# Isolation and Characterization of Omnipotent Suppressors in the Yeast *Saccharomyces cerevisiae*

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ABSTRACT

Approximately 290 omnipotent suppressors, which enhance translational misreading, were isolated in strains of the yeast *Saccharomyces cerevisiae* containing the $\psi^+$ extrachromosomal determinant. The suppressors could be assigned to 8 classes by their pattern of suppression of five nutritional markers. The suppressors were further distinguished by differences in growth on paromomycin medium, hypertonic medium, low temperatures (10°), nonfermentable carbon sources, α-aminoadipic acid medium, and by their dominance and recessiveness. Genetic analysis of 12 representative suppressors resulted in the assignment of these suppressors to 6 different loci, including the three previously described loci *SUP35* (chromosome *IV*), *SUP45* (chromosome *II*) and *SUP46* (chromosome *II*), as well as three new loci *SUP42* (chromosome *IV*), *SUP43* (chromosome *XV*) and *SUP44* (chromosome *VII*). Suppressors belonging to the same locus had a wide range of different phenotypes. Differences between alleles of the same locus and similarities between alleles of different loci suggest that the omnipotent suppressors encode proteins that effect different functions and that altered forms of each of the proteins can effect the same function.

THE omnipotent suppressors of *Saccharomyces cerevisiae* suppress certain UAA, UAG and UGA nonsense mutations, as well as certain frameshift and other types of mutations (INGE-VECHTOMOV and ANDRIANOVA 1970; HAWTHORNE and LEUPOLD 1974; GERLACH 1975; CHATTOO *et al.*, 1979a; CULBERTSON, GABER and CUMMINS 1982). In addition to suppression, the omnipotent suppressors exhibit a wide range of pleiotropic effects, including, cycloheximide dependency, respiratory deficiency, sensitivity to high and low temperatures, high osmotic pressure and aminoglycoside antibiotics (GERLACH 1975; MASUREKAR *et al.* 1981; SURGUCHOV *et al.* 1984). Recessive omnipotent suppressors have been isolated at several different loci, including; *SUP35* (also denoted *SUP2*, *SUF12*, *SUPP* and *SAL3*), *SUP45* (also denoted *SUP1*, *SUPQ* and *SAL4*) (INGE-VECHTOMOV and ANDRIANOVA 1970; HAWTHORNE and LEUPOLD 1974; GERLACH 1975; COX 1977; CULBERTSON, GABER and CUMMINS 1982), *sup111*, *sup112* and *sup113* (ONO *et al.* 1982, 1984). Dominant suppressors have been isolated at the single locus, *SUP46* (ONO, STEWART and SHERMAN 1981). The suppression by omnipotent suppressors is due to increased levels of misreading, as measured by *in vitro* translation assays (MASUREKAR *et al.* 1981; SURGUCHOV *et al.* 1984; EUSTICE *et al.* 1986). In addition, mutations in the *TEF2* gene, which codes for elongation factor-1α (SANDBAKEN and CULBERTSON, 1988), and mutations in the *CRL* genes (cycloheximide resistant temperature sensitive lethal), which resemble both *gcn* (general control of amino acid biosynthesis) and omnipotent suppressor mutations, also cause increased levels of translational misreading (MCCUSKER and HABER 1988a, b).

We have isolated an extensive number of omnipotent suppressors, characterized them phenotypically and genetically mapped 12 of the suppressors in an attempt to identify new suppressor loci. In order to obtain a broader range of suppressors at new loci, suppressors were isolated in strains containing the $\psi^+$ cytoplasmic determinant. The $\psi^+$ factor enhances the activity of UAA and certain frameshift suppressors (COX 1965, 1971; CULBERTSON *et al.* 1977; LIEBMAN and SHERMAN 1979) as well as enhancing the phenotypic suppression of nonsense mutations by paromomycin (PALMER, WILHELM and SHERMAN 1979a). Similarly, ALL-ROBYN *et al.* (1990) recently isolated omnipotent suppressors in $\eta^+$ (eta$^+$) strains and showed that $\eta^+$ increased the level of suppression of certain nonsense mutations by some omnipotent suppressor alleles. Our collection contains dominant or semidominant alleles of the previously described suppressors, *SUP35*, *SUP45* and *SUP46*, plus alleles of three previously unidentified suppressors, which we have named *SUP42*, *SUP43* and *SUP44*. These new suppressors behave similarly to *SUP35*, *SUP45* and *SUP46*.

