The Dominant *PNM2*$^-$ Mutation Which Eliminates the ψ Factor of *Saccharomyces cerevisiae* Is the Result of a Missense Mutation in the *SUP35* Gene

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ABSTRACT

The *PNM2*$^-$ mutation of *Saccharomyces cerevisiae* eliminates the extrachromosomal element ψ. *PNM2* is closely linked to the omnipotent suppressor gene *SUP35* (also previously identified as *SUP2, SUF12, SAL3* and *GST1*). We cloned *PNM2*$^-$ and showed that *PNM2* and *SUP35* are the same gene. We sequenced the *PNM2*$^-$ mutant allele and found a single G → A transition within the N-terminal domain of the protein. We tested the effects of various constructs of *SUP35* and *PNM2*$^-$ on ψ inheritance and on allosuppressor and antisuppressor functions of the gene. We found that the C-terminal domain of *SUP35* protein (SUP35p) could be independently expressed; expression produced dominant antisuppression. Disruption of the N-terminal domain of *PNM2*$^-$ destroyed the ability to eliminate ψ. These results imply that the domains of SUP35p act in an antagonistic manner: the N-terminal domain decreases chain-termination fidelity, while the C-terminal domain imposes fidelity. Two transcripts were observed for *SUP35*, a major band at 2.4 kb and a minor band at 1.3 kb; the minor band corresponds to 3′ sequences only. We propose a model for the function of *SUP35*, in which comparative levels of N- and C-terminal domains of SUP35p at the ribosome modulate translation fidelity.

THE ψ factor of *Saccharomyces cerevisiae* affects translation fidelity by acting to enhance the efficiency of weak tRNA suppressors of nonsense (Cox 1965, 1971; Liebman *et al.* 1975; Liebman and Sherman 1979; Ono *et al.* 1979a,b). ψ also has some effect on certain frameshift suppressors and on phenotypic suppression of nonsense mutations by paromomycin (see review by Cox *et al.* 1988). *In vivo*, ψ is most commonly assayed by its ability to enhance the activity of the serine-inserting ochre suppressor *SUQ5*^{oc}(SUP16), by which phenotype it was first identified; *SUQ5*^{oc} can suppress efficiently only in a [ψ^+] background.

Suppressor tRNA-mediated read-through of nonsense codons in *in vitro* translation experiments can be observed only in ψ^+ cell-free extracts (Tuite *et al.* 1983, 1987). The experiments suggest that [ψ^+] and [ψ^-] strains differ in the presence (in [ψ^-]) or absence (in [ψ^+]) of a protein fidelity factor that loosely binds to ribosomes. The ψ "gene" is not thought to code for that protein, but to control its presence or absence (Cox *et al.* 1988).

ψ is different from other factors that affect translational suppression (except for η, Liebman and All-Robyn 1984) in that it is inherited as an extrachromosomal genetic factor: in a cross between a [ψ^+] strain and a [ψ^-], all spores are [ψ^+]. It appears as if a ψ^+ gene is carried on a plasmid or other extrachromosomal nucleic acid. However, in spite of numerous searches (McCready and McLaughlin 1977; Lund 1982; Tuite *et al.* 1982), no extrachromosomal location has been identified as the carrier of the ψ gene.

At least two nuclear genes, *PNM1* and *PNM2*, are known to affect ψ inheritance (Young and Cox 1971). The *PNM*$^-$ mutations which have been analyzed genetically are dominant. They appear to have two effects: they inactivate ψ^+, and they also appear to block its replication. Thus in a cross between a *PNM1*$^-$ or *PNM2*$^-$ strain and a [ψ^+] strain, the diploid has the [ψ^-] phenotype, but if sporulated immediately will give rise to some [ψ^+] spores. ψ^+ determinants are only diluted out by subsequent cell divisions of the diploid, and eventually sporulation produces only 4:0 ψ^-:ψ^+ tetrads (Young and Cox 1971; McCready *et al.* 1977). Identification of the *PNM* genes and their products should help us understand ψ.

Recessive nuclear mutations also occur which enhance the efficiency of tRNA suppressors. They define five "allosuppressor" loci, *SAL1–SAL5* (Cox 1977). *PNM2* is closely linked to one of these loci, *SAL3*. In this report we show that a *sal3* mutation cannot be separated from *PNM2* by genetic mapping. Nevertheless, it is possible to isolate *sal3* mutations in *PNM2*$^-$ mutant strains without affecting the PNM2 phenotype. The wild-type *SAL3* gene, cloned by Crouzet and Tuite (1987), is identical to the gene identified as *SUP35* (Hawthorne

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TABLE 1

Yeast strains used in this work

Name	Genotype	Source
Haploid strains		
483/1a	*MATα ade2-1^{oc} his5-2 lys1-1 ura3-1 can1-100 SUQ5^{oc} PNM2$^-$ [ψ$^-$]*	B. S. Cox
483/1a *sal3*	*MATα ade2-1^{oc} his5-2 lys1-1 trp5-48 ura3-1 can1-100 SUQ5^{oc} PNM2$^-$ sal3-6 [ψ$^-$]*	B. S. Cox
818/17b	*MATa ade2-1^{oc} aro1D leu2-3,112 his3-11,15 trp4 pet14 ura3-1 SUQ5^{oc} [ψ$^-$]*	This study
831/3b *sal3*	*MATα ade2-1^{oc} his3-11,15 leu2-3,112 sal3-6 ura3-1 SUQ5^{oc} [ψ$^-$]*	This study
831/4c	*MATα ade2-1^{oc} aro1D his3-11,15 sec1 trp4 ura3-1 SUQ5^{oc} [ψ$^+$]*	This study
783/4c	*MATα ade2-1^{oc} his3-11,15 leu2-3,112 ura3-1 SUQ5^{oc} [ψ$^+$]*	B. S. Cox
783/4c *sal3*	*MATα ade2-1^{oc} his3-11,15 leu2-3,112 sal3-7 ura3-1 SUQ5^{oc} [ψ$^-$]*	Derived from 783/4c
MT152/1d	*MATa ade2-1^{oc} his5-2 leu1 kar1-1 [ψ$^+$]*	M. F. Tuite
MT152/3a	*MATα ade2-1^{oc} leu1 kar1-1 [ψ$^+$]*	M. F. Tuite
MT152/1b	*MATa ade2-1^{oc} kar1-1 [ψ$^+$]*	M. F. Tuite
Diploid strains		
829	Mating of 483/1a *sal3* × 818/17b	
831	*MATa/MATα ade2-1^{oc}/ade2-1^{oc} SUQ5^{oc}/SUQ5^{oc} his3-11,15/his3-11,15 ura3-1/ura3-1 sal3-6/+ PNM2$^-$/+ aro1D/+ pet14/+ trp4/+ +/leu2-3,112 +/sec1 [ψ$^-$]*	Derived from segregants from 829

and Leupold 1974), *SUP2* (Inge-Vectomov and Adrianova 1970; Kushnirov *et al.* 1988), *SUF12* (Wilson and Culbertson 1988), and *GST1* (Kikuchi *et al.* 1988), and has been mapped to chromosome *IV*. We therefore used the *SAL3* clone provided by M. F. Tuite to carry out a chromosome walk from *SAL3* to the *PNM2*$^-$ gene using a genomic library made from a *PNM2*$^-$ strain. We found that in spite of their apparent functional independence, *SAL3* and *PNM2*$^-$ were coded by the same length of DNA, *i.e.*, *sal3* and *PNM2*$^-$ are mutations of the same gene.

