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Exceptional codon recognition by the glutamine tRNAs in *Saccharomyces cerevisiae*

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Recently, it was shown that wild-type glutamine tRNAs in yeast cause low-level nonsense suppression that can be enhanced by increasing glutamine tRNA gene copy number. In order to investigate glutamine tRNA behavior further, anticodon mutations that confer nonsense suppression were identified in yeast *sup70* gene, which codes for glutamine $tRNA_{CAG}$. In this study we show that suppressors derived by mutation severely limit growth such that suppressor-bearing spores germinate but arrest cell division at approximately the 50 cell stage. Analysis of a *sup70* deletion was used to establish that growth limitation results from loss of wild-type glutamine $tRNA_{CAG}$ function. By exploiting the growth inhibition of *sup70* alleles, some exceptional codon recognition properties of glutamine tRNAs were revealed. Our results indicate that amber suppressor glutamine $tRNA_{UAG}$ can translate 5′-CAG-3′ glutamine codons with low efficiency in the presence of an A/C mismatch at the first position of the codon, suggesting that reading may occur at a low level by a two-out-of-three reading mechanism. In addition, when glutamine $tRNA_{CAA}$ is over-expressed *in vivo*, it translates 5′-CAG-3′ codons using a mechanism that resembles prokaryotic-like U/G wobble, which normally does not occur in yeast. Our studies also suggest that the yeast glutamine tRNA suppressors could potentially be exploited to express ciliated protozoan genes that normally contain internal 5′-UAG-3′ and 5′-UAA-3′ codons.

Key words: genetic code/glutamine tRNA/translation termination/yeast

Introduction

Glutamine tRNAs exhibit unconventional properties in a variety of organisms that reveal how some aspects of the present-day genetic code might have evolved. The most extreme form of exceptional tRNA behavior has been found in the ciliated protozoa, including *Paramecium, Tetrahymena* and *Stylonychia*, where the otherwise universal stop codons 5′-UAG-3′ and 5′-UAA-3′ are normally translated as glutamine (Caron and Meyer, 1985; Fox, 1985; Helftenbein, 1985; Horowitz and Gorovsky, 1985; Prat *et al.*, 1986; Preer *et al.*, 1985; Cupples and Pearlman, 1986; Grivell, 1986). In *Tetrahymena* the departure from the universal genetic code is made possible by the presence of two novel glutamine tRNAs containing anticodon nucleotides complementary to 5′-UAG-3′ and 5′-UAA-3′ stop codons (Kuchino *et al.*, 1985; Hanyu *et al.*, 1986). This explains why 5′-UGA-3′ serves as the only available signal for translational termination in the ciliates.

Other studies show that glutamine tRNAs can interact with specific codons through unconventional anticodon:codon pairing relationships. For example, glutamine $tRNA_{CAA}$ may act as an *in vivo* amber suppressor in mouse cells infected with Moloney murine leukemia virus (Mo-MuLV), allowing read-through of a 5′-UAG-3′ amber codon within the viral transcript to produce a single polyprotein (Philipson *et al.*, 1978; Varmus, 1985; Yoshinaka *et al.*, 1985; Kuchino *et al.*, 1987). Recognition of the viral 5′-UAG-3′ codon by glutamine $tRNA_{CAA}$ (anticodon 3′-GUU_m-5′) may require wobble pairing at both the first and third anticodon positions. The features of tRNA structure and/or mRNA context that permit such unusual codon recognition are not yet clear.

In the yeast *Saccharomyces cerevisiae*, the two wild-type glutamine tRNA isoacceptors that read 5′-CAG-3′ and 5′-CAA-3′ glutamine codons suppress 5′-UAG-3′ and 5′-UAA-3′ nonsense mutations, respectively, when the tRNA genes are placed on multi-copy plasmids (Calderon *et al.*, 1984; Pure *et al.*, 1985; Weiss and Friedberg, 1986). More importantly, it has also been shown that wild-type glutamine $tRNA_{CAG}$ confers inefficient but detectable 5′-UAG-3′ amber suppression when expressed at normal physiological levels (Weiss *et al.*, 1987). Naturally-occurring amber suppression in normal cells has been attributed to unconventional wobble pairing between a G at the third position of the anticodon and a U at the first position of the codon. Based on these results, the inefficient amber suppressor glutamine $tRNA_{CAG}$ in yeast appears to have the properties expected of a proposed evolutionary progenitor for the efficient amber suppressor glutamine $tRNA_{UAG}$ in ciliates (Hanyu *et al.*, 1986; Weiss *et al.*, 1987).

In this study we describe genetic experiments designed to provide additional information on the decoding properties of the glutamine tRNAs in yeast. Genetic analyses of glutamine tRNA function were initially made possible through the isolation of growth-limiting nonsense suppressor mutations in the *sup70* gene, which encodes glutamine $tRNA_{CAG}$. Once the *sup70* gene was cloned, it was possible to evaluate suppression and growth inhibition through the *in vitro* construction and *in vivo* phenotypic analysis of a *sup70* deletion. The results of these studies show that glutamine tRNAs exhibit additional, unexpected properties that allow recognition of specific codons in violation of the standard rules for genetic code translation.

