FRAMESHIFT SUPPRESSION IN *SACCHAROMYCES CEREVISIAE* VI. COMPLETE GENETIC MAP OF TWENTY-FIVE SUPPRESSOR GENES

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ABSTRACT

Five previously unmapped frameshift suppressor genes have been located on the yeast genetic map. In addition, we have further characterized the map positions of two suppressors whose approximate locations were determined in an earlier study. These results represent the completion of genetic mapping studies on all 25 of the known frameshift suppressor genes in yeast.——The approximate location of each suppressor gene was initially determined through the use of a set of mapping strains containing 61 signal markers distributed throughout the yeast genome. Standard meiotic linkage was assayed in crosses between strains carrying the suppressors and the mapping strains. Subsequent to these approximate linkage determinations, each suppressor gene was more precisely located in multi-point crosses. The implications of these mapping results for the genomic distribution of frameshift suppressor genes, which include both glycine and proline tRNA genes, are discussed.

IN the yeast *Saccharomyces cerevisiae*, 25 recombinationally distinct suppressor mutations have been identified among revertants of +1 frameshift mutations at the *his4* locus (CULBERTSON *et al.* 1977; CUMMINS *et al.* 1980; GABER and CULBERTSON 1982b; CULBERTSON, GABER and CUMMINS 1982). Twenty-two of these suppressors have been classified into two groups that differ by their ability to exhibit specific suppression of +1 G:C insertion mutations in either glycine or proline codons (DONAHUE, FARABAUGH and FINK 1981). The remaining three suppressors exhibit a wider range of specificity, including the ability to cross-suppress +1 frameshift mutations in different types of codons and, in some cases, the ability to cross-suppress nonsense mutations (CULBERTSON, GABER and CUMMINS 1982).

By analogy with bacterial systems (RIDDLE and CARBON 1973), we anticipated that at least some of the codon-specific frameshift suppressors might correspond to genes encoding altered glycine and proline tRNAs. The altered tRNAs produced in these strains might contain four-base anticodons that could permit suppression of appropriate +1 frameshift mutations. This has been confirmed by molecular cloning and DNA-sequencing studies of the frameshift suppressors

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SUF16-1, which encodes a four-base anticodon glycine tRNA (GABER and CULBERTSON 1982a), and *SUF2-1*, which encodes a four-base anticodon proline tRNA (CUMMINS, DONAHUE and CULBERTSON 1982).

These genetic and biochemical studies indicate that the genetic map positions of many of the codon-specific yeast frameshift suppressor genes may correspond to the locations of glycine and proline tRNA genes. The codon-nonspecific suppressors probably correspond to other classes of tRNA and/or non-tRNA genes.

In order to facilitate additional genetic and molecular studies on gene families that mediate suppression of frameshift mutations, we have sought to determine the map locations of all of the suppressors. The advantages of information gained by these genetic mapping studies are threefold. Knowledge of the chromosome locations of each suppressor provides precise information on the genomic distribution of gene families involved in frameshift suppression, which may prove particularly useful in the analysis of potentially reiterated tRNA gene families. Second, knowledge of map position is useful in classifying dominant suppressor mutations that are not amenable to complementation analysis. Third, knowledge of map position can be useful in molecular cloning studies. For example, cloning of the *SUF16* gene was facilitated by determining that this suppressor was located in a chromosome region that had previously been cloned (GABER and CULBERTSON 1982a,b; HITZEMAN, CLARKE and CARBON 1980).

A large number of mapping techniques are currently available in yeast. Numerous genes have been positioned on the yeast genetic map through the use of specialized chromosome localization techniques. Some of these mapping strategies take advantage of the aberrant segregation patterns observed upon sporulation of specifically constructed aneuploid strains enabling the assignment of genes to particular chromosomes (MORTIMER and HAWTHORNE 1973; CULBERTSON and HENRY 1973; WICKNER 1979; CUMMINS *et al.* 1980). Several other methods used to determine the chromosomal locations of genes have relied on phenotypic analysis of vegetative cells after mitotic recombination or after induction of chromosome loss (NAKAI and MORTIMER 1969; LIRAS *et al.* 1978; KAWASAKI 1979; MORTIMER, CONTOPOULOU and SCHILD 1981). Recently, two additional approaches to genetic mapping have been developed, one on the basis of internuclear chromosome transfer in yeast heterokaryons (DUTCHER 1981) and the other on the basis of meiotic chromosome segregation analysis in recombination-deficient strains (KLAPHOLTZ and ESPOSITO 1982).

Despite the general utility of such mapping strategies, several of these methods that have been tested in this laboratory have not proved to be efficient approaches to the mapping of frameshift suppressor genes (CUMMINS *et al.* 1980; see Discussion). In order to simplify the mapping of frameshift suppressors, we have devised an alternative mapping strategy by constructing a set of "mapping strains" containing *leu2-3*, a suppressible frameshift mutation (CULBERTSON *et al.* 1977), and 61 signal markers distributed throughout the yeast genome. Using these strains it has been possible to efficiently locate frameshift suppressor genes by conventional recombination analysis in tetrads derived from diploids

of normal chromosome constitution. In this study five previously unmapped suppressor genes have been located on the genetic map. In addition, we have further characterized the map positions of two suppressors whose approximate locations were reported in a previous study (GABER and CULBERTSON 1982b). This communication represents the completion of genetic mapping studies on all 25 suppressor genes.

MATERIALS AND METHODS

Yeast strains, genetic methods, and media: Multiply marked strains used to locate the suppressor genes on the genetic map are listed in Table 1. Genetic markers were obtained from the Berkeley Stock Center with the exception of *MAL2* and *pet9* (from D. HAWTHORNE), *ser2* (from D. MEHNERT), *MAL3* (from G. KAWASAKI), *tsl1* (from D. KABACK), *pet11* (from T. COOPER), *cyc1-13* and *rad7* (from F. SHERMAN), *HO* (from J. HICKS), and *met2-2* (from G. FINK). Genetic methods and nomenclature are those described in the Cold Spring Harbor Yeast Course Manual (SHERMAN, FINK and LAWRENCE 1971).

The methods for scoring the segregation of genetic markers and the media used in this study are generally those previously reported (GABER and CULBERTSON 1982b). The segregation of *cyc1* was scored as described by SHERMAN *et al.* (1974).

The radiation-sensitive *rad7* mutant was scored as follows. Individual spores of each tetrad derived from sporulated diploids heterozygous for *rad7* were crossed with *rad7* tester strains of appropriate mating type such that diploids could be selected by complementation of auxotrophic markers in the cross. Suspensions of the diploids were spotted onto YEPD medium, irradiated with ultraviolet light for 62.5 sec at a dose of 0.8 ergs/mm^2/sec, and incubated for 24 hr at 30°. Diploids homozygous for *rad7* could be readily distinguished from diploids heterozygous for *rad7* by their decreased ability to grow on YEPD medium after irradiation.

