The *SUP35* Omnipotent Suppressor Gene Is Involved in the Maintenance of the Non-Mendelian Determinant [*psi*$^+$] in the Yeast *Saccharomyces cerevisiae*

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ABSTRACT

The *SUP35* gene of yeast *Saccharomyces cerevisiae* encodes a 76.5-kD ribosome-associated protein (Sup35p), the C-terminal part of which exhibits a high degree of similarity to EF-1α elongation factor, while its N-terminal region is unique. Mutations in or overexpression of the *SUP35* gene can generate an omnipotent suppressor effect. In the present study the *SUP35* wild-type gene was replaced with deletion alleles generated *in vitro* that encode Sup35p lacking all or a part of the unique N-terminal region. These 5′-deletion alleles lead, in a haploid strain, simultaneously to an antisuppressor effect and to loss of the non-Mendelian determinant [*psi*$^+$]. The antisuppressor effect is dominant while the elimination of the [*psi*$^+$] determinant is a recessive trait. A set of the plasmid-borne deletion alleles of the *SUP35* gene was tested for the ability to maintain [*psi*$^+$]. It was shown that the first 114 amino acids of Sup35p are sufficient to maintain the [*psi*$^+$] determinant. We propose that the Sup35p serves as a trans-acting factor required for the maintenance of [*psi*$^+$].

THE phenomenon of informational suppression is successfully exploited as a tool for elucidating the genetic control of translational ambiguity in both prokaryotic and eukaryotic cells. Nonsense suppressors, the best studied class of informational suppressors of the yeast *Saccharomyces cerevisiae*, can be divided into two categories, codon-specific and codon-nonspecific (omnipotent). As a rule, the nonsense suppressors with precise codon specificity arise by anticodon mutations of tRNA genes (PIPER *et al.* 1976; GOODMAN *et al.* 1977; BROACH *et al.* 1981). In contrast, omnipotent suppressors are presumed not to be mutations in tRNA genes because of their lack of codon specificity (HAWTHORNE and LEUPOLD 1974; LIEBMAN and ALL-ROBYN 1984; ONO *et al.* 1984). It was shown that mutations in these genes may cause an increased level of translational ambiguity (SURGUCHOV *et al.* 1980; MASUREKAR *et al.* 1981). The function of the gene products in protein synthesis of only a few of the omnipotent suppressors are known (MASUREKAR *et al.* 1981; SURGUCHOV *et al.* 1984; EUSTICE *et al.* 1986; ALL-ROBYN *et al.* 1990). Another approach to identifying protein components involved in the control of translational accuracy consists in the identification of *trans*-acting mutations that modify the expression of suppressors (for reviews, see SHERMAN 1982; SURGUCHOV *et al.* 1984; HINNEBUSCH and LIEBMAN 1991). In addition to nuclear mutations, cytoplasmically inherited factors [*psi*$^+$] and [*eta*$^+$] modify the action of suppressors. In spite of extensive studies, the physical entities corresponding to these cytoplasmic determinants are still unknown [for reviews, see COX *et al.* (1988) and HINNEBUSCH and LIEBMAN (1991)]. Mutations in nuclear *PNM* genes have been described that convert a [*psi*$^+$] cytoplasmic genome to [*psi*$^-$] (YOUNG and COX 1971; MCCREADY *et al.* 1977).

Herein we provide evidence that the omnipotent suppressor *SUP35*, also called *SUP2*, *SUPP*, *SAL3*, *SUF12* and *GST1* (INGE-VECHTOMOV and ANDRIANOVA 1970; HAWTHORNE and LEUPOLD 1974; GERLACH 1975; CROUSET and TUITE 1987; CULBERTSON *et al.* 1982; KIKUCHI *et al.* 1988), participates in maintenance of [*psi*$^+$] in yeast cells. It was shown earlier that the *SUP35* gene encodes a 76.5-kD ribosome-associated protein in which the C-terminal part, beginning from methionine-254, exhibits a high degree of similarity to EF-1α elongation factor (KUSHNIROV *et al.* 1988; WILSON and CULBERTSON 1988; DIDICHENKO *et al.* 1991). Mutations in or overexpression of this gene decrease translational fidelity, resulting in an omnipotent suppressor effect (SURGUCHOV *et al.* 1984; CHERNOFF *et al.* 1992). Overexpression of *SUP35* also drastically reduces the growth rate of [*psi*$^+$] strains possibly indicating that the combination of different factors increasing translational ambiguity, such as the [*psi*$^+$] determinant and extra copies of the *SUP35* gene, leads to a level of inaccuracy incompatible with cell viability (DAGKESAMANSKAYA and TER-AVANESYAN 1991).