Results

A growth-limiting amber suppressor defines the sup70 locus

A mutation in the structural gene for glutamine $tRNA_{CAG}$ was initially identified in a collection of dominant, amber nonsense suppressors obtained by reversion of the amber mutation *trp1-1^{am}* in the diploid strain IEY68 (Table I; Edelman, 1987). The revertants were screened by tetrad analysis to identify suppressors that inhibited growth in haploid spore progeny. One Trp^+ diploid that later proved to carry an altered glutamine $tRNA_{CAG}$ consistently yielded tetrads containing two viable Trp^- spores and two spores that failed to form normal-sized colonies (Figure 1A). This result suggested that the revertant contained a suppressor of *trp1-1^{am}* that co-segregated with a recessive growth defect. Microscopic examination of the suppressor-bearing spores indicated that they successfully germinated but underwent limited growth, forming micro-colonies that terminated cell division at approximately the 50 cell stage.

In order to analyze inviable spores derived from the revertant, matings were performed between ungerminated haploid spores and vegetative haploid cells of a strain carrying *trp1-1^{am}*. Approximately half of the diploids resulting from the spore-cell matings were Trp^+ in phenotype and were therefore derived by mating with the haplo-inviable spores. These Trp^+ diploids had normal growth rates, confirming that the spores carried a dominant amber suppressor that co-segregated with a recessive growth defect. In additional genetic crosses, the mutation conferring the Trp^+ phenotype was shown to be unlinked to the *trp1* locus, confirming that reversion was caused by an extragenic suppressor of *trp1-1^{am}*. The suppressor, referred to in previous communications as *sup60-1^{am}* (Winey *et al.*, 1989; Sandbaken and Culbertson, 1988), has been renamed *SUP70-1^{am}* to avoid conflict with pre-existing nomenclature for leucine-inserting nonsense suppressors (Liebman *et al.*, 1984).

Table I. List of strains

Strain	Genotype
S288C	*MATα CUP1 gal2 mal SUC2*
IEY68	*MATa/α his4-38/HIS4$^+$ leu2-3/leu2-3 trp1-1^{am}/trp1-1^{am} ura3-52/ura3-52 lys1-1^{oc}/lys1-1^{oc} met14/MET14$^+$*
IEY75	*MATa leu2-3 ura3-52 trp1-1^{am} lys1-1^{oc}*
IEY83	*MATa/α his4-38/HIS4$^+$ leu2-3/leu2-3 trp1-1^{am}/trp1-1^{am} ura3-52/ura3-52 lys1-1^{oc}/lys1-1^{oc} SUP70-1^{am}/sup70$^+$*
IEY98	*MATa/MATα rna1/RNA1$^+$ ura3-52/ura3-52 ade4/ADE4$^+$ SUP8/sup8$^+$ sup70$^+$/sup70$^+$::URA3$^+$ lys1-1^{oc}/lys1-1^{oc}*
IEY122	*MATa/α his4-38/HIS4$^+$ leu2-3/leu2-3 trp1-1^{am}/trp1-1^{am} ura3-52/ura3-52 lys1-1^{am}/lys1-1^{am} met14/MET14$^+$ sup70$^+$/Δsup70::LEU2$^+$*
IEY151	*MATα ura3-52 trp1-1^{am} leu2-3 met14 SUP70-1^{am} REV1-1*
IEY154	*MATα sup70$^+$/sup70$^+$::[pIE14(URA3$^+$ SUP70-1^{am})] ura3-52 trp1-1^{am} lys1-1^{oc} leu2 ade2-1^{oc}*
IEY159	*MATa sup70$^+$/sup70$^+$::[pIE14(URA3$^+$ SUP70-1^{am})] ura3-52 trp1-1^{am} lys1-1^{oc} leu2 ade2-1^{oc}*
IEY166	*MATα ura3-52 leu2-3 trp1-1^{am} REV1-1*
IEY167	*MATa ura3-52 trp1-1^{am} leu2-3 lys1-1^{oc} met14 REV1-1*
IEY198	*MATa/MATα ura3-52/ura3-52 lys1-1^{oc}/lys1-1^{oc} ade2-1^{oc}/ade2-1^{oc} trp1-1^{am}/trp1-1^{am} met8-1^{am}/met8-1^{am} SUP70-2^{oc}/sup70$^+$*

