FRAMESHIFT SUPPRESSION IN *SACCHAROMYCES CEREVISIAE*. III. ISOLATION AND GENETIC PROPERTIES OF GROUP III SUPPRESSORS

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ABSTRACT

Suppressors of ICR-induced mutations that exhibit behavior similar to bacterial frameshift suppressors have been identified in the yeast *Saccharomyces cerevisiae*. The yeast suppressors have been divided into two groups. Previous evidence indicated that suppressors of one group (Group II: *SUF1*, *SUF3*, *SUF4*, *SUF5* and *SUF6*) represent mutations in the structural genes for glycyl-tRNA's. Suppressors of the other group (Group III: *SUF2* and *SUF7*) were less well characterized. Although they suppressed some ICR-revertible mutations, they failed to suppress Group II frameshift mutations. This communication provides a more thorough characterization of the Group III suppressors and describes the isolation and properties of four new suppressors in that group (*SUF8*, *SUF9*, *SUF10* and *suf11*).——In our original study, Group III suppressors were isolated as revertants of the Group III mutations *his4–712* and *his4–713*. All suppressors obtained as ICR-induced revertants of these mutations mapped at the *SUF2* locus near the centromere of chromosome III. Suppressors mapping at other loci were obtained in this study by analyzing spontaneous and UV-induced revertants of the Group III mutations. *SUF2* and *SUF10* suppress both Group III *his4* mutations, whereas *SUF7*, *SUF8*, *SUF9* and *suf11* suppress *his4–713*, but not *his4–712*. All of the suppressors except *suf11* are dominant in diploids homozygous for *his4-713*. The suppressors fail to suppress representative UAA, UAG and UGA nonsense mutations.—— *SUF9* is linked to the centromere of chromosome *VI*, and *SUF10* is linked to the centromere of chromosome *XIV*. A triploid mapping procedure was used to determine the chromosome locations of *SUF7* and *SUF8*. Subsequent standard crosses revealed linkage of *SUF7* to *cdc5* on chromosome *XIII* and linkage of *SUF8* to *cdc12* and *pet3* on chromosome *VIII*.

ACRIDINE half-mustards (ICR compounds) are highly mutagenic in both prokaryotes and eukaryotes. Mutations induced by these compounds have been described in T4 bacteriophage (STREISINGER *et al.* 1966), *Salmonella*

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typhimurium (AMES and WHITFIELD 1966), *Saccharomyces cerevisiae* (BRUSICK 1970; CULBERTSON *et al.* 1977), *Podospora anserina* (PICARD 1973), *Neurospora crassa* (MALLING 1967) and *Drosophila melanogaster* (CARLSON and OSTER 1962). Extensive biochemical and genetic studies on ICR-induced mutations in *S. typhimurium* show that a large proportion are +1 G/C insertions in G/C-rich regions (YOURNO and HEATH 1969; YOURNO 1971). Mutations of this type shift the reading frame of the message out of phase beyond the point of the insertion and result in the production of a nonfunctional protein.

ICR-induced revertants of +1 G/C insertions in the Salmonella histidine operon frequently carry mutations mapping at sites external to the operon that confer a His^+ phenotype (RIDDLE and ROTH 1970). These external suppressor mutations map in tRNA genes, and altered forms of tRNA have been shown to mediate suppression frameshift mutations (RIDDLE and ROTH 1972a,b). Direct confirmation of the role of tRNA in frameshift suppression was obtained by the demonstration that strains of Salmonella carrying the frameshift suppressor *sufD* produce a glycyl-tRNA with the nucleotide quadruplet CCCC at the anticodon position, instead of CCC normally found in wild type (RIDDLE and CARBON 1973). The addition of this extra base is presumed to permit recognition of the four-base codon GGGN and thereby correct the reading frame. A second class of frameshift suppressors was shown to alter the chromatographic behavior of prolyl-tRNA. These suppressors are also thought to act by recognition of a four-base codon. These results demonstrate that acridine half-mustards derive their powerful mutagenic activity in part from an ability to promote the insertion of G/C base pairs in DNA.

A detailed analysis of ICR-induced mutations at the *his4* locus in *Saccharomyces cerevisiae* showed that they have properties similar to those of bacterial frameshift mutations (CULBERTSON *et al.* 1977). Thirty-nine mutants were divided into five groups on the basis of reversion, complementation, suppression and biochemical tests. Eighteen mutations of Group II and two mutations of Group III have the properties expected of frameshift mutations: (1) polarity, (2) abolition of polarity by internal suppressors, (3) failure to be suppressed by nonsense suppressors or failure to corevert with nonsense mutations, (4) high-frequency reversion in the presence of ICR compounds, (5) low-frequency reversion in the presence of alkylating agents, and (6) suppression by dominant external suppressors. Mutations of Group II and III are distinguished genetically in that they are suppresed by different sets of external suppressors.

Transfer RNA's from a wild-type strain and strains carrying Group II suppressors (*SUF1*, *SUF3*, *SUF4*, *SUF5* and *SUF6*) were compared by column chromatography in order to determine whether tRNA was involved in suppression (CULBERTSON *et al.* 1977). Three glycyl-tRNA isoaccepting species, $tRNA^{GLY1}$, $tRNA^{GLY2}$ and $tRNA^{GLY3}$, were identified by their order of elution on a Sepharose-4B column. Evidence was obtained that *SUF5* may be the structural gene for $tRNA^{GLY1}$, whereas *SUF1*, *SUF4* and *SUF6* may code for $tRNA^{GLY3}$. On the basis of these experiments, we concluded that Group II mutations at the

his4 locus are frameshift mutations and the Group II external suppressors are frameshift suppressors.

In our original study, two suppressors of Group III mutations, *SUF2* and *SUF7*, were identified that failed to suppress Group II *his4* alleles. In this communication, we report the isolation and characterization of four new suppressors (*SUF8*, *SUF9*, *SUF10* and *suf11*) that show allele-specific suppression of Group III mutations. Five of the six suppressors have been mapped and define new chromosomal genes not identified in previous suppressor studies.

MATERIALS AND METHODS

Yeast strains and genetic methods: Some of the *Saccharomyces cerevisiae* strains used to isolate and characterize Group III suppressors are listed in Table 1. All strains were originally derived from S288Cα, referred to throughout as "wild type." Genetic methods and nomenclature are those described in the Cold Spring Harbor Yeast Course Manual (SHERMAN, FINK and LAWRENCE 1971).