Appropriate strains containing the *HO* gene, which confers homothallism, were constructed by spore to cell matings as described in HICKS and HERSKOWITZ (1976). The *HO* phenotype was scored in tetrads by assaying for both mating ability and sporulation. Spore clones containing *HO* were identified by their ability to sporulate and their ability to mate weakly with both *Mata* and *MATα* mating-type tester strains.

Genetic mapping: Standard linkage values were derived from tetrad data by using the equation X (in centiMorgans, cM) = 50 [tetratype asci + 6(nonparental ditype asci)]/total asci (PERKINS 1949). Gene order in multi-point crosses was generally determined by analyzing recombinant asci containing cross-overs in the regions of interest. In some crosses the suppressors were tested for centromere linkage by using the equation:

$$\text{tetratype asci/total asci} = X + Y - 3/2\,(XY),$$

where X is the second division segregation frequency of the centromere-linked markers *trp1* (0.45) or *leu1* (3.9) and Y is the second division segregation frequency of the suppressor (PERKINS 1949).

RESULTS

General mapping strategy

We have developed a mapping strategy involving the construction of a set of "mapping strains" containing the suppressible frameshift mutation *leu2-3* and 61 signal markers distributed throughout the yeast genome (Table 1, Figure 1). Using these strains, it has been possible to efficiently locate frameshift suppressor genes by conventional recombination analysis in tetrads derived from diploids of normal chromosome constitution.

The signal markers incorporated into the mapping strains were chosen on the basis of their known map positions in order to maximize the probability that a

TABLE 1

Mapping strains

Strain	Genotype	Other auxotrophic phenotypes[a]
A141-1D	*leu2-3 met1 ade6 cdc11 pet17 lys11 his1 MAT***a**	
A141-37C	*leu2-3 met1 ade6 cdc11 pet17 lys11 his1 MATα*	
A298-65C	*leu2-3 pet2 arg4 ade8 aro1C trp4 rna3 MAT***a**	Met$^-$ Thr$^-$
A298-61D	*leu2-3 pet2 arg4 ade8 aro1C trp4 rna3 MATα*	Ura$^-$ Thr$^-$
A343-1A	*leu2-3 pet14 arg1 lys7 ura1 ade3 met6 MAT***a**	
A343-6A	*leu2-3 pet14 arg1 lys7 ura1 ade3 met6 MATα*	
A236-57B	*leu2-3 trp1 met4 aro7 his3 lys11 SUC2 MAL3 can1 MAT***a**	
A236-24C	*leu2-3 trp1 met4 aro7 his3 lys11 SUC2 MAL3 can1 MATα*	
A331-4D	*leu2-3 trp2 his6 ura1 ino1 MAL1 tsl1 MAT***a**	
A331-4B	*leu2-3 trp2 his6 ura1 ino1 MAL1 tsl1 MATα*	
A250-19B	*leu2-3 asp5 ilv3 MAL4 SUC MAT***a**	
A256-99A	*leu2-3 trp5 lys9 met10 ade1 pet9 MAT***a**	
A333-1B	*leu2-3 trp5 his5 lys9 met10 ade1 thr4 cdc9 MATα*	
A193-16C	*leu2-3 met13 ade2 cdc4 pet3 ura4 his4-15 lys2 MAT***a**	
A193-23A	*leu2-3 met13 ade2 cdc4 pet3 ura4 his4-15 lys2 MATα*	
A121-3A	*leu2-3 met14 ade5 pet8 ura3 his7 lys1 MAT***a**	
A121-3D	*leu2-3 met14 ade5 pet8 ura3 his7 lys1 matα*	
A334-49B	*leu2-3 pha2 petX prt1 arg8 MAT***a**	His$^-$ Ura$^-$
A334-27B	*leu2-3 pha2 petX prt1 arg8 MATα*	

[a] The genotypic identities of mutations conferring auxotrophic phenotypes in some of the strains are unknown.

given suppressor mutation would exhibit linkage to at least one of these markers (Figure 1). Assuming that linkage can be detected across a minimal 100-cM interval flanking a signal marker, it is possible with these mapping strains to monitor approximately 90% of the known genetic intervals in the yeast genome using tetrad analysis to establish linkage. The mapping strains listed in Table 1 were constructed so none of the strains contain multiple markers conferring the same phenotype, thus obviating the need for complementation testing in tetrads to determine the segregation of markers. In addition, most of the signal markers were tested by one criterion or more to verify their map positions and correct identities. These criteria included demonstration of linkage to a neighboring marker, centromere linkage, or complementation tests (see Table 2).

In general, the procedure for establishing the initial map location of a suppressor was to cross a *leu2-3 SUFX* strain by a set of mapping strains and to analyze tetrads from these crosses. Since the mapping strains all contain *leu2-3*, the diploids resulting from the crosses are all homozygous for *leu2-3*, a frameshift mutation that is suppressed by mutations in 19 of the 25 known suppressor genes (CUMMINS *et al.* 1980; GABER and CULBERTSON 1982b; CULBERTSON, GABER and CUMMINS 1982). Thus, in most cases a suppressor phenotype can be unambiguously scored in tetrads as a 2+:2− segregation on medium