The *SUP35* gene codes for a 77-kD protein of 685 amino acids. The N-terminal 253 amino acids of the protein contains the repeating domain motif Gln-Gly-Gly-Tyr-Gln-Gln-Tyr-Asn-Pro. The C-terminal two thirds of the protein, amino acids 254–685, has strong sequence homology with yeast elongation factor EF-1α (Kushnirov *et al.* 1988; Wilson and Culbertson 1988; Kikuchi *et al.* 1988). This paper describes the sequence of the *PNM2*$^-$ mutant gene and establishes by artificially recombined *PNM2*$^-$ and wild-type sequences that a single missense mutation in the N-terminal-coding domain is responsible for the dominant PNM2 phenotype. We also show that just as the PNM2 phenotype requires only an intact N-terminal domain, so *SAL3* function only requires translation of an intact C-terminal domain and that this can be achieved in the absence of the putative upstream promoter of the gene. The separation of the gene into ψ$^-$-determining (*PNM2*$^-$) and allosuppressor (*SAL3*) domains corresponds to the two functional domains demonstrated by Ter-Avanesyan *et al.* (1993), one promoting "fidelity" and the other "infidelity" in chain-termination at nonsense codons.

We also show that of the two transcripts reported from the *SUP35* gene, a major species of 2.4 kb and a minor species of 1.3 kb (Surguchov *et al.* 1986; Kikuchi *et al.* 1988) the smaller is homologous only to sequences from the 3′ end of the gene. We propose a model for the function of *SUP35*, in which comparative levels of N- and C-terminal domains of *SUP35* protein (SUP35p) at the ribosome modulate chain-termination fidelity.

MATERIALS AND METHODS

Strains: The *PNM2*$^-$ library was cloned into *Escherichia coli* DH5α (*supE*44, Δ*lac*U169(ϕ80*lacZ*ΔM15) *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi*-1 *relA*1). Plasmids were routinely maintained in DH5α or MC1061 (*araD139*, Δ(*ara-leu*)*7696*, Δ(*lac*)*l74*, *galU*, *galK*, *hsdR2*(r_{K-}, m_{K+}), *mcrB1*, *rpsL*(str^r)); except when single-stranded template was required for sequencing, when JM109 (*recA*1 *supE*44 *endA*1 *hsdR*17 *gyrA*96 *relA*1 *thi* Δ(*lac-proAB*) was used.

Yeast strains used in these experiments are listed in Table 1. Strains which are *ade2-1^{oc} SUQ5^{oc}* [ψ$^+$] are white and adenine-independent; strains which are *ade2-1^{oc} SUQ5^{oc}* [ψ$^-$] are red and adenine-requiring. Cells with genotype *ade2-1^{oc} SUQ5^{oc} sal3* [ψ$^-$] are white, because of the allosuppressor effect of the *sal3* mutation.

Growth media: *E. coli* was grown in L broth or L agar. Ampicillin was added to 100 mg/liter. X-gal (5-bromo-4-chloro-3-indolyl-β-D-galacoside) was made as a fresh stock solution and added to cooled molten L agar before pouring plates at 10 mg/liter to test plasmid phenotypes.

The complete medium for growth of yeast was 1/4YEPD, 0.25% yeast extract, 1% Bacto-peptone and 4% dextrose. The lower concentration of yeast extract and higher concentration of glucose compared with standard yeast media (YEPD, 1% yeast extract, 1% Bacto-peptone, 2% dextrose) ensures that *ade2*-based adenine-requiring strains are red even when petite (Cox *et al.* 1980), since adenine remains limiting for growth. Omission medium was 0.67% yeast nitrogen base without amino acids, and 2% dextrose. Bases and amino acids were added at 2 mg/liter. Diploids were sporulated on 1% potassium acetate, 2% agar, buffered at pH7.

When necessary, [ψ$^+$] strains were converted to [ψ$^-$] by growth on 4 mM guanidine HCl-YEPD (Tuite *et al.* 1981). All media were from Difco. Purified agar was from Oxoid.

Vectors: All DNA manipulations were carried out according to the protocols described in Sambrook *et al.* 1989. All plasmid vectors used have *LEU2* as the selectable marker for yeast transformations and *amp^r* as that for *E. coli*.

The plasmid YEp13 (Broach *et al.* 1979), a 2 μm origin-based yeast shuttle vector, was used to make the library for isolating the *PNM2*$^-$ gene.

For sequencing reactions, the *PNM2*$^-$ gene was cloned into the integrating plasmid pRS305 (Sikorski and Hieter 1989).

To test the phenotypes of various gene constructs, two different yeast shuttle vectors were used: the 2 μm-based multicopy vector YEp366 (MYERS *et al.* 1986); and the low copy number, CEN6-containing pRS315 (SIKORSKI and HIETER 1989). Both plasmids contain a unique *Xba*I site, into which gene constructs were cloned.

Transformations: *E. coli* was transformed according to the method of HANAHAN 1983.

Yeast transformations were carried out using the lithium acetate method of ITO *et al.* 1983. Transformants were selected for leucine prototrophy. Single clones of transformants were streaked on nonselective YEPD and then screened for suppressor effects and ψ phenotype by replica plating on 1/4YEPD, and on −Leu omission plates.

Genetic methods: A *sal3 PNM2*⁻ strain was constructed in order to map the *PNM2*⁻ mutation. A *sal3* mutation was isolated in the *PNM2*⁻ strain 483/1a, *MATα, ade2-1*^{oc} *his5-2 lys1-1 trp5-48 can1-100 ura3-1 SUQ5*^{oc} *PNM2*⁻ [ψ^-]. Adenine-independent revertants were selected on omission medium and, after subculturing, were tested by complementation with a set of *sal* mutants of opposite mating-type, representing the five *SAL* loci previously identified (COX 1977). A revertant complementing all the testers except *sal3 MAT***a** was chosen for crosses.

This strain was crossed with a strain 818/17b, *MAT***a**, *ade2-1*^{oc} *aro1D leu2-3,112 his3-11,15 trp4 pet14 ura3-1 SUQ5*^{oc} [ψ^-] to introduce relevant markers on chromosome *IV*. Diploids were isolated by micromanipulation of zygotes from mating mixtures of cells. The phenotype of the diploid was red and adenine-requiring, since *sal3* mutations are recessive. A second diploid, 831, was made with segregants from this cross (Table 1).

Tetrad analysis was carried out by micromanipulation of ascospores from sporulating cultures after digestion with 10 μg/ml zymolyase. Tetrads were dissected and scored for segregation of the markers for which the diploid was heterozygous.

Auxotrophic markers were scored by replica-plating cultures to appropriate omission media. The *pet14* gene was scored by replica-plating to YEP medium with glycerol as the sole carbon source. *Sec* mutants were scored by their inability to grow at 34°. *Sal3* mutant segregants were white and adenine-independent.

PNM2⁻ spore clones were identified by complementation tests scoring for the color of the diploids, as follows. Red segregants were *SAL*⁺, and were crossed with red, *ade2-1*^{oc} [ψ^+] strains of opposite mating-type. The diploids formed are white if the spore is *SAL*⁺ *PNM*⁺ because the diploid is [ψ^+], and in this background the *SUQ5*^{oc} gene becomes active and suppresses the *ade2-1*^{oc} mutation. Diploids are red if the segregant is *SAL*⁺ *PNM2*⁻, because the presence of *PNM2*⁻ makes the diploid [ψ^-].

White segregants were *sal3*, and were complemented with white, *ade2-1*^{oc} *SUQ5*^{oc} [ψ^+] strains. Diploids formed from a *sal3 PNM*⁺ parent are [ψ^+] and remain white; diploids from a *sal3 PNM2*⁻ parent are [ψ^-] and red.