TABLE 1

List of strains

Strain	Genotype*	Ploidy	Source
5588–8A	*his4–713 ade2* **a**	1n	G. FINK
5595–9D	*his4–713* α	1n	G. FINK
5569–1A	*his4–712 met8–1 leu2–1* **a**	1n	G. FINK
RG747	*his4–713 aro7(XVI) trp1(IV) cdc14(VI) pet17(XV)* **a**	1n	R. GABER
RG748	*his4–713 aro7(XVI) trp1(IV) cdc14(VI) pet17(XV)* α	1n	R. GABER
RG751	*his4–713 ilv3(X) arg1(XVIII) leu1(VII) pet8(XIV)* **a**	1n	R. GABER
RG752	*his4–713 ilv3(X) arg1(XVIII) leu1(VII) pet8(XIV)* α	1n	R. GABER
RG755	*his4–713 ade1(I) ura3(V) lys2(II) thr1(VIII)* **a**	1n	R. GABER
RG756	*his4–713 ade1(I) ura3(V) lys2(II) thr1(VIII)* α	1n	R. GABER
RG759	*his4–713 ura1(XI) lys1(IX) met2(XVII) ade2(XV)* **a**	1n	R. GABER
RG760	*his4–713 ura1(XI) lys1(IX) met2(XVII) ade2(XV)* α	1n	R. GABER
RG838	*his4–713 met2(XVII) ura1(XI) lys1(IX) rna1(XIII)* **a**	1n	R. GABER
RG839	*his4–713 met2(XVII) ura1(XI) lys(IX) rna1(XIII)* α	1n	R. GABER
CC666	*his4–713* **a** *ade2 lys2 SUF7* *his4–713* α *ade2 lys2 SUF7* *his4–713* α *ade2 lys2 SUF7*	3n	C. CUMMINS
CC1059	*his4–713* **a** *leu2 trp1 SUF8* *his4–713* α *leu2 trp1 SUF8* — – — *trp1 SUF8*	3n-1(III)	C. CUMMINS
H151–2A	*his4 leu2 thr4 MAL2* α *his4 leu2 thr4 MAL2* α	2n	J. HICKS
XJ9	*his4 leu2 crp1–13* **a** *lys2 ade6* *his4 leu2 crp1–13* **a** *lys2 ade6*	2n	J. HICKS
MC305	*ade2–1 met⁻ trp1–1 lys1–1 leu2–2* **a**	1n	M. CULBERTSON
GF276	*leu2–1 met8–1* α	1n	G. FINK
GF38	*his4–260* **a**	1n	G. FINK

* Roman numerals in parentheses indicate chromosome location.

Media: The following types of standard media were used: YEPD, which contains 2% bactopeptone, 1% yeast extract, 2% glucose, 2% agar; YEPDG, which contains 2% Bactopeptone, 1% yeast extract, 2% glycerol (v/v), 0.1% glucose, 2% agar; KAC (sporulation medium), which contains 1% potassium acetate, 0.1% glucose, 1.25 g/l yeast extract, 2% agar; minimal medium, which contains 6.7 g/l Difco Yeast Nitrogen Base, 2% glucose, 2% agar. Synthetic complete medium contains the components of minimal medium plus adenine, uracil, lysine, histidine, leucine, tryptophan, methionine, tyrosine, phenylalanine, arginine and threonine. Purines, pyrimidines and amino acids were added to concentrations given in the Cold Spring Harbor Yeast Course Manual (SHERMAN, FINK and LAWRENCE 1971).

The segregation of auxotrophic markers in crosses was scored on synthetic complete media lacking the appropriate purine, pyrimidine or amino acid requirement. In genetic mapping experiments, petite (*pet*) mutants were scored on YEPDG, and cell division (*cdc*) mutants were scored at 37° on YEPD. The temperature-sensitive mutants, *rna1* and *rna2*, were scored at 37° on YEPD.

Isolation of Group III suppressors: His+ revertants of strains 5588-8A (*his4–713*), 5595–9D (*his4–713*) and 5569–1A (*his4–712*) were isolated as follows: Single colonies from each strain were isolated on YEPD plates. Cells from isolated colonies were inoculated into culture tubes containing 3 ml YEPD broth and grown overnight with shaking at 30° to a density of 2×10^8 cell/ml. In this procedure, the cloning of independent lines prior to mutagenesis insured that mutants obtained from different culture tubes were of independent origin. The cells were centrifuged, washed twice with sterile water and concentrated 10-fold by resuspension in 0.3 ml of water. 0.1 ml aliquots were spread on plates to select for His+ revertants (minimal + adenine for strain 5588–8A, minimal for strain 5595–9D, or minimal + methionine + leucine for strain 5569–1A). The plates were either incubated without mutagenesis, irradiated with UV for 25 sec at a dose that gave 50% survival, or treated with ICR-170 as described in CULBERTSON *et al.* (1977).

Spectrum of suppression: Group III suppressors were analyzed for their ability to suppress representatives of previously identified groups of ICR-induced mutations at the *his4* locus (CULBERTSON *et al.* 1977). To test each new suppressor, strains were constructed carrying a suppressor and *his4–29*, an in-frame deletion of the entire *his4A* and *his4B* regions (FINK and STYLES 1974). Suppression of *his4A* and *his4B* mutations was examined by crossing *his4–29 SUFX* strains to *his4A-x suf+* and *his4B-x suf+* strains. Since *his4–29* fails to recombine with all known sites in *his4A* and *his4B* and is not itself suppressed, the appearance of His+ spores from these crosses signals suppression. Suppression of *his4C* mutations was tested by standard ascal dissection of crosses heterozygous for a suppressible allele and the allele to be tested (*e.g.*, *his4–713 SUFX* × *his4C-x suf+*). A 2 His+:2 His− segregation in these crosses signals suppression of the allele in question.

Group III suppressors were also tested to determine whether they could suppress the well-characterized nonsense mutations, *trp1–1* (UAG), *met8–1* (UAG), *lys1–1* (UAA), *ade2–1* (UAA), *leu2–1* (UAA), *his4–260* (UGA) and *leu2–2* (UGA) (HAWTHORNE 1969; FINK 1966; FINK, unpublished). Strains carrying the suppressors to be tested were crossed with strains MC305, GF276 and GF38 (Table 1), which carry the nonsense mutations.

Assay for increased efficiency of suppression in [PSI+] strains: The ability of the non-Mendelian [PSI] element to increase the efficiency of Group III suppressors was tested using the same strategy employed previously for Group II suppressors (CULBERTSON *et al.* 1977). The method involves construction of [PSI−] strains that carry an appropriate combination of a suppressor and a weakly suppressed *his4* allele. We have found that certain such combinations result in temperature-sensitive growth on minimal medium and that temperature-independent growth can be restored by the addition of histidine to minimal medium. These strains have a His+ phenotype at 30° and a His− phenotype at 37°. Increased efficiency of suppression can be assayed by crossing temperature-sensitive *his4-x SUFX* [PSI−] strains with *his4-x suf+* [PSI+] strains. Increased efficiency of suppression due to [PSI] is signalled by the loss of the temperature-sensitive phenotype on minimal medium in *his4-x SUFX* [PSI+] spores derived from these crosses.

Genetic mapping procedures: Centromere linkage was initially determined in crosses heterozygous for the centromere-linked suppressor *SUF2* and heterozygous for each new suppressor (*his4–713 SUF2* × *his4–713 SUFX*). Previous mapping studies show that the percent second-division segregation (% SDS) of *SUF2* is 1.20, and the suppressor is located on the right arm of chromosome *III* (CULBERSON *et al.* 1977). Centromere-linkage values for the new suppressors were calculated from the equation:

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

where X is the second-division segregation frequency of *SUF2* and Y is the second-division segregation frequency of the other suppressor in the cross (PERKINS 1949). Other centromere-linked genes used to map centromere-linked frameshift suppressors were *trp1* (0.45% SDS), *cdc4* (18.6), *pet8* (% SDS too low to measure), *rna2* (7.9), *met14* (2.2), *cdc5* (2.9) and *ade1* (10.1) (HAWTHORNE and MORTIMER 1960; MORTIMER and HAWTHORNE 1973).

Two of the dominant Group III suppressors, *SUF7* and *SUF8*, were mapped by a variation of previously published methods employing spore segregants from triploid strains (MORTIMER and HAWTHORNE 1973; WICKNER 1979). Triploid strains were constructed that were homozygous for the Group III suppressible allele *his4–713* and each of the suppressors to be mapped (*his4–713/his4–713/his4–713 SUFX/SUFX/SUFX*). Triploid strain construction was accomplished by first isolating diploid strains capable of mating. *his4–713/his4–713 SUFX/SUFX* **a**/α diploids were plated for single colonies on YEPD and tested by replica-plating for the ability to mate with a confluent lawn of **a** or α mating-type tester strains. Spontaneous maters were detected in populations of **a**/α nonmating diploids at approximate frequencies of 10^{-3} to 10^{-4} maters per total colonies screened.