## MATERIALS AND METHODS

**Genetic methods:** The yeast strains used in this study are described in Table 1. Since the majority of our suppressor

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**TABLE 1**

**Yeast strains**

| Strain | Genotype | Source |
|---|---|---|
| D1142-1A | *ψ⁺ MATa lys2-187 met8-1 leu2-1 aro7-1 his4-166 ura3-1 cyc1-72* | This study |
| D1150-1D | *ψ⁺ MATα lys2-187 met8-1 leu2-1 aro7-1 his4-166 can1-100 trp1-289 cyc1-72* | This study |
| B-7316 | *ψ⁺ MATa SUP35 lys2-187 met8-1 leu2-1 aro7-1 his4-166 ura3-1 cyc1-72* | This study |
| B-7331 | *ψ⁺ MATα SUP35 lys2-187 met8-1 leu2-1 aro7-1 his4-166 can1-100 trp1-289 cyc1-72* | This study |
| B-7329 | *ψ⁺ MATα SUP42 lys2-187 met8-1 leu2-1 aro7-1 his4-166 can1-100 trp1-289 cyc1-72* | This study |
| B-7332 | *ψ⁺ MATα SUP42 lys2-187 met8-1 leu2-1 aro7-1 his4-166 can1-100 trp1-289 cyc1-72* | This study |
| B-7317 | *ψ⁺ MATa SUP43 lys2-187 met9-1 leu2-1 aro7-1 his4-166 ura3-1 cyc1-72* | This study |
| B-7334 | *ψ⁺ MATα SUP43 lys2-187 met8-1 leu2-1 aro7-1 his4-166 can1-100 trp1-289 cyc1-72* | This study |
| B-7319 | *ψ⁺ MATa SUP44 lys2-187 met8-1 leu2-1 aro7-1 his4-166 ura3-1 cyc1-72* | This study |
| B-7323 | *ψ⁺ MATa SUP45 lys2-187 met8-1 leu2-1 aro7-1 his4-166 ura3-1 cyc1-72* | This study |
| B-7324 | *ψ⁺ MATa SUP46 lys2-187 met8-1 leu2-1 aro7-1 his4-166 ura3-1 cyc1-72* | This study |
| B-7325 | *ψ⁺ MATa SUP46 lys2-187 met8-1 leu2-1 aro7-1 his4-166 ura3-1 cyc1-72* | This study |
| B-7326 | *ψ⁺ MATa SUP46 lys2-187 met8-1 leu2-1 aro7-1 his4-166 ura3-1 cys1-72* | This study |
| B-7327 | *ψ⁺ MATa SUP46 lys2-187 met8-1 leu2-1 aro7-1 his4-166 ura3-1 cyc1-72* | This study |
| B-7924 | *MATa his7 ura4 ade8 aro1C trp4 rna3 lys2 leu2-3 ade5* Pet⁻ | This study |
| B-7925 | *MATα his7 ura4 ade8 aro1C trp4 rna3 lys2 leu2-3 arg4* Pet⁻ | This study |
| B-7926 | *MATα cdc28-1 glc1 lys2-187 tyr1 cyc1-72* | This study |
| B-7927 | *MATα aro7-1 trp1-289 lys2-187 cdc37-1* | This study |
| B-7928 | *MATα ade5 lys5 met13 cyh2 trp5 leu1* | This study |
| B-7929 | *MATa ade5 lys5 met13 cyh2 trp5 leu1* | This study |
| SR672-1 | *MATa cdc37-1 ura1* | Stock center |
| K381-15C | *MATa ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1* | S. KLAPHOLZ |
| K381-10A | *MATα ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1* | S. KLAPHOLZ |
| K382-23A | *MATa ura3 can1 cyh2 ade2 his7 hom3 spo11* | S. KLAPHOLZ |
| K382-19D | *MATa ura3 can1 cyh2 ade2 his7 hom3 tyr1 spo11* | S. KLAPHOLZ |
| SL818-2C | *MATa SUP39 met8-1 leu2-1 aro7-1 trp1-1 ade3-26 ilv1-1 his5-2* Lys⁻ | S. LIEBMAN |