Ade2-1 SUQ5^{oc} *PNM*⁺ [ψ^-] strains were identified by cross-streak tests with strains of genotype: *MAT***a** or *MATα, ade2-1*^{oc} *kar1-1* [ψ^+]; the cross-streak was [ψ^+] and white. [ψ^+] strains were identified either by a cross-streak test or by streaking for single colonies on 4 mM guanidine HCl-YEPD medium. This medium causes [ψ^+] strains to become [ψ^-] and red. When the cross-streak test was ambiguous, diploids were isolated by micromanipulation to determine complementation.

Construction of the *PNM2*⁻ library: DNA was prepared from the yeast strain 483/1a (*MATα ade2-1*^{oc} *his5-2 lys1-1 ura3-1 can1-100 SUQ5*^{oc} *PNM2*⁻ [ψ^-]) by the method of CRYER *et al.* (1975). The DNA was incompletely digested with *Sau*3A and the digests were fractionated on 10–40% sucrose/1 M NaCl gradients. Fractions were assayed on agarose gels; those fractions containing fragments greater than 9 kb were pooled and the DNA collected by ethanol precipitation.

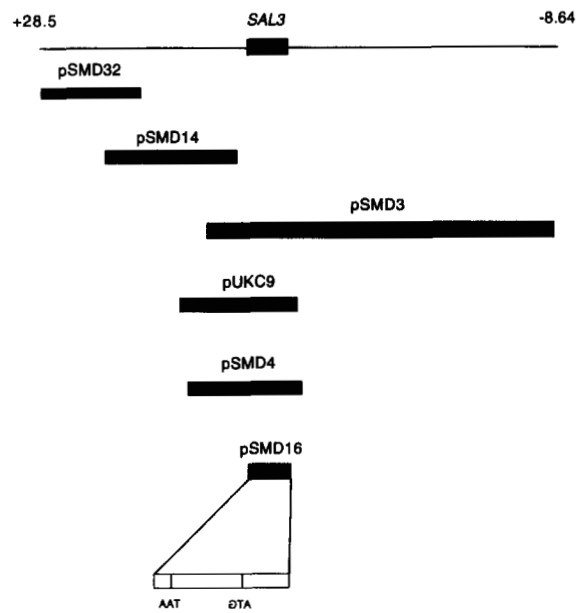

FIGURE 1.—A diagram of the clones obtained in the chromosome walk and their relationship to the *SUP35* (*SAL3*) gene. The plasmid pUKC9 from M. F. TUITE was used to obtain *SAL3* from the library (see text).

The DNA was ligated into YEp13, which had been linearized with *Bam*HI and treated with bacterial alkaline phosphatase. Ligation mixes were used to transform *E. coli* DH5α, selecting for ampicillin resistance. About 15,000 independent colonies were recovered from three separate ligation reactions. They were pooled by scraping them off the plates into 50% glycerol and stored at −70°.

Colony hybridization: *E. coli* colony hybridization was according to GRUNSTEIN and HOGNESS (1975).

Chromosome walking: The plasmid pUKC9 contained the wild-type *SAL3* gene cloned into pMA9 vector, and was a kind gift from M. F. TUITE. Nitrocellulose filters were probed with ^{35}S-labeled nick-translated *Pst*I fragments purified from pUKC9, containing sequences specific to the *SAL3* gene. Among the positive clones selected was pSMD3, which contained a 17-kb fragment insert.

pSMD3 was transformed into strain 831/3b (*MATα ade2-1*^{oc} *his3-11,15 leu2-3,112 ura3-1 SUQ5*^{oc} *sal3-6* [ψ^-]) to test for its ability to complement the *sal3-6* mutation and into strain 831/4c (*MATα ade2-1*^{oc} *aro1D his3-11,15 sec1 trp4 ura3-1 SUQ5*^{oc} [ψ^+]) to test for *PNM2*⁻ activity. Both recipient strains are white, ADE⁺, and become red, ade⁻ by complementation of *sal3-6* or through elimination of ψ^+, respectively. pSMD3 complemented *sal3* and showed *PNM2*⁻ activity (see RESULTS). pSMD3 was subcloned by double digestion with *Xba*I and *Pvu*II, with the *Xba*I site blunt-ended. Fragments were ligated into YEp13 cut with *Pvu*II. Subclone pSMD16 is a 3.37-kb *Pvu*II/*Xba*I fragment which complemented *sal3* and was *PNM2*⁻. The relationships among these fragments are diagrammed in Figure 1.

DNA sequencing: The *Pvu*II site on the *Pvu*II/*Xba*I fragment (of subclone pSMD16) carrying the *PNM2*⁻ mutant gene was converted into an *Xba*I site using a *Pvu*II-to-*Xba*I linker. The sequence was then inserted into the *Xba*I site on the mul-

tiple cloning site of the vector pRS305. The gene was isolated in both orientations in plasmids pSM107 and pSM108.

DNA sequencing was carried out using Sequenase Version 2.0 (U.S. Biochemical Corp.) by the SANGER *et al.* (1977) dideoxynucleotide method, and using single-stranded template prepared from plasmids pSM107 and pSM108. The T3 and T7 primers were used, as well as a series of primers chosen from the published sequence of the *SUF12* gene (WILSON and CULBERTSON 1988), and from our own sequence data as it was generated.

Deletion analysis: pSMD16 was cut with *Sal*I and religated, to produce construct pΔT15. This deletion removed the 3'-coding region of the *SAL3* gene from nucleotide +1444. pSMD16 was cut with *Hin*dIII and religated to produce construct pΔT5; this deletion removed virtually the entire coding region, from the *Hin*dIII site at nucleotide +98. These constructs are diagrammed in Figure 2. The deletion constructs, pSMD16, and pUKC9 were all transformed into 783/4c (*MATα ade2-1^{oc} his3-11,15 leu2-3,112 ura3-1 SUQ5^{oc}* [ψ^+]) to test [ψ] phenotypes conferred by the plasmids, and into 783/4c *sal3* (*Matα ade2-1^{oc} his3-11,15 leu2-3,112 ura3-1 SUQ5^{oc} sal3-7* [ψ^-]) to test for ability to complement the *sal3* phenotype.

Plasmid constructs: The wild-type *SUF12* gene, a kind gift from MICHAEL CULBERTSON, was cloned into the vector pRS315 to form plasmid pSM138. pSM138 was the starting material for all plasmids derived from the wild-type *SUP35* sequence.

pSM139 was constructed by cutting pSM138 with *Pst*I, to remove a 1.23-kb fragment containing upstream untranslated sequence as well as coding sequences to nucleotide +126 (amino acid 42), and then religating.

pMN140 was constructed after an intermediate cloning step. pSM138 was cut with *Sma*I and *Xho*I, which removed a *Hin*dIII site on the multiple cloning site of the vector. Fragment overhang was filled in using DNA polymerase and nucleotides, and the plasmid was religated. This plasmid was cut with *Hin*dIII to remove the 335-bp *Hin*dIII fragment in the coding region of *SUF12* (nt +98 through +433), and then religated, to form plasmid pMN140.

pMN140 contained a frameshift mutation within the coding region, which produced a stop codon 23 amino acids downstream of the frameshift. To restore the correct reading frame, pMN140 was linearized with *Hin*dIII, and the ends were chewed back with mung bean nuclease. Blunted ends were religated; this construct was named plasmid pMN340.

Plasmids derived from the *PNM2*$^-$ sequence used pSM128 as starting material, which had the *PNM2*$^-$ gene cloned into the *Xba*I site of YEp366. pMN129 was constructed by digesting pSM128 with *Pst*I, to remove the 1.23-kb fragment containing upstream untranslated sequence and coding sequences to nucleotide +126 (amino acid 42), and then religating.