Diploid maters were tested to determine their chromosome constitutions prior to use in the construction of triploids. The 2 most frequent events whereby nonmating **a**/α cells can acquire the ability to mate are (1) mitotic nondisjunction resulting in 2n–1 monosomic segregants that are hemizygous for the mating-type locus (*MAT1*) on chromosome *III*, or (2) mitotic recombination resulting in homozygosity of the *MAT1* locus. These 2 events can be distinguished by crossing the diploid maters to diploid tester strains that are homozygous for the opposite mating type and for the recessive chromosome *III* markers *thr4* and/or *leu2* (strains H151–2A and XJ9, Table 1).

If a mater arose by mitotic nondisjunction, it would carry only one copy of chromosome *III* (*i.e.*, *LEU2+*/0, *THR4+*/0, **a**/0, or α/0). Crosses by H151–2A or XJ9 would produce tetraploids carrying 3 copies of chromosome *III* (4n–1) with the *leu2* or *thr4* markers in the +/—/— configuration. Sporulation of the 4n–1 tetraploids would result in tetrads that give 2+:2− segregations for the Leu or Thr auxotrophic phenotypes. This result was taken as evidence that the mater arose by mitotic nondisjunction and subsequent chromosome loss. Maters of this type have chromosome constitution 2n–1 and contain only one copy of chromosome *III*.

By contrast, a mater that arose by mitotic recombination would carry 2 copies of chromosome *III* (*i.e.*, *LEU2+/LEU2+*, *THR4+/THR4+*, **a**/**a**, or α/α. Crosses with HI51–2A or XJ9 would produce tetraploids carrying 4 copies of chromosome *III* with the *leu2* and *thr4* markers in the +/+/—/— configuration. Sporulation of the tetraploids would result in tetrads that give 4+:0−, 3+:1− and 2+:2− segregations for the Leu or Thr phenotypes. This result was taken as evidence that the mater arose by mitotic recombination resulting in homozygosity of *MAT1*. Maters of this type have a normal 2n diploid chromosome constitution.

Triploids homozygous for *his4–713* and the suppressor to be mapped were constructed by crossing diploid maters of known chromosome *III* constitution with haploid *his4–713 SUFX* strains. The homozygous triploids were sporulated and random spores capable of mating were recovered. Since sporulation of triploids in yeast requires that 6 chromatids for each independently segregating chromosome must be distributed among the 4 meiotic products, each spore has an equal probability of carrying 1 or 2 copies of each chromosome. Among the mixed aneuploid segregants of these triploids, some would be expected to carry 1 copy of the chromosome bearing the suppressor, while others would be expected to carry 2 copies. The 2 types of spores were

distinguished in crosses with haploid strains carrying *his4–713 suf+* and recessive signal markers on each of the 17 chromosomes. Spores carrying 1 copy of the chromosome carrying the suppressor were identified in these crosses by a 2+:2- segregation for the suppressor phenotype (His+), whereas spores carrying 2 copies were identified by 4+:0-, 3+:1- and 2+:2- segregations for the suppressor phenotype. By including signal markers in these crosses to monitor the segregation of each chromosome, it was possible to eliminate chromosomes as candidates for the location of the suppressor. For example, in crosses where the suppressor segregated aberrantly, chromosomes identified by markers that segregated 2+:2- were considered unlikely candidates for the location of the suppressor.

This triploid procedure usually led to the elimination of most of the chromosomes as possible locations for the suppressor. The remaining chromosomes were examined by standard meiotic linkage analysis, using diploids homozygous for *his4–713*, heterozygous for the suppressor and heterozygous for markers on the chromosomes to be tested. Once linkage was established, map distances in centimorgans (cM) were calculated from linkage data by the equation $X(\mathrm{cM}) = 50[\text{tetratype asci} + 6 \text{ (nonparental ditype asci)}]/\text{total asci}$ (PERKINS 1949).

RESULTS

Isolation of Group III suppressors: The results of a detailed analysis of 180 independent His+ revertants of strains carrying *his4–712* and *his4–713* are presented in Table 2. Each revertant was first analyzed by determining whether the event responsible for the His+ phenotype was linked or unlinked to the *HIS4* locus. Diploids were constructed by crossing each revertant strain to a strain carrying a wild-type *HIS4* gene and an *ade2* mutation. Random spores were isolated by treating sporulated diploids with gluculase and plating the cultures on YEPD medium. Since the diploids were heterozygous for the *ade2* mutation, it was possible to identify haploid spore clones by the red pigmentation produced in haploid *ade2* segregants. At least 40 spore clones from each diploid were examined.

External suppressors of *his4–712* or *his4–713* are expected to recombine with the *HIS4* locus during meiosis, resulting in the generation of His- segregants among haploid spore clones derived from diploids formed in *his4* revertant × wild-type *HIS4+* crosses. If an external suppressor shows no linkage to the *HIS4* locus, 25% of the random spores are expected to have a His- phenotype, whereas linkage would reduce the frequency of His- segregants to an extent dependent on the map distance between the suppressor and *HIS4*. External suppressors tightly linked to *HIS4*, internal second-site suppressors and wild-type revertants are not expected to generate His- segregants at a high enough frequency to be detected in small samples of random spores. In order to simplify presentation of the data, we have classified revertants failing to generate His- segregants as internal *his4* mutations; such revertants were not analyzed further. Revertants that yielded His- segregants were assumed to carry an external suppressor and were analyzed as described below.

The ratio of internal mutations to external suppressors depended on the particular *his4* allele reverted and the method of induction. Table 2 shows that all UV-induced and spontaneous revertants of *his4–712* were internal (14/14 and 3/3, respectively), whereas all ICR-170-induced revertants of *his4–712* were external (13/13). Most of the UV-induced revertants of *his4–713* were internal

TABLE 2

Analysis of his4–712 *and* his4–713 *revertants*

Method of induction	*his4* allele	Numbers of independent isolates studied									Reversion frequency*
		External revertants						Total external revertants	Total internal revertants	Total revertants	
		SUF2	*SUF7*	*SUF8*	*SUF9*	*SUF10*	*suf11*				
UV-irradiation	*his4–712*	0	0	0	0	0	0	0	14	14	54
	his4–713	1	0	1	1	1	0	4	61	65	875
Spontaneous	*his4–712*	0	0	0	0	0	0	0	3	3	2
	his4–713	0	5	6	10	0	1	22	17	39	202
ICR-170	*his4–712*	13	0	0	0	0	0	13	0	13	
	his4–713	46	0	0	0	0	0	46	0	46	

* Spontaneous reversion frequencies are expressed as the number of revertants per 10^8 viable cells plated. UV-induced reversion frequencies are expressed as the number of revertants per 10^8 survivors obtained following a 25 sec exposure to UV at a dose of 800 ergs/cm^2/sec; this dose gives 50% survival. The method used for ICR-170 mutagenesis in which the mutagen is spotted directly on a petri plate containing a lawn of cells does not permit precise calculation of reversion frequency in response to a given dose. However, we estimate that the frequency of ICR-170 induced revertants among survivors of mutagenesis is in excess of 100-fold above the spontaneous reversion frequency for *his4–712* and *his4–713*.

