GENETIC MAPPING OF NONSENSE SUPPRESSORS IN YEAST[1]

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THE super-suppressors in yeast are defined as allele specific suppressors which act upon mutants at two or more loci (HAWTHORNE and MORTIMER 1963). The properties of the suppressible alleles indicate that they are nonsense mutants (MANNEY 1964; HAWTHORNE and FRIIS 1964; FINK 1966), and thus the super-suppressors (nonsense suppressors) are analogous to the amber and ochre suppressors of *Escherichia coli*. To date, 22 different nonsense suppressors have been isolated. These suppressors can be assigned to 10 phenotypic classes on the basis of the spectra of mutant alleles suppressed. However, as many as 8 genetically distinct suppressors are found within a single phenotypic class. Thus the action spectrum alone is insufficient for the identification of a given suppressor. To facilitate the identification and description of the nonsense suppressors, the following studies were undertaken to locate the suppressor genes on the linkage map of yeast.

MATERIALS AND METHODS

The suppressors were isolated in haploid stocks carrying from 5 to 13 mutants known to be suppressible. Clones isolated as revertants on medium lacking a given nutrilite were tested for the other requirements. The suppressor mutations generally caused the simultaneous reversion of two or more of the mutant requirements. Representative clones for each pattern of reversion were then crossed to strains bearing new sets of suppressible alleles, and tetrad analyses were performed so that each suppressor was screened against the 18 suppressible alleles used in the classification scheme. To facilitate scoring of the suppressors in the tetrad analyses, most hybrids were constructed with at least one suppressible allele homozygous. The other hybrids were heterozygous for 12 or more suppressible alleles and it was therefore possible to recognize the two suppressor-free segregants in each ascus without ambiguity. Over 200 suppressor isolates were examined in this manner.

The media and techniques employed have been described in our earlier publications (HAWTHORNE and MORTIMER 1960; MORTIMER and HAWTHORNE 1966). The gene symbols used also correspond to the ones described in these reports. A new technique to determine if two dominant suppressors are allelic was devised for this study. In this method, which does not involve tetrad analysis, an unknown suppressor is mated with a set of stocks that carry known suppressors and in addition a suppressible allele of the gene conferring canavanine resistance. The genotypes and phenotypes of the parents are, for example: S_1 *cana*$^r{}_1$ (sensitive) $\times$ S_x *CANA*$^s{}_1$ (sensitive). Recovery of canavanine resistant clones from platings of spores from these hybrids was considered sufficient evidence for recombination between the suppressors because only suppressor-free segregants will permit expression of the *cana*$^r{}_1$ mutation. For example, the frequencies of canavanine

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RESULTS

The action spectra for the 10 classes of suppressors are presented in Table 1.

TABLE 1

Classification of nonsense suppressors in Saccharomyces based on their action spectra

Alleles	Class I	II	Ochre III	IV	V	Ochre-amber VI	VII	VIII	Amber IX	X
$ad_{5,\ 7-63}$	±	—	±	±	—	±	—	—	—	—
tr_{5-2}	+	±	—	—	—	—	—	—	—	—
ur_{4-1}	+	+	—	—	—	—	—	—	—	—
ad_{2-1}	+	±	±	—	—	±	—	—	—	—
$cana_{1-52}$	+	+	+	—	—	±	—	—	—	—
ty_{1-1}	±	±	+	±	—	—	—	—	—	—
ly_{1-1}	+	+	+	±	—	—	—	—	—	—
ly_{2-1}	+	+	+	+	—	—	—	—	—	—
hi_{5-2}	+	+	+	+	—	—	—	—	—	—
ad_{6-3}	+	+	+	+	—	±	—	—	—	—
ga_{1-32}	+	+	+	+	±	±	—	—	—	—
met_{4-1}	+	+	+	+	—	±	±	—	—	—
hi_{4-1}	+	+	+	+	—	±	±	±	—	—
le_{2-1}	+	+	+	+	±	+	+	+	—	—
is_{1-1}	+	+	+	+	±	+	+	+	—	—
ty_{7-1}	—	—	—	—	—	±	+	+	+	+
ty_{6-1}	—	—	—	—	—	+	—	±	+	+
tr_{1-1}	—	—	—	—	—	—	+	±	+	—

For the nutritional requirements, + = good growth from replica prints by 2 days; ± = visible growth by 3 to 5 days; and — = no signs of growth by 5 days. For canavanine resistance, the converse holds.

resistant spores from diploids heterozygous for two suppressors compared to diploids homozygous for a suppressor were found to be in the order of 10^{-1} and 10^{-5}, respectively.