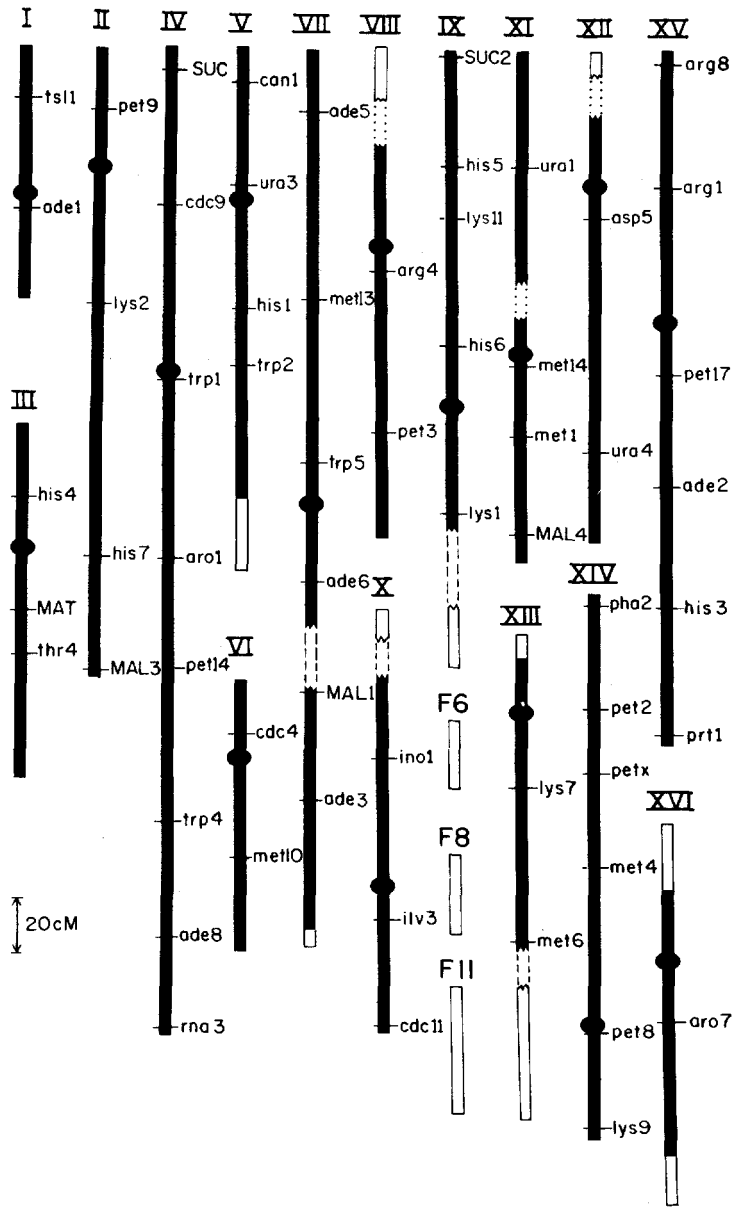

FIGURE 1.—The figure shows a schematic representation of the yeast genetic map showing the locations of genetic markers incorporated into the mapping strains. The map is based on data presented by MORTIMER and SCHILD (1980) as modified by KLAPHOLTZ and ESPOSITO (1982). Dark circles represent the locations of the centromeres of each chromosome. Dark bars indicate the genetic intervals of each chromosome in which genetic linkage could be detected relative to at least one signal marker. Dark bars cover a genetic distance of 100 cM surrounding each signal marker. The open bars represent regions of the genome not covered by any signal marker. Dashed and dotted lines represent linkages established by mitotic and aneuploid analyses, respectively. The signal markers cover approximately 90% of the known genome.

TABLE 2

Verification of genetic markers in mapping strains

Strain	Marker	Chromosome location	Method of verification[a]	Strain	Marker	Chromosome location	Method of verification[a]
A141-1D and A141-37C	*met1*	XI-R	1	A256-99A	*trp5*	VII-L	2
	ade6	VII-R	1		*lys9*	XIV-R	1,3
	cdc11	X-R	1		*met10*	VI-R	1
	pet17	XV-R	1		*ade1*	I-R	3
	lys11	IX-L	3		*pet9*	II-L	1,2
	his1	V-R	1				
				A333-1B	*trp5*	VII-L	2
A298-65C and A298-61D	*pet2*	XIV-L	1		*his5*	IX-L	1,3
	arg4	VIII-R	2		*lys9*	XIV-R	1,3
	ade8	IV-R	1		*met10*	VI-R	1
	aro1C	IV-R	1		*ade1*	I-R	3
	trp4	IV-R	1		*thr4*	III-R	1,3
	rna3	IV-R	1		*cdc9*	IV-L	1
A343-1A and A343-6A	*pet14*	IV-R	1	A193-16C and A193-23A	*met13*	VII-L	1
	arg1	XV-L	1,3		*ade2*	XV-R	1,3
	lys7	XIII-R	3		*cdc4*	VI-L	1
	ura1	XI-L	3		*pet3*	VIII-R	1
	ade3	VII-R	1		*ura4*	XII-R	3
	met6	XIII-R	4		*his4-15*	III-L	1
					lys2	II-R	1
A236-57B and A236-24C	*trp1*	IV-R	2				
	met4	XIV-L	1	A121-3A and A121-3D	*met14*	XI-R	2
	aro7	XVI-R	1		*ade5*	VII-L	1
	his3	XV-R	1,3		*pet8*	XIV-R	2
	lys11	IX-L	3		*ura3*	V-L	2
	SUC2	IX-L	4		*his7*	II-R	1
	MAL3	II-R	1		*lys1*	IX-R	3
	can1	V-L	1				
				A334-49B and A334-27B	*pha2*	XIV-L	1
A331-4D and A331-4B	*trp2*	V-R	1		*petX*	XIV-L	1
	his6	IX-L	2,3		*prt1*	XV-R	1
	ura1	XI-L	3		*arg8*	XV-L	1
	ino1	X-L	3				
	MAL1	VII-R	1		*MAT*	III-R	3
	tsl1	I-L	4				
A250-19B	*asp5*	XII-R	2				
	ilv3	X-R	2				
	MAL4	XI-R	1				
	SUC	IV-R	4				

[a] Verification was accomplished by: 1, demonstration of linkage to a neighboring marker; 2, demonstration of centromere-linkage; 3, complementation tests; 4, marker assumed to be correct, but no verification test was performed.

lacking leucine and recombination relative to the signal markers can be determined. The remaining suppressors, which fail to suppress *leu2-3*, have been mapped by using other strategies (CUMMINS *et al.* 1980; this study).

In the initial screening procedure, a *leu2-3 SUFX* strain was crossed with a

set of eleven mapping strains and a sample of 24 tetrads was analyzed from each diploid (a total of 264 tetrads for each suppressor). When the suppressor was scored relative to the signal markers, deviations from the 1PD:1NPD:4T ratio expected of unlinked genes were examined further by analysis of additional tetrads. Finally, the location of each suppressor was determined in multipoint crosses to establish the order of genes in the region of interest. For most cases in the sections that follow, only the results of the final multi-point crosses are presented.

Genetic mapping of frameshift suppressors

Detailed mapping data for *SUF1*, *SUF3*, *SUF4*, *SUF6*, *suf11*, *SUF23* and *SUF25* are presented below and summarized in Table 3. Mapping data for 18 other frameshift suppressors have been presented in previous communications (CULBERTSON *et al.* 1977; CULBERTSON, UNDERBRINK and FINK 1980; CUMMINS *et al.* 1980; GABER and CULBERTSON 1982b; CULBERTSON, GABER and CUMMINS 1982). The map locations for all 25 frameshift suppressors are summarized in Table 4 and Figure 2.

Mapping of SUF1: This suppressor was originally located on the left arm of chromosome XV by observing linkage of *SUF1* to *arg8* in a cross between a *leu2-3 SUF1* strain and the mapping strain A334-49B (Table 1). Subsequently, tetrads were analyzed from a three-point cross heterozygous for *SUF1*, *arg8* and *arg1* to determine the order of genes in this region (see cross number RG2852 in Table 3). The tetrad data indicate a map distance of 23.5 cM for the *SUF1-arg8* marker pair. Although the calculated map distance for *SUF1-arg1* marker pair is greater than 50 cM, a significant deviation from a 1:1 ratio (43:19, $\chi^2 = 9.74$) for parental and nonparental ditype asci indicates that these two markers are linked.

