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UGA Suppressors in *Saccharomyces cerevisiae*: Allelism, Action Spectra and Map Positions

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ABSTRACT

Sixty independent UGA suppressors of *Saccharomyces cerevisiae* have been studied. They are dominant and are divided into 16 groups (loci) by recombination. Suppressors representing these loci are divided into two classes by action spectra; four in class 1 (a broad action spectrum) and 12 in class 2 (a narrow action spectrum). Class 1 suppressors are less frequent in terms of not only total number but also number per locus than class 2 suppressors, indicating difference in either or both mutation frequency and selective pressure between suppressors of the two classes. Two of the class 1 suppressors, *SUP152* and *SUP161*, do not recombine with *SUP28* and *SUP33*, leucine-inserting UAA suppressors, respectively, indicating that they are mutations in genes coding for $tRNA^{Leu}_{UUA}$. Of the remaining two class 1 suppressors, *SUP160* which causes lethality in the ψ^+ cytoplasm is mapped on chromosome *XV* very close to the centromere, and *SUP165* on the right arm of chromosome *XIV* 44 cM distal to *lys9*. Of the class 2 suppressors, ten do not recombine with one or another of previously known UGA suppressors. The remaining two class 2 suppressors, *SUP154* and *SUP155*, are mapped on the left and right arms of chromosome *VII*, respectively.

THE study of *Saccharomyces cerevisiae* nonsense suppressors was initiated by HAWTHORNE and MORTIMER (1963). Since then many investigators have isolated numerous nonsense suppressors in this organism. The *S. cerevisiae* nonsense suppressors are divided into two categories, codon-specific and codon-nonspecific (omnipotent). Generally speaking, studies of the codon-specific suppressors have been more systematic and extensive than those of the codon-nonspecific suppressors (*cf.* SHERMAN 1982). However, our knowledge on UGA suppressors has been much limited (*cf.* SHERMAN, ONO and STEWART 1979; SHERMAN 1982).

Interestingly, most of the nonsense mutations isolated early in the study are either UAA or UAG (*cf.* HAWTHORNE and LEUPOLD 1974). The paucity of UGA mutations is more apparent as more loci are studied. For examples, although several mutations in the *CYC1* locus are shown to be UAA or UAG, none is clearly identified as UGA (*cf.* SHERMAN, ONO and STEWART 1979). Studies of the *lys2* and *can1* mutants also indicate that UGA mutations are much less frequent than UAA and UAG mutations (CHATTOO *et al.* 1979; ONO, ISHINO and SHINODA 1983). Since the potential for isolating suppressors is a function of the availability of the mutations on which suppressors act, it is obvious why the study of UGA suppressors has been slow in *S. cerevisiae*.

To overcome this problem, HAWTHORNE (1976) has undertaken efforts to convert UAA to UGA mutations using mutagens which specifically induce base transitions. He has obtained several presumed UGA mutations, and has subsequently isolated suppressors that act specifically on them. In this way, 15 presumed UGA suppressors are characterized and mapped (HAWTHORNE 1981). Since some of them are positioned close to UAA suppressors which cause insertion of serine or leucine, it is concluded that they are UGA suppressors derived from $tRNA^{Ser}_{UCA}$ or $tRNA^{Leu}_{UUA}$, and eventually that the mutations on which they act are UGA mutations. Independently, GESTLAND and WILLS (1979) have shown that a suppressor specific for *leu2-2* and *his4-166* causes readthrough of UGA codon during *in vitro* protein synthesis. Moreover, HÖTTINGER *et al.* (1982) have introduced a cloned *Schizosaccharomyces pombe* UGA suppressor, which is a mutation in a $tRNA^{Ser}$ gene, into *S. cerevisiae*, and have found that it suppresses *leu2-2* and *his4-166*. The study has clearly shown that *leu2-2* and *his4-166* are UGA mutations.

We previously isolated two suppressors specific for *leu2-2* and *his4-166*, which were used to identify UGA mutations of the *LYS2* and *CAN1* loci (CHATTOO *et al.* 1979; ONO, ISHINO and SHINODA 1983). Therefore, we thought it would be worth to undertake a systematic search of UGA suppressors using newly defined UGA mutations. Since it is of interest to see whether the ψ^+ factor affects UGA suppressors, we selected revertants from ψ^- and ψ^+ strains containing the UGA mutations as well as UAA and UAG mutations. In

this report, we describe characteristics of the suppressors responsible for UGA-specific reversion.