The suppressible alleles used in the classification are, in many cases, the "type alleles" for the loci in question and therefore are in general circulation. Two nonsense codons, differing by a single base, are represented in this selection of alleles. All but three mutants have one nonsense codon, the ochre codon, which was also carried by the original class of suppressible mutants: ad_{2-1}, ad_{6-3}, hi_{5-2}, tr_{5-2}, etc. (HAWTHORNE and MORTIMER 1963). The three mutants tr_{1-1}, ty_{6-1}, and ty_{7-1} have the amber codon. This classification is based on mutagenesis studies which have shown that only the latter mutants contain a G-C pair at the mutant site (HAWTHORNE, in preparation). If we assume that UAA and UAG are the nonsense codons transcribed from the suppressible alleles, the patterns of suppression are consistent with the predictions of the "wobble" hypothesis (CRICK 1966). There are ochre-specific suppressors, classes I-V; amber-specific suppressors, classes IX and X; and suppressors which act upon both nonsense codons, classes VI-VIII.

The nonsense suppressors studied are listed in Table 2. New designations are

TABLE 2

Centromere-linkage data for the nonsense suppressors

Class	Suppressor	Synonyms and references	First division segregation asci	Second division segregation asci	Percent second division segregation
I	S_1	S_a, S_c (2, 4)	36	66	65
I	S_2	S_b (2), S_k (1)	37	79	68
I	S_3	S_l (1)	181	70	68
I	S_4	S_m (1), S_i (6)	47	62	57
I	S_5	S_n (1)	45	114	72
I	S_6	S_o (1)	68	91	57
I	S_7	S_p (1)	116	122	51
I	S_8	S_q (1)	26	60	70
II	S_{11}	S_d (3, 4), S (5)	308	20	6
III	S_{15}		16	18	53
IV	S_{20}	S_e (6)	16	39	71
IV	S_{21}		9	33	79
IV?	S_{25}	S_s (1)	25	40	62
V	S_{30}		42	5	10
V	S_{31}		10	6	38
VI	S_{35}	S_f (4)	14	35	72
VII	S_{40}		24	44	65
VIII	S_{45}	S_k (6)	44	80	65
IX	S_{50}	S_h (4)	35	72	67
IX	S_{51}		36	0	0
IX	S_{11A}		45	6	12
X	S_{60}		3	3	50

References: (1) GILMORE 1967; (2) HAWTHORNE and MORTIMER 1963; (3) MANNEY 1964; (4) MORTIMER and HAWTHORNE 1966; (5) OSHIMA and OSHIMA 1966; (6) HAWTHORNE, personal communications.

used to provide a more systematic nomenclature based on the above classification. Suppressors belonging to a given class are numbered sequentially, and intervals in the listing are provided to allow for new suppressors in these classes. Where the suppressor has been described in the literature, the synonyms and references are given.

Centromere linkage data are also provided in Table 2. The percent second division segregation of the different suppressors was determined by tetrad analysis. The first division spore array in each ascus was deduced from the segregation of known centromere-linked genes included in the crosses for this purpose. A second division segregation frequency significantly less than 66.7 percent is indicative of centromere linkage.

The suppressors showing centromere linkage S_3, S_4, S_6, S_7, S_{11}, S_{15}, S_{30}, S_{31}, S_{51}, and S_{11A} were tested for linkage with other centromere-linked genes. The data for cases in which linkage was found are given in Table 3. Evidence for linkage between some other suppressors and marker genes are also presented in Table 3.