Hybrid gene constructs were made as follows. pMN240 was made by linearizing pMN140 with *Hin*dIII and then ligating the 335-bp *Hin*dIII fragment from the *PNM2*$^-$ sequence. This hybrid contained wild-type sequence except for the 335-bp *Hin*dIII fragment. pMN228 was from pMN129 linearized with *Pst*I and then ligated with the 1.23-kb *Pst*I fragment from pSM138. This construct contained wild-type sequence from the upstream *Xba*I site to the *Pst*I site at nucleotide +126, and then *PNM2*$^-$ sequence. pMN238 was from pSM139 linearized with *Pst*I, and then ligated with the 1.23-kb *Pst*I fragment from pSM138. This construct contained *PNM2*$^-$ sequence from the upstream *Xba*I site to the *Pst*I site at nucleotide +126, and then wild-type *SUF12* sequence. Diagrams of all these constructs are shown in Figure 2.

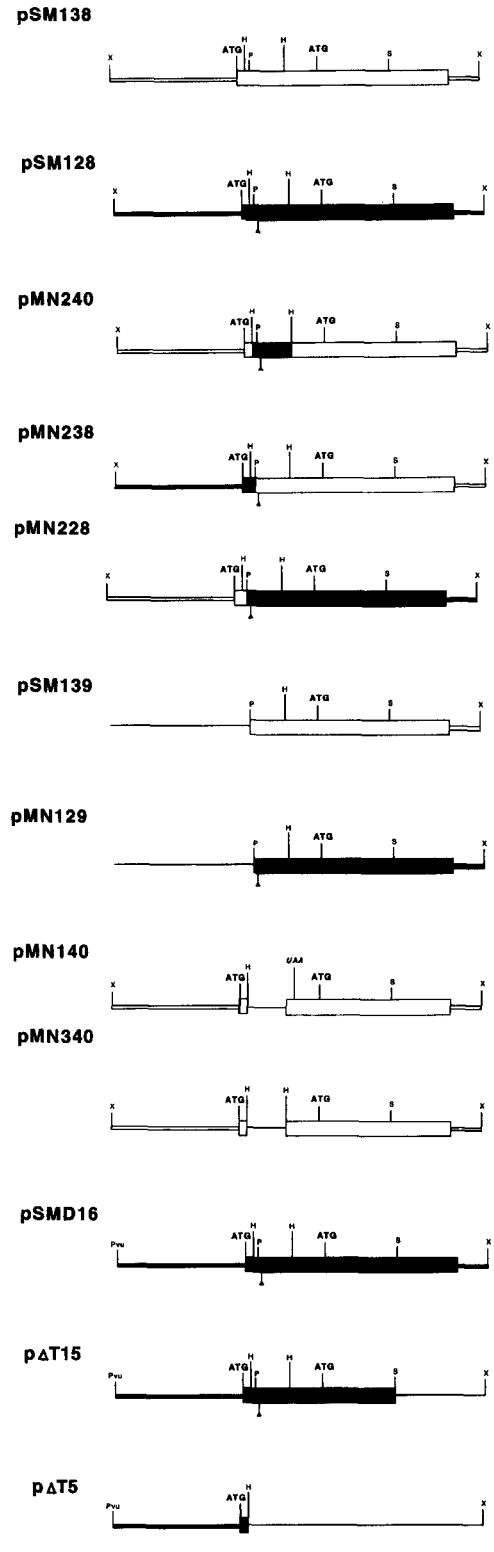

FIGURE 2.—Plasmid constructs used to test PNM and SAL phenotypes (see text and Table 3). All sequences derived from wild type *SUP35* are shown as open boxes, all those derived from *PNM2*$^-$ are shaded. Lines signify deleted sequences; the site of the mutation in the *PNM2*$^-$ sequence is marked (△). Restriction sites shown are H, *Hin*dIII; P, *Pst*I; S, *Sal*I; and X, *Xba*I. ATG denotes the translation start site, codon +1, as well as ATG at codon +254. UAA denotes a premature translation termination introduced by the deletion.

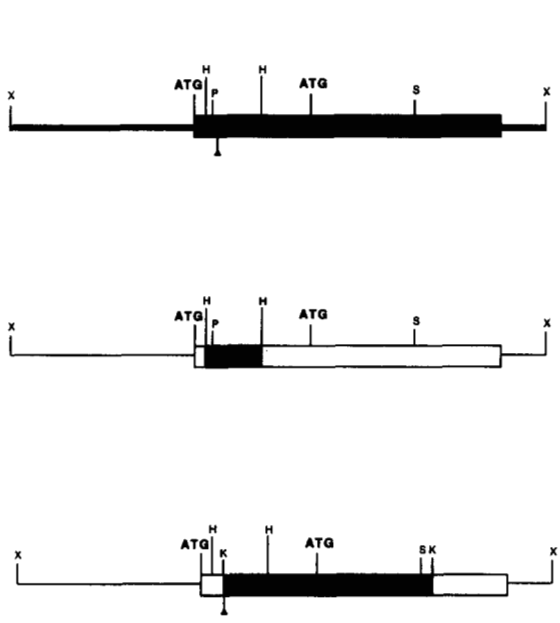

FIGURE 3.—Fragments used as probes in Northern blot analyses shown as solid blocks within the *PNM2*$^-$ (A, C) or *SUP35* (B) gene diagram. Restriction sites shown are H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; S, *Sal*I; and X, *Xba*I. ATG denotes the translation start site, codon +1, as well as ATG at codon +254.

Constructs were transformed into the yeast strains 783/4c [ψ^+] and 783/4c *sal3* to test [ψ] phenotype and *sal3* complementation, respectively.

Northern blot analysis: Total RNA was extracted from isogenic ψ^+ and ψ^- strains of MT152/1d (*ade2-1 his5-2 leu1 kar1-1*), according to the method of SCHMITT *et al.* (1990). Equal amounts of extracted RNA were run on agarose gels and blotted onto Hybond N (Amersham) nylon membrane. Blotted RNA was cross-linked to the membrane using ultraviolet radiation.

The blot was probed, sequentially, with full-length 3370-bp *Xba*I fragment containing the *PNM2*$^-$ sequence; with 335-bp *Hin*dIII fragment from *SUF12*; with 1343-bp *Kpn*I fragment from *PNM2*$^-$; and with a 600-bp *Xba*I/*Pst*I fragment from the yeast actin gene. The blot was stripped of all radioactivity after each reaction. Figure 3 shows the location of these fragments.

RESULTS

Linkage of *sal3* and *PNM2*$^-$: The data from the tetrads is presented in Table 2. No recombinants between *sal3* and *PNM2*$^-$ were found. The few segregants which gave a result suggesting that they might be recombinant were re-tested by single-cell matings to remove any ambiguity over the diploid phenotype.

The tetrad data suggest an order for the genes in this region of chromosome *IV*: *TRP4–PET14–SAL3/PNM2–SEC1–ARO1D* with the distances indicated in Table 2 and shown in Figure 4. This order is in agreement with the latest genetic map (MORTIMER *et al.* 1991), and establishes the location of *SAL3* with respect to *SEC1*, which is ambiguous in the published map. From our data, the order which requires the fewest double crossovers is *SAL3–SEC1–ARO1D*. This order is confirmed by the observation that in the six tetrads which had a crossover between *SAL3* and *SEC1*, *SAL3* and *ARO1D* segregated as tetratypes and *SAL3* and *PET14* as parental ditypes, as expected if this is the correct order.