(61/64), roughly half of the spontaneous revertants were external (22/39) and all of the ICR-170-induced revertants of *his4–713* were external (46/46).

A random-spore procedure similar to that described above was used to determine whether the external suppressors obtained were synonymous with the previously identified suppressor, *SUF2* (CULBERTSON *et al.* 1977). External revertants of *his4–712* and *his4–713* were crossed with strains carrying *SUF2* and the appropriate suppressible *his4* allele. The crosses were scored for the appearance of His⁻ segregants among random spores. Among spontaneous and UV-induced external suppressors, only one behaved as an allele of the *SUF2* locus. This is of interest in view of the fact that all ICR-170-induced external suppressors of *his4–712* and *his4–713* were alleles of *SUF2*.

The three UV-induced and 22 spontaneous external suppressors that recombined with *SUF2* were examined in pairwise crosses to determine the number of suppressor genes represented. An analysis of the His phenotypes of random spores from these crosses demonstrated that the suppressors could be divided into five groups capable of recombination with each other. Representative suppressors of each group were examined by tetrad analysis in pairwise crosses and in crosses with *SUF2*. The results given in Table 3 demonstrate that representative sup-

TABLE 3

Linkage relations among Group III suppressors

Cross*	*SUFX* × *SUFY*†			Linkage between suppressors	Centromere linkage
	4+:0– PD	2+:2– NPD	3+:1– T		
SUF2 × *SUF7*	5	6	14	unlinked	*SUF7* linked§
SUF2 × *SUF8*	3	5	15	unlinked	*SUF8* unlinked
SUF2 × *SUF9*	11	10	4	unlinked	*SUF9* linked
SUF2 × *SUF10*	12	13	0	unlinked	*SUF10* linked
SUF2 × *suf11*‡	1	2	7	unlinked	*suf11* unlinked
SUF7 × *SUF8*	3	4	16	unlinked	—
SUF7 × *SUF9*	5	4	14	unlinked	—
SUF7 × *SUF10*	5	4	13	unlinked	—
SUF7 × *suf11*	9	6	15	unlinked	—
SUF8 × *SUF9*	3	3	12	unlinked	—
SUF8 × *SUF10*	5	4	15	unlinked	—
SUF8 × *suf11*	1	1	7	unlinked	—
SUF9 × *SUF10*	7	6	5	unlinked	—
SUF9 × *suf11*	0	0	10	unlinked	—
SUF10 × *suf11*	5	5	18	unlinked	—

* Centromere linkage was determined on the basis that *SUF2* is tightly linked to the centromere of chromosome *III* (CULBERTSON *et al.* 1977) (see MATERIALS AND METHODS). Centromere linkage is indicated by a deficiency of tetratype asci in crosses involving *SUF2*.

† All crosses were homozygous for the suppressible marker, *his4–713*, and the segregation of the suppressors was scored on medium lacking histidine. PD = parental ditype, NPD = nonparental ditype, T = tetratype.

‡ Lowercase letters denote that *suf11* is recessive.

§ Although the sample is small and the deviation from a 1:1:4 ratio is not statistically significant, subsequent experiments described in text confirm that *SUF7* is centromere-linked.

pressors of each group recombine with each other and with *SUF2*, thereby defining six Group III suppressor loci designated *SUF2*, *SUF7*, *SUF8*, *SUF9*, *SUF10* and *suf11*. The most frequent classes of suppressors obtained as spontaneous or UV-induced external revertants of *his4–713* were alleles of *SUF7*, *SUF8* and *SUF9* (Table 2). The centromere linkage data shown in Table 3 for the new suppressors are discussed in a subsequent section.

Dominance of the suppressors and the spectrum of suppression: Since the suppressors were obtained as revertants of *his4–713*, we first determined whether they were capable of suppressing the other Group III *his4* allele (Table 4). *his4–713 SUFX* strains were crossed with *his4–712 suf*$^+$ strains, and suppression of *his4–712* was analyzed in tetrads from these heteroallelic crosses. If *his4–712* was suppressed, a 2 His$^+$:2 His$^-$ segregation would be expected, since *his4–713* and *his4–712* map close together in the *his4C* region and do not recombine at an appreciable frequency during meiosis (CULBERTSON *et al.* 1977). If a suppressor failed to suppress *his4–712*, the expected segregation patterns would be 0:4, 1:3 and 2:2 (His$^+$:His$^-$) in ratios dependent on the recombination frequency between the suppressor and *his4–713*. The results of these crosses demonstrate that *SUF2* and *SUF10* suppress *his4–712*, whereas *SUF7*, *SUF8*, *SUF9* and *suf11* do not.

The suppressors were tested for dominance or recessiveness in diploids homozygous for *his4–713*; *SUF2* and *SUF10* were also tested in diploids homozygous for *his4–712*. *SUF2*, *SUF7*, *SUF8*, *SUF9* and *SUF10* were dominant in *his4–713* homozygotes, whereas *suf11* was recessive and is therefore designated by lower-case letters. Although *SUF2* and *SUF10* behaved as dominant suppressors in *his4–713* homozygotes, they were recessive in *his4–712* homozygotes. We have adopted the convention that suppressor genotypes are written in upper-case letters if the suppressor exhibits dominant suppression of at least one mutation.

Standard crosses were used in conjunction with the *his4–29* deletion heterozygote patch test (MATERIALS AND METHODS) to determine whether the Group III

TABLE 4

Suppression of ICR-induced his4 *alleles by Group III suppressors**

his4 allele and genotype	*SUF2*	*SUF7*	*SUF8*	*SUF9*	*SUF10*	*suf11*
Group I:						
his4–506	—	—	—	—	—	—
Group II:						
his4–519, –38, –504, –204, –212, –210	—	—	—	—	—	—
Group III:						
his4–713	+	+	+	+	+	+
his4–712	±	—	—	—	±	—
his4–713/his4–713 SUFX/suf$^+$	+	±	±	±	+	—
his4–712/his4–712 SUFX/suf$^+$	—	—	—	—	—	—
Group IV:						
his4–706	—	—	—	—	—	—

* "+" designates visible growth on -his media after 48 hr, incubation at 30°.
"±" designates visible growth on -his media after 72 hr, incubation at 30°.
"—" designates no growth on -his media after 120 hr, incubation at 30°.

suppressors are capable of suppressing other groups of ICR-170-induced mutations (CULBERTSON *et al.* 1977). Group I mutations have been defined as only those that revert spontaneously. Group II mutations are externally suppressible frameshift mutations. Group IV mutations do not revert with ICR-170, and Group I and Group IV are not externally suppressible. *SUF2*, *SUF7*, *SUF8*, *SUF9*, *SUF10* and *suf11* failed to suppress *his4–506* (a Group I mutation), *his4–519*, *–38*, *–504*, *–204*, *–212*, *–210*, *leu2–3* (Group II frameshift mutations) and *his4–706* (a Group IV mutation). Suppressions of Group II frameshift mutations, *SUF1*, *SUF3*, *SUF4*, *SUF5* and *SUF6*, fail to suppress *his4–713* and *his4–712*, demonstrating a lack of cross-suppression between suppressors of Group II and Group III.