MATERIALS AND METHODS

Yeast strains and genetic markers: Some of strains and nonsense mutations used in this study are listed in Tables 1 and 2, respectively. The *can1-1001* mutation was defined as UGA by its response to a suppressor that acted on *leu2-2* and *his4-166* (CHATTOO *et al.* 1979; ONO, ISHINO and SHINODA 1983). The *leu2-1'*, *his5-2'* and *lys1-1'* UGA mutations were converted from UAA mutations (HAWTHORNE 1976). The *ade5,7-143* and *gal10-1* UGA mutations were obtained by HAWTHORNE (1976). UGA suppressors (HAWTHORNE 1976, 1981) and serine- or leucine-inserting UAA suppressors (ONO, STEWART and SHERMAN 1979a, b; ONO *et al.* 1981) were described previously. The ψ^+ cytoplasmic determinant was first described as an allosuppressor of *SUP16(SUQ5)*, a UAA suppressor (COX 1965), but it was later shown to act as a suppressor of UAA (LIEBMAN, STEWART and SHERMAN 1975; LIEBMAN and SHERMAN 1979) and UGA (ONO *et al.* 1986) mutations. Weak suppression of UAG mutations by ψ^+ was also observed (ONO *et al.* 1986).

Most of the markers used for mapping UGA suppressors were supplied by the Yeast Genetic Stock Center, University of California, Berkeley, California, (MORTIMER 1984). They were combined in a few strains to facilitate analyses (B. ONO and Y. ISHINO-ARAO, unpublished data).

Yeast growth media and growth conditions: Standard yeast growth media were used (SHERMAN, FINK and LAWRENCE 1974). Media for testing various nutritional and resistant/sensitive markers were described previously (ONO *et al.* 1985).

Isolation of revertants: Revertants were selected from strains IS110-18A (ψ^-) and YO6-15C (ψ^+) by various combinations of the included nonsense markers. Cells were spread on selective plates, and the plates were irradiated with UV light at a dose of 50% survivor. After incubation at 30° for 5–10 days, the resultant colonies were picked and subcloned to obtain pure clones. The revertants were classified into two groups according to their modes of reversion; some reverted for mutations of a single nonsense codon (UAA, UAG or UGA), while others reverted for mutations of all three nonsense codons (UAA, UAG and UGA); it should be stressed that no revertant reverting for mutations of two of the three nonsense codons was obtained by the present selection scheme. In this study, we made genetic analyses of the UGA-specific revertants.

Yeast genetic procedures: Standard yeast genetic procedures were used (SHERMAN, FINK and STEWART 1974). Random-spore analysis was achieved by crossing two strains of opposite mating types (**a** and α), and selecting diploid by complementation of appropriate markers, for example *ade2-1* and *ura4-1*, or by zygote isolation with a micromanipulator. The diploid was made homozygous for *leu2-2* in order to detect UGA suppressors. The diploid was sporulated and treated with diluted snail gut juice. After gentle sonication, the suspension was spread on SC medium containing half the usual concentration of adenine sulfate (10 μg/ml) and containing L-canavanine sulfate (60 μg/ml) instead of L-arginine sulfate (CAD medium). The plate was incubated at 30° for 5 days. Red colonies were picked (32 colonies per cross) and tested for their requirement for leucine. Since the diploid was heterozygous for both *can1-100* and *ade2-1*, canavanine resistance and redness are indicators that the colony derives from a spore. In this circumstance, if a diploid is homozygous for a certain UGA suppressor, then all spores are leucine-independent because they contain the suppressor. In contrast, if the diploid is heterozygous for two independently segregating UGA suppressors, then a quarter of the spores are leucine-dependent because of the absence of suppressor. When *ade2-1* was not usable due to the genetic makeup of a diploid, an increased number (up to 320 per cross) of colonies were tested for their leucine requirement. For representative diploids, tetrad analyses were achieved in order to confirm the results of the random-spore analyses.