Our previous studies revealed at least two functional domains within Sup35p. Deletion analysis of the *SUP35* gene shows the essential function of the protein to be contained within the evolutionarily conserved C-terminal region. The N-terminal region of the protein, which varies evolutionarily both in length and sequence in *S. cerevisiae*, *Pichia pinus* and man (KUSHNIROV *et al.* 1990; HOSHINO *et al.* 1989) is not essential. Overexpression of this N-terminal region or the entire Sup35p leads to omnipotent suppression and

reduced growth of [*psi*$^+$] strains. The expression of the C-terminal portion of Sup35p, on single or multicopy plasmids, causes an antisuppressor effect (Ter-Avanesyan *et al.* 1993).

In this study we demonstrate that deletions within the N-terminal region of Sup35p cause elimination of the [*psi*$^+$] determinant. We suggest that Sup35p serves as a *trans*-acting factor required for the maintenance of [*psi*$^+$].

MATERIALS AND METHODS

Strains and plasmids: The following segregants of the diploid strain H19 described in (Dagkesamanskaya and Ter-Avanesyan 1991) were used: 1A-H19 (*MATα ade2-1 lys1-1 his3-11, 15 leu2-3, 112 SUQ5* [*psi*$^+$]); 5V-H19 (*MAT***a** *ade2-1 can1-100 leu2-3, 112 ura3-52 SUQ5* [*psi*$^+$]). A strain 10B-H49 of genotype *MATα ade2-1 SUQ5 leu2-3,112 lys1 his3-11,15 kar1-1* [*rho*$^+$] [*psi*$^+$] was derived from a cross between the strain CKO1 (*MAT***a** *leu1 kar1-1 can*^{R} [*psi*$^+$] [*rho*$^+$]) that was obtained from P. M. Lund (Oxford, Great Britain) and 1A-H19 [*psi*$^+$]. The [*psi*$^-$] derivatives of all of these strains were also used.

All deletion alleles used in this study, with the exception of *sup35-ΔN2* and *sup35-ΔN3*, were obtained by the deletion of internal restriction fragments of *SUP35* gene as shown in Figures 1 and 3. In the case of of 3′-terminal *SUP35* deletion constructs, translation terminated in adjacent plasmid DNA thus adding to the protein-encoded extensions of 7–17 amino acids of non-functional sequence. The *sup35-ΔN2* and *sup35-ΔN3* deletion alleles were generated by joining the *SUP35* promoter to the *Eco*RV or *Hpa*I restriction sites of the *SUP35* gene so that translation can start from ATG codons corresponding to Met-124 or Met-254, respectively. All deletion constructs, with the exception of *ΔBst-Hin*d, were able to direct the synthesis of corresponding truncated versions of Sup35p, as was shown by Western blot analysis (Ter-Avanesyan *et al.* 1993). The 5′-deletion alleles *sup35-ΔN3*, *sup35-ΔN2* and *sup35-ΔNB* (Figure 1) used in gene-replacement experiments were cloned in the plasmid pFL44 (F. Lacroute, Gif sur Yvette, France). Other deletion constructs of the *SUP35* gene were cloned in the plasmids pEMBLyex4 (Cesareni and Murray 1987) and pRG415 (R. Gaber, Evanston, Illinois). For details, see Ter-Avanesyan *et al.* (1993). The YEp13 plasmid have been described elsewhere (Broach *et al.* 1979).