The above search for linkage between the centromere-linked suppressors and the centromere markers of the established chromosomes resulted in the identifi-

TABLE 3

Tetrad data indicating gene to gene linkage

Linkage group	Gene pair	PD	NPD	T
II	S_{45}–ly_2	55	1	48
II	S_{45}–ty_1	76	2	27
III	S_{21}–hi_4	9	2	33
VI	S_{11}–hi_2	65	1	41
VI	S_{11}–S_{11A}	35	0	0
VI	S_6–hi_2	18	0	5
VI	S_6–met_{10}	16	0	0
VIII	S_{31}–ar_4	6	0	2
X	S_4–is_3	27	0	11
X	S_7–is_3	32	0	22
X	S_4–S_7	47	11	121*
X	S_{30}–is_3	14	0	3
X	S_{51}–is_3	17	0	2
XV	S_3–p_{17}	15	0	7
XVI	S_{15}–ty_7	28	0	6
Frag. 1	S_{20}–ad_2	15	2	35
Frag. 1	S_{20}–hi_8	6	3	16
Frag. 2	S_{35}–ty_2	65	1	37
Frag. 2	S_{35}–thr_2	46	0	4
Frag. 2	S_{40}–ty_6	52	0	15
Frag. 5	S_{25}–met_1	55	0	8
Frag. 5	S_{25}–MA_4	9	0	14
Frag. 6	S_{50}–th_1	41	0	3
Frag. 6	S_{50}–py_2	44	0	9

* GILMORE (1966).

cation of two new chromosomes, XV and XVI. The centromere of chromosome XV is marked by S_3 and p_{17} (petite) located on opposing arms. Although there are only 22 tetrads from the S_3 by p_{17} cross, all 15 parental ditype asci were first division segregation; of the 7 tetratype asci, 3 were first division for S_3, 2 were first division for p_{17}, and 2 were second division for both markers. Both S_3 and p_{17} have been checked against the centromere markers of chromosomes I to XIV and no linkage was observed (Table 4). The centromere marker that identifies chromosome XVI is ty_7 which shows 52% second division segregation; S_{15} is located on the same arm. The sequence *centromere*-ty_7-S_{15} was tentatively chosen upon inspection of 6 tetrads that were tetratype for these two genes: 3 were first division for ty_7, 1 was first division for S_{15}, and 2 were second division for both markers. The tetrad data that exclude linkage of ty_7 with the centromere markers of the other chromosomes are given in Table 4.

Sixteen nonsense suppressors have been placed on the linkage maps (Figure 1). Other than the inclusion of the suppressor loci and the centromere markers for chromosomes XV and XVI, p_{17} and ty_7, the maps are essentially those of our previous publication (MORTIMER and HAWTHORNE 1966). The gene ol_1 (oleic-acid requirement) which was located previously on chromosome VII (RESNICK

TABLE 4

Ascus-type ratios (PD:NPD:T) for new centromere markers in combination with centromere markers on the established linkage groups

Chromosome	Centromere marker	New centromere markers								
		p_{17}			S_3			ty_7		
I	ad_1	9	10	11	28	15	18	7	11	12
II	ga_1	19	27	19	24	21	16	57	60	107
III	a	30	25	47	22	19	25	64	50	156
IV	tr_1	22	41	29	29	33	15	111	117	244
V	ur_3	10	8	13	4	6	5	5	6	20
VI	S_{11}	24	13	20	15	12	14	14	16	30
VII	le_1	..	..	..	27	26	21	..	..	..
	tr_5	7	12	19	..	..	..	31	31	98
	ad_6	6	8	16	..	..	..	39	26	106
VIII	ar_4	1	1	5	17	17	19	7	12	40
IX	ly_1	9	14	26	18	28	62	32	36	106
X	is_3	18	15	27	21	11	13	11	9	18
XI	met_{14}	23	19	24	20	10	13	9	16	23
XII	thr_5	16	12	31	14	10	11	7	7	27
XIII	ly_7	7	7	16	2	4	12	6	7	17
XIV	p_8	1	3	3	10	10	9	8	7	28
XV	p_{17}	..	..	..	15	0	7	10	9	34
XVI	ty_7	10	9	34	9	8	24	..	..	..