The sup70 gene encodes glutamine $tRNA_{CAG}$ and maps on chromosome 13

In order to clone the *SUP70-1^{am}* gene, a yeast genomic library was constructed by inserting *Hin*dIII restriction fragments derived from the *SUP70-1^{am}/sup70$^+$* heterozygous diploid strain IEY83 into the shuttle plasmid YCp50 (Table I; Table II). The plasmid library was used to transform the diploid strain IEY68 (Table I) to a Ura^+ phenotype. Ura^+ transformants were then screened to identify those that were Trp^+ in phenotype on the assumption that the *SUP70-1^{am}* allele on the YCp50 plasmid would suppress the *trp1-1^{am}* mutation in strain IEY68. Among 5000 transformants screened, one Ura^+ Trp^+ transformant exhibited mitotic co-instability and meiotic co-inheritance of the Ura^+ and Trp^+ phenotypes, suggested that the plasmid in this transformant, designated pIE17, carried an amber suppressor.

pIE17 was transferred to *E.coli* strain 6507, and was found to contain a 7.6 kb yeast *Hin*dIII fragment. A 4.6 kb *Hin*dIII–*Bam*HI fragment from this region (Figure 2) was

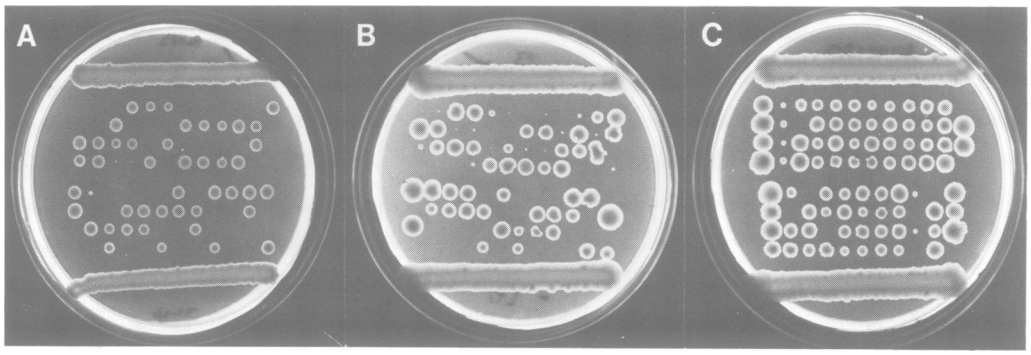

Fig. 1. Phenotypes of *sup70* alleles in tetrads. The upper half of each plate contains tetrads from the diploid IEY83 (Table I), which is heterozygous for *SUP70-1^{am}/sup70$^+$*. The lower half of each plate contains tetrads from the diploid IEY122 (Table I), which is heterozygous for *Δsup70$^+$::LEU2$^+$/sup70$^+$*. Panel (**A**) shows growth at 30°C. Spores carrying the amber suppressor or null *sup70* alleles formed micro-colonies of ~50 cells, but failed to grow into visible colonies. Panel (**B**) shows growth at 18°C. Spores carrying the amber suppressor form small but visible colonies, whereas spores carrying the null allele form only non-visible micro-colonies. Panel (**C**) shows growth at 30°C in tetrads from strains IEY83 and IEY122 that had been transformed with the multi-copy plasmid pIE30 (Table II), which carries a glutamine $tRNA_{CAA}$ gene. In most of the tetrads all four spores formed visible colonies. This result indicates that growth limitation caused by mutations in the *sup70* gene can be alleviated by over-expression of glutamine $tRNA_{CAA}$.

subcloned into YCp50 to generate plasmid pIE22 (Table II). This plasmid conferred amber suppression upon transformation of a haploid yeast strain containing *trp1-1^{am}*, indicating that the gene of interest resided within the *Hin*dIII–*Bam*HI fragment. We determined the DNA sequence of an internal *Eco*RI–*Xho*I fragment representing

Table II. Description of plasmids

Plasmid	Vector	Functional yeast DNA	*sup70* DNA insert	Source
YCp50	–	*CEN4 URA3$^+$*	–	M.Johnston
YIp5	–	*URA3$^+$*	–	D.Botstein
pGT20	pBR322	Gln-tDNA$_{CAA}$	–	J.Carbon
EC402	YIp5	*URA3$^+$*, 2μ DNA	–	E.Craig
pIE17	YCp50	*CEN4, URA3$^+$, SUP70-1^{am}*	*Hin*dIII–*Hin*dIII	This study
pIE22	YCp50	*CEN4, URA3$^+$, SUP70-1^{am}*	*Bam*HI–*Hin*dIII	This study
pIE24	YIp5	*URA3$^+$, SUP70-1^{am}*	*Bam*HI–*Hin*dIII	This study
pIE28	YIp5	*URA3$^+$, sup70$^+$*	*Bam*HI–*Hin*dIII	This study
pIE30	EC402	*URA3$^+$*, 2μ DNA, Gln-tDNA$_{CAA}$	*Bam*HI–*Hin*dIII	This study
pIE32	YCp50	*CEN4, URA3$^+$*, Gln-tDNA$_{CAA}$	*Bam*HI–*Hin*dIII	This study
pIE33	EC402	*URA3$^+$*, 2μ DNA, *sup70$^+$*	*Bam*HI–*Hin*dIII	This study
pIE34	YCp50	*CEN4, URA3$^+$, sup70$^+$*	*Bam*HI–*Sal*I	This study
pIE35	pBR322	*Δsup70$^+$::LEU2$^+$*	*Bam*HI–*Hin*dIII	This study
pIE37	YIp5	*URA3$^+$, SUP70-2^{oc}*	*Bam*HI–*Hin*dIII	This study
pIE45	YCp50	*CEN4, URA3$^+$, SUP70-1^{am}*	*Sau*3A–*Eco*RV	This study
pIE46	EC402	*URA3$^+$*, 2 μ DNA, *SUP70-1^{am}*	*Sau*3A–*Eco*RV	This study