Group III suppressors were examined for their ability to suppress UAG, UAA and UGA nonsense mutations. Tetrads were analyzed from crosses heterozygous for *his4–713*, a suppressor, and the nonsense mutations *trp1–1*, *met8–1*, *lys1–1*, *ade2–1*, *leu2–1* and *leu2–2* (see MATERIALS AND METHODS; Table 1). The presence of a suppressor in these crosses was indicated by 4:0, 3:1 and 2:2 (His^+:His^-) segregations on histidineless medium. Since all of the phenotypes corresponding to the nonsense mutations segregated 2^+:2^- in these crosses, it was concluded that none were suppressed by *SUF2*, *SUF7*, *SUF8*, *SUF9*, *SUF10* or *suf11*. The suppressors were also tested for their ability to suppress the UGA mutation *his4–260* by analysis of tetrads from *his4–713 SUFX* × *his4–260 suf*$^+$ crosses. The observed 0:4, 1:3, and 2:2 (His^+:His^-) segregations show that *his4–260* is not suppressed by Group III suppressors. From these analyses, we conclude that *SUF2*, *SUF7*, *SUF8*, *SUF9*, *SUF10* and *suf11* fail to suppress these UAG, UAA and UGA nonsense mutations; therefore, they are not likely to be nonsense or omnipotent suppressors.

Efficiency of suppression in [PSI$^+$*] strains*: Three of the Group II suppressors described previously (*SUF1*, *SUF4* and *SUF6*) exhibit increased efficiency of suppression in the presence of the cytoplasmically inherited [PSI] element (CULBERTSON *et al.* 1977). This element was originally identified by the increased efficiency of suppression observed in [PSI$^+$] strains carrying the serine-inserting ochre suppressor *SUQ5* (SUQ5≡SUP15≡SUP16) (COX 1965; LIEBMAN, STEWART and SHERMAN 1975). We have tested Group III suppressors for increased efficiency of suppression in [PSI$^+$] strains using an assay that measures increased suppression at an elevated temperature (see MATERIALS AND METHODS). Since the assay requires construction of suppressor-bearing strains that are phenotypically His^+ at 30°, but His^- at 37°, the Group III suppressors were examined for suppression of *his4–713* and *his4–712* at those two temperatures. We found that *his4–712* strains carrying *SUF2* or *SUF10* exhibit temperature-sensitive growth on minimal medium. *SUF7*, *SUF8*, *SUF9* and *suf11* exhibit this phenotype in strains carrying *his4–713*. Strains carrying the appropriate combinations of suppressors and suppressible *his4* alleles were crossed with *his4–712 suf*$^+$ [PSI$^+$] or *his4–713 suf*$^+$ [PSI$^+$] strains, and tetrads were analyzed at 30° and 37°. In each cross, a 2 His^+:2 His^- segregation was observed at 30° and a 0 His^+:4 His^- segregation was observed at 37°. Since the crosses were homozygous for *trp5–48*,

a UAA mutation that is suppressed by [PSI] (Cox 1965; LIEBMAN and SHERMAN 1979), it was possible to follow the non-Mendelian segregation of the element. The results demonstrate that [PSI] fails to increase the efficiency of Group III suppressors to an extent that can be detected by this assay. Thus, the influence of [PSI] on suppressors of ICR-induced mutations appears to be limited to the Group II suppressors *SUF1*, *SUF4* and *SUF6* (CULBERTSON *et al.* 1977).

Mapping of SUF9 *and* SUF10: *SUF2* was used as a centromere-linked marker (CULBERTSON *et al.* 1977) in crosses designed to identify other centromere-linked suppressors. As shown in Table 3, reduced second-division segregation indicated by deficiencies in the expected frequency of tetratype asci (3 His^{+}:1 His^{-}) were observed in crosses of *SUF2* × *SUF7*, *SUF2* × *SUF9* and *SUF2* × *SUF10*. Since *SUF9* and *SUF10* appeared to be tightly linked to their respective centromeres in these crosses, an attempt was made to map these suppressors in standard crosses that were heterozygous for centromere-linked markers on as many chromosomes as possible.

Table 5 and Figure 1 describe the results of crosses in which linkage was detected between *SUF9* and *cdc4* (chromosome *VI*) and between *SUF10*, *rna2* and *pet8* (chromosome *XIV*). In the cross showing linkage between *SUF9* and *cdc4*, the inclusion of two additional centromere-linked markers on other chromosomes (*trp1* on chromosome *IV* and *met14* on chromosome *XI*) permitted the unambiguous identification of crossovers between *SUF9* and the centromere and between *cdc4* and the centromere. In seven of 10 tetrads where *cdc4* recombined with the

TABLE 5

Mapping of SUF9 *and* SUF10

Gene pair*	PD	NPD	T	Gene-pair distance (cM)	FDS:SDS	% SDS
SUF9–trp1	25	28	23†	unlinked	53:23	30
SUF9–cdc4	57	1	18	16		
SUF9–met14	26	19	31	unlinked	45:31	41
cdc4–trp1	29	33	14†	unlinked	62:14	18
cdc4–met14	30	23	23	unlinked	53:23	30
met14–trp1	32	39	5	unlinked	71:5	7
SUF10–trp1	160	153	18	unlinked	313:18	5.4
SUF10–pet8	327	0	4	0.6		
SUF10–rna2	313	0	18	2.7		
pet8–trp1	165	151	15	unlinked	316:15	4.5
pet8–rna2	284	0	47	7.1		
trp1–rna2	141	131	59	unlinked	272:59	18

* The two crosses were *his4–713 trp1 SUF9* × *his4–713 cdc4 met14* and *his4–713 SUF10 rna2 trp1* × *his4–713 pet8*.

† Ten of the 14 tetrads that were tetratype for the *cdc4–trp1* marker pair were tetratype for the *cdc4–met14* marker pair, but parental ditype or nonparental ditype for the *trp1–met14* marker pair. Eighteen of the 23 tetrads that were tetratype for the *SUF9–trp1* marker pair were tetratype for the *SUF9–met14* marker pair, but were parental or nonparental ditype for the *trp1–met14* marker pair.

cM = centimorgan. FDS = first-division segregation. SDS = second-division segregation.

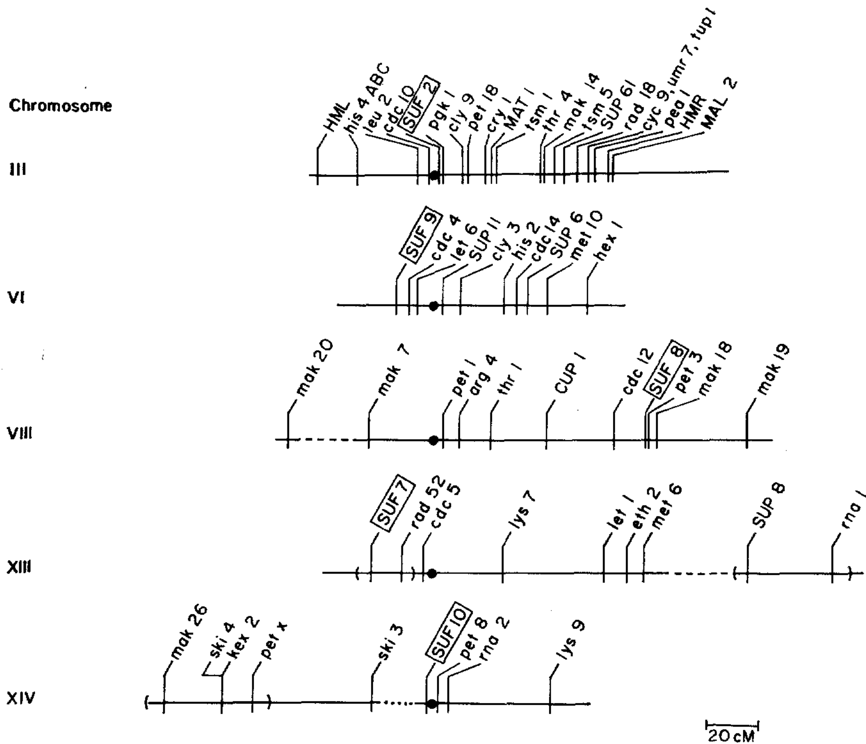

FIGURE 1.—Genetic map showing the locations of *SUF2*, *SUF7*, *SUF8*, *SUF9* and *SUF10*. Gene order for markers within parentheses has not been established. Dashed lines indicate mitotic linkage. Dotted lines indicate trisomic linkage. Mapping data for *SUF2* were published previously (CULBERTSON *et al.* 1977). See text for a discussion of the discrepancy between the *cdc5*-centromere distance obtained in this study (1cM) and that obtained by MORTIMER and HAWTHORNE (1973).