Genetic methods: Standard yeast genetic procedures of crossing, sporulation and tetrad analysis were used to construct the appropriate strains and analyze gene segregation. Nutritional markers were scored by growth on synthetic (SC) medium lacking specific amino acids or nucleic acid bases. Sporulation and YPD media were also used (Sherman *et al.* 1986). Yeast strains were cured of the [*psi*$^+$] determinant by growth on YPD medium supplemented with 5 mM guanidine hydrochloride (GuHCl). The [*psi*$^-$] colonies of *ade2-1 SUQ5* carrying strains were chosen by pink color and adenine requirement because the serine-inserting dominant suppressor *SUQ5* (also called *SUP16*) cannot suppress the *ade2-1* ochre mutation in the absence of the [*psi*$^+$] determinant (Cox 1965; Liebman *et al.* 1975; Ono *et al.* 1979). Strains to be scored for [*psi*] were crossed to *SUQ5* [*psi*$^-$] tester and the efficiency of *SUQ5* was examined in the meiotic progeny. If *SUQ5* was efficient enough to suppress *ade2-1* in the progeny of such a cross, the tested strain was scored as [*psi*$^+$]. The meiotic segregation of the *kar1* phenotype was determined by the strong-weak complementation test of Conde and Fink (1976). All segregants of the diploid heterozygous for the *kar1-1* mutation were crossed with *MAT***a** or *MATα ade8* testers. *kar1* strains

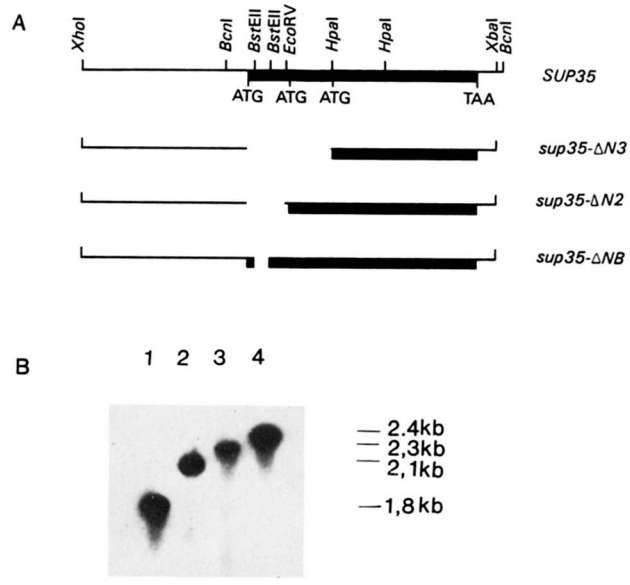

Figure 1.—Construction of deletion alleles of the chromosomal *SUP35* gene. (A) 5′-Deletion alleles used for replacement of the wild-type *SUP35* gene. The solid bar represents the coding sequence of the *SUP35* gene, lines (——) noncoding regions. The restriction map of the *SUP35* gene is shown at the top of this picture. The first, second and third in-frame ATG codons and terminator TAA codon are shown under the restriction map. The *Xho*I-*Xba*I fragments carrying the *sup35-ΔN3*, *sup35-ΔN2* or *sup35-ΔNB* alleles were integrated into a chromosome through recombination at free DNA ends (see text for details). (B) Southern blot analysis. DNA from the *sup35-ΔN* deletion mutants 1-5V-H19 (lane 1), 2-5V-H19 (lane 2), 3-5V-H19 (lane 3) and original strain 5V-H19 (lane 4) was isolated, digested with *Bcn*I and hybridized to the M13 single stranded probe, containing the *Bcn*I-*Xba*I fragment of the *SUP35* gene. A 2.4-kb fragment is observed for the strain 5V-H19 corresponding to the wild type *SUP35* gene and a 2.3-kb band is present in strain 3-5V-H19, a 2.1-kb band in strain 2-5V-H19 and a 1.8-kb band in strain 1-5V-H19 as expected for the *sup35-ΔNB*, *sup35-ΔN2* and *sup35-ΔN3* alleles, respectively.