Restriction fragments listed under '*sup70* DNA insert' are indicated on the restriction map in Figure 1. Plasmids containing 2μ DNA carry the origin of replication from the endogenous 2μ yeast plasmid. *CEN4* centromeric sequences confer single-copy, Mendelian segregation.

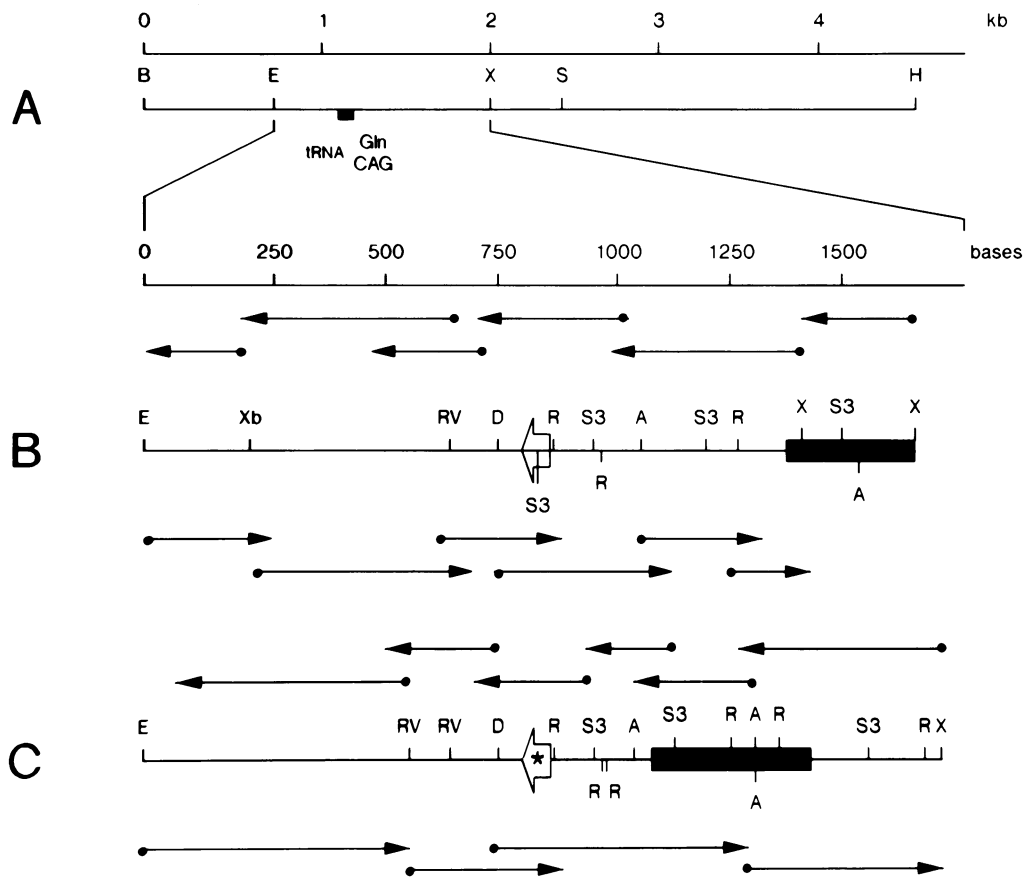

Fig. 2. Restriction map of the *sup70* region. Panel (**A**) shows the restriction map for the 4.6 kb *Bam*HI–*Hin*dIII fragment, which was derived from the original 7.6 kb *Hin*dIII–*Hin*dIII clone. Panels (**B**) and (**C**) show expanded restriction maps for *sup70$^+$* DNA from plasmid pIE28 and for *SUP70-1^{am}* DNA from plasmid pIE22 (Table II), respectively. The open arrows indicate the location and direction of transcription of the tRNA gene. The asterisk in panel (C) indicates the location of the suppressor mutation, which abolishes a *Sau*3A restriction site. Thick lines in panels (B) and (C) indicate the presence of delta (δ) or tau (τ) repeated genetic elements. Narrow arrows indicate the direction and extent of DNA sequence analysis. Restriction sites are designated as follows: A = *Alu*I, B = *Bam*HI, D = *Dra*I, E = *Eco*RI, H = *Hin*dIII, R = *Rsa*I, RV = *Eco*RV, S = *Sal*I, S3 = *Sau*3A, X = *Xho*I, Xb = *Xba*I.