centromere, *SUF9* also recombined with the centromere. In 18 tetrads where *SUF9* recombined with the centromere, six had the parental ditype configuration and 12 had the tetratype configuration for the *SUF9-cdc4* marker pair. These results are consistent with the gene order *SUF9-cdc4*-centromere. The calculated distance of 9 cM between *cdc4* and the centromere is in close agreement with the results of MORTIMER and HAWTHORNE (1973).

The percent second-division segregation for the *SUF9-trp1* marker pair is 30%, suggesting a *SUF9*-centromere distance of 15 cM. This value is somewhat anomalous compared to the *SUF9-cdc4* and the *cdc4*-centromere distances (16 cM and 9 cM, respectively). The anomaly is probably due to differences in the two methods used to calculate map distances. The calculation of map distance using percent second-division segregation (frequency of tetratype asci) is subject to error resulting from undetected double crossovers, whereas the linkage equation (PERKINS 1949; see MATERIALS AND METHODS) accounts for single and double crossovers. On this basis, the *SUF9*-centromere distance of 15 CM is probably

an underestimate. When all crossovers are considered, the distance is approximately 20 CM.

The nonsense suppressor *SUP11* is located 3 CM from the centromere on the opposite chromosome arm (HAWTHORNE and MORTIMER 1968). Recombination between the two suppressors was demonstrated in a cross of *SUF9* × *SUP11*-**o** in which the diploid was homozygous for *his4–713* (to permit scoring of *SUF9*) and homozygous for *ade2–1* (to permit scoring of *SUP11*-**o**). The combined results show that *SUF9* is not an allele of the *SUP11* locus.

SUF10 was mapped in a cross that was heterozygous for the chromosome *XIV* markers, *pet8* and *rna2*, and the centromere-linked chromosome *IV* marker, *trp1* (Table 5; Figure 1). Only four tetrads recombinant for the marker pair *SUF10-pet8* were observed in 331 asci. The orientation of markers in one tetrad suggested that a double exchange had occurred with crossovers in both the *SUF10-pet8* and the *pet8-rna2* intervals. The orientation of markers in the other three tetrads suggested that in each case a single exchange had occurred between *SUF10* and the centromere. In these tetrads, the *SUF10-trp1*, *SUF10-pet8* and *SUF10-rna2* marker pairs were in the tetratype configuration, but the *rna2-trp1*, *rna2-pet8* and *pet8-trp1* pairs were in the parental configuration. These results suggest that *SUF10* and *rna2* are on opposite sides of the centromere. Since no crossovers were observed between *pet8* and the centromere, two alternative gene orders are possible: *SUF10*-centromere-*pet8-rna2* or *SUF10-pet8*-centromere-*rna2*. *SUF10* is 0.6 cM from the centromere.

Aneuploid mapping of SUF7 *and* SUF8: The triploid mapping method described in MATERIALS AND METHODS was used to determine the chromosome locations of *SUF7* and *SUF8*. This procedure involves the construction of triploids homozygous for a suppressible *his4* allele and the suppressor to be mapped. Caution must be exercised in obtaining mating diploids used to construct the triploids. The appropriate mating diploids were isolated as spontaneous mitotic segregants from nonmating diploids. Since maters can arise by several different mechanisms, including mitotic nondisjunction and mitotic recombination, the tetraploid testcrosses described in MATERIALS AND METHODS were used to determine the chromosome constitutions of spontaneous maters prior to their use in triploid strain construction.

We find that in most diploid strains, chromosome *III* nondisjunction resulting in hemizygosity of the mating-type locus (*MAT1*) is as frequent an event as mitotic recombination resulting in homozygosity of *MAT1*. The 2n-1 (*CIII*) nondisjunctants can be used to construct 3n-1 (*CIII*) triploids for mapping purposes, provided that the suppressor to be mapped is not on chromosome *III*. In the mapping studies reported below, we have used a 3n triploid to map *SUF7* and a 3n-1 (*CIII*) triploid to map *SUF8*.

The results of the aneuploid mapping experiments are shown in Table 6. Forty-eight mating spores derived by sporulation of the *SUF7* triploid were crossed with a *his4–713* suf^+ strain. Analysis of tetrads from these crosses revealed that 43 of the spores carried one copy of the suppressor (2^+:2^- segregation) and five carried two copies of the suppressor (4^+:0^-, 3^+:1^- and 2^+:2^-

TABLE 6

Aneuploid mapping of dominant suppressors

Suppressor	Total spores analyzed	Total *SUFX/SUFX* aneuploids	Aneuploid spore number	Chromosome signal markers*																	Proposed chromosome location
				I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*XIII*	*XIV*	*XV*	*XVI*	*XVII*	
SUF7	48	5†	*SUF7/SUF7-1*	+	nt	—	—	—	—	—	—	+	+	—	nt	nt	—	—	—	—	
			SUF7/SUF7-2	—	nt	—	—	—	—	—	—	nt	—	nt	—	nt	—	—	—	nt	*XIII*
			SUF7/SUF7-3	+	nt	—	—	—	+	—	—	—	—	+	—	+	—	—	—	—	
SUF8	23	3†	*SUF8/SUF8-1*	+	—	—	—	+	—	—	+	—	—	—	nt	—	—	—	—	—	*VIII*
			SUF8/SUF8-2	—	+	—	—	—	—	—	+	+	—	—	—	—	—	—	—	—	

As described in the text, we analyzed three spores from the *SUF7* triploid and two spores from the *SUF8* triploid because they were aneuploid for the chromosome carrying the suppressor (*e.g.*, *SUF7/SUF7* or *SUF8/SUF8*). The analysis indicated which other markers segregate aberrantly as the result of aneuploidy.

* "+" indicates aneuploid segregation for the chromosomes listed. "—" indicates euploid segregation for the chromosomes listed. "nt" indicates that the chromosome was not tested. The chromosome signal markers used were as follows: *I (ade1)*, *II (lys2, met8)*, *III (MAT1)*, *IV (trp1)*, *V (ura3)*, *VI (cdc14)*, *VII (leu1)*, *VIII (thr1, arg4)*, *IX (lys1)*, *X (ilv3)*, *XI (ura1)*, *XII (asp5)*, *XIII (rna1)*, *XIV (pet8)*, *XV (pet17, ade2)*, *XVI (aro7)*, *XVII (met2)*.