yielded only rare complementing diploids in the mixed culture and were easily distinguishable from the *KAR1* segregants that gave a massive complementation reaction. Nonsuppressive petites in transformants of the strain 1-5V-H19 were obtained by ethidium bromide treatment (Goldbring *et al.* 1970). For performing "cytoduction" experiments, strains of opposite mating type, one of which carries the *kar1-1* mutation that blocks karyogamy, were mated by mixing them together on the surface of a YPD plate and incubated for 1 day at 30°. This mixture was then spread on appropriate medium and cytoductants were selected as described in detail in Results.

Yeast transformation: DNA transformation of lithium acetate-treated yeast was done as described by Ito *et al.* (1983).

Manipulations with DNA and RNA: Isolation of plasmid and yeast chromosomal DNA and total cellular RNA was performed as given elsewhere (Maniatis *et al.* 1982; Hoffman and Winston 1987; Sherman *et al.* 1986). Restriction endonuclease cleavage, ligation and other enzymatic procedures as well as Southern and Northern blot analyses were done as described by Maniatis *et al.* (1982) and Sherman *et al.* (1986). The DNA and RNA blots were hybridized to M13 single-stranded probe carrying the *SUP35* fragment, labeled by primer extension according to Messing (1983).

RESULTS

Construction of deletions in the chromosomal *SUP35* gene: The sequence of Sup35p can be divided into three regions (KUSHNIROV *et al.*, 1988). The C-terminal region (amino acids 254–685) shows similarity to elongation factor EF-1α, while the N-terminal (amino acids 1–123) and middle (amino acids 124–253) regions are unique and differ significantly in their amino acid content. The beginning of the middle and C-terminal regions are defined by the second (Met-124) and third (Met-254) methionines in the protein. Transformation of the 5V-H19 [*psi*$^+$] strain with multicopy or centromeric plasmids carrying either one of the 5′-deletion alleles of the *SUP35* gene (termed *sup35-ΔN3*, *sup35-ΔN2*, or *sup35-ΔNB* or in common *sup35-ΔN*, Figure 1), causes a non-suppressed phenotype, resulting in pink colonies and adenine auxotrophy (TER-AVANESYAN *et al.*, 1993).

To further examine the properties of the deleted versions of the *SUP35* gene, the chromosomal wild-type *SUP35* gene was replaced with the *sup35-ΔN3*, *sup35-ΔN2* or *sup35-ΔNB* allele. This was performed by co-transformation of the [*psi*$^+$] haploid strain 5V-H19 with the *LEU2*-carrying YEp13 plasmid and DNA fragments obtained after *Xho*I and *Xba*I digestion of the plasmids pFL44-3ATG, pFL44-2ATG and pFL44-Δ*Bst* carrying alleles *sup35-ΔN3*, *sup35-ΔN2* and *sup35-ΔB*, respectively (Figure 1). Approximately 5–10% of leucine-independent colonies in each transformation experiment were pink and adenine-requiring. Three of them (1-5V-H19, 2-5V-H19 and 3-5V-H19 presumably carrying *sup35-ΔN3*, *sup35-ΔN2* and *sup35-ΔNB*, respectively) were studied in detail. Plasmid-less colonies of these transformants were selected after streaking on non-selective YPD medium. The color of the colonies did not depend on the presence of the YEp13 plasmid. Because these transformants were pink and adenine-requiring, we expected them to carry the antisuppressor *sup35-ΔN* alleles instead of the wild-type *SUP35* gene. This was proved by Southern analysis (Figure 1).