isolates at the six different loci described here were either dominant or semidominant for suppression, we have used the upper case designation for mutant alleles of *SUP35*, *SUP42*, *SUP43*, *SUP44*, *SUP45* and *SUP46* throughout this paper. Media and genetic procedures including crosses, sporulation, dissection, tetrad analysis and scoring nutritional markers have been described (SHERMAN, FINK and HICKS 1987). Suppressors were selected on various omission media, each lacking a different combination of two amino acids required by the strains. Suppression of suppressible markers was tested by spotting on the appropriate omission medium and incubating at 30° for 5 days. Sensitivity of the suppressors to various agents or conditions was measured on the following media: α-aminoadipate medium described by CHATTOO *et al.* (1979b); high osmotic media containing YPD (1% Bacto-yeast extract, 2% Bacto-peptone and 2% dextrose) and 1.0 to 1.5 M ethylene glycol; cold temperatures on YPD medium incubated at 4–10°; and paromomycin (a gift from Warner Lambert) media containing YPD and 0.5 to 5.0 mg/ml paromomycin. The parental strains D1142-1A and D1150-1D grew at concentrations of 2.5 mg/ml paromomycin but were inhibited at 5.0 mg/ml. In contrast, the growth of hypersensitive suppressors was inhibited at 0.5 to 1.0 mg/ml and resistant suppressors grew at 5.0 mg/ml.

**Chromosomal assignment of suppressors:** The chromosomal assignment of some of the dominant paromomycin-sensitive suppressors was achieved using the benomyl method described by WOOD (1982b). Diploid strains were treated with benomyl (a gift from E. I. Dupont de Nemours) and samples were plated on YPD medium containing 2.5 mg/ml paromomycin. Paromomycin resistant colonies were isolated and tested on omission media for the expression of auxotrophic markers.

## RESULTS

**Isolation and characterization of omnipotent suppressors:** A total of 294 omnipotent suppressors were isolated by selecting for revertants of strains D1142-1A and D1150-1D (Table 1) on nine types of omission media, each lacking one of the following combinations of amino acids: lysine and methionine; lysine and tyrosine; lysine and leucine; lysine and histidine; methionine and leucine; methionine and histidine; tyrosine and leucine; tyrosine and histidine; leucine and histidine. These conditions selected for the isolation of omnipotent suppressors, since revertants must concomitantly suppress two different types of the following mutations: *met8-1* (UAG), *aro7-1* (UAG); *leu2-1* (UAA); *his4-166* (UGA) and *lys2-187* [not a nonsense mutation (CHATTOO *et al.* 1979a)]. Revertants were isolated as spontaneous isolates (50%) or induced using either ultraviolet light (500 ergs $mm^{-2}$) (20%) or X-ray (15 kR) treatments (30%). The suppressors were subcloned, then tested on omission media for their ability to suppress all five suppressible markers. While all isolates suppressed *lys2-187* and *met8-1*, they differed in their ability to suppress the other three mutations and could be divided into eight classes based on differences in their pattern of suppression (Table 2). The majority of the isolates suppressed either all five suppressible markers or all the markers except for *his4-166* (classes I and II). The remainder

**TABLE 2**

**Phenotypic characterization of omnipotent suppressors**

| Class | Suppression[a] | | | | | | | Dominance[c] | | | Paromomycin[d] | | | | Cold[g] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *lys2-187* | *met8-1* (UAG) | *leu2-1* (UAA) | *aro7-1* (UAG) | *his4-166* (UGA) | No. | α-aa[b] (−) | Dom | Sd | Rec | sens. | res. | Osm. sens.[e] | Resp. def.[f] | (−) | (+) |
| I | + | + | + | + | + | 96 | 20 | 39 | 55 | 2 | 30 | 23 | 22 | 2 | 11 | 9 |
| II | + | + | + | + | − | 116 | 13 | 53 | 43 | 20 | 28 | 27 | 25 | 1 | 20 | 6 |
| III | + | + | + | − | − | 29 | 0 | 21 | 6 | 2 | 13 | 4 | 11 | 9 | 9 | 1 |
| IV | + | + | − | − | − | 18 | 0 | 15 | 1 | 2 | 1 | 2 | 3 | 0 | 0 | 0 |
| V | + | + | − | + | − | 18 | 4 | 12 | 6 | 0 | 2 | 9 | 2 | 1 | 5 | 1 |
| VI | + | + | + | − | + | 12 | 1 | 8 | 1 | 1 | 2 | 3 | 3 | 2 | 2 | 0 |
| VII | + | + | − | − | + | 4 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| VIII | + | + | − | + | + | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Totals | | | | | | 294 | 38 | 150 | 117 | 27 | 76 | 68 | 66 | 15 | 47 | 17 |

[a] Suppression of *lys2-187*, *met8-1* (UAG), *leu2-1* (UAA), *aro7-1* (UAG) and *his4-166* (UGA) was measured by spotting isolates on the appropriate omission media and incubating for 5 days at 30° (+ = suppression, − = no suppression).

[b] Isolates unable to utilize α-aminoadipate as sole nitrogen source.

[c] Dominance and recessiveness of suppression of *lys2-187*, *met8-1*, *leu2-1*, *aro7-1* and *his4-166*: dominant (Dom), semidominant (Sd), recessive (Rec).