The distribution of the three markers in this region did not permit a determination of gene order by standard analysis of recombinant asci because of the high frequency of recombination and hence the high probability of numerous multiple cross-overs in each marked interval. However, the genetic distances between markers were suggestive of the correct order. Since *SUF1* exhibits closer linkage to *arg8* and *arg1* than do *arg8* and *arg1* to each other, *SUF1* is probably located between *arg8* and *arg1*. The most probable gene order for this chromosome region is therefore *arg8-SUF1-arg1* (Figure 2).

Mapping of SUF3: This suppressor was originally located on the right arm of chromosome *IV* by observing linkage of *SUF3* to *trp4* and *ade8* in a cross between a *leu2-3 SUF3* strain and the mapping strain A298-65C (Table 1). Subsequently, tetrads were analyzed from a four-point cross heterozygous for *SUF3*, *trp4*, *ade8* and *rna3* to determine the order of genes in this region (see cross number LM370 in Table 3). Among 53 tetrads that were tetratype for the *SUF3-trp4* marker pair, 9 were disregarded because of gene conversion of the *ade8* marker and 5 were disregarded because of multiple crossing-over. Of the remaining 39 tetrads, 36 were parental ditype for the *SUF3-ade8* marker pair and tetratype for the *trp4-ade8* marker pair. Three had the reverse configuration, being parental ditype for the *trp4-ade8* marker pair and tetratype for the *SUF3-ade8* marker pair. Since these latter tetrads comprise a minority class, they are

TABLE 3

Genetic mapping of suppressors

Suppressor	Cross no.[a]	Gene pair	PD[b]	NPD[b]	T[b]	Total	Gene pair distance (cM)	FDS:SDS[b]	%SDS[b]
SUF1	RG2852	*SUF1* × *arg1*	43	19	150	212	62.3		
		SUF1 × *arg8*	113	1	90	204	23.5		
		arg1 × *arg8*	32	26	157	215	72.8		
SUF3	LM370	*SUF3* × *trp4*	124	0	53	177	15.0		
		SUF3 × *ade8*	87	0	69	156	22.1		
		SUF3 × *rna3*	25	11	127	163	59.2		
		trp4 × *ade8*	59	4	121	184	39.4		
		trp4 × *rna3*	27	20	130	177	70.6		
		ade8 × *rna3*	61	7	91	159	41.8		
SUF4	LM720	*SUF4* × *ser2*	57	21	161	239	60.0		
		SUF4 × *ade3*	69	17	172	258	53.1		
		ser2 × *ade3*	230	0	32	262	6.1		
SUF6	IE593	*SUF6* × *met2*	185	0	15	200	3.8		
		SUF6 × *pha2*	88	9	98	195	39.0		
		SUF6 × *pet2*	147	1	52	200	14.5		
		met2 × *pha2*	80	11	104	195	43.6		
		met2 × *pet2*	162	1	37	200	10.8		
		pet2 × *pha2*	63	17	110	200	53.0		
suf11	LM203	*suf11* × *ade2*	138	5	201	344	33.6		
		suf11 × *pet17*	278	0	66	344	9.6		
		suf11 × *cdc21*	342	0	2	344	0.3		
		pet17 × *cdc21*	277	0	66	343	9.6		
		suf11 × *trp1*	87	63	192	342		150:192	56.1
		suf11 × *leu1*	77	69	194	340		146:194	57.1
		trp1 × *leu1*	138	169	33	340		307: 33	9.7
		pet17 × *ade2*	100	7	236	343	40.5		
		cdc21 × *ade2*	139	4	201	344	32.7		
		pet17 × *leu1*	84	78	153	315		162:153	48.6
		pet17 × *trp1*	93	80	138	311		173:138	44.4
		cdc21 × *trp1*	87	61	203	351		148:203	57.8
		cdc21 × *leu1*	78	66	204	348		144:204	58.6
SUF23	RG3381	*ilv3* × *cyc1*	166	1	179	346	26.7		
		cyc1 × *SUF23*	336	0	16	352	2.3		
		SUF23 × *cdc11*	253	1	110	364	15.9		
		ilv3 × *cdc11*	110	8	246	364	40.4		
	RG3628	*SUF23* × *rad7*	266	0	0	266	0.0		
		SUF23 × *cyc1*	253	0	6	259	1.2		
SUF25	RG3582	*SUF25* × *HO*	176	0	66	242	13.6		
		SUF25 × *cdc9*	100	4	136	240	33.3		
		cdc9 × *HO*	80	14	148	242	47.9		

[a] Genotypes of the crosses listed in the table are as follows: RG2852: *leu2-3 SUF1 arg8 MAT***a** ×

leu2-3 arg1 lys7 ura1 met6 ade3 MATα; LM370: *leu2-3 trp4 ade8 rna3 MATa* × *leu2-3 ade3 SUF4 MATα*; LM720: *leu2-3 ser2 MATa* × *leu2-3 ade3 SUF4 MATα*; IE593: *leu2-3 can1-101 pet2 met2 pha2 MATα* × *leu2-3 his4-519 can1-101 SUF6 MATa*; LM203: *his4-713 pet17 trp1 leu1 suf11 MATa* × *his4-713 ade2 cdc21 MATα*; RG3381: *leu2-3 SUF23 ilv3 cdc11 MATa* × *leu2-3 cyc1-13 MATα*; RG3628: *leu2-3 his4-519 SUF23 ura3 MATa* × *leu2-3 his4-519 cycl-13 rad7-1 MATα*; RG3582: *leu2-3 SUF25 cdc9 MATa* × *leu2-3 HO ade6 MATα*.

PD = parental ditype; NPD = nonparental ditype; T = tetratype; SDS = second division segregation frequency; FDS = first division segregation frequency.