The *sup35-ΔN* mutations cause elimination of the [*psi*$^+$] determinant: All independently obtained *sup35-ΔN* mutants were crossed with the [*psi*$^+$] and [*psi*$^-$] variants of the tester strain 1A-H19. Hybrids formed by these crosses were pink and unable to grow on adenine omission medium. Therefore the antisuppressor effect of all three *sup35-ΔN* alleles is a dominant trait. Diploids formed between strains carrying *sup35-ΔN* alleles and either [*psi*$^+$] or [*psi*$^-$] versions of the strain 1A-H19 were expected to have the same genotype: [*psi*$^+$] *ade2-1/ade2-1 SUQ5/SUQ5 sup35-ΔN/SUP35*, since all gene replacement experiments were performed in the [*psi*$^+$]-carrying strain 5V-H19. Since all of these diploids were heterozygous for one of the antisuppressor *sup35-ΔN* alleles and homozygous for the *SUQ5* allele, the diploids should segregate 2 suppressed:2 nonsuppressed spores in every tetrad, and all segregants were expected to be [*psi*$^+$]. In fact, the diploids formed by crosses with the [*psi*$^+$] tester yielded, as a rule, tetrads that segregated 2 Ade$^+$:2 Ade$^-$ rather than 4 Ade$^+$:0 Ade$^-$. That the adenine-requiring segregants from these tetrads carried *sup35-ΔN* deletion alleles was verified in several cases by Southern blot analysis (data not shown). Surprisingly, the diploids from crosses with the [*psi*$^-$] tester gave no suppressed segregants (Table 1). Nonsuppressed segregants derived from the crosses to the [*psi*$^+$] tester appeared to be [*psi*$^-$], rather than the expected [*psi*$^+$]. This was shown as follows. The *sup35-ΔN*-carrying adenine-requiring segregants from several tetrads showing monogenic segregation for adenine auxotrophy were crossed again with the [*psi*$^-$] tester strain 1A-H19. The diploids formed by these crosses did not segregate spore cultures with the suppressed phenotype. The control crosses with the [*psi*$^+$] tester resulted in [*psi*$^+$] diploids, since they produced as a rule tetrads with 2 Ade$^+$:2 Ade$^-$ segregation (data not shown).

TABLE 1

data from crosses of the *sup35-ΔN* mutants with the [*psi*$^+$] and [*psi*$^-$] variants of strain 1A-H19

Strain	Cross with	Segregation in tetrads (Ade$^+$:Ade$^-$) 2:2	1:3	0:4
1-5V-H19	[*psi*$^+$]	77	14	1
1-5V-H19	[*psi*$^-$]	0	0	30
2-5V-H19	[*psi*$^+$]	6	2	0
2-5V-H19	[*psi*$^-$]	0	0	13
3-5V-H19	[*psi*$^+$]	10	3	0
3-5V-H19	[*psi*$^-$]	0	0	15

Strains 1-5V-H19, 2-5V-H19 and 3-5V-H19 carry *sup35-ΔN3*, *sup35-ΔN2* and *sup35-ΔNB* chromosomal deletion alleles, respectively. The deviation from the 2 Ade$^+$:2 Ade$^-$ segregation pattern observed in the progeny of diploids formed in crosses with the [*psi*$^+$] tester resembles the excess of [*psi*$^-$] spore cultures usually yielded by diploids heterozygous for the recessive *pnm* mutations (COX *et al.*, 1980).

Taken together, these observations suggest that the *sup35-ΔN* deletion alleles cause elimination of the [*psi*$^+$] determinant in haploid strains. The elimination of the [*psi*$^+$] factor is a recessive trait, since crosses between the *sup35-ΔN*-bearing strains and the [*psi*$^+$] tester produced diploids that could segregate white and adenine-independent spore cultures and therefore carried the [*psi*$^+$] determinant. This property of deletion mutations in the *SUP35* gene makes them similar to the recessive *pnm* mutations already described (COX *et al.* 1980).