approximately half of the yeast DNA insert in plasmid pIE22. The sequence revealed the presence of a tDNA coding region corresponding to a tRNA with the anticodon 3′-AUC-5′, which could potentially read a 5′-UAG-3′ amber codon. This finding coupled with an analysis of sequence flanking the tRNA gene suggested that the *SUP70-1*^{am} allele was derived by a single base change from a previously identified wild-type glutamine $tRNA_{CAG}$ gene containing a 3′-GUC-5′ anticodon (Calderon *et al.*, 1984; Lin *et al.*, 1986; Weiss and Friedberg, 1986).

To confirm that *SUP70-1*^{am} is a mutation in a gene encoding glutamine $tRNA_{CAG}$, the wild-type allele was cloned by the integration/excision method (Roeder and Fink, 1980). DNA sequence analysis of the cloned *sup70*$^{+}$ gene in plasmid pIE28 (Table II; Figure 2) revealed a tDNA coding region identical to that of the *SUP70-1*^{am} allele except that it contained the anticodon sequence 3′-GUC-5′ (Figure 3A). Therefore, amber suppression results from a G to A transition at position 36 in the anticodon of glutamine $tRNA_{CAG}$ (Figure 3B).

We also showed that the cloned suppressor gene corresponds to the genetic locus defined by *SUP70-1*^{am}. The *Hin*dIII−*Bam*HI fragment carrying the glutamine $tRNA_{UAG}$ amber suppressor gene was first inserted into YIp5 to construct the integrative vector pIE24 (Table II; Figure 2). Site-directed chromosomal integration of pIE24 was accomplished by cutting the plasmid at a unique *Xho*I site within the yeast DNA insert followed by transformation of strain IEY167 to a Trp^{+} Ura^{+} phenotype. The transformants were analyzed in crosses homozygous for *REV1-1*, a mutation described in more detail below, which restores viability in segregants carrying *SUP70-1*^{am} and which permits appropriate phenotypic analysis without interference due to growth inhibition.

Transformants of strain IEY167 were crossed with strain IEY166 (Table I). Tetrads derived from each cross contained two Trp^{+} Ura^{+} and two Trp^{-} Ura^{-} spores, indicating that in each case the plasmid had integrated into a chromosome. In crosses with strain IEY151 (Table I), tetrads contained two Trp^{+} Ura^{+} and two Trp^{+} Ura^{-} spores. The absence of Trp^{-} (*sup70*$^{+}$) recombinant spores indicates that the site of pIE24 integration is closely linked to the *sup70* locus. Since integration occurs by homologous recombination in yeast (Hicks *et al.*, 1978), this results suggests that *sup70* is synonymous with the cloned glutamine $tRNA_{CAG}$ gene.

The map location of the *sup70* gene was initially determined to be on chromosome 13 using the 2μ plasmid-induced chromosome breakage method of Falco and Botstein (1983). Subsequently, linkage was demonstrated between the *sup70* locus and the chromosome 13 markers *rna1*, *SUP8* and *ade4* (Table III). For the purpose of genetic mapping, the *sup70*$^{+}$ locus was marked by a linked, integrated copy of the *URA3*$^{+}$ gene. The analysis of recombinants between *URA3*$^{+}$ and chromosome 13 markers suggested the gene order *CEN13-rna1-SUP70-SUP8-ade4*. *SUP70* is 5.0 cM from *SUP8* and 32.8 cM from *rna1*.

At least eight genes encode two closely related glutamine tRNA isoacceptors

We attempted to determine the number of redundant gene copies encoding glutamine $tRNA_{CAG}$ using Southern hybridization. Genomic DNA from strain S288C was digested with a variety of enzymes, separated by agarose