TABLE 4

Map locations for frameshift suppressors

Suppressor	Group specificity[a]	Chromosome location	Flanking markers	Reference[b]
SUF1	II	XV-L	*arg8*	1
SUF2	III	III-R	*cdc10, pgk1*	2
SUF3	II	IV-R	*trp4, ade8*	1
SUF4	II	VII-R	*ade3*	1
SUF5	II	XV-R	*ade2, ade9*	3
SUF6	II	XIV-L	*met2, kar1*	1
SUF7	III	XIII-L	*SUF22, cdc5*	4
SUF8	III	VIII-R	*cdc12, pet3*	4
SUF9	III	VI-L	*cdc4*	4
SUF10	III	XIV-L	*pet8*	4
suf11	III	XV-R	*pet17, cdc21*	1
suf12	Nonspecific	IV-R	*hom2, arg82*	5
suf13	Nonspecific	XV-R	*tra3, prt1*	5
suf14	Nonspecific	XIV-L	*petX, met4*	5
SUF15	II	VII-R	*ser2, rad2*	6
SUF16	II	III-R	*pgk1, pet18*	6
SUF17	II	XV-L	*arg1, SUP3*	6
SUF18	II	VI-R	*SUP11, cly3*	6
SUF19	II	V-L	*mak10, min1*	6
SUF20	II	VI-R	*CEN6, SUP11*	6
SUF21	II	XVI-R	*chl1, CEN16*	6
SUF22	II	XIII-R	*SUF7*	6
SUF23	II	X-R	*cyc1, rad7*	6
SUF24	II	IV-R	*lys4*	6
SUF25	II	IV-L	*cdc9, HO*	6

[a] Consult the discussion for a description of group specificity.

[b] References to published mapping data are as follows: 1, this study; 2, CULBERTSON *et al.* (1977); 3, CULBERTSON, UNDERBRINK and FINK (1980); 4, CUMMINS *et al.* (1980); 5, CULBERTSON, GABER and CUMMINS (1982); 6, GABER and CULBERTSON 1982b.

probably the result of multiple crossing-over. These results suggest that *SUF3* is located between *trp4* and *ade8*. In addition, the data are in agreement with the previously established *trp4-ade8-rna3* gene order (MORTIMER and SCHILD 1980). The most likely order of genes for this region is *trp4-SUF3-ade8-rna3* (Figure 2).

Mapping of SUF4: In the original mapping crosses, this suppressor was tentatively assigned to the right arm of chromosome *VII* on the basis of a deficiency of nonparental ditype asci for the *SUF4-ade3* marker pair in a cross between a *leu2-3 SUF4* strain and the mapping strain A343-1A (Table 1).

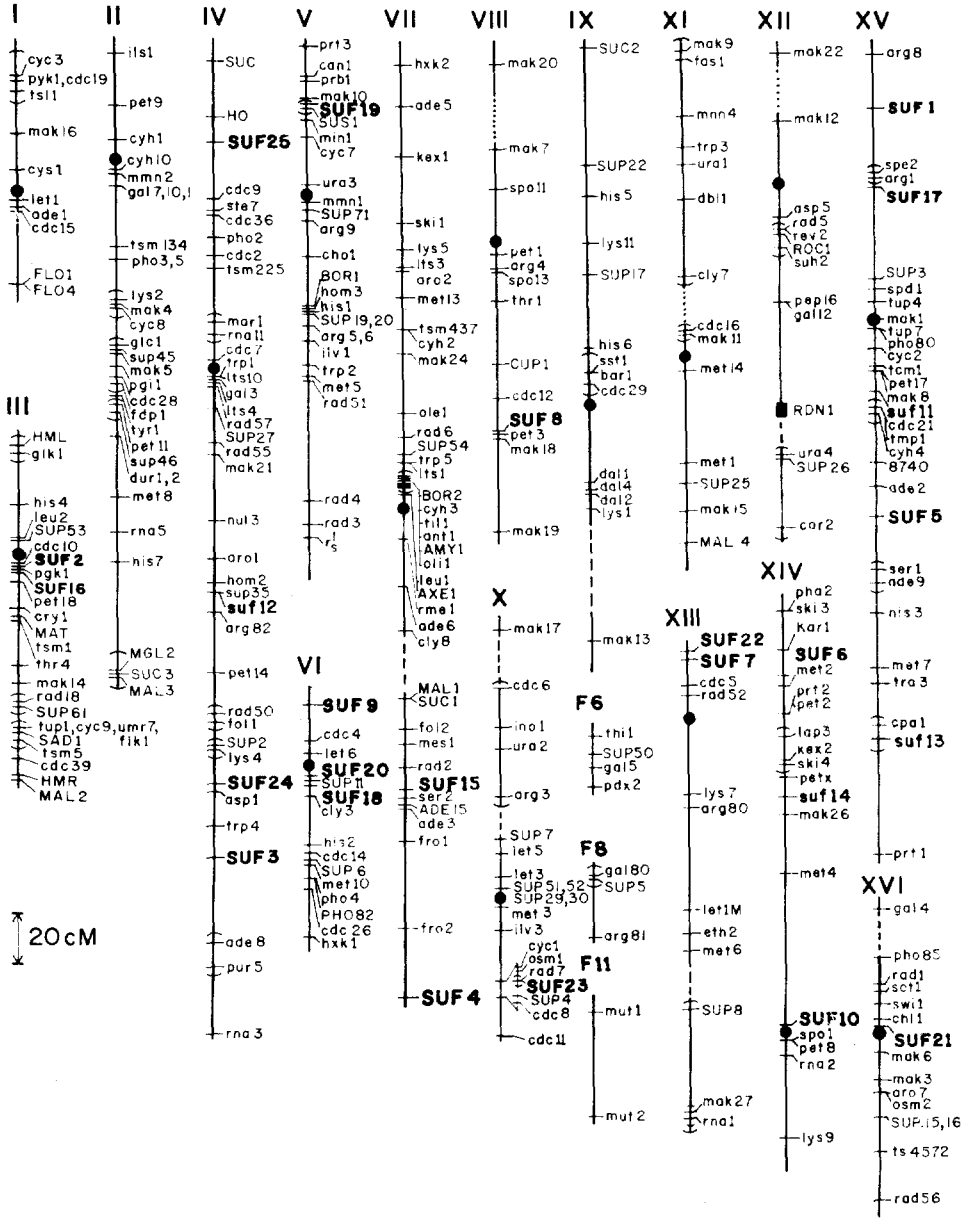

FIGURE 2.—Genetic map of the yeast genome showing the locations of 25 frameshift suppressor genes. The map is based on data presented by MORTIMER and SCHILD (1980) as modified by KLAPHOLTZ and ESPOSITO (1982). Dashed and dotted lines represent linkages established by mitotic and aneuploid analyses, respectively. When the orientation of two or more genes relative to outside markers is in question, these genes have been enclosed within parentheses.

Subsequently, a three-point cross heterozygous for *SUF4*, *ade3* and *ser2* was analyzed to confirm linkage of *ade3* and *SUF4* and to determine the order of genes in this region (see cross number LM720 in Table 3).

Data from this cross indicate that the genetic distance between *ade3* and

SUF4 calculated by the formula of PERKINS (1949) is 53 cM. A χ^2 value of 31.44 shows that the deviation from the 1PD:1NPD:4T ratio expected for unlinked genes is statistically significant. This result indicates the likelihood of linkage between *ade3* and *SUF4*.