[d] Isolates hypersensitive (sens.) (0.5–1.0 mg/ml) or resistant (res.) (5.0 mg/ml) to paromomycin.

[e] Isolates sensitive to high osmotic pressure (1.0–1.5 M ethylene glycol).

[f] Isolates which are respiratory deficient (measured as the inability to utilize glycerol as sole carbon source).

[g] Isolates with diminished (−) or enhanced (+) growth at cold temperatures (5–10°).

of the isolates were grouped into 6 classes which differed in their ability to suppress *leu2-1*, *aro7-1* and *his4-166*. Analysis of the number of isolates in each class allowed us to rank the suppressible markers in the following order, from most suppressible to least suppressible: *lys2-187* = *met8-1* > *leu2-1* > *aro7-1* > *his4-166*.

The different patterns of suppression reflect either different levels of suppression or different amino acid replacements. Variations in the levels of suppression of the *lys2-187* mutation can be tested on a medium supplemented with lysine and containing α-aminoadipate as the principal nitrogen source. In contrast to *LYS2*$^+$ normal strains, *lys2*$^-$ strains are able to utilize α-aminoadipate as a principal nitrogen source (CHATTOO *et al.* 1979b). Apparently the anabolism of high levels of α-aminoadipate through the biosynthetic pathway of lysine causes the accumulation of a toxic intermediate and *lys2* mutants block the formation of this intermediate (ZARET and SHERMAN 1985). Furthermore, the degree of growth on α-aminoadipate medium is related to the degree of the *lys2* block. In contrast to the *lys2-187* parental strain, 38 of the 294 suppressors were unable to grow on α-aminoadipate medium, suggesting that the levels of suppression were higher in these strains. However, the isolates which suppress the *lys2-187* mutation best, do not all show a similar high level of suppression of the other suppressible mutations since about half of the α-aminoadipate sensitive isolates do not suppress one or more of the suppressible markers: *leu2-1*, *aro7-1* and *his4-166* (see Table 2). Nevertheless the efficiency of omnipotent suppression is specific for each of the suppressible mutations.

The isolates were tested for dominance or recessiveness of suppression by crossing them to either D1142-1A or D1150-1D and checking the suppression spectrum of the diploids. Most isolates were either dominant (150) or semidominant (117) for suppression with only a small number (27) being recessive (Table 2). Isolates were classed as semidominant if they suppressed *lys2-187* and *met8-1* dominantly but suppressed one or more of *leu2-1*, *aro7-1* and *his4-166* recessively. There was no correlation between the class of suppression spectrum of the isolates and their dominance or recessiveness.

As shown in Table 2, the isolates exhibited a variety of pleiotropic effects in addition to suppression. Due to the large number of mutants isolated in this study, most strains were characterized without any genetic backcrossing. These strains may contain secondary mutations which may account for some of the presumably pleiotropic effects that were uncovered. However, in the 12 strains which were mapped by tetrad analysis (Table 4), those pleiotropic phenotypes present in each strain segregated with the suppressor. In addition, other workers have reported similar pleiotropic effects for various omnipotent suppressors (GERLACH 1975; MASUREKAR *et al.* 1981; SURGUCHOV *et al.* 1984).

About 25% of the isolates were hypersensitive to the aminoglycoside, paromomycin. Paromomycin and other aminoglycosides increase the level of misreading in *in vitro* translation assays using yeast ribosomes (PALMER, WILHELM and SHERMAN, 1979b; SINGH, URSIC and DAVIES 1979) and can also phenotypically suppress some nonsense and certain other mutations

suppressible by omnipotent suppressors (CHATTOO *et al.* 1979a; PALMER, WILHELM and SHERMAN, 1979b; SINGH, URSIC and DAVIES 1979). Paromomycin hypersensitive alleles of *SUP35*, *SUP45* and *SUP46* have been isolated previously (MASUREKAR *et al.* 1981; MIRONOVA *et al.* 1982). However, in addition to the paromomycin hypersensitive isolates, we also uncovered an equal number of isolates which were resistant to paromomycin. Whereas the normal strains grew and were inhibited on media containing 2.5 mg/ml and 5.0 mg/ml of paromomycin respectively, the hypersensitive isolates were unable to grow at paromomycin concentrations of 0.5 to 1.0 mg/ml, and resistant isolates grew at levels of 5.0 mg/ml.