† Two *SUF7* aneuploid spores and one *SUF8* aneuploid spore were not analyzed due to extremely poor spore viability in crosses.

segregations). Three of the five spores crarying two copies of *SUF7* were analyzed in detail to determine which chromosomes were aneuploid and which were euploid. Analysis of spore *SUF7/SUF7–1* (Table 6) revealed that chromosomes *I* (*ade1*), *IX* (*lys1*) and *X* (*ilv3*) were present in two copies. Chromosomes *XII* (*asp5*) and *XIII* (*rna1*) were not tested due to unresolved difficulties in scoring the segregation of *asp5* and *rna1* in these crosses. Chromosome *II* (*lys2*) was not tested because the *SUF7* triploid was homozygous for *lys2* (see Table 1). All other chromosomes segregated 2:2 in crosses involving this spore and were therefore eliminated as candidates for the location of the suppressor. Analysis of the other two spores, *SUF7/SUF7–2* and *SUF7/SUF7–3*, demonstrated that chromosomes *I*, *IX*, *X* and *XII* were present in one copy. Therefore, these chromosomes were eliminated as candidates. The only chromosomes remaining as possibilites for the location of the suppressor were chromosomes *II* and *XIII*. It was subsequently shown by standard meiotic analysis that *SUF7* is linked to the chromosome *XIII* marker, *lys7*.

Three of the 23 spores derived by sporulation of the *SUF8* triploid showed aneuploid segregation for the suppressor phenotype when crossed with a *his4–713 suf*$^+$ strain (Table 6). Two of these spores were analyzed in detail to determine their chromosome constitutions. Analysis of spore *SUF8/SUF8–1* revealed that chromosomes *I* (*ade1*), *V* (*ura3*) and *VIII* (*thr1*) were present in two copies. Again, difficulties were encountered in scoring *asp5* (chromosome *XII*). However, it was possible to score *rna1* in these crosses and to rule out chromosome *XIII* as a candidate for the location of the suppressor. Since the *SUF8* triploid was homozygous for *trp1* (see Table 1), we were unable to score the segregation of chromosome *IV* directly. However, an indirect test was performed in which spore *SUF8/SUF8–1* (*trp1* or *trp1/trp1*) was crossed with a *TRP1*$^+$ strain, and four Trp$^+$ segregants (*TRP1*$^+$ or *TRP1*$^+$*/trp1*) from four different tetrads of this cross were recrossed with a *TRP1*$^+$ strain. The absence of trp$^-$ segregants in these secondary crosses indicated that chromosome *IV* was probably present in one copy in the original spore and therefore does not carry the suppressor. The seecond spore, *SUF8/SUF8–2*, contained one copy of chromosomes *I*, *V* and *XII* and two copies of chromosome *VIII*. These results suggest that the suppressor maps on chromosome *VIII*. It has subsequently been shown by standard meiotic analysis that *SUF8* is linked to the chromosome *VIII* marker, *cdc12*.

Meiotic mapping of SUF7 *and* SUF8: The mapping data and chromosome locations of *SUF7* and *SUF8* are presented in Table 7 and Figure 1. The location of *SUF7* on chromosome *XIII* was determined in a cross involving five heterozygous markers, *ade1*, *trp1*, *cdc5*, *lys7* and *SUF7*. *ade1* and *trp1* are centromere-linked markers on chromosomes *I* and *IV*, respectively. Five tetrads recombinant for the *cdc5-trp1* marker pair were analyzed in detail. Three of these tetrads had the orientation of markers consistent with a single crossover between *trp1* and its centromere. In one tetrad, the orientation of markers suggested that a double exchange had occurred on chromosome *XIII*. One crossover occurred between *cdc5* and the centromere on one arm and the other crossover between *lys7* and the centromere on the other arm. The marker orientation in the remaining tetrad sug-

TABLE 7

Mapping of SUF7 *and* SUF8

Gene pair*	PD	NPD	T	Gene-pair distance (cM)	FDS:SDS	% SDS
SUF7–cdc5	93	1	76	24		
SUF7–trp1	52	41	85	unlinked	93:85	48
cdc5–trp1†	95	73	5	unlinked	168:5	2.9
SUF7–lys7	45	11	117	>50		
cdc5–lys7	56	0	64	27		
lys7–trp1	49	48	79	unlinked	97:79	45
SUF8–cdc12	229	0	79	12.8		
SUF8–pet3	305	0	3	0.5		
cdc12–pet3	227	0	81	13.1		

* The two crosses were *his4–713 trp1 ade1 cdc5* × *his4–713 lys7 SUF7* and *his4–713 cdc12 pet3* × *his4–713 SUF8*. In the *SUF7* cross, *ade1* was used as an additional centromere-linked marker (data not shown).

† Consult the text for a discussion of the discrepancy between the *cdc5*-centromere distance obtained in this study (1 cM) and that obtained in MORTIMER and HAWTHORNE 1973.

cM = centimorgan. FDS = first-division segregation. SDS = second-division segregation.

gested that a single crossover had occurred between *cdc5* and the centromere. Since this tetrad had the parental configuration for the *cdc5-SUF7* marker pair and a tetratype configuration for the *cdc5-lys7* marker pair, the probable gene order is *SUF7-cdc5*-centromere-*lys7*. The *SUF7*-centromere distance of 24 cM calculated from these data is consistent with data presented earlier suggesting centromere linkage of this suppressor in a cross of *SUF2* × *SUF7*. The only discrepancy in our data, compared with previously published data, is in regard to the distance of *cdc5* from the centromere. A distance of only 1 cM was calculated in this study as compared to 14 cM in MORTIMER and HAWTHORNE (1973). The reason for this difference might be explained by the fact that the original *cdc5* strain (ts473, obtained from the Berkeley Collection) contained at least two, and possibly three, mutations conferring a temperature-sensitive phenotype. We were successful in separating two of the mutations, but neither one mapped at the location previously described for *cdc5*. It is possible that the mutation designated *cdc5* in the study, which maps 1 cM from the chromosome *XIII* centromere, is not the same mutation as that reported in the previous mapping study. The map order of *rad52* and *SUF7*, with respect to the centromere, has not been established.

The location of *SUF8* was determined in a three-point cross that included the suppressor and the chromosome *VIII* markers *pet3* and *cdc12* (Table 7; Figure 1). Linkage analysis revealed that *SUF8* maps at a position 0.5 cM from *pet3* and 12.8 cM from *cdc12*. The gene order was established by examining three tetrads that were recombinant for the *SUF8-pet3* marker pair. One of these tetrads had the orientation of markers consistent with a double exchange in which one crossover had occurred in the *SUF8-pet3* interval and the other in the *cdc12-SUF8* interval. In the other two tetrads, the orientation of markers suggested that a single exchange had occurred between *SUF8* and *pet3*. In both tetrads, the *cdc12-*

pet3 marker pair was in the tetratype configuration, and the *SUF8-cdc12* marker pair was in the parental configuration. Therefore, the probable gene order is *cdc12-SUF8-pet3*. The orientation of *cdc12* and *pet3* with respect to the centromere was established previously (WICKNER 1979).

The map location of *suf11* has not yet been determined. Since this suppressor is recessive, an aneuploid mapping protocol differing from that described above for dominant suppressors will be required.

Stability of Group III suppressors: An analysis of Group II suppressors revealed that one subset of related suppressors (*SUF1*, *SUF4* and *SUF6*) showed a high degree of phenotypic instability (CULBERTSON, UNDERBRINK and FINK 1980). In this analysis of Group III suppressors, we have not observed any significant instability for *SUF2*, *SUF7*, *SUF8*, *SUF9* and *suf11*. However, in crosses heterozygous for *SUF10* (*his4–713 SUF10* × *his4–713 suf*$^+$), tetrads that segregate 0 His$^+$:4 His$^-$ spores are not uncommon. The possibility that these aberrant tetrads result from instability of *SUF10* is being investigated.