Among 32 tetrads from this cross that were tetratype for the *ser2-ade3* marker pair, 18 were disregarded because of multiple crossing-over. Of the remaining 14 tetrads, 12 were parental ditype for the *SUF4-ade3* marker pair and tetratype for the *SUF4-ser2* marker pair. Two had the reverse configuration, being parental ditype for the *SUF4-ser2* marker pair and tetratype for the *SUF4-ade3* marker pair. Since these latter tetrads comprise a minority class, they are probably the result of multiple crossing-over. These results suggest that the gene order is *ser2-ade3-SUF4*.

The gene order for *SUF4* and two other markers shown to be located in this region, *fro1* and *fro2*, has not been determined. For the previously established gene sequence *ade3-fro1-fro2*, the map distances between *ade3* and *fro1* and between *fro1* and *fro2* are 15.3 cM and 22.0 cM, respectively (THORNTON 1978). Since the map distance between *ade3* and *SUF4* is 53 cM, the most probable gene order is *ade3-fro1-fro2-SUF4*. The most likely gene order for the entire region is *ser2-ADE15-ade3-fro1-fro2-SUF4*.

Mapping of SUF6: The chromosomal location of this suppressor was originally determined before the construction of the mapping strains listed in Table 1. When a *leu2-3 SUF6* strain was crossed with a *leu2-3 suf*$^+$ *met2* strain, *SUF6* was found to be closely linked to *met2*. However, the analysis of this cross was complicated by the fact that the *met2* mutation, designated *met2-2*, is suppressed by *SUF6*. As a result of suppression, recombination between the suppressor and the *met2* locus was signaled by the appearance of 3Met$^+$:1Met$^-$ asci. To distinguish between gene conversion of *met2-2* and reciprocal recombination between *SUF6* and *met2*, spore clones were progeny-tested to determine which spores were phenotypically Met$^+$ as the result of suppression. The results of this preliminary mapping study indicated that the 3Met$^+$:1Met$^-$ asci were the consequence of reciprocal recombination. *SUF6* was shown to reside approximately 4 cM from the *met2* locus.

The position of *SUF6* relative to neighboring markers on the left arm of chromosome XIV was determined by analyzing tetrads from a four-point cross heterozygous for *SUF6*, *met2-2*, *pet2* and *pha2* (see cross number IE593 in Table 3). In order to score the segregation of *SUF6* and the suppressible *met2-2* mutation, the cross was also homozygous for two other frameshift suppressible mutations, *leu2-3* and *can1-101*. The latter mutation confers resistance to canavanine, but is suppressed to a sensitive phenotype in the presence of *SUF6* (CULBERTSON, UNDERBRINK and FINK 1980). Since recombination between *SUF6* and *met2-2* results in a 3Met$^+$:1Met$^-$ segregation, the *can1-101* mutation was employed to determine whether these asci might be the result of gene conversion of *met2-2* rather than reciprocal recombination between *met2* and *SUF6*. LEU$^+$ Can^s Met$^+$ spore clones from each 3Met$^+$:1Met$^-$ ascus were plated on CAN medium. Several canavanine-resistant revertants derived from each spore clone were picked and in all cases revertants from one spore clone per tetrad were found to be Leu$^-$ Met$^-$ in phenotype. Since this scheme for selecting loss of

suppressor function resulted in a Met^- phenotype, the original spores must have contained *met2-2*. All of the $3Met^+:1Met^-$ asci were therefore the result of reciprocal recombination rather than gene conversion.

Among 15 tetrads that were tetratype for the *SUF6-met2* marker pair, all 15 were parental ditype for the *pet2-met2* marker pair and tetratype for the *SUF6-pet2* marker pair. This result suggests that the gene order is *SUF6-met2-pet2*. The data from this cross were also consistent with *pha2* being on the same side of *met2* as *SUF6*, but at a more distal position (43.6 cM). Since *kar1* is also located on the same side as *SUF6* approximately 25 cM from *met2* (DUTCHER, as reported in MORTIMER and *Schild* 1980) and since *SUF6* is less than 4 cM from met2, the most likely gene order is pha2-kar1-SUF6-met2-pet2.

Recent evidence indicates that markers formerly assigned to chromosome *XVII* are actually located on the left arm of chromosome *XIV* (KLAPHOLTZ and ESPOSITO 1982; CULBERTSON, GABER and CUMMINS 1982). Thus, *SUF6* is now known to be located on this chromosome arm. The genetic map shown in Figure 2 shows the new configuration of markers. By combining the above results with previously published data (KLAPHOLTZ and ESPOSITO 1982; MORTIMER and SCHILD 1980) the most likely gene order for the entire chromosome region is *pha2-ski3-kar1-SUF6-met2-pet2* (Figure 2).

Mapping of suf11: It was not possible to map this suppressor by using the mapping strains listed in Table 1 because *suf11* fails to suppress *leu2-3* (CUMMINS *et al.* (1980). *suf11* was located fortuitously on the right arm of chromosome XV by observing linkage of the suppressor to *pet17* in a cross between a *his4-713 suf11* strain and a *his4-713 pet17* strain, in which the *his4-713* mutation is suppressed by *suf11*. Subsequently, tetrads were analyzed from a four-point cross heterozygous for *suf11*, *pet17*, *cdc21* and *ade2* to determine the order of genes in this region (see cross number LM203 in Table 3). Since *pet17* exhibits linkage to the chromosome XV centromere, two centromere-linked markers were also included in heterozygous condition in the cross to determine second division segregation frequencies. Among two tetrads that were tetratype for the *suf11-cdc21* marker pair, both were parental ditype for the *suf11-pet17* marker pair and tetratype for the *pet17-cdc21* marker pair. In addition, analysis of marker configurations relative to *trp1* and *leu1* indicated that in both tetrads *pet17* and *suf11* failed to recombine with the centromere whereas *cdc21* and *ade2*, the more distal markers, both recombined with the centromere. These results suggest that *suf11* is located between pet17 and cdc21. suf11 is loosely centromere linked, giving a second division segregation frequency of approximately 57% (28.5 cM). Another marker in this region, *mak8*, is tightly linked to *pet17*. These markers failed to recombine in a sample of 61 tetrads analyzed by WICKNER and LEIBOWITZ (1976). By combining these data with the previously established gene order (see MORTIMER and SCHILD 1980), the most likely order of genes for the entire region is (*pet17-mak8*)-*suf11-cdc21* (Figure 2).

Mapping of SUF23: In a previous study (GABER and CULBERTSON 1982b) this suppressor was located on the right arm of chromosome X by observing linkage with *ilv3* in a cross between a *leu2-3 SUF23* strain and the mapping strain A250-19B (Table 1). Furthermore, through the analysis of two three-point crosses the

suppressor was shown to map between *ilv3* and *SUP4*. The position of *SUF23* relative to other markers in the *ilv3-SUP4* region, including *cyc1*, *osm1* and *rad7*, was unknown.