Alleles of *SUP35* and *SUP45* have also been described which are respiratory deficient, and sensitive to high osmotic pressure, and to high or low temperatures (GERLACH 1975; SURGUCHOV *et al.* 1984). We also uncovered a small number of isolates that were respiratory deficient, as determined by their inability to utilize glycerol as a carbon source. In addition, some isolates were sensitive to high osmotic pressure (1.0–1.5 M ethylene glycol) and cold temperatures (5–10°). None of our isolates were sensitive to growth at high temperatures; however, a small number grew better at 5–10° than the original strains.

There was no relationship between the pattern of suppression and the different phenotype characteristics. Isolates ranged from those which exhibited no other phenotype besides suppression to isolates with any number of different combinations of the phenotypes shown in Table 2.

**Chromosomal assignment of suppressors:** The suppressors could not be unambiguously assigned to complementation groups by testing heterozygous diploids for their suppression capabilities, since even the most recessive mutations exhibited some degree of dominance. Therefore, isolates with the easily selectable dominant phenotype of paromomycin hypersensitivity were chosen and subdivided into groups using the rapid chromosomal assignment method described by WOOD (1982b). This method relies on mitotic chromosome loss induced by benomyl to uncover recessive auxotrophic markers. Isolates were crossed to mapping strains (B-7924 and B-7925) containing auxotrophic markers on chromosomes II (*his7*) and IV (*trp4*), as well as other chromosomes. Diploids were heterozygous for a suppressor mutation and retained their dominant phenotype of hypersensitivity to paromomycin. These diploids were treated with benomyl, then grown on medium containing paromomycin (2.5 mg/ml) to select for strains which had lost the chromosome carrying the suppressor mutation. Loss of the suppressor mutation results in loss of the paromomycin hypersensitivity of the diploid since the recessive wild-type gene ($sup^+$) is now homozygous. Recessive auxotrophic mutations on the same chromosome will also be expressed if the suppressor-bearing chromosome is lost. Suppressors were assigned to a chromosome by testing diploid isolates which were able to grow at paromomycin concentrations of 2.5 mg/ml for the concomitant expression of any auxotrophic mutations. A total of 22 of the suppressors were assigned to 4 different groups using this method. A number of the suppressors expressed either the *his7* (five suppressors) or *trp4* (six suppressors) mutation and were assigned to chromosome *II* and *IV*, respectively. This result was expected because both *SUP45* and *SUP46* map on chromosome *II* and *SUP35* maps on chromosome *IV*. However, the rest of the suppressors were not assigned to either chromosome *II* or *IV* but could be divided into two more groups on the basis of their chromosomal assignment. These two groups of suppressors appeared to be two new suppressors. One group of seven suppressors was erroneously assigned to chromosome *XII* since they expressed the *ura4* mutation and the other group of four suppressors could not be assigned to any chromosome using the benomyl procedure and various mapping strains. The two new suppressors were denoted *SUP43* (unassigned) and *SUP44* (chromosome *XII*). However, our assignment of *SUP44* to chromosome *XII* by the benomyl mapping method was incorrect. Meiotic mapping showed no linkage between *SUP44* and various genes on chromosome *XII* (data not shown); however, there was linkage between the *SUP44* allele in B-7319 and genes on chromosome *VII* (Table 3). The inaccurate assignment of *SUP44* to chromosome *XII* may have been due to the high frequency of multiple chromosome losses induced by benomyl (WOOD 1982a).

**Meiotic mapping of suppressors:** Twelve suppressors were mapped by crossing to several strains and assaying for the suppressors by either paromomycin sensitivity or by suppression of suppressible mutations. The genetic mapping of these 12 suppressors defined six different suppressor loci (Table 3). Of the five isolates assigned to chromosome II, one (B-7323) mapped at the *SUP45* locus (centromere proximal to both *tyr1* and *cdc28*), while four mapped at *SUP46* (centromere distal to *cdc28* and *tyr1*, but closely linked to *tyr1*) (Tables 3 and 4). This mapping data shows that the order of genes on chromosome *II* is *SUP45*, *cdc28*, *tyr1* and *SUP46*. This corresponds well with previously reported mapping data for *SUP45* and *SUP46* (ONO, STEWART and SHERMAN 1981; ONO *et al.* 1984). Isolates assigned to chromosome *IV* mapped at two different loci; two of the suppressors mapped at *SUP35* (linked to *cdc37*) and two mapped at a new locus unlinked to *cdc37* but linked instead to *trp1*. This new suppressor was denoted *SUP42*.