For mapping a UGA suppressor, diploids were constructed in a way that they were homozygous for *leu2-2* and heterozygous for various markers as well as for the suppressor of interest. The diploids were sporulated, and tetrads were examined. Linkage was evaluated by statistical significance of deviation from P:N = 1:1, where P and N represent parental ditype and nonparental ditype tetrads, respectively (MORTIMER and HAWTHORNE 1975). When linkage was found, the map distance was expressed in term of centimorgan (cM) units (PERKINS 1949).

RESULTS

Allelism tests of UGA suppressors: Thirty-two (YOA-1 through YOA-32) and 28 (YOZ-1 through YOZ-28) UGA-specific revertants derived from IS110-18A and YO6-15C, respectively, were subjected to the present study. These revertants contained dominant mutations responsible for reversion (data not shown).

First, YOZ-2 was shown to have a single suppressor by crossing it to IS110-18A and analyzing the tetrads of resultant diploid, and the suppressor was designated *SUP150*. Second, YOZ-2 was crossed to YOA-1 through YOA-32. The resultant diploids were subjected to random-spore analyses (see MATERIALS AND METHODS). Revertants which gave rise to no leucine-dependent spore were assumed to have *SUP150*. One of them, YOA-6, was assigned as a standard strain of *SUP150* after it was shown to have a single suppressor. One of the remaining revertants, YOA-1, was assigned as a standard strain of *SUP151* after it was shown to have a single suppressor. Third, suppressors in the YOZ series were classified into three groups using YOA-6 (*SUP150*) and YOA-1 (*SUP151*); those did not recombine with *SUP150*, those did not recombine with *SUP151*, and those recombined with both *SUP150* and *SUP151*. YOZ-3 and YOZ-1 representing the second and third groups were assigned as standard strains of *SUP151* and *SUP152*, respectively, after they were shown to have single suppressors. Then, using YOZ-1 (*SUP152*) the above mentioned steps were repeated. The cycle was repeated until all revertants were classified by suppressors; when no corresponding suppressor was found in one revertant set, a standard strain was constructed using the standard strain in the other set. For example, no revertant in the YOA series contained *SUP152*. Then, strain YO19-15C was constructed by crossing YOZ-1 (*SUP152*) with strain IS110-18A, sporulating the diploid, and dissecting the

TABLE 1

Yeast strains

Strain	Genotype	Reference
IS110-18A	*ψ⁻ MATa lys2-101 leu2-2 his4-166 trp5-48 ilv1-2 ura4-1 met8-1 aro7-1*	This study
YO6-15C	*ψ⁺ MATα lys2-101 leu2-2 his4-166 ade2-1 can1-100 ilv1-2 met8-1 aro7-1*	This study
3133-3C	*ψ⁻ MATa ade5,7-143 gal10-1 his5-2′ leu2-1′ ilv1-1 lys1-1 ura4-1 met8-1 trp1-1 can1-52 aro1*	D. C. HAWTHORNE (personal communication)
IS98-1C	*ψ⁻ MATa can1-1001 met8-1*	This study
IS482-2B	*ψ⁻ MATa leu2-2*	This study
IS482-1A	*ψ⁻ MATα leu2-2*	This study

TABLE 2

Nonsense mutations

Nonsense codon	Mutations
UGA	*lys2-101 leu2-2 his4-166 can1-1001 leu2-1′ his5-2′ ade5,7-143 gal10-1*
UAA	*trp5-48 leu2-1 ilv1-2 can1-100 ade2-1 ura4-1*
UAG	*met8-1 aro7-1*

TABLE 3

Linkage between UGA suppressor loci

Suppressor pair	Segregation P	N	T	cM	Deduced linkage
SUP157-SUP163	13	0	2	7	*SUP157-SUP163*
SUP162-SUP155	30	3	35	39	*SUP162-(SUP155, SUP166)*
SUP162-SUP166	13	1	33	41	
SUP155-SUP166	30	0	1	2	