Rescue of [*psi*$^+$] by the expression of truncated versions of Sup35p: The results presented above suggest that the N-terminal part of Sup35p is necessary for [*psi*$^+$] maintenance. The identification of the minimal region of Sup35p sufficient to maintain the [*psi*$^+$] determinant was performed as follows (Figure 2). First, the [*rho*$^+$] and [*psi*$^+$] determinants were transferred by cytoduction from the [*rho*$^+$] [*psi*$^+$] *kar1-1* strain, 10B-

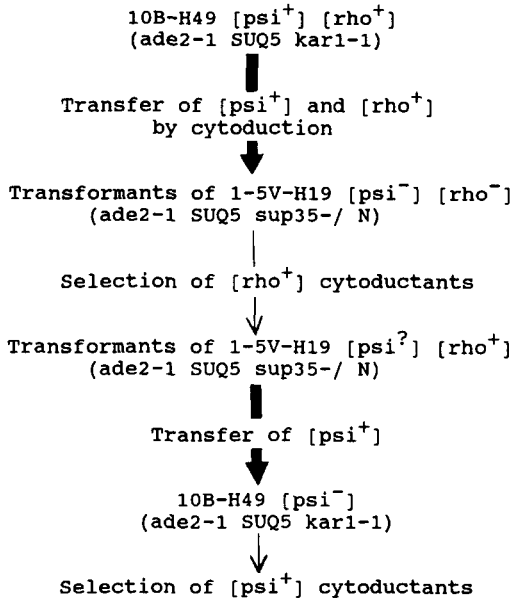

FIGURE 2.—Scheme of cytoduction experiments performed to identify the plasmids with the *sup35* deletion mutations capable of rescuing the [*psi*$^+$] determinant. Genotypes of strains are presented in MATERIAL AND METHODS. Cytoductants were selected as described in the text. [*psi*$^?$], the [*psi*] status is to be determined.

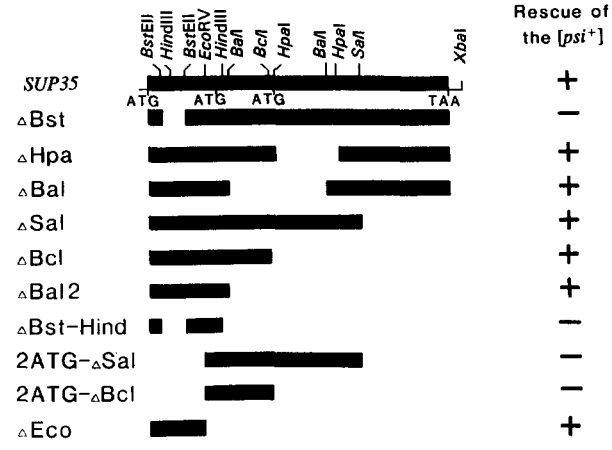

FIGURE 3.—The ability of the *sup35* deletion constructs to rescue the [*psi*$^+$] determinant. Coding regions of the constructs are represented by solid bars. "+," ability to rescue the [*psi*$^+$] determinant; "−," inability to rescue the [*psi*$^+$]. For other details, see the legend to Figure 1.