TABLE 2

Tetrad data from meiosis of diploids 829 and 831

Gene pair	PD[a]	NPD[a]	TT[a]	Distance (cM)[b]
PNM2$^-$*-sal3*	137	0	0	
sal3-sec1	81	0	5	2.9
sal3-pet14	69	1	27	17.3 (0.85)
sal3-aro1D	92	1	20	12.0 (2.0)
sal3-trp4	24	15	68	58.6[c]
sec1-pet14	49	0	2	15.4
sec1-aro1D	69	0	16	9.4
sec1-trp4	14	14	50	60.3[c]
pet14-aro1D	56	2	38	26.9 (0.74)
pet14-trp4	19	16	50	48.3[c]
aro1D-trp4	25	10	70	69.0[c]

[a] PD, parental ditype; NPD, nonparental ditype; TT, tetratype.
[b] The map distances shown are based on PERKINS' (1949) formula, as modified by MA and MORTIMER (1983). Interference was calculated by the method of PAPAZIAN (1952) where appropriate and is shown in parentheses.
[c] Estimates; the tetrad data involving *TRP4* gives anomalous values in all pairwise combinations, there being too many nonparental ditypes. We attribute this to there having been, in the sporulating population, a clone of cells in which the coupling relationship had been changed to a repulsion by a mitotic crossover. We have calculated map distance using tetratypes only to estimate crossovers, using PAPAZIAN's equations and an interference value of 0.6.

The maximum distance, x, the *SAL3* and *PNM2*$^-$ mutations are apart at the 1% level of probability is given by either $x = 1 - p^{1/T}$ (JEFF KING, personal communication), or alternatively by $x = -\ln(p)/T$ (B. S. COX, unpublished) where p is the chosen level of probability and T the number of tetrads in an analysis where only parental ditypes were observed. In either case, the answer is 1.7 cM, which in most of the genome corresponds to about 5 kb (MORTIMER *et al.* 1989).

Isolation of the *PNM2*$^-$ gene by chromosome walking: We cloned the gene for *PNM2*$^-$ by chromosome walking from *sal3* using a *PNM2*$^-$ genomic library. Plasmid pSMD3 complemented *sal3*; in addition, this plasmid gave transformant colonies from *ade2-1*^{oc} *SUQ5*^{oc} [ψ^+] recipients that were white with numerous red sectors. The chromosome walk was extended in either direction to overlap a total of 37 kb on either side of *SAL3*. Appropriate clones were tested for their ability to complement other markers mapping in this region of chromosome *IV*. Of those tested, namely *sec1*, *sec5*, *sec7*, *hom2*, *aro1D*, *cdc37* and *pet14*, we indentified only one, namely *sec1*, 25 kb downstream of *sal3* (pSMD32, Figure 1).

A plasmid containing *PNM2*$^-$ would convert a white *ade2-1*^{oc} *SUQ5*^{oc} [ψ^+] recipient to red [ψ^-]. In diploids heterozygous for the *PNM2*$^-$ gene, the diploid is red and the meiotic segregants [ψ^-] (YOUNG and COX 1971;

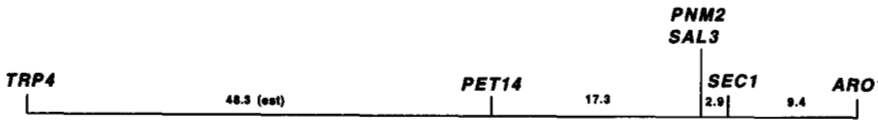

FIGURE 4.—A map of the *SAL3/PNM2* region of chromosome *IV* based on the tetrad data in Table 2.

MCCREADY *et al.* 1977); in other words, the PNM2$^-$ phenotype is dominant. The recovery of transformant colonies which were not wholly red was therefore unexpected. A segregation analysis was carried out on transformant colonies by replating them on 1/4 YEPD and replica-plating the colonies to −leu omission medium and to 1/4 YEPD 4 mM guanidine HCl.

Each transformant segregated four types of colony, namely (1) white with numerous red sectors (mottled): these were all Leu$^+$; (2) white with no red sectors: these were all leu$^-$; (3) and (4), wholly red: these were either Leu$^+$ or leu$^-$. Replicas of colonies onto guanidine HCl medium were all red; indicating that, like the parent recipient, the white, adenine-independent phenotype was dependent on the ψ^+ factor. Samples of wholly red segregants were tested by complementation and found to be [ψ^-].

We deduced that a cell receiving this plasmid could grow into a colony segregating [ψ^-] cells. Only [plasmid$^+$] cells could segregate both ψ^+ and ψ^- cells, giving the mottled phenotype. At the same time, and independently of the segregation of ψ, [plasmid$^-$] cells would segregate. If they were still [ψ^+] when this happened, they would stay [ψ^+] and give white leu$^-$ colonies. [ψ^-] cells growing into red colonies could either have the plasmid and be Leu$^+$, or lose it and become leu$^-$.

We took these observations as *prima facie* evidence that pSMD3 contained the *PNM2*$^-$ gene, as it was able to convert [ψ^+] recipient cells to [ψ^-]. First, [plasmid$^+$] cells always segregated red [ψ^-] progeny: all Leu$^+$ segregants were either wholly red or mottled; conversely, all wholly white [ψ^+] segregants were leu$^-$. Secondly, the red, unsuppressed phenotype persisted in the absence of the plasmid. This indicated permanent loss of ψ (*PNM2*$^-$), and not merely a dominant antisuppressor function determined by the plasmid.

Various subclones of pSMD3 were made and isolated in order to locate *PNM2*$^-$ more precisely. Subclones chosen on the basis of their restriction maps were transformed into an *ade2-1*^{oc} *SUQ5*^{oc} [ψ^+] strain. One of these subclones, pSMD16, which contained a 3.37-kb insert, gave the same phenotype as pSMD3, namely mottled colonies of mixed red and white sectors.

Deletion analysis of pSMD16: To confirm that the PNM2 and SAL3 phenotypes were both determined by the cloned sequences, two deletions of pSMD16 were made. One, between the *Sal*I sites at +1444 on the *SAL3* sequence and that in the vector removes the 615 3′-terminal coding bases and all the 3′ non-coding region and the other, using *Hin*dIII and religating removes all the 3′ coding and non-coding sequence from +98. The first of these deletions, pΔt15, retains the *PNM2*$^-$ determinant but loses the ability to complement *sal3*. The second, pΔt5, loses both properties (Table 3). This confirms that both phenotypes are dependent on the integrity of the same open reading frame, that of the *SAL3* (*SUP35*) gene. The properties of pΔt15 suggest that different domains of the gene determine *PNM2*$^-$ and *SAL3*.

Sequence of *PNM2*$^-$ gene: The entire 3.37-kb *Xba*I fragment carrying the *PNM2*$^-$ mutant gene was sequenced using plasmids pSM107 and pSM108. This sequence was compared to the published sequence of the wild-type *SUF12* gene (WILSON and CULBERTSON 1988) which included 378 bases upstream of the start of the coding region, and 213 bases downstream of the termination codon. We also re-sequenced the copy of wild-type *SUF12* gene sent to us by Dr. Michael Culbertson, using plasmid pSM138, from the beginning of the coding region to 473 bases upstream of the start codon. There were four differences found between the *PNM2*$^-$ mutant sequence and the published *SUF12* wild-type sequence. Of these, an extra T at position −376, an extra C at position +2098, and an extra A at +2130, all found in the *PNM2*$^-$ sequence, appeared in both our wild-type (*SUF12*) sequence and in the published sequences for *GST1* and *SUP2* (KIKUCHI *et al.* 1988; KUSHNIROV *et al.* 1988). The only consistent difference between the wild-type and *PNM2*$^-$ sequences was a G → A transition at position +173 in the coding region for the N-terminal domain of the protein. The consequence of the mutation was to change the amino acid sequence at position 58 from glycine (*SUP35*) to aspartate (*PNM2*$^-$).