TABLE 4

Classification of UGA suppressors by action spectra

Nonsense mutation	Suppression[a] by Class 1		Class 2		
	SUP160 *SUP165* *SUP85*[b]	*SUP152* *SUP161*	*SUP150* *SUP154* *SUP71*[b]	*SUP151* *SUP153* *SUP154* *SUP155* *SUP156*	*SUP157* *SUP158* *SUP159* *SUP162* *SUP163*
lys2-101[c]	+	+	+	+	
leu2-2[c]	+	+	+	+	
his4-166[c]	+	+	+	+	
can1-1001[d]	+	+	−	−	
leu2-1′[e]	+	NT[f]	+	NT	
his5-2′[e]	+	NT	+	NT	
ade5,7-143[e]	+	NT	−	NT	
gal10-1[e]	+	NT	−	NT	

[a] Suppression was tested in the *ψ⁺* cytoplasm, except for *SUP160* which was tested in the *ψ⁻* cytoplasm.

[b] Suppressors described by HAWTHORNE (1976, 1981).

[c] Suppression was evaluated by nutritional requirements of the revertans.

[d] Suppression was evaluated in *can1-1001/can1-100* heteroallelic diploids.

[e] Suppression was evaluated by nutritional requirements of segregants derived from the diploids constructed with the standard revertants (or their derivatives) and strain 3133-3C.

[f] NT, not tested.

resultant asci. In case of *SUP160*, a standard strain, YO62-10A, was constructed by crossing YOA-9 with YO9-4C (*ψ⁻*), instead of YO6-15C (*ψ⁺*), because this suppressor was lethal in the *ψ⁺* cytoplasm.

After completion of the entire random-spore analysis, the 60 suppressors were divided into 15 loci; *SUP150* through *SUP163* plus *SUP165* (*SUP164* was first thought to comprise a distinct locus, but it was later shown to be identical with *SUP162*). The two UGA suppressors we described previously, *SUPG1* and *SUPG2* (ONO, ISHINO and SHINODA 1983), did not recombine with *SUP155* and *SUP161*, respectively. The result of the random-spore analysis was confirmed by tetrad analyses of diploids made by crossing representative strains. In the course of the tetrad analyses, however, we distinguished *SUP166* from *SUP155* (described later). After all, 16 loci of UGA suppressors were revealed in the present study.

During the random-spore analyses, we noticed that certain combinations of the revertants gave rise to less recombinants than the others. Tetrad analyses clearly showed linkage of two pairs of suppressors; *SUP157-SUP163* and *SUP155-SUP162* (Table 3). In addition, it was shown that a cross between revertants containing "*SUP155*" suppressors gave rise to a rare recombinant (Table 3). From this result, we distinguished *SUP166* from *SUP155*.

Action spectra of UGA suppressors: Action spectra of the UGA suppressors representing 16 loci were examined (Table 4). The action on the *can1-1001* UGA mutation was tested by crossing the standard strains of mating type *α* with strain IS98-1C and by examining canavanine resistance of the resultant diploid. Diploids containing *SUP152*, *SUP160*, *SUP161* and *SUP165* were sensitive to canavanine, indicating that these suppressors suppressed *can1-1001*. In con-

TABLE 5

Distribution of UGA suppressors in different loci

Cyto-plasm	No. of suppressors[a] in Class 1				Class 2											
	SUP152	*SUP160*	*SUP161*[b]	*SUP165*	*SUP150*	*SUP151*	*SUP153*	*SUP154*	*SUP155*[b]	*SUP156*	*SUP157*	*SUP158*	*SUP159*	*SUP162*	*SUP163*	*SUP166*
ψ^-	0	1	0	0	4	3	1	4	2	3	4	0	2	2	1	1
ψ^+	1	0	1	1	2	5	3	0	1	2	4	5	1	4	2	0
Total	1	1	1	1	6	8	4	4	3	5	8	5	3	6	3	1

[a] For distinction between classes 1 and 2, see Table 4.
[b] Suppressors *SUP161* and *SUP155* correspond to *SUPG1* and *SUPG2* (ONO, ISHINO and SHINODA 1983), respectively.

trast, diploids containing other suppressors grew on the canavanine medium, indicating that these suppressors did not act on *can1-1001* in heterozygous condition. The action on the UGA mutations described by HAWTHORNE (1976) was examined by crossing the standard strains with strain 3133-3C and examining the tetrads of the resulted diploids. It should be emphasized that suppressors that suppressed *can1-1001* suppressed *ade5,7-143* and *gal10-1*, while those did not suppress *can1-1001* did not suppress *ade5,7-143* and *gal10-1*.