and MORTIMER 1966) also is presented on the map. One ambiguity in the earlier map regarding the order of closely linked genes has been resolved. On chromosome II, p_{11} is now located between ty_1 and met_8; the tetrad anaylsis of a three point cross gave: 52(PD), 0(NPD), and 12(T) asci for ty_1–p_{11}, 41(PD), 0(NPD), and 17(T) asci for p_{11}–met_8, and 36(PD), 1(NPD), and 33(T) asci for ty_1–met_8. However, there are several new cases of ambiguity in gene order. On chromosome VI, S_6 is closely linked to met_{10} with no crossovers obtained in 16 tetrads analyzed. On chromosome X, S_{51} showed first division segregation in all 36 tetrads analyzed, so its order with respect to the centromere and is_3 is not known. On fragment 1, S_{20} has not been mapped against ac_4. It has been placed further from ad_2 than ac_4 on the basis of linkage data from separate analyses. On fragment 2, S_{40} has not been checked against thr_2. It has been tentatively placed proximal to $ty_{2,4,6}$ on the basis of its lower second division segregation frequency. All the other sequences depicted were chosen to minimize the number of multiple exchanges.

DISCUSSION

With so many different nonsense suppressors in yeast, a phenotypic characterization of a given suppressor isolate is a necessary first step in its identification. This involves testing the action of the suppressor upon a set of suppressible alleles. To make this classification useful to other researchers, we have used suppressible alleles that have been widely circulated: ad_{2-1}, ad_{6-3}, hi_{4-1}, is_{1-1},