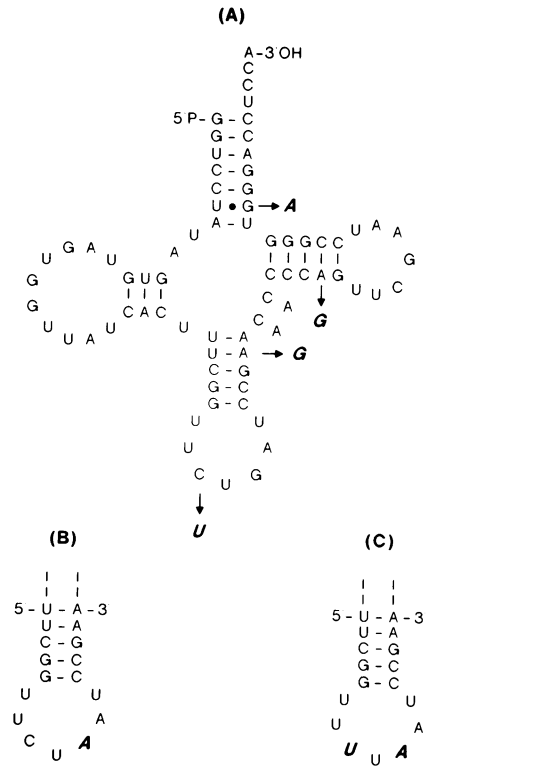

Fig. 3. Clover-leaf structures of wild-type and mutant glutamine tRNAs. Panel (**A**) shows the wild-type glutamine $tRNA_{CAG}$. The 3′-terminal CAA residues are not encoded in the gene. Base modifications are not shown. Arrows pointing towards residues shown in boldface represent alternate nucleotides found in glutamine $tRNA_{CAA}$. Panel (**B**) shows the anticodon stem/loop region of the *SUP70-1*^{am} product, where the nucleotide change that confers amber suppression is shown in boldface. Panel (**C**) shows the anticodon stem/loop region of the *SUP70-2*^{oc} product, where the two nucleotide changes that confer ochre suppression are shown in boldface.

Table III. Genetic mapping of the *SUP70* locus

Marker pair	PD	NPD	TT	Total	Distance (cM)
rna1 - URA3^{+}	56	3	60	119	32.8
SUP8 - URA3^{+}	107	0	12	119	5.0
SUP8 - ade4	84	1	36	121	17.4

The *SUP70* locus was marked by integration of the *URA3*$^{+}$ gene at this locus. PD = parental ditype ascus, NPD = non-parental ditype ascus, TT = tetratype ascus. Tetrads were derived from the diploid strain IEY98 (Table I). The map distance in centiMorgans (cM) was calculated using the formula of Perkins (1949).

gel electrophoresis, transferred to nitrocellulose and probed with a labeled *Sau*3A−*Eco*RI fragment containing the 3′ half of the *SUP70-1*^{am} gene plus 3′ flanking sequences (Figure 4). This particular probe was selected to avoid inclusion of sequence elements located to the 5′ side of the tRNA gene that are known to be repeated throughout the yeast genome (Figure 2). In those lanes in which sufficient resolution was achieved, a minimum of eight bands was detected.

This result could be a misleading indicator of glutamine $tRNA_{CAG}$ gene copy number for two reasons: (i) the presence of non-tDNA repeated sequences in the 3′ flanking region of the probe, or (ii) cross-hybridization of the probe with other tRNA isoacceptors. Hybridization experiments using probes that contain only the 3′ flanking region yielded

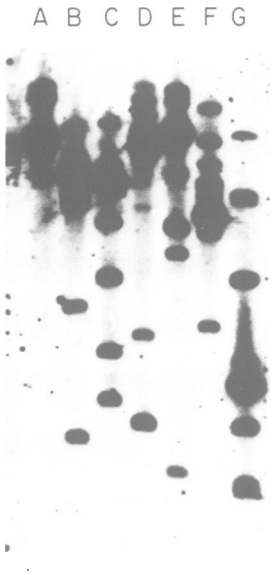

Fig. 4. Copy number of genes encoding the glutamine tRNAs. 5 μg of yeast DNA from strain S288C (Table I) were digested with (A) *Bam*HI, (B) *Hin*dIII, (C) *Eco*RI, (C) *Xho*I, (E) *Pst*I, (F)*Xba*I, or (G) *Xho*I + *Eco*RI. Digested DNA was size fractionated by agarose gel electrophoresis and transferred to a nitrocellulose filter. The filter was probed with a ^{32}P-labeled *Sau*3A−*Eco*RI fragment from plasmid pIE22 (Table I, Figure 1), which carries the *SUP70-1^{am}* allele (glutamine tRNA$_{CAG}$). An identical pattern was observed using glutamine tRNA$_{CAA}$ gene probes, indicating cross-hybridization between the two families of genes (see text). The results show that there are at least eight genes encoding the two isoacceptors.

a single band regardless of the enzyme used to digest genomic DNA, indicating that the observed banding patterns shown in Figure 4 were not due to the presence of repeated sequences 3′ to the tRNA gene.

Previously published DNA sequence data indicate that glutamine tRNA$_{CAG}$ and glutamine tRNA$_{CAA}$ differ in sequence at only four positions including the anticodon (Figure 3) (Calderon *et al.*, 1984; Lin *et al.*, 1986; Weiss and Friedberg, 1986). Thus, it seemed likely that some of the bands observed in Figure 4 might result from cross-hybridization. Attempts to distinguish tDNA$_{CAG}$ from tDNA$_{CAA}$ by altering hybridization conditions or by using restriction enzymes that cut within one but not the other tDNA coding sequence gave equivocal results. However, banding patterns identical to those shown in Figure 4 were generated using a previously cloned tDNA$_{CAA}$ probe (Tschumper and Carbon, 1982). This result suggests that cross-hybridization is occurring, and that there are at least eight genes encoding the two isoacceptors combined.