Since HAWTHORNE (1976, 1981) divided UGA suppressors into two groups by the action on *ade5,7-143* and *gal10-1*, we examined action of his representative suppressors, *SUP85* (group I) and *SUP71* (group II), on *can1-1001*. Apparently, suppression of *can1-1001* well coincided with that of *ade5,7-143* and *gal10-1*, which eventually led us to a conclusion that the classes described by us were same with those described by HAWTHORNE (1976, 1981).

Distribution of UGA suppressors in 16 loci: Distribution of the 60 UGA suppressors in different loci is shown in Table 5. All class 1 loci contained single members while all class 2 loci, except for *SUP166*, contained more than one member. Distinction of the two classes are obvious in this respect too. Although *SUP154* was not found in the ψ^+ revertants, we attribute it to a statistical error due to a small sample size since this suppressor is not detrimental in ψ^+ strains. In contrast, absence of *SUP160* in the ψ^+ revertants was attributed to its lethality in the ψ^+ cytoplasm. The reason why *SUP158* was not found in the ψ^- revertants is unknown; it is also attributable to a statistical error.

Mapping of the UGA suppressors: HAWTHORNE (1976, 1981) has isolated UGA suppressors using a different set of UGA mutations. Since he has distinguished and mapped 15 loci, we first examined correspondence of our UGA suppressors to his UGA suppressors (Table 6). Ten suppressors were shown to be identical. That is, six UGA suppressors we obtained were new. It should be emphasized that *SUP166* did not recombine with *SUP77* at all, while *SUP155* recombined with *SUP77* at a very low frequency. This result also indicates that *SUP166* is close but distinct from *SUP155*.

TABLE 6

Correspondence between the newly obtained UGA suppressors and the previously known UGA suppressors

Suppressor pair	Segregation P	N	T	Suppressor pair	Segregation P	N	T
SUP150-SUP71	15	0	0	*SUP158-SUP75*	51	0	0
SUP151-SUP78	7	0	0	*SUP159-SUP80*	15	0	0
SUP153-SUP79	25	0	0	*SUP163-SUP73*	23	0	0
SUP155-SUP77	46	0	2	*SUP164-SUP76*	15	0	0
SUP156-SUP72	10	0	0	*SUP166-SUP77*	21	0	0
SUP157-SUP74	24	0	0				

According to the genetic code, genes coding for $tRNA^{Leu}_{UAA}$ and $tRNA^{Ser}_{UCA}$ can mutate to UAA and UGA suppressors via different mutations in anticodons. So that, we next examined correspondence of the six new UGA suppressors to the serine- or leucine-inserting UAA suppressors. Instead of the UAA suppressors themselves, we used markers known to be closely linked to them. The signal markers were as follows: *aro7* (*SUP16*), *his5* (*SUP17* and *SUP22*), *his1* (*SUP19*), *ura4* (*SUP26*), *trp1* (*SUP27*), *lys9* (*SUP28*), *met3* (*SUP29*) and *trp3* (*SUP33*). The primary revertants were crossed to either strain IS482-2B or strain IS482-1A depending on the mating type. The resultant diploids were sporulated, the asci were dissected. A segregant which had few unnecessary markers was picked from each cross, and it was crossed to strains containing the signal markers in various combinations. The resultant diploids were examined by random-spore analysis achieved by the same way as mentioned above (Table 7). The *SUP152* and *SUP161* suppressors did not recombine with the *SUP28* and *SUP33* leucine-inserting UAA suppressors, respectively. Since *SUP32* UAA suppressor was not testable by this method, we tested recombination of this suppressor to each of UGA suppressors and found no linkage (data not shown).

Mapping of the new UGA suppressors is summarized in Table 8. *SUP160* was mapped on chromosome *XV* very close to the centromere. *SUP165* showed

TABLE 7

Correspondence between UGA suppressors and UAA suppressors

Gene pair	Segregation[a] P	N	T	cM
SUP152-lys9	27	0	0*	0
SUP152-SUP28	51	0	0*	0
SUP161-trp3	10	2	26**	50
SUP161-SUP33	21	0	0*	0

[a] P, N and T represent parental ditype, nonparental ditype and tetratype tetrads, respectively. * and ** indicate statistical significance of linkage at the 1% and 2.5% levels, respectively.