In this study we report the results of two additional multi-point crosses that further define the location of *SUF23*. The first cross was heterozygous for *SUF23*, *cyc1*, *ilv3* and *cdc11* and the second cross was heterozygous for *SUF23*, *cyc1* and *rad7* (see cross numbers RG3381 and RG3628 in Table 3).

Among 16 tetrads derived from cross RG3381 that were tetratype for the *SUF23-cyc1* marker pair, 5 contained multiple cross-overs and were therefore not used to establish gene order. The remaining 11 tetrads were parental ditype for the *ilv3-cyc1* and *SUF23-cdc11* marker pairs and tetratype for the *ilv3-SUF23*, *cyc1-cdc11* and *ilv3-cdc11* marker pairs. These results suggest that *SUF23* is located between *cyc1* and *cdc11*. By combining these data with previous results that established the position of *SUF23* relative to *SUP4* (GABER and CULBERTSON 1982b), *SUF23* must be located between *cyc1* and *SUP4*.

Since this region contains *osm1* and *rad7*, a second three-point cross was analyzed (cross number RG3628 in Table 3). Among 266 tetrads derived from this cross, no recombinants were detected for the *SUF23-rad7* marker pair. Although the failure to detect recombination precludes an unambiguous determination of gene order, this result establishes the likelihood of a maximum genetic distance of 0.19 cM ($1/(266 + 1) \times 50$) between *SUF23* and *rad7*.

Before this study, the gene order *cyc1-osm1-rad7-SUP4* was unambiguously determined by tetrad analysis, deletion mapping, and by correlating deletion endpoints relative to appropriate restriction sites located on cloned DNA fragments from the *cyc1* region (LAWRENCE *et al.* 1975; SINGH and SHERMAN 1978; STILES, CARDILLO and SHERMAN, as reported in MORTIMER and SCHILD, 1980). Although meiotic tetrad data are not available for the *osm1-rad7* marker pair, the observation of recombination in deletion mapping studies of the *cyc1-osm1-rad7* region compared with the absence of detectable recombination between *SUF23* and *rad7* in a large sample of tetrads suggests the likelihood that *SUF23* rather than *osm1* resides at a position which is closer to *rad7*. Upon considering all of these data, the most likely gene order for the entire region is *ilv3-cyc1-osm1-(rad7-SUF23)-SUP4-cdc11* (Figure 2).

Mapping of SUF25: In a previous study this suppressor was located on the left arm of chromosome *IV* by observing linkage with *cdc9* (GABER and CULBERTSON 1982b). In the previous study the analysis of a three-point cross heterozygous for *SUF25*, *cdc9* and *trp1* gave ambiguous results that precluded a determination of gene order. This problem has been resolved by analyzing another three-point cross heterozygous for *SUF25*, *cdc9* and *HO* (see cross number RG3582 in Table 3). Among 66 tetrads that were tetratype for the *SUF25-HO* marker pair, 27 were disregarded because of multiple crossing-over or gene conversion of *cdc9*. Of the remaining 39 tetrads, 30 were parental ditype for the *SUF25-cdc9* marker pair and tetratype for the *cdc9-HO* marker pair. Nine tetrads had the reverse configuration, being parental ditype for the *cdc9-HO* marker pair and tetratype for the *SUF25-cdc9* marker pair. This latter ascal type is probably the result of multiple crossing-over, since it is a minority class.

These results suggest that the order of genes in this region is *cdc9-SUF25-HO* (Figure 2).

DISCUSSION

Genetic mapping of frameshift suppressor genes

This communication describes the completion of genetic mapping studies on 25 frameshift suppressor genes in *Saccharomyces cerevisiae*. All of the suppressors have been assigned to their respective chromosomes and, where possible, the gene order relative to neighboring genetic markers has been determined.

The most difficult task in mapping frameshift suppressor genes was in determining the initial chromosome assignments. Although tetrad analysis greatly facilitates the ease with which the order of genes can be determined on the chromosomes in this organism, the ability to rapidly assign large numbers of genes to their respective chromosomes has traditionally proved more difficult. This difficulty can be attributed primarily to the large number of independently segregating, cytologically invisible chromosomes (16) in yeast and the high rate of meiotic recombination (total genome length of >3000 cM) (MORTIMER and SCHILD 1980; KLAPHOLTZ and ESPOSITO 1982).

Several different chromosome localization methods were employed in earlier frameshift suppressor mapping studies. Although theoretically sound, their efficiency and overall usefulness was disappointing. For example, although *SUF7* and *SUF8* were correctly assigned to their respective chromosomes by using a triploid mapping procedure, (CUMMINS *et al.* 1980) attempts to map other dominant frameshift suppressors by using triploids failed to provide an unambiguous chromosome assignment. In addition, an attempt was made to determine the chromosome location of *SUF3* (L. MATHISON, unpublished data) using the mapping method of heat-shock-induced chromosome loss in diploid strains homozygous for *cdc6* or *cdc14* (KAWASAKI 1979). It was found that all of the chromosomes could be eliminated as a candidate for the location of the suppressor. Nonetheless, tetrad data presented in this communication show that *SUF3* is in fact located on chromosome *IV*.

Since the expression of certain frameshift suppressor genes may be sensitive to chromosome dosage effects, any mapping method resulting in aneuploidy could be subject to error. For example, it is possible that certain suppressors are expressed as recessive rather than dominant mutations in aneuploids. This could result from the altered dosage of various genes affecting the balance of components required for protein synthesis. Thus, the apparent loss of a suppressor could result from loss of the relevant chromosome or to loss of dominance caused by aneuploidy.

In order to map mutations requiring specific genetic backgrounds for their expression, it may be necessary to use a protocol that does not perturb the normal chromosome constitution of the cell. In this communication we have developed a mapping method that satisfies this requirement. A set of "mapping strains" was constructed that contains a total of 61 signal markers distributed

throughout most of the yeast genome. Using these strains it has been possible to efficiently locate frameshift suppressor genes by conventional recombination analysis in tetrads derived from diploids of normal chromosome constitution.

By assuming that linkage could be detected within at least a 100-cM interval flanking each signal marker and by selecting markers that insure a broad distribution across the genome, the chance of successfully mapping a mutation by using these mapping strains is approximately 90%.

Distribution of frameshift suppressor genes

Table 4 summarizes the distribution of frameshift suppressors in the yeast genome. These suppressors have been classified according to genetic and biochemical criteria into three major groups.