DISCUSSION

External suppressors of ICR-induced mutations at the his4 *locus*: Evidence was presented in a previous communication that ICR-induced, ICR-revertible mutations at the *his4* locus in yeast exhibit behavior similar to bacterial frameshift mutations (CULBERTSON *et al.* 1977). An extensive analysis of ICR-induced, spontaneous and UV-induced revertants of these mutations revealed an important feature anticipated from the analysis of frameshift mutations in bacteria—the existence of dominant external suppressors of the mutations.

Seven suppressors were divided into two groups based on their spectra of suppression. Five suppressors were classified in one group (Group II: *SUF1*, *SUF3*, *SUF4*, *SUF5* and *SUF6*) since they suppress the same set of 18 ICR-induced *his4* alleles. Column chromatography of tRNA from strains carrying these suppressors revealed that *SUF5* contains a chromatographically altered species of tRNA^{GLY1}, and *SUF1*, *SUF4* and *SUF6* have reduced levels of tRNA^{GLY3} isoacceptor activity. These results suggest that Group II suppressors are frameshift suppressors, some of which may act in a manner analogous to the *sufD* suppressor in Salmonella (RIDDLE and ROTH 1972b; RIDDLE and CARBON 1973). Although the detailed mechanism of suppression by *sufD* is unclear (KURLAND 1979), the simplest model invokes direct reading of the four-base codon GGGN by an altered glycyl-tRNA containing a quadruplet CCCC anticodon (RIDDLE and CARBON 1973).

Two of the original suppressors (Group III: *SUF2* and *SUF7*) failed to suppress Group II frameshift mutations at the *his4* locus, but were found to suppress two other ICR-induced, ICR-revertible mutations, *his4–712* and *his4–713*. In this communication, the genetic properties of these suppressors and the isolation and properties of four new Group III suppressors are described. Cross-suppression of Group II and Group III *his4* mutations by the two groups of suppressors has not been observed. It is tempting to speculate that Group III suppressors in yeast are

analogous to the Salmonella suppressors associated with structurally altered prolyl-tRNA's (RIDDLE and ROTH 1972b).

Isolation of Group III suppressors: Suppressor mutations mapping at six loci, *SUF2*, *SUF7*, *SUF8*, *SUF9*, *SUF10* and *suf11*, were obtained among revertants of the Group III mutations, *his4–712* and *his4–713*. ICR-induced revertants of these *his4* alleles invariably contain suppressor mutations mapping at the *SUF2* locus near the centromere of chromosome *III*. Mutations at other suppressor loci were obtained by spontaneous or UV-induced reversion of *his4–713*. *SUF7*, *SUF8* and *SUF9* were the most commonly represented suppressors among these revertants. The reason for the induction of suppressor mutations exclusively at the *SUF2* locus by ICR-170 is unknown. Similar results were obtained in an analysis of ICR-induced revertants of Group II frameshifts at the *his4* locus in which suppressors were found to map primarily at the *SUF3* and *SUF5* loci (CULBERTSON, UNDERBRINK and FINK 1980).

Properties of Group III suppressors: Suppression and dominance tests were performed on representative mutations mapping at each of the six suppressor loci. *SUF2*, *SUF7*, *SUF8*, *SUF9*, *SUF10* and *suf11* suppress *his4–713*; however, *his4–712* is suppressed only by *SUF2* and *SUF10*. All of the Group III suppressors failed to suppress Group II ICR-induced frameshift mutations, as well as ICR-induced, ICR-nonrevertible mutations in other groups previously described (CULBERTSON *et al.* 1977). Since the Group II frameshift suppressors do not suppress *his4–712* and *his4–713*, these studies define two groups of suppressors that fail to exhibit cross-suppression. In addition, the Group III suppressors were tested for their ability to suppress nonsense mutations. They failed to suppress representative UAA, UAG and UGA mutations and are therefore not likely to be nonsense or omnipotent suppressors.

With the exception of the recessive suppressor *suf11*, all of the Group III suppressors are dominant in diploids homozygous for *his4–713*. The two suppressors of *his4–712*, *SUF2* and *SUF10*, are recessive in *his4–712* homozygotes. Since *his4–712* is the more weakly suppressed of the two Group III mutations in haploid strains, the dominant or recessive phenotypes of these suppressors might be explained by different efficiencies of suppression of the two suppressible alleles. These results serve to emphasize that dominance or recessiveness of a suppressor cannot be used in any rigorous sense to support or refute models of the molecular mechanism of suppression.

The results of mapping experiments show that *SUF7* is located 24 cM from the centromere on the left arm of chromosome *XIII*. *SUF8* is located between *cdc12* and *pet3* on the right arm of chromosome *VIII*. *SUF9* is located 20 cM from the centromere on the left arm of chromosome *VI*. *SUF10* is located 0.6 cM from the centromere on the left arm of chromosome *XIV*. *SUF2* was previously mapped and is located 0.6 cM from the centromere on the right arm of chromosome *III* (CULBERTSON *et al.* 1977). On the basis of these mapping studies, we can state that Group III suppressors represent a new class of suppressors not previously identified, since they do not map at previously described nonsense, missense or omnipotent suppressor loci.

TABLE 8

Properties of Group II and Group III suppressors

Suppressor locus	Group suppressed at *his4*	Independent isolates studied	ICR-170 inducible	Centromere linkage	Chromosome location	Dominant (+) or recessive (−)	Increased efficiency in [PSI+] strains	Lethal combinations	Spontaneous reversion frequency	tRNA affected
SUF1	II	3	NO†	NO	NT	+*	YES	*SUF4, SUF6*	HIGH	GLY3
SUF3	II	58	YES	NO	NT	+	NO	NONE	LOW	?
SUF4	II	1	NO	NO	NT	+	YES	*SUF1, SUF6*	HIGH	GLY3
SUF5	II	19	YES	NO	*XV*	+	NO	NONE	LOW	GLY1
SUF6	II	1	NO	NO	NT	+	YES	*SUF1, SUF4*	HIGH	GLY3
SUF2	III	60	YES	YES	*III*	+	NO	NONE	LOW	?
SUF7	III	5	NO	YES	*XIII*	+	NO	NONE	LOW	?
SUF8	III	7	NO	NO	*VIII*	+	NO	NONE	LOW	?
SUF9	III	11	NO	YES	*VI*	+	NO	NONE	LOW	?
SUF10	III	1	NO	YES	*XIV*	+	NO	NONE	?	?
suf11	III	1	NO	NO	NT	—	NO	NONE	LOW	?

NT = not tested.
* A suppressor is listed as dominant if it shows dominant suppression of at least one *his4* allele.
† Among 73 ICR-induced corevertants analyzed, one was an allele of *SUF1*. We cannot rule out the possibility that this suppressor was of spontaneous origin (CULBERTSON, UNDERBRINK and FINK 1980).

Mechanism of suppression: A summary comparing the properties of Group II and Group III suppressors is provided in Table 8. The Group II suppressors have been characterized as the probable structural genes for glycyl-tRNA's, based on column chromatography of the tRNA's (CULBERTSON *et al.* 1977). Group III suppressors were examined by a similar chromatographic analysis to determine whether prolyl-tRNA's might be involved in suppression. These experiments were unsuccessful in showing any differences in isoacceptor activity or chromatographic behavior between isoaccepting species of prolyl-tRNA derived from wild-type strains and strains carrying the suppressors. We are currently addressing the biochemical basis of Group III suppression by methods more sensitive than column chromatography. At present, we do not know the molecular mechanism by which these suppressors act.

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