TABLE 8

Mapping of the new UGA suppressor loci

Chromosome	Gene pair	Segregation[a] P	N	T	cM	Deduced gene order
XV	*SUP160-pet17*	18	0	12*	20	*(cen15, SUP160)-pet17*
	SUP160-cen15	26		1	2	
XIV	*SUP165-lys9*	17	3	20*	44	*cen14-pet8-lys9-SUP165*
	SUP165-pet8	3	3	11		
V	*ura3-SUP150*	70	0	26*	14	*ura3-SUP150-cen5*
	SUP150-cen5	182		7[b]	2	
	ura3-cen5	67		29	15	
VII	*cyh2-SUP154*	13	0	12*	24	*cyh2-SUP154-trp5-leu1-cen7*
	SUP154-trp5	49	0	27*	18	
	SUP154-leu1	22	1	38*	36	
	cyh2-trp5	10	2	13**	44	
	trp5-leu1	21	0	13*	19	

[a] P, N and T represent parental ditype, nonparental ditype and teratype tetrads, respectively. Figures indicated at the space between P and N represent sums of tetrads showing P or N segregation between the gene of interest and a centromere marker (*trp1*); thus, they represent FD tetrads. * and ** indicate statistical significance of linkage at the 1% and 2.5% levels, respectively.

[b] Two of these tetrads were from a cross where *ura3* was heterozygous; both of which were parental ditype for *SUP150-ura3* and second division segregation for both *SUP150* and *ura3* indicating that *ura3* is distal to *SUP150*.

statistically significant linkage to both *lys9* and *pet17* (at levels of 1% and 5%, respectively), but we negated the latter linkage because the suppressor did not show linkage to *ade2* and *cen15*, markers flanking *pet17*. Thus, we placed *SUP165* on the right arm of chromosome *XV*; since the suppressor did not show linkage to *pet8*, it should be distal to *lys9*. *SUP150* was located close to the centromere of chromosome *V*. By comparing the present data with those in literature (MORTIMER and SCHILD 1980), we placed it on the left arm (Table 8). This conclusion was supported by the observation that two tetrads showing second division segregation for *SUP150* showed parental ditype segregation for the suppressor and *ura3*. Since this suppressor did not recombine with *SUP71* (Table 6), we contend that *SUP150* and *SUP71* are identical. *SUP154* was mapped on the left arm of chromosome *VII* between *cyh2* and *trp5*. *SUP155* was located on the right arm of chromosome *VII* at a position very close to *SUP166* (*SUP77*) as described before (Table 6).

TABLE 9

***S. cerevisiae* UGA suppressors**

Class	Suppressor	Chromosome arm[a]	Corresponding suppressor[b]
1-1	*SUP160*	*XV*-C	
	SUP165	*XIV*-R	
1-2	*SUP152*	*XIV*-R	*SUP28* (UAA-LEU)
	SUP161	*XI*-L	*SUP33* (UAA-LEU)
2	*SUP150*	*V*-C(L)	*SUP71* (UGA)
	SUP151	*XIII*-R	*SUP78* (UGA)
	SUP156	*II*-R	*SUP72* (UGA)
	SUP157	*X*-L	*SUP74* (UGA)
	SUP163	*X*-L	*SUP73* (UGA)
	SUP158	*XI*-L	*SUP75* (UGA)
	SUP159	*IV*-R	*SUP80* (UGA)
	SUP162	*VII*-R	*SUP76* (UGA)
	SUP153	*XIII*-L	*SUP79* (UGA)
	SUP166	*VII*-R	*SUP77* (UGA)
	SUP155	*VII*-R	
	SUP154	*VII*-L	

[a] C, R and L represent "centromere," "right" and "left."
[b] LEU indicates "leucine-inserting."

DISCUSSION

S. cerevisiae suppressors responsible for reversion of *lys2-101*, *leu2-2* and *his4-166* UGA mutations have been genetically characterized. Sixty UGA-specific revertants have been shown to contain dominant suppressors. These suppressors were divided into sixteen recombinationally distinguishable loci. They are listed in Table 9; suppressors relevant to the following discussion are also included.