A SUP70 ochre suppressor allele limits growth

We reasoned that it should be possible to derive an ochre suppressor allele of *sup70* by as single nucleotide change of C to U at position 34 in the 3′-AUC-5′ anticodon of *SUP70-1^{am}*. Unlike the behavior of bacterial ochre suppressors, the resulting anticodon in yeast would not be expected to simultaneously read amber codons (Guthrie and Abelson, 1982). Mutations at *sup70* that confer ochre suppression were isolated in strain IEY159 (Table I), which contains the *sup70^{+}* allele on chromosome 13 along with an adjacent, integrated copy of plasmid pIE24, which carries *SUP70-1^{am}* and URA3^{+} (Table II). This strain also contains both amber (*trp1-1*) and ochre (*lys1-1* and *ade2-1*) mutations, allowing selection for co-reversion of two ochre mutations concomitant with the loss of amber suppression.

A Lys^{+} Ade^{+} Trp^{-} revertant of spontaneous origin was obtained in this strain and crossed with strain IEY154, which contains an integrated copy of plasmid pIE24[*SUP70-1^{am}*] at the *sup70^{+}* locus (Tables I and II). Tetrad analysis of the sporulated diploid showed that the Trp^{+} phenotype conferred by the *SUP70-1^{am}* allele was in complete repulsion with co-segregating Lys^{+} Ade^{+} phenotypes. This result indicates that ochre suppression is due to a single mutation linked to the *sup70* locus and presumably in the integrated copy of the *SUP70-1^{am}* gene.

The ochre suppressor allele, designated *SUP70-2^{oc}*, was cloned by chromosomal excision of plasmid pIE24 followed by transformation of *E.coli* strain 6507 to ampicillin resistance. A plasmid designated pIE37 was recovered and shown to confer ochre suppression upon re-transformation of strain IEY68 (Table I). DNA sequence analysis revealed that the glutamine tRNA gene on this fragment contains a C to U transition at position 34 (Figure 3C). The corresponding tRNA contains the anticodon sequence 3′-AUU-5′, which would be expected to read 5′-UAA-3′ ochre codons. The ochre suppressor tRNA thus differs from the wild-type 3′-GUC-5′ anticodon at both the first and third anticodon nucleotides (positions 36 and 34, respectively).

In order to examine the growth effects of *SUP70-2^{oc}* in haploid spores, a heterozygous *SUP70-2^{oc}/sup70^{+}* diploid containing the *lys1-1^{oc}2* ochre mutation in homozygous condition was constructed and sporulated. Each tetrad contained two viable Lys^{-} spores and two spores that failed to form normal-sized colonies. Like spores containing *SUP70-1^{am}*, the slow-growing spores in these tetrads formed micro-colonies that terminated growth at approximately the 50 cell stage. In a spore-cell lethal rescue experiment similar to that described above for *SUP70-1^{am}*, the growth limitation in haploid spores was shown to be caused by the presence of the ochre suppressor mutation. Thus, both *SUP70-1^{am}* and *SUP70-2^{oc}* nonsense suppressors confer a similar growth-limiting phenotype in haploid strains.

Suppression of growth limitation

In order to simplify genetic analysis of the recessive lethal suppressors, we identified an unlinked dominant mutation that suppressed the recessive growth defect associated with *SUP70-1^{am}* without affecting the dominant amber suppressor phenotype. The mutation was identified among fast-growing revertants arising from the micro-colonies derived from *SUP70-1^{am}* lethal spores. One revertant, designated IEY151, was crossed with strain IEY75 (Table I). Among twenty tetrads from the diploid, four tetrads contained four viable spores of which two were Trp^{+} (parental ditype); twelve tetrads contained three viable spores of which one was Trp^{+} (tetratype); and four tetrads contained two viable spores of which none were Trp^{+} (non-parental ditype). This result is consistent with the independent segregation of *SUP70-1^{am}* and a second mutation, designated *REV1-1*, that restores viability to spores carrying *SUP70-1^{am}*. This conclusion was confirmed in backcrosses between IEY151 and each of the two *sup70^{+}* spores of a non-parental ditype tetrad. Each tetrad contained two Trp^{+} and two Trp^{-} spores all of which were viable, indicating

that the crosses were homozygous for *REV1-1*. Similar genetic crosses revealed that *REV1-1* also suppresses the growth defect in strains carrying *SUP70-2^{oc}*. Thus, the *REV1-1* mutation provides a way to study *SUP70* suppressors in diagnostic crosses without interference from the growth limitation observed in *rev1$^+$ SUP70* suppressor strains.