Two of the *SUP43* isolates, which could not be

**TABLE 3**

**Meiotic mapping of omnipotent suppressors**

| Strains | Suppressor[a] | No. of alleles mapped[b] | Chromosome[c] | Gene pair | Tetrads[d] P | N | T | cM[e] |
|---|---|---|---|---|---|---|---|---|
| B-7323 | *SUP45* | 1 | II | *SUP45-cdc28* | 21 | 0 | 11 | 17 |
| | | | | *SUP45-tyr1* | 19 | 1 | 9 | 26 |
| | | | | *cdc28-tyr1* | 33 | 0 | 7 | 9 |
| B-7327 | *SUP46* | 4 | II | *SUP46-tyr1* | 40 | 0 | 3 | 4 |
| | | | | *SUP46-cdc28* | 34 | 0 | 14 | 15 |
| | | | | *cdc28-tyr1* | 36 | 0 | 9 | 10 |
| B-7316 | *SUP35* | 2 | IV | *SUP35-cdc37* | 10 | 0 | 6 | 18 |
| | | | | *SUP35-trp1* | 5 | 5 | 11 | |
| | | | | *cdc37-trp1* | 4 | 7 | 9 | |
| B-7332 | *SUP42* | 2 | IV | *SUP42-trp1* | 14 | 0 | 9 | 20 |
| | | | | *SUP42-cdc37* | 3 | 5 | 15 | |
| | | | | *cdc37-trp1* | 7 | 7 | 13 | |
| B-7319 | *SUP44* | 1 | VII | *SUP44-met13* | 22 | 0 | 3 | 6 |
| | | | | *SUP44-cyh2* | 23 | 1 | 6 | 20 |
| | | | | *met13-cyh2* | 20 | 1 | 6 | 23 |
| B-7334 | *SUP43* | 2 | XV | *SUP43-pet17* | 6 | 0 | 18 | 37 |
| | | | | *SUP43-ade2* | 8 | 7 | 30 | |

Suppressors were crossed to the following strains for tetrad analysis: *SUP45* and *SUP46* to B-7926; *SUP35* and *SUP42* to B-7327 and SR672-1; *SUP44* to B-7328 and B-7329; and *SUP43* to K381-15C, K381-10A, K382-19D and K382-23A.

[a] The suppressor contained in each of the strains mapped.

[b] The number of alleles which we have mapped to each of the suppressor loci.

[c] The chromosome on which each of the suppressor loci map.

[d] The results of our tetrad analyses (P = parental, N = nonparental and T = tetratype).

[e] The distance ($x$) in centiMorgans between various genes, calculated by

$$x = \frac{100}{2}\left[\frac{T + 6N}{P + N + T}\right].$$

assigned to a chromosome by the benomyl mapping method, were found to map on chromosome *XV*. Tetrad analysis showed that *SUP43* was linked to *pet17* but not to *ade2*, which is centromere distal to *pet17*. This locates *SUP43* centromere proximal to *pet17*.

A suppressor isolated by ALL-ROBYN *et al.* (1990) is allelic to our *SUP44* isolate, B-7319, since crosses between B-7319 and their suppressor yielded only parental ditype tetrads (16/16) (ALL-ROBYN *et al.* 1990). These authors assigned their *SUP44* isolate to chromosome VII by OFAGE mapping of a cloned *SUP44* gene. Our *SUP44* isolate (B-7319) also mapped on chromosome *VII*, between *met13* and *cyh2*, but closely linked to *met13* (Table 3). This corresponds to the map position of a previously isolated suppressor, *SUP38* (B. ONO, unpublished results) (MORTIMER and SCHILD 1985), suggesting that *SUP44* and *SUP38* may be allelic.

Another unmapped suppressor, *SUP39*, isolated by ALL-ROBYN *et al.* (1990) does not map at *SUP35*, *SUP43*, *SUP44*, *SUP45* or *SUP46* (ALL-ROBYN *et al.* 1990). B-7329 was crossed to SL818-2C to test for linkage between *SUP42* and *SUP39*. Tetrad analysis of this cross showed that these two suppressors are not linked (2P:9N:14T).

Our mapping data shows that the omnipotent suppressor loci *SUP42*, *SUP43* and *SUP44* do not correspond to the *SUP35*, *SUP39*, *SUP45* or *SUP46* loci. In addition, they do not map near *sup111*, *sup112* or *sup113*, which map on chromosome *VIII*, the right arm of chromosome *VII* and chromosome *XIII*, respectively (ONO *et al.* 1986). *SUP42* and *SUP43* are new suppressor loci while *SUP44* maps close to and may be allelic to *SUP38* (B. ONO, unpublished data) (MORTIMER and SCHILD 1985).

The phenotypes of 12 suppressors, mapped to 6 different loci are summarized in Table 4. Different alleles can have very different suppression spectra, suppression efficiencies and various other phenotypes. While these different phenotypic characteristics are allele-specific, they appear to be a common trait of mutants isolated at all six omnipotent suppressor loci described here.