Confirmation of mutation: Various deletion and recombinant constructs of the wild-type and *PNM2*$^-$ versions of the gene were made in order to confirm that this mutation alone is responsible for the PNM2 phenotype. Table 3 gives the results of transformations of strain 783/4c, *ade2-1*^{oc} *SUQ5*^{oc} [ψ^+], with various plasmid constructs. Transformants segregated red, white, or sectored red and white colonies on 1/4 YEPD. As before these were replica-plated to −Leu omission medium. Transformants with wild-type *SUP35* (pSM138) segregated only suppressed (white) [ψ^+] colonies. Transformants with *PNM2*$^-$ (pSM128) showed the PNM phenotype; that is, they segregated non-suppressed (red) colonies with (Leu$^+$) or without (leu$^-$) plasmid and few or no wholly white Leu$^+$, [ψ^+] colonies.

To confirm that the 335-bp *Hin*dIII fragment containing the G_{173} → A transition caused the PNM phenotype, a sequence substitution was constructed which was wild-type except for the 335-bp *Hin*dIII fragment

TABLE 3

Results of transformations of strain *783/4c* [ψ^+] with various plasmid constructs

Plasmid[a]		Red	Sectored red/white	White	ψ phenotype[b]	Sal phenotype[c]
pSM138	Leu$^+$	0	0	133		
	leu$^-$	0	0	143	ψ^+	SAL3$^+$
pSM128	Leu$^+$	72	227	0		
	leu$^-$	76	12	4	PNM2$^-$	SAL3$^+$
pMN240	Leu$^+$	63	215	7		
	leu$^-$	22	5	52	PNM2$^-$	SAL3$^+$
pMN238	Leu$^+$	0	0	40		
	leu$^-$	0	0	369	ψ^+	SAL3$^+$
pMN228	Leu$^+$	9	136	2		
	leu$^-$	49	4	28	PNM2$^-$	SAL3$^+$
pSM139	Leu$^+$	0	501	0		
	leu$^-$	0	0	95	ψ^+; ASU	SAL3$^+$
pMN129	Leu$^+$	22	412	0		
	leu$^-$	0	0	116	ψ^+; ASU	SAL3$^+$
pMN140	Leu$^+$	0	0	363		
	leu$^-$	0	0	84	ψ^+	sal3
pMN340	Leu$^+$	1	390	0		
	leu$^-$	0	0	107	ψ^+; ASU	SAL3$^+$
pSMD16	Leu$^+$	1	18	0		
	leu$^-$	3	0	32	PNM2$^-$	SAL3$^+$
pΔT15	Leu$^+$	4	59	0		
	leu$^-$	5	0	10	PNM2$^-$	sal3
pΔT5	Leu$^+$	0	0	40[d]		
	leu$^-$	0	0	0	ψ^+	sal3

[a] For diagrams of plasmid constructs, refer to Figure 2.
[b] The phenotype of transformants of *783/4c* [ψ^+].
[c] The phenotype of transformants of *783/4c sal3–7* [ψ^-].
[d] Sectored Leu$^+$/leu$^-$.

(plasmid pMN240). Transformants with pMN240 showed phenotypes identical to transformants with plasmid pSM128 carrying the *PNM2*$^-$ mutant gene. To show that the effect was independent of the promoter sequences, transformants were made with pMN228 (wild-type promoter to +98) and pMN238 (*PNM2*$^-$ promoter to +98). The phenotypes were determined not by the source of the promoter but by the identity of the sequence at position +173. We conclude that the G → A transition mutation at position +173 of the coding sequence is responsible for the phenotype of *PNM2*$^-$.

A separation of *PNM2*$^-$ and antisuppressor effects: The plasmid constructs pSM139 and pMN129 were identical deletions of promoter sequences, as well as amino acids 1–42 of the expressed proteins, from *SUP35*$^+$ and *PNM2*$^-$, respectively. Since these constructs removed the promoters, no expression of the full-length proteins was expected. Nevertheless, transformants with these constructs produced similar results, predominantly sectored red and white colonies (Table 3). Unlike the transformants obtained with the other *PNM2*$^-$ constructs, the red segregants were all Leu$^+$ and the white all leu$^-$. In other words, no [ψ^-] segregants were observed: loss of the plasmid resulted in recovery of suppression. Neither of these constructs therefore conferred the PNM2 phenotype, even though the pMN129 construct contained the G_{173} → A mutant sequence. It appears that expression of an intact N-terminal domain, containing the G_{173} → A mutation, is required for the elimination of ψ.

On the other hand both constructs, regardless of the presence of the G_{173} → A mutation, confer a dominant antisuppressor (ASU) phenotype. The distinction between an dominant antisuppressor (ASU) and a PNM2 phenotype in these assays is that cells transformed with *PNM2*$^-$ segregate red colonies which may be Leu$^+$ or leu$^-$. With the ASU plasmids, all Leu$^+$ colonies are wholly red and wholly [ψ^+]; when they lose the plasmid they produce white [ψ^+] sectors.

These results implied that the C-terminal portion of the *SUP35/PNM2*$^-$ gene, downstream of nucleotide +173, could be expressed in the absence of the upstream promoter sequence, and that the expressed protein is identical for both *SUP35* and *PNM2*$^-$ sequences. This was confirmed by complementation of the *sal3* mutation with these constructs (see below).

Deletion of the 335-bp *Hin*dIII fragment from the coding region of the *SUP35* coding sequence, and then religation, produced a frameshift mutation, and a (premature) termination signal 23 amino acids downstream.

Transformants with plasmid pMN140, containing this construct remained white (suppressed) and [ψ^+]. The construct was modified to remove the frameshift. Transformants with this in-frame *Hin*dIII deletion in the *SUP35* sequence, pMN340, produced the dominant antisuppressor ASU, [ψ^+] phenotype (Table 3). Both plasmids were constructed from wild-type *SUP35* sequence, so transformants were expected to remain [ψ^+]; both constructs have disrupted N-terminal regions. However, while the pMN340 construct would produce protein with an intact C-terminal region, the frameshift mutation and downstream nonsense codon produced in the pMN140 construct would prevent expression of the C-terminal region of *SUP35* protein.

Transformations with pSM139, pMN129 and pMN340 showed that a dominant antisuppressor (ASU) phenotype is produced where an intact C-terminal region of *SUP35* is expressed even where there is no expression of intact N-terminal sequences. The phenotype is dominant, since the transformed strains retained a wild-type chromosomal copy of *SUP35*.

Complementation of *sal3* mutation: The *SUP35/PNM2* gene is identical with the gene previously identified as *SAL3* (Crouzet and Tuite 1987). We tested the plasmid constructs for their ability to complement a *sal3* mutation. The strain 783/4c *ade2-1^{oc} SUQ5^{oc} sal3-7* [ψ^-] is white because the *sal3* mutation increases the efficiency of the weak ochre suppressor *SUQ5^{oc}*; complementation of *sal3* results in red colonies. Table 3 shows which gene constructs were able to complement *sal3*. Wild-type *SUP35* (pSM138, pMN238, pMN240, pMN340) or mutant *PNM2$^-$* (pSMD16, pSM128, pMN229) sequences were *SAL3$^+$*, even without upstream promoters (pSM139, pMN129). Introduction of a *Hin*dIII deletion produced a frameshift and termination codon in the N-terminal coding regions and prevented complementation of *sal3* (pMN140), but removing the frameshift restored it (pMN340). Deletion of the coding sequences of *PNM2$^-$* downstream of the *Sal*I site at +1444 removed the ability to complement *sal3* (pΔT15), as did deletion of most of the coding region downstream of the *Hin*dIII site at nt +98 (pΔT5).

