growth, while less efficient UAG-specific suppressors allowed normal cell growth on complete medium. In contrast, most of the suppressors tested which acted on amber markers as well as the ochre allele *leu2–1* but not *his5–2* or *lys1–1* were recessive. The action of these suppressors on *cyc1–179* ranged from moderately efficient to very inefficient, and they were nearly all associated with diminished cell growth. Taken alone, the dominance results are compatible with a suggestion that the suppressors are all altered tRNA's and that the distinction between recessiveness and dominance is only a distinction between very weak suppressors and moderate to efficient suppressors. However, this hypothesis is not consistent with the observation that some UAG-specific dominant suppressors are less efficient than some recessive suppressors of *leu2–1*. Probably the mechanism of suppression by the recessive suppressors of *leu2–1* is

different from that of the UAG-specific suppressors. SMIRNOV *et al.* (1974) have shown that recessive suppressors s_1 and s_2 most likely result from a mutant protein participating in or affecting the termination of proteins on ribosomes. These suppressors, which were isolated by INGE-VECHTOMOV and ANDRIANOVA (1970), have been divided into mutants of two genes. They appear similar to the recessive omnipotent suppressors reported by HAWTHORNE and LEUPOLD (1974), and later by GERLACH (1975), that are thought to be capable of acting on UAA, UAG and UGA. These suppressors have also been divided into mutants of two genes, although suppressors with different phenotypic properties including semi-dominance (GERLACH 1975) have been found at the same locus. Possibly many or all of the present recessive suppressors of *leu2–1* are alleles of these two genes.

All of the highest efficiency suppressors isolated in this study from a suppressor-free strain appear to be alleles of one or another of the eight class I, set I UAA suppressors, which are the most efficient UAA suppressors and which cause the insertion of tyrosine (GILMORE, STEWART and SHERMAN 1971; LIEBMAN, STEWART and SHERMAN 1975b). In our ψ^- strains in the absence of modifiers or secondary mutations, these UAA suppressors were found to act on several *cyc1* ochre mutants with 10% to 15% efficiency, while the corresponding UAG suppressors acted on *cyc1–76* and *cyc1–179* with approximately 75% efficiency. The following representative amber suppressor alleles have been shown to cause the insertion of tyrosine at the amber codon in *cyc1–179*: *SUP6–***a***2*, *SUP7–***a***2* (SHERMAN *et al.* 1973), *SUP2–***a***2*, *SUP4–***a***4*, *SUP5–***a***2* and *SUP11–***a***2*. Technical difficulties prevented us from showing that *SUP3–***a***2* and *SUP8–***a***2* likewise cause the insertion of tyrosine at amber codons, but there is no reason to believe that they do not. Since many amino acid replacements at the amber codon in the *cyc1–179* allele are known to lead to functional iso-1-cytochrome *c* (STEWART and SHERMAN 1972), it is noteworthy that of the 1088 suppressor mutations isolated directly from wild-type genes, only tyrosine inserters acted efficiently on the *cyc1–179* allele. Since the low-efficiency amber suppressors exhibit diverse patterns of action on the nutritional markers, these suppressors probably insert amino acids other than tyrosine. Thus, the restriction of high-efficiency suppressors to tyrosine inserters seems a significant observation. Specifically, serine insertion is known to be acceptable to the function of at least one of the genes used for selection, *tyr7–1*, since the serine-inserting UAA suppressor, *SUQ5–***o**, acts on the ochre *tyr7–1,***o** allele (LIEBMAN, unpublished results) which was obtained by mutation of the amber codon in *tyr7–1* (MAGNI, personal communication). Furthermore, serine is probably acceptable for all the nutritional amber markers used in the selection since they are all acted on by the amber suppressor, *SUQ5–***o***1–***a***1*, which was obtained by mutation of the serine-inserting UAA suppressors, *SUQ5–***o**.

The dissimilarity between serine and tyrosine suggests that other amino acids as well are probably acceptable replacements for the amber markers. Thus probably the selection scheme used in this study would have detected highly efficient amber suppressors that inserted amino acids other than tyrosine. Apparently, the tyrosine-inserting UAG-suppressors, just like the tyrosine-inserting UAA-sup-

pressors, are the most efficient suppressors compatible with survival in haploid yeast. Amber suppressors that are even more efficient, and that may insert other amino acids, are probably lethal (LIEBMAN and SHERMAN 1976) just as highly efficient UAA suppressors are lethal (Cox 1971; LIEBMAN, STEWART and SHERMAN 1975b). It was recently demonstrated that serine is inserted by the highly efficient UAG suppressor *SUP-RL1*, which is lethal in haploids but which can be maintained in the heterozygous condition (BRANDRISS, STEWART, SHERMAN and BOTSTEIN, manuscript in preparation). Chromosome mapping studies have established that this suppressor is not an allele of the serine-inserting suppressor *SUQ5* (BRANDRISS, SOLL and BOTSTEIN 1975; LIEBMAN, STEWART and SHERMAN 1975a).

Suppressors from among classes 4 through 12 act on the amber allele *cyc1–179* with too low an efficiency to make it technically feasible to determine the amino acids that they insert. However, several of these suppressors act with a higher efficiency on the *cyc1–176* amber mutant and work is now in progress to determine the amino acids inserted by these less efficient suppressors.

Although *SUQ5–o1–a1* can efficiently suppress *cyc1–179*, *SUQ5–a* suppressors were not uncovered among the 1088 amber suppressors directly isolated by mutations of wild-type genes. Perhaps *SUQ5–a* alleles differ from the wild-type gene by more than a single base change. Indeed, if yeast suppressors generally result from mutations in tRNA anticodons, one would not expect a specific serine tRNA species to be capable of mutating to both a UAA and a UAG suppressor. We suggest that the wild-type *SUQ5* gene can mutate to a *SUQ5–o* allele by a single base change and that the UAA suppressor can in turn mutate to *SUQ5–a* by another single change. In contrast, a tyrosine tRNA could be converted to either a UAA or a UAG suppressor by different single-base changes at the anticodon.

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