## DISCUSSION

Many different factors are involved in ensuring the accurate translation of mRNA in yeast. Mutations with increased levels of misreading can arise in various genes including the omnipotent suppressor genes. Our search for omnipotent suppressors has expanded the number of genes involved in translational fidelity by three new genes; *sup42*, *sup43* and *sup44*. These new omnipotent suppressor genes do not map at any previously described loci and are located on chromosomes *IV*, *XV* and *VII*, respectively.

In other studies of omnipotent suppressors, recessive alleles of *SUP35* and *SUP45* or dominant alleles of *SUP46* are the predominant or exclusive isolates (INGE-VECHTOMOV and ANDRIANOVA 1970; HAWTHORNE and LEUPOLD 1974; GERLACH 1975; CULBERTSON, GABER and CUMMINS 1982; ONO, STEWART and SHERMAN 1981). The exception is a study by ONO *et al.* (1982) designed to isolate inefficient suppressors in which recessive alleles of *sup111*, *sup112* and *sup113* were isolated. In contrast, our study resulted in mostly dominant and semidominant alleles of *SUP35*, *SUP45*, *SUP46*, *SUP42*, *SUP43* and *SUP44*. The dominance or recessiveness of omnipotent suppressors does not appear to be locus-dependent as previously suggested (ONO *et al.* 1984), since both dominant or semidominant and recessive alleles of *SUP35* and *SUP45* have been isolated. In addition dominant and semidominant alleles of *SUP42*, *SUP43*, *SUP44* and *SUP46* have also been isolated. The differences in the range of suppressor loci isolated in various studies and the predominance of either dominant or recessive alleles is probably due to differences in the initial strains. Some of these differences include the

**TABLE 4**

**Phenotypes of mapped suppressors**

| Strain | Suppressor | Suppression[a] | | | | | Dom.[b] | Pm. sens.[c] | α-aa[d] | Resp. def.[e] | Osm. sens.[f] | Cold[g] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *lys2-187* | *met8-1* | *leu2-1* | *aro7-1* | *his4-166* | | | | | | |
| B-7316 | *SUP35* | + | + | + | + | + | Sd | S | S | + | + | + |
| B-7331 | *SUP35* | + | + | − | − | − | Dom | S | + | + | + | 0 |
| B-7329 | *SUP42* | + | + | + | + | − | Dom | S | + | + | S | 0 |
| B-7332 | *SUP42* | + | + | + | + | − | Sd | S | + | + | + | 0 |
| B-7317 | *SUP43* | + | + | + | + | + | Sd | S | + | + | + | 0 |
| B-7334 | *SUP43* | + | + | + | + | − | Sd | S | + | + | + | 0 |
| B-7319 | *SUP44* | + | + | + | + | + | Dom | S | + | + | + | 0 |
| B-7323 | *SUP45* | + | + | + | + | + | Sd | S | + | + | + | 0 |
| B-7324 | *SUP46* | + | + | + | + | − | Dom | S | + | + | S | − |
| B-7325 | *SUP46* | + | + | + | + | − | Dom | S | S | + | + | − |
| B-7326 | *SUP46* | + | + | + | + | + | Sd | S | S | + | + | − |
| B-7327 | *SUP46* | + | + | + | − | − | Sd | S | + | + | + | − |

[a] Suppression of *lys2-187*, *met8-1* (UAG), *leu2-1* (UAA), *aro7-1* (UAG) and *his4-166* (UGA) was measured by spotting isolates on the appropriate omission media and incubating for 5 days at 30°. (+ = suppression, − = no suppression).
[b] Dominance and recessiveness of suppression: dominant (Dom), semidominant (Sd).
[c] Hypersensitivity to paromomycin at 0.5–1.0 mg/ml.
[d] Suppressors unable to utilize α-aminoadipate as sole nitrogen source (+ = growth, S = no growth).
[e] Suppressors which are respiratory deficient (measured as the inability to utilize glycerol as sole carbon source).
[f] Suppressors which are sensitive to high osmotic pressure (1.0–1.5 M ethylene glycol).
[g] Suppressors with enhanced (+) or diminished (−) growth at 5–10° (0 = growth similar to parental strains).

isolation of suppressors in either $\psi^-$ or $\psi^+$ strains and the use of different suppressible mutations to select for suppressors. Since we used $\psi^+$ strains and 5 suppressible mutations with a range of suppressibility (see Results), we were able to select for a broader range of suppressors with presumably different efficiencies of suppression.