H49, to the *sup35*-Δ*N3*-bearing strain, 1-5V-H19, that had been transformed with plasmids that carry different truncated versions of the *SUP35* gene (Figure 3). [*rho*$^+$] and [*psi*$^+$] show high coincidence of transfer (COX *et al.* 1988). Therefore, cytoductants and diploids were selected from the mating mixtures of cells by transfer to histidine omission medium containing glycerol as a sole carbon source. Respiratory competent colonies phenotypically indistinguishable in other respects from the transformants of strain 1-5V-H19, were scored as cytoductants. Surprisingly, none of the cytoductants obtained showed the noticeable decrease in growth rate expected for [*psi*$^+$] strains carrying *SUP35*-containing multicopy plasmids (DAGKESAMANSKAYA and TER-AVANESYAN 1991; TER-AVANESYAN *et al.* 1993), although some of them possessed this non-Mendelian determinant as will be demonstrated below. This means that chromosomal antisuppressor *sup35*-Δ*N3* allele ameliorated the deleterious effect of overexpression of Sup35p or its C-terminally truncated versions in [*psi*$^+$] strains, possibly due to reduction of the high translational ambiguity caused by the combined action of multicopy *SUP35* plasmids and [*psi*$^+$]. The [*psi*] status of the cytoductants was revealed in the second round of cytoduction experiments (Figure 2). Only those [*rho*$^+$] cytoductants of transformants that could transfer [*psi*$^+$] by cytoduction to the [*psi*$^-$] tester strain, 10B-H49, were considered to possess the [*psi*$^+$] determinant. Mating of the transformants with strain 10B-H49 allowed us to isolate cytoductants selectively, since only those cells of the recipient strain 10B-H49 that received the [*psi*$^+$] determinant could grow on the adenine omission medium. Transformants of strain 1-5V-H19 and diploid cells cannot grow on this medium because they carry the dominant antisuppressor mutation *sup35*-Δ*N3*. Strain 10B-H19 is Ade$^-$ because *SUQ5* cannot suppress the *ade2-1* ochre mutation in the absence of [*psi*$^+$]. The selectivity of this system allowed us to develop a qualitative test for [*psi*$^+$] transfer by cytoduction (Figure 4). The adenine-independent clones selected in these experiments possess the same nuclear genotype as the tester strain 10B-H49 and can be easily converted to the adenine-dependent phenotype after curing of the [*psi*$^+$] determinant by plating on GuHCl-containing medium. Results of the study of the ability of different truncated versions of Sup35p to rescue the [*psi*$^+$] determinant in the *sup35*-Δ*N3*-carrying strain are summarized in Figure 3. It is noteworthy that in spite of the fact that most of experiments described above were performed with multicopy plasmids, the results did not depend on the plasmid copy number, since the centromeric plasmid carrying the "ΔSal" construct could efficiently rescue the [*psi*$^+$] determinant in the 1-5V-H19 strain (data not shown).

DISCUSSION

Our previous studies have shown that single or multicopy plasmids carrying the *sup35*-Δ*N* alleles generate in haploid strains an antisupressor effect but do not cause [*psi*$^+$] elimination (TER-AVANESYAN *et al.* 1993). In this report we have shown that replacement of the chromosomal wild-type *SUP35* gene with the *sup35*-Δ*N* deletion alleles in the *ade2-1 SUQ5* [*psi*$^+$] strain causes, in addition to a previously observed non-suppressed phenotype, a loss of the [*psi*$^+$] determinant. These effects are expressed differently in heterozygous diploids: the antisuppressor effect is dominant while the elimination

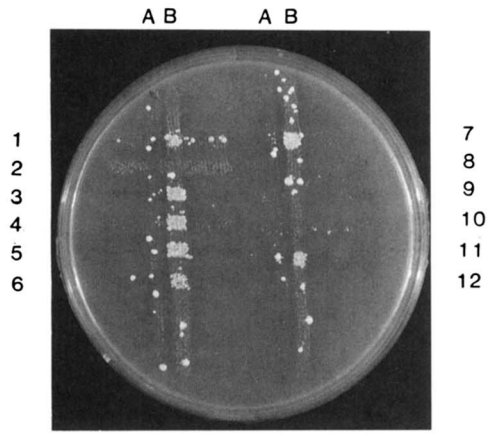

FIGURE 4.—An illustration of the qualitative tests to determine the capacity of the plasmids to support maintenance of [*psi*$^+$] determinant in strain 1-5V-H19. The vertical streaks: (A) 1A-H19 (*MAT*α *ade2-1 SUQ5* [*psi*$^-$]); (B) 10B-H19 (*MAT*α *ade2-1 SUQ5 kar1-1* [*psi*$^-$]). The strains streaked horizontally are cytoductants of transformants of 1-5V-H19 (*MAT***a** *ade2-1 SUQ5 sup35-ΔN3*) with multicopy plasmids carrying wild-type *SUP35* gene and *SUP35* deletion constructs being tested for ability to transfer [*psi*$^+$]: 1, *SUP35* gene; 2, "ΔBst"; 3, "ΔHpa"; 4, "ΔBal"; 5, "ΔBal2"; 6, "ΔSal"; 7, "ΔBcl"; 8, "ΔBst-Hind"; 9, "2ATG-ΔSal"; 10, "2ATG-ΔBcl"; 11, "ΔEco"; 12 is the transformant carrying the control plasmid pEMBLyex. The complete genotypes of strains mentioned above are presented in MATERIAL AND METHODS. The appearance of [*psi*$^+$] cytoductants depends upon the presence of the *kar1-1* mutation in the tester strain.