Group II-specific suppressors: Mutant alleles mapping at 16 of the suppressor loci specifically suppress group II frameshift mutations that are known to contain +1 G:C base pair insertions in glycine codons (GABER and CULBERTSON 1982b; DONAHUE, FARABAUGH and FINK 1981). Four out of five of the original suppressors (*SUF1, SUF4, SUF5* and *SUF6*) have been shown to affect either the chromatographic behavior or isoacceptor activity of glycine tRNA (CULBERTSON *et al.* 1977). In addition, an allele of another suppressor gene, *SUF16-1*, has been shown by DNA sequence analysis to encode a glycine tRNA containing a nucleotide insertion in the anticodon (GABER and CULBERTSON 1982a). This suppressor tRNA can read a mutant four-base glycine codon and thereby restore the normal reading frame to the message. These results strongly suggest that the Group II-specific suppressors represent a dispersed family of glycine tRNA genes.

Two observations make it unlikely that these genes encode a single glycine tRNA isoacceptor. It has been shown by Sepharose-4B chromatography of glycine tRNAs that different suppressor mutations, each affecting a single peak of glycine tRNA isoacceptor activity, alter the elution profiles of two of the three detectable peaks of isoacceptor activity (CULBERTSON *et al.* 1977). In addition, these suppressors have been classified genetically into two subclasses on the basis of differential recognition of different four-base glycine codon sequences, suggesting that the wild-type tRNA anticodons from which these suppressors were mutationally derived differ from each other in the wobble position (GABER and CULBERTSON 1982b). These results suggest the existence of multiple glycine tRNA isoacceptors that can be mutationally altered to act as frameshift suppressors.

Group III-specific suppressors: Mutant alleles mapping at six of the suppressor loci specifically suppress group III frameshift mutations that are known to contain +1 G:C base pair insertions in proline codons (CUMMINS *et al.* 1980; DONAHUE, FARABAUGH and FINK 1981). These suppressors have been classified genetically into two subclasses on the basis of differential suppression of the frameshift mutations *his4-712* and *his4-713*, both of which contain 5′-CCCU-3′ four-base proline codons, but which differ by virtue of their positions in the *his4C*-coding sequence. Suppressor mutations in two genes, *SUF2* and *SUF10*, are capable of suppressing both *his4* mutations. Because the *SUF2-1* allele has

been shown by DNA sequence analysis to encode an altered proline tRNA containing a nucleotide insertion in the anticodon and because *SUF2* and *SUF10* suppressors are similar in phenotype, it appears likely that both genes may encode proline tRNAs.

The four suppressors comprising the second group III-specific subclass suppress *his4-713*, but fail to suppress *his4-712*. The inability of suppressors in this latter subclass to suppress *his4-712* might result from an inhibitory influence of sequences surrounding this mutation. Such "context" effects have been shown to influence the efficiency of nonsense suppression in *Salmonella* (BOSSI and ROTH 1980). This model does not exclude the possibility that the latter subclass of suppressors may encode proline tRNAs, but such tRNAs would most likely consist of proline isoacceptors other than that encoded by *SUF2*.

A second explanation assumes that suppressors of the latter subclass do not act at the same four-base sequence as that which is suppressed by *SUF2* and *SUF10*. They could encode tRNAs other than proline isoacceptors and act at a nearby codon or one directly adjacent to the *his4-713* proline codon. A similar mechanism of action has been proposed to explain the behavior of the *sufJ* suppressor of *Salmonella* (BOSSI and ROTH 1981). A test to distinguish between these models is currently in progress through an attempt to isolate a suppressor gene representative of the latter subclass.

Non-group-specific suppressors: Suppressor mutations mapping at three unlinked genes, *suf12*, *suf13* and *suf14*, result in the expression of novel phenotypes (CULBERTSON, GABER and CUMMINS 1982). These suppressors are unlikely to be structural mutations in glycine or proline tRNA genes because they cross-suppress different types of frameshift or nonsense mutations.

Genetic mapping studies indicate that *suf12* may be located at the same position as the "omnipotent" suppressor *sup35* (HAWTHORNE and MORTIMER 1968; CULBERTSON, GABER and CUMMINS 1982). Alleles of *sup35* exhibit phenotypic variation and are known to cross-suppress certain nonsense mutations belonging to different classes (HAWTHORNE and LEUPOLD 1974). Since alleles of *suf12* also exhibit phenotypic variation and cross-suppress several types of frameshift and nonsense mutations, they bear a striking resemblance to *sup35* alleles. Although it has not been possible to perform rigorous allelism tests, the phenotypic similarity of these suppressors and their close proximity on the genetic map suggest the possibility that *sup35* and *suf12* may be synonymous.

The behavior of *suf12* and *sup35* alleles is atypical of mutations in genes that encode suppressor tRNAs. Because several lines of evidence implicate *sup35* as a gene that possibly encodes a ribisomal protein, mutations at this locus may resemble the ribosomal ambiguity mutations in bacteria that cause generalized misreading of the genetic code (GORINI 1974; LIEBMAN and CAVENAGH 1979; LIEBMAN, CAVENAGH and BENNETT 1980; LIEBMAN and CAVENAGH 1981). However, definitive identification of the *sup35* gene product is still lacking. In addition, *suf12* could represent an adjacent rather than a synonymous gene.

The molecular basis of suppression by *suf13* and *suf14* loci is unknown. These suppressors do not map in the vicinity of any previous described suppressor locus.

Gene copy number: Because two frameshift suppressor genes, *SUF2* and *SUF16*, have been shown to encode tRNAs, it is less surprising that a large number of different suppressors have been isolated. It has been shown, for example, that tyrosine-inserting nonsense suppressors mapping at eight unlinked loci encode a single isoacceptor of tyrosine tRNA (HAWTHORNE and MORTIMER 1968; OLSON *et al.* 1977). Thus, the large number of frameshift suppressor genes may reflect the fact that tRNA genes can be present in multiple copies in the genome.

The kinetics of frameshift suppressor induction described in previous studies suggest that additional mutant searches would probably uncover additional suppressor genes (CUMMINS *et al.* 1980; GABER and CULBERTSON 1982b). Furthermore, these genetic studies were not performed in a manner that would have distinguished between suppressor mutations in closely linked genes and linked mutations in a single gene. Although the two suppressor genes sequenced to date were not present as tandem repeats, (CUMMINS, DONAHUE and CULBERTSON 1982; GABER and CULBERTSON 1982a), it is not known at this time whether the remaining suppressor loci harbor single or repeated gene copies. For these reasons it is not known with certainty how many potential suppressor genes might exist for each class in the yeast genome.

Using cloned DNA fragments carrying known suppressor genes or in some cases using purified tRNAs as hybridization probes, it should be possible to determine the genomic copy number of various suppressor gene classes. With hybridization data and further DNA sequencing studies it should ultimately be possible to assess the degree of mutational saturation of the gene classes represented by frameshift suppressors *SUF1* through *SUF25*.

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