There is a range of allele-specific pleiotropic phenotypes which appears to be common to all omnipotent suppressor loci. However, some of these pleiotropic phenotypes, such as respiration deficiency may be due to secondary mutations in some of the suppressor isolates. The omnipotent suppressor mutants described in this study can exhibit very different suppression spectra and suppression efficiencies, as well as being dominant, semidominant or recessive for suppression. In addition, mutants can be respiration deficient, show sensitivity to high osmotic pressure and can exhibit a range of sensitivities and resistance to aminoglycoside antibiotics and diminished and enhanced growth at cold temperatures. These pleiotropic effects do not appear to be locus-specific since different alleles can exhibit very different phenotypes (Tables 2 and 5). In addition, the different suppression and phenotypic patterns can not be explained simply by differences in the efficiency of suppression. This diversity of phenotypes of different alleles at the six omnipotent suppressor loci described here suggests that these omnipotent suppressors code for proteins that effect different functions and that altered forms of each of the proteins can effect the same function.

In a previous study, we showed that *SUP35*, *SUP44*, *SUP45* and *SUP46* mutants are also similar biochemically. In *in vitro* translation assays, using poly(U) as template and ribosomal components isolated from various mutants, the misreading defect of these suppressors was localized to the 40 S subunit (EUSTICE *et al.* 1986). This result plus other data suggests that the *sup35*, *sup44*, *sup45* and *sup46* genes may code for ribosomal proteins (40S subunit) or for enzymes which modify ribosomal proteins. However, *SUP43* mutants differ from the other suppressors. Ribosomes from two alleles of *SUP43* did not show increased levels of misreading of the poly(U) template *in vitro* (EUSTICE *et al.* 1986). This difference may be due to differences in specificity, or the *sup43* gene may not code for either a ribosomal protein or for an enzyme which modifies a ribosomal protein. *In vitro* translation data for *sup42* mutants is not available since this suppressor was only recently identified.

Recent cloning and sequencing data show that the *sup35* and *sup45* genes have open reading frames which are too large to be ribosomal proteins (BREINING, SURGUCHOV and PEIPERSBERG 1984; HIMMELFARB, MAICAS and FRIESEN 1985; BREINING and PEIPERSBERG 1986; KUSHNIROV *et al.* 1988; WILSON and CULBERTSON, 1988). In addition, the *sup35* gene sequence has some homology to elongation factor 1α (KUSHNIROV *et al.* 1988; WILSON and CULBERTSON 1988). This suggests that these two genes code for translation factors rather than ribosomal proteins. Since the misreading defect of mutants in all these genes is localized to the 40S subunit, these factors appear to be closely associated to this subunit. Addi-

tionally, certain alleles of *SUP35* have a 40S ribosomal protein with an altered electrophoretic mobility on two-dimensional acrylamide gels (EUSTICE *et al.* 1986). As more of the omnipotent suppressor genes are cloned and sequenced, we should gain further insight into the complex mechanism which ensures the accurate translation of mRNA into protein in yeast.

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## ADDENDUM

Another similar study of omnipotent suppressors describes the isolation and mapping of the two suppressors, *SUP138* and *SUP139* (ONO *et al.* 1989). Table 5 summarizes the different omnipotent suppressors and their alleles.

**TABLE 5**

**Omnipotent suppressors and their alleles**

| Omnipotent suppressor | Other names | References |
|---|---|---|
| *SUP35* | *SUP2, SUPP, SUP36, SUF12, SAL3* | INGE-VECHTOMOV and ADRIANOVA (1970), HAWTHORNE and LEUPOLD (1974), GERLACH (1975), COX (1977), CULBERTSON, GABER and CUMMINS (1982), ONO *et al.* (1984) |
| *SUP39* | | ALL-ROBYN *et al.* (1990) |
| *SUP42* | | This study |
| *SUP43* | | This study |
| *SUP44* | *SUP38, SUP138* | This study, ALL-ROBYN *et al.* (1990), ONO *et al.* (1990) |
| *SUP45* | *SUP1, SUPQ, SUP47, SAL4, MOS1* | INGE-VECHTOMOV and ADRIANOVA (1970), HAWTHORNE and LEUPOLD (1974), GERLACH (1975), COX (1977), CULBERTSON, GABER and CUMMINS (1982), ONO *et al.* (1984), GELUGNE and BELL (1988) |
| *SUP46* | | ONO, STEWART and SHERMAN (1981) |
| *sup111* | | ONO *et al.* (1986) |
| *sup112* | | ONO *et al.* (1986) |
| *sup113* | | ONO *et al.* (1986) |
| *SUP139* | | ONO *et al.* (1990) |