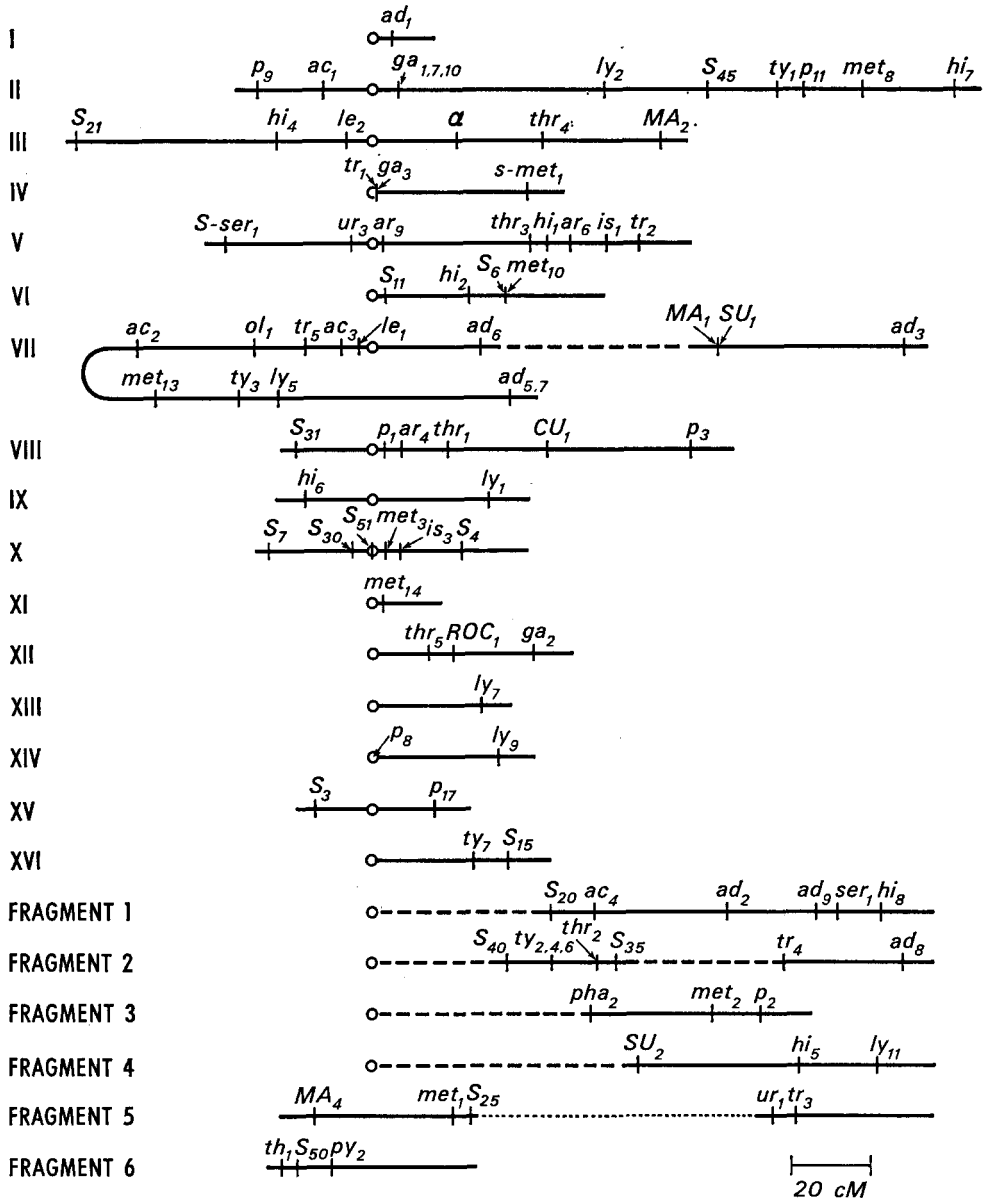

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FIGURE 1.—Genetic map of Saccharomyces showing location of sixteen nonsense suppressors.

le$_{2-1}$, *ly*$_{1-1}$, *ly*$_{2-1}$, *tr*$_{1-1}$, *ty*$_{1-1}$ and *ur*$_{4-1}$. Although this scheme (Table 1) is presented mainly as a tool, it is hoped that it may reflect physiological similarities for suppressors within a given class and differences for those in different classes.

The classification of suppressors presented in this study is dependent upon the

rather arbitrary choice of alleles. Only 3 amber mutants were included in the set of 18 alleles. It is likely that as additional amber mutants are utilized, more classes of amber suppressors will be distinguished. There is also the possibility that additional classes of ochre-specific suppressors will be discovered. GILMORE (1967) has presented a suppressor classification scheme that includes 8 classes based on the patterns of suppression of 5 ochre alleles. Class I in both classification schemes includes the same set of suppressors. However, no other correspondence in numbering of classes has been found.

Another group of allele-specific locus-nonspecific suppressors in *Saccharomyces cerevisiae* has been identified by INGE-VECHTOMOV (1965, 1966). These suppressors were classified as dominant, semi-dominant, and recessive and were located at at least six, four, and two loci, respectively. Unfortunately, these suppressors have not yet been tested for allelism with the suppressors S_1 to S_{60}.

A centromere-linked suppressor isolated by OSHIMA and OSHIMA (1966) had been proposed as a marker to identify a new chromosome. However, we have identified this suppressor (obtained through the courtesy of Y. OSHIMA) to be allelic with S_{11} which has been located near the centromere of chromosome VI (MORTIMER and HAWTHORNE 1966).

The suppressible alleles have the properties of nonsense mutants and it is proposed, as already demonstrated for the corresponding bacterial system, that the nonsense suppressor mutations in yeast occur in genes that transcribe transfer RNA. A mutational event in the triplet that corresponds to the anticodon can result in a tRNA that reads nonsense instead of, or in addition to, its normal codon. The genes that transcribe tyrosyl-tRNA, in particular, can mutate by single base changes to ochre-, ochre-amber-, or amber-specific suppressors. Thus one would predict alleles at certain suppressor loci that are characterized by different action spectra. One such example has been obtained in this study; an amber suppressor in class IX was found to be allelic with an ochre suppressor, S_{11}, in class II. In this case the designation assigned this suppressor was S_{11A} rather than one corresponding to its phenotype.

We are indebted to DR. R. A. GILMORE for making available to us his series of class I suppressors which were used for allelism tests with our isolates.

SUMMARY

Linkage studies with nonsense suppressors, undertaken as an adjunct to their description, have resulted in the location of 16 of the 22 investigated on 11 different linkage groups. Two new chromosomes have been identified during these studies. This provides evidence for at least 16 chromosomes in the haploid genome of *Saccharomyces cerevisiae*.

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