Crouzet and Tuite 1987 showed that complementation of the *sal3* phenotype required both an intact *SUP35* C-terminal sequence and an intact *Hin*dIII fragment sequence. We have now shown that the latter effect is due only to the introduction of a frameshift and termination codon as a result of making the *Hin*dIII deletion. The deletion itself is irrelevant to *SAL3* expression. It does however, effectively prevent N-terminal function and a concomitant result is the dominant ASU phenotype.

To summarize, the constructs which complemented the *sal3* mutation did so by providing an intact C terminus. Translation of the C-terminal sequence alone conferred the dominant antisuppressor (ASU) phenotype: all constructs which complement *sal3* where the N-terminal domain is not expressible were ASU.

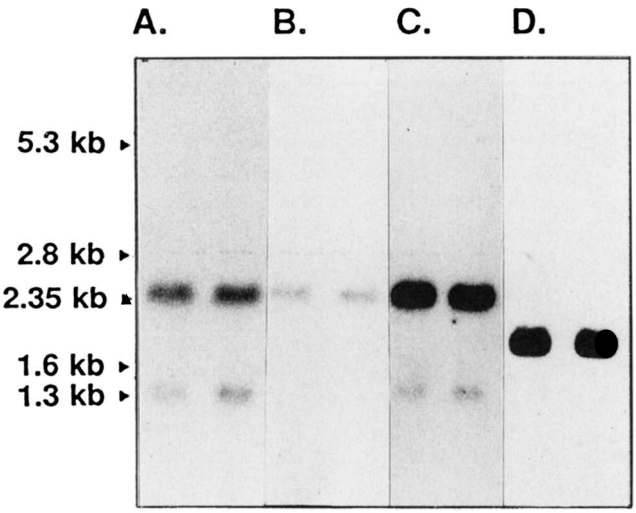

Figure 5.—Total RNA was extracted from isogenic ψ^+/ψ^- strains, blotted, and probed, sequentially, (A) with the full-length 3370-bp *Xba*I fragment containing the *PNM2$^-$* sequence; (B) with the 335-bp *Hin*dIII fragment from *SUP35*; and (C) with the 1343-bp *Kpn*I fragment from *PNM2$^-$*. (For constructs, refer to Figure 3.) Lane (D) was probed with yeast actin gene sequence, and was a loading control.

Taken together, the complementation of *sal3* and the ASU phenotypes suggest that the C-terminal domain sequences of *SUP35* can be transcribed and translated in the absence of the upstream non-coding regions; we infer a promoter region within the N-terminal domain coding region. This putative promoter may depend on sequences within the *Hin*dIII fragment for its activity, since there is no ASU or *SAL3$^+$* activity from pMN140, which has this piece deleted. However, the entire *Hin*dIII fragment sequence may not be necessary, since deletions of the *Pst*I fragment, which partially overlaps into the *Hin*dIII sequence, complement *sal3* and are ASU.

Northern blot analysis: The results of Northern blot analysis of total RNA from isogenic ψ^+/ψ^- strains are shown in Figure 5. When the blot was probed with the full-length (3370 bp) *Xba*I fragment containing the *PNM2$^-$* sequence, it showed two transcripts, a major band at 2.4 kb and a minor band at 1.3 kb. Other investigators, using probes derived from the wild-type *SUP35* sequence, have reported similar results (Surguchov *et al.* 1986; Kikuchi *et al.* 1988; Kushnirov *et al.* 1990). It has been speculated (Kushnirov *et al.* 1990) that the 2.4-kb band mRNA codes for the complete protein, beginning at codon +1; while the smaller 1.3-kb transcript codes for a polypeptide copy of the C-terminal domain of the protein, beginning at the ATG codon at +254.

When the blot was probed with the 335-bp *Hin*dIII fragment from *SUP35*, only the larger 2.4-kb band was

lit; this suggests that the smaller transcript did not contain the *Hin*dIII sequence. Both transcripts hybridized with the 1343-bp *Kpn*I fragment from *PNM2*⁻; this probe spans the presumed initiation site of the second transcript. The differences in hybridization are not due to differences in amounts of RNA present, since both lanes showed equivalent amounts of actin mRNA. We interpret this as preliminary evidence that the short transcript contains C-terminal sequences. Two transcripts would account for expression of the N-terminal deletion constructs (pSM139 and pMN129), which otherwise lack presumed promoter sequences.

DISCUSSION

We cloned a nuclear gene required for the maintenance of the extrachromosomal element ψ, *PNM2*. We have presented evidence that it is identical with the *SAL3* gene cloned by CROUZET and TUITE (1987), the *SUF12* gene cloned by WILSON and CULBERTSON (1988), the *SUP2* gene cloned by KUSHNIROV *et al.* (1988) and the *GST1* gene cloned by KIKUCHI *et al.* (1988) and that it is also known as *SUP35* (HAWTHORNE and LEUPOLD 1974). *PNM2/sal3* was mapped to chromosome *IV*, 2.9 cM from the *sec1* gene and 11.5 cM from *aro1*, with the most likely order being *SAL3(SUP35)–sec1–aro1*.

We cloned and sequenced the mutant gene *PNM2*⁻ and found the only difference between mutant and wild-type sequences is a missense mutation, a G → A transition at position +173 within the N-terminal coding region of the gene. The mutation predicts a non-conservative glycine to aspartate change at amino acid 58 of the *PNM2*⁻-encoded protein.

Data presented in this study show that this difference alone is responsible for the PNM2 phenotype. Hybrid constructs involving *PNM2*⁻ and wild-type sequences (pMN240, pMN238 and pMN228, Table 3) show that the PNM2 phenotype is produced only when plasmids include the mutation. We propose that the structural change caused by this mutation alters the affinity of *SUP35* protein (SUP35p) for the ψ element and leads to the eventual elimination of ψ.

The sequence of *SUP35* shows that the open reading frame is organized into at least two domains, with an N-terminal domain (amino acids 1–253) characterized by a 9-amino acid motif repeated four times; and a C-terminal domain (amino acids 254 (=ATG) – 685) which shows considerable homology with EF-1α (WILSON and CULBERTSON 1988; KUSHNIROV *et al.* 1987, 1988). The change predicted by the *PNM2*⁻ mutation is in the N-terminal domain and predicts a non-conservative change Gly → Asp in the first of the repeats.

Analysis of the clone of *PNM2*⁻ reported here contributes to our knowledge of the expression and function of these domains, as we discuss below.

Expression: *Sal3* allosuppressor mutations were complemented by both the wild-type and *PNM2*⁻ mutant versions of the *SUP35* gene (Table 3, pSM128 and pSM138). We show that an intact 3′ end of the open reading frame is necessary and sufficient for *SAL3* function (Table 3, pΔt15, pSM139, pMN129), but is not necessary for the *PNM2*⁻ function.