The UGA suppressors are divided into two classes according to their action spectra (Table 3); suppressors in class 1 are presumably more efficient than those in class 2 because they have a broader action spectrum. Since *SUP152* and *SUP161* do not recombine with *SUP28* and *SUP33*, respectively, *SUP152* and *SUP28*, likewise *SUP161* and *SUP33*, are likely to be different mutations in the same gene, which eventually leads to a speculation that the genes code for $tRNA^{Leu}_{UUA}$. From our observation and that of HAWTHORNE (1981), it becomes apparent that only class 1 UGA suppressors correspond to serine- or leucine-inserting UAA suppressors. It is also obvious that not all class 1 UGA suppressors correspond to UAA suppressors. The class 1 UGA suppressors are divided into two subclasses (1-1 and 1-2) by this criterion (Table 9). It should be noted that *SUP160*, which causes lethality in the ψ^+ cytoplasm, belongs to class

1-1. Thus, we assume that subclass 1-1 suppressors are more efficient than subclass 1-2 suppressors.

According to the genetic code, the following tRNAs can mutate to UGA suppressors via single base substitutions in the anticodon: $tRNA^{Cys}_{UGU}$ and $tRNA^{Trp}_{UGG}$ (the first bases), $tRNA^{Leu}_{UUA}$ and $tRNA^{Ser}_{UCA}$ (the second bases), and $tRNA^{Arg}_{CGA}$, $tRNA^{Arg}_{AGA}$ and $tRNA^{Gly}_{GGA}$ (the third bases). Biochemical analyses have indicated that $tRNA^{Trp}_{UGG}$ and $tRNA^{Arg}_{AGA}$ are coded for by four and eight genes, respectively (*cf.* GUTHRIE and ABELSON 1982). On the other hand, it is expected that $tRNA^{Trp}_{UGG}$ mutates to a UAG suppressor, and $tRNA^{Leu}_{UUA}$ and $tRNA^{Ser}_{UCA}$ to UAA suppressors, via different single base substitutions. Unfortunately, no UAG suppressor causing insertion of tryptophan has been identified. In contrast, systematic and exhaustive surveys of UAA suppressors have defined four and six loci giving rise to serine- and leucine-inserting UAA suppressors, respectively (GILMORE, STEWART and SHERMAN 1971; ONO, STEWART and SHERMAN 1979a, b; ONO *et al.* 1981).

For UAA or UAG suppressors, it has been known that suppressors of different action spectra cause insertion of different amino acids (*cf.* SHERMAN, ONO and STEWART 1979; SHERMAN 1982). Therefore, it is reasonable for us to assume that class 1 and class 2 UGA suppressors cause insertion of different amino acids. Moreover, it has been known that tyrosine-inserting UAA suppressors, arisen by mutations at the first base of anticodon, are more efficient than serine- or leucine-inserting UAA suppressors, arisen by mutations at the second base of anticodon. The absence of glutamate-, lysine- or glutamine-inserting UAA suppressors arisen by mutations at the third base of anticodon is attributed to their extreme inefficiency. From these informations, we predict that class 1-1 UGA suppressors are mutations at the first base of anticodons of $tRNA^{Cys}_{UGU}$ and $tRNA^{Trp}_{UGG}$, and that class 2 UGA suppressors are mutations at the third base of anticodons of $tRNA^{Arg}_{CGA}$, $tRNA^{Arg}_{AGA}$ and $tRNA^{Gly}_{GGA}$. Since recovery of class 1 UGA suppressors is far from complete (Table 4), further search for suppressors in this class is needed. By assuming the same mutant frequency for suppressors in classes 1-1 and 1-2, we expect existence of about six loci that give rise to class 1-1 UGA suppressors. On the other hand, we presume the number of loci giving rise to class 2 UGA suppressors is twelve, or does not exceed twelve by very much, since the recovery of them are complete or nearly complete (Table 5). Since a single species of tRNA is coded for by one to ten genes in *S. cerevisiae* (*cf.* GUTHRIE and ABELSON 1982), we predict that the classes 1-1 and 2 UGA suppressor loci as a whole code for one to two and three to four species of tRNA, respectively.

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