of the [*psi*$^+$] determinant is a recessive trait, *i.e.*, heterozygous diploids possess an antisuppressor phenotype but carry the [*psi*$^+$] determinant. Dominant and recessive mutations causing either an antisuppressor phenotype or [*psi*$^+$] loss have been described in *S. cerevisiae* (MCCREADY and COX 1973; MCCREADY *et al.* 1977; COX *et al.* 1980). The *sup35-ΔN* alleles differ from these mutations by their ability to cause both effects simultaneously.

It seems likely that the dominant antisuppressor effect is not the direct consequence of mutations that simply delete or reduce some of the *SUP35* functions. In contrast, the recessive *pnm*-like effect suggests the loss of some function that is essential for [*psi*$^+$] maintenance. We suggest that the Sup35 protein serves as a *trans*-acting factor essential for the [*psi*$^+$] maintaining machinery. Deletion analysis performed in this study revealed that the first 114 amino acids of Sup35p (*e.g.*, the "ΔEco" construct) are sufficient for maintenance of the [*psi*$^+$] determinant. This sequence also represents the smallest region of Sup35p whose overexpression generates an omnipotent suppressor effect. Thus, this study has revealed a specific role of this domain of Sup35p in maintaining the [*psi*$^+$] determinant. These results are in agreement with the data obtained recently in the laboratory of B. S. COX (Oxford, Great Britain). These authors have cloned and sequenced the *PNM2* gene, which was found to be identical to the *SUP35* omnipotent suppressor. A mutation in this gene was identified causing amino acid substitution at position 58 of the encoded protein (S. M. DOEL, C. R. NIERRAS, S. J. MCCREADY and B. S. COX, personal communication). This confirms our conclusion about the critical role of the N-terminal domain of Sup35p in the maintenance of the [*psi*$^+$] determinant. It is noteworthy that in contrast to the *sup35-ΔN* deletion mutations, the [*psi*$^+$] loss caused by the *PNM2* point mutation is a dominant trait and a heterozygous diploid gradually loses over successive generations the ability to produce [*psi*$^+$] spores (MCCREADY *et al.* 1977). Another feature that distinguishes the *sup35-ΔN* deletion alleles from the *PNM2* point mutation is that, unlike deletion mutations, point mutation in the N-terminal part of the protein does not generate an antisuppressor phenotype. It is possible to explain the dominant phenotype of the *PNM2* point mutation by the assumption that the mutant protein acts by titration of its wild-type counterpart or some factor(s) essential for the maintenance of [*psi*$^+$]. It should be also noted that expression of Sup35p is necessary but not sufficient for the [*psi*$^+$] phenotype since strains carrying the wild-type *SUP35* gene may either possess or not the [*psi*$^+$] determinant. Moreover, the expression of the *SUP35* gene does not depend on the [*psi*] status of the cell, as was determined by RNA blot analysis (not shown).

Simultaneous but separate expression of both the N- and C-terminal domains of Sup35p is not the same as the expression of the entire protein, since segregants combining the [*psi*$^+$] determinant, chromosomal *sup35-ΔN3* allele and plasmid-borne alleles of the *SUP35* gene encoding a C-terminally truncated proteins (*e.g.*, the "ΔEco," "ΔBal," "ΔBcl" or "ΔSal" constructs) possess an antisuppressor phenotype. Therefore, for the Sup35 protein to possess the wild-type activity the integrity of these domains is required. In spite of this, the N-terminal domain of the Sup35 protein expressed alone allows maintenance of the [*psi*$^+$] determinant.

In conclusion we would like to emphasize that the C-terminal domain of Sup35p is essential for cell viability while the N-terminal domain of this protein is essential for [*psi*$^+$] "viability."

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