Expression of an intact N-terminal domain of *SUP35* was not merely sufficient but was necessary for *PNM2*⁻ function. These two phenotypes therefore correspond to the two predicted domains of the protein. Disruptions of the N-terminal domain, which prevent its transcription by deleting the promoter region and which delete the coding region to amino acid 42, led to the loss of ability to eliminate ψ (Table 3, pMN129). However, such a disruption, whether of the *PNM2*⁻ gene or of wild-type (pMN139), still allowed *SAL3*⁺ function and produced a novel phenotype; namely, dominant antisuppression. Dominant antisuppression was also observed by TER-AVANESYAN *et al.* (1994) in *SUQ5* [ψ^+] strains transformed with wild-type *SUP35* clones containing deletions within the N-terminal-coding domain, but with an intact promoter. It is clear that the C-terminal domain has both antisuppressor and *SAL3*⁺ activities. This is confirmed by a construct, pMN140, in which deletion of a *Hin*dIII fragment within the N-terminal domain introduces a frameshift and a stop codon. This would prevent translation of the C-terminal coding domain. Transformants with this plasmid show neither the antisuppressor nor the SAL3⁺ phenotypes. Both are restored in pMN340, in which the frameshift has been corrected.

It seems from the expression of *SAL3*⁺ and ASU in pMN129 and pMN139 that the promoter regions upstream of the N-terminal domain are not necessary for expression of the C-terminal domain. This implies the existence of a secondary promoter within the remaining N-terminal domain in these constructs. The operation of this promoter may depend on the presence of the *Hin*dIII fragment, because in pMN140, everything is present except this. The existence of this promoter as a functioning structure is consistent with the observation of two mRNA transcripts, the smaller of which hybridizes only to sequences from the 3′ end of the gene.

To summarize: (i) transcription of the N-terminal-coding domain is necessary and sufficient for expression of the PNM2 phenotype, and requires the presence of the upstream promoter; (ii) expression of the *SAL3* function depends on transcription and translation of the C-terminal-coding domain. This may be achieved either by the upstream promoter or by means of a promoter within the N-terminal-coding domain; and (iii) when only the C-terminal-coding domain is expressed an antisuppressor phenotype is seen.

Function: *SUP35* is an essential gene. The C-domain is sufficient to complement the lethality of gene disruptions (M. D. TER-AVANESYAN, personal communication). The C-domain if overexpressed relative to the N-domain

produces antisuppression and it is sufficient for complementation of *sal3* mutations.

The N-domain is not essential for the viability of the cell. It is necessary for the maintenance of ψ^+ (TER-AVANESYAN *et al.* 1994). A point mutation within it, *PNM2*$^-$, causes the loss of ψ. When it is overexpressed compared to the C-domain, omnipotent suppression is observed (TER-AVANESYAN *et al.* 1994; this report). Finally we have shown that it is a promoter for a transcript from which the C-domain can be translated.

The simplest explanation for the dominant PNM2 phenotype of the $G_{173} \rightarrow A$ mutation cloned from a *PNM2*$^-$ strain is that the N terminus of *SUP35* protein (SUP35p) directly binds either the ψ factor or some other factor necessary for maintaining ψ so as to prevent its replication. TER-AVANESYAN *et al.* (1994) have data showing that deletions in the N-terminal domain (NΔ) have a recessive *pnm* effect; that is, in the presence of an intact N-terminal sequence, ψ^+ is maintained, but cells become [ψ^-] when NΔ is homozygous or segregates in haploids. This is unequivocal evidence that an intact N terminus is necessary for the maintenance of ψ^+. They also show that expression of a portion of N-domain which includes the *Hin*dIII fragment sequences is sufficient to maintain ψ. Taken together, the dominant and recessive phenotypes associated with this domain suggest a direct interaction with ψ.

We can collate all these functions of *SUP35* and its domains if we consider the primary role of the gene to be in translation termination. We note that nearly all the suppressor phenotypes assayed concern read-through of stop codons (HAWTHORNE and LEUPOLD 1974; HINNEBUSCH and LIEBMAN 1992; but see CULBERTSON *et al.* 1982). If the EF-1α-like C-domain promotes efficient translation termination at nonsense codons the properties of mutations in this gene can be explained as follows. Overexpression of C-domain, either by incorporation on a multicopy plasmid or by deletion of N-domain sequences promotes termination with the phenotypic effect of antisuppression. Mutations in this domain either cause omnipotent suppression (*sup35*) or allosuppression (*sal3*) (read-through in the absence or presence of mutant tRNA).

If this is the function of the C-domain, it leaves unexplained the utility of the N-domain. On the face of it, it is maladaptive, since all it seems to do is serve the purpose of interfering with normal translation termination, promoting suppression when overexpressed. We propose that it has a role in context recognition. All the termination codons assayed genetically are out of any context for normal translation termination. The N-domain could have the function of recognizing termination contexts and promoting termination by the C-domain when they are present or aborting termination when they are absent. Thus increasing the amount of SUP35p (or of N-domain) increases the chances of abortion of chain-termination at nonsense mutations, hence promoting read-through. Conversely, deletion or depletion of N-domain, or overexpression of C-domain alone increases chain-termination at nonsense codons, giving an antisuppressor phenotype. From this model we would expect underexpression of C-domain to produce an allosuppressor effect and this may be what happens in *sal3* mutants.

The role of ψ in the cell can be explained as follows. There are two versions of C-domain in the cell: it occurs either as complete protein with an N-terminal domain *in situ* (NCp) or as C-domain alone (Cp), translated from the smaller mRNA transcript. The balance between read-through and chain-termination would be regulated by the relative amounts of the two forms as we suggest above. The effect of the ψ factor would be to alter this ratio to produce relatively less Cp in [ψ^+] cells. The way in which ψ acts to affect NCp:Cp ratios we cannot say. It could be at the level of protein-binding, for example by ψ acting as a sink for Cp, or it could affect the relative activity of the two promoters, or the amounts or availability for translation of the two transcripts. Phenomenologically, ψ^+ is a dominant allosuppressor.

An alternative model for the role of ψ, which does not depend on any role for the putative translation product of the C-terminal sequences is as follows. *SAL3* is needed for faithful termination and for maintenance of ψ, which binds to it. In [ψ^+] cells this binding titrates SUP35p away from termination and enhances suppression. *PNM2*$^-$ is a dominant interfering allele which binds ψ poorly and interferes with ψ binding to the wild-type protein present in heterozygotes without affecting the termination activity of SUP35p. This gives the classic dominant antisuppression/ψ-elimination phenotype. It also explains why in high copy-number, *PNM2*$^-$ becomes only partially dominant for ψ elimination: overproduction would compensate for poor binding and so allow maintenance. Finally, it allows for all the effects to explained by the binding of ψ to the site indicated by the *PNM2*$^-$ mutation in the N-terminal region. We are indebted to one of the referees of this paper for this neat idea.

The most potent argument against the direct role of SUP35p in translation termination is that some mutants of the gene allow frameshift suppression, which ostensibly has nothing to do with termination (CULBERTSON *et al.* 1982). However, some frameshift mutations generate nearby in-frame termination codons, and the fate of a ribosome-mRNA complex at such points is either termination or stalling due to the lack of cognate aminoacyl tRNA. Stalling would be greater if the termination factor were NCp, where the N-domain has no context to recognize. Stalling could lead to frameshift read-through, as it does in the case of translation of Ty RNAs (BELCOURT and FARABAUGH 1990). This predicts that the *suf12* mutation might be found in the N-domain of

SUP35; and indeed, WILSON and CULBERTSON (1988) found that complementation of *suf12* was not possible in a deletion construct from which C-terminal expression could occur. This construct was the equivalent of pMN139 in this study. Biochemical evidence that SUP35p is a termination rather than an elongation factor is provided by the observation that it is expressed in a ratio of approximately 1:20 with ribosomes, which puts it close to a stoichiometric ratio with polysomes (DIDICHENKO *et al.* 1991).

To date, *PNM2*⁻ is the only mutation of *SUP35* that has been sequenced. Knowledge of the other specific mutations in other alleles of *SUP35* should provide a means for analyzing our model.

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