Nonsense suppression by SUP70 alleles

The ability of *SUP70* alleles to suppress a variety of nonsense mutations was determined in strains containing single integrated copies of either *SUP70-1^{am}* or *SUP70-2^{oc}* (Table IV). In general, we found that like other yeast nonsense suppressors *SUP70-2^{oc}* was specific for ochre alleles and failed to suppress amber mutations. This result is indicative of the restricted wobble characteristic of yeast nonsense suppressors (Guthrie and Abelson, 1982). Furthermore, *SUP70-1^{am}*-mediated suppression in a strain containing a single integrated gene copy was more efficient than suppression in a strain containing multiple copies of the wild-type *sup70$^+$* gene (Weiss *et al.*, 1987).

Loss of sup70 function limits growth

In order to determine the cause of growth limitation in haploid spores carrying *SUP70* nonsense suppressor mutations, diploid strain IEY122 (Table I) was constructed which is heterozygous for *sup70$^+$* and a null allele (deletion), designated *Δsup70::LEU2$^+$* (Materials and methods). IEY122 was sporulated and tetrads were analyzed. Each tetrad contained two Leu$^-$ spores that grew normally and two spores that formed microcolonies of ~50 cells before terminating cell division. The null allele therefore appeared to confer a growth defect similar to that observed for the *SUP70-1^{am}* and *SUP70-2^{oc}* alleles.

The growth defect of the *sup70* deletion could be alleviated by the introduction of a wild-type *sup70$^+$* gene. In one experiment, tetrads from a transformant of IEY122 containing pIE34, a centromere-containing *URA3$^+$ sup70$^+$* plasmid (Table II), segregated 4$^+$:0$^-$, 3$^+$:1$^-$, and 2$^+$:2$^-$ for viability. The recovery of viable Leu$^+$ spores containing pIE34 indicates that viability can be restored in strains carrying a null allele by the presence of the *sup70$^+$* gene on a single copy plasmid. Viability was restored in a similar manner by the presence of the *sup70$^+$* gene on the multicopy 2μ plasmid pIE33 (Table II).

Since the *Δsup70::LEU2$^+$* allele consists of a deletion of 1.1 kb of DNA flanking the *sup70* gene (Materials and methods), it was necessary to determine whether growth limitation results from the absence of *sup70$^+$* function or from the loss of potentially essential flanking DNA sequences. Restoration of normal growth by the introduction of *sup70$^+$* on a plasmid did not rule out a possible role for flanking sequences because the plasmids used in the experiments described above carried all of the flanking DNA that was deleted in the *Δsup70::LEU2$^+$* mutation.

We devised two strategies to address this problem. First, we crossed a strain carrying the *REV1-1* mutation, which supresses the growth limitation caused by *SUP70* nonsense suppressors, as described above, with a haploid strain carrying the integrated *Δsup70::LEU2$^+$* allele and a *sup70$^+$* gene on a plasmid. Spore segregants were identified that carried *REV1-1* and *Δsup70::LEU2$^+$* but which lacked the *sup70$^+$*-bearing plasmid. These segregants had normal growth rates, indicating that *REV1-1* suppresses the growth defect associated with a deletion of both the *sup70$^+$* gene and flanking sequences. *REV1-1* therefore appears to bypass the requirement of *sup70$^+$* function during growth.

Table IV. Suppression of nonsense mutations

Nonsense mutation	Multi-copy *sup70$^+$*	*SUP70-1^{am}*	*SUP70-2^{oc}*
UAG alleles			
trp1-289	++	++	−
met8-1	++	++	−
ilv1-1	+	++	−
aro7-1	+	++	−
tyr7-1	+	++	−
lys2-801	+	++	−
trp1-1	+/−	+	−
ade3-26	−	+	−
his4-580	−	+/−	−
UAA alleles			
arg4-17			+
leu2-1			+
lys2-1			+
lys1-1			+
ade2-1 (growth)			+
ade2-1 (color)			+/−
ade2-101 (growth)			+
ade2-1 (color)			+/−
his5-2			−

Suppression was tested by replica plating haploid strains carrying a suppressor and one or more nonsense mutations from plates containing YEPD medium to plates containing media lacking appropriate amino acids or purines. The extent of suppression was scored in terms of the amount of time required for visible growth to occur, as follows: ++: dense growth after one day; +: sparse growth after one day; +/−: visible growth after 2−3 days; −: no visible growth. For *ade2-1* and *ade2-101*, colony color also was monitored as an indication of suppression, where +/− indicates pink colony color as compared with wild-type white or *ade2* mutant red colony color. Colony color was assayed in ψ$^+$ strains (see Cox, 1971).

Since *REV1-1* also suppresses the growth defect caused by *SUP70* amber and ochre suppressors, which have identical surrounding sequences and differ only by single nucleotide substitutions within the *sup70* gene, we believe that the growth defect in strains carrying *Δsup70::LEU2$^+$*, *SUP70-1^{am}*, or *SUP70-2^{oc}* is probably caused by the loss of *sup70* function rather than by the loss of flanking sequences. The same conclusion was reached using an alternate strategy described below.

The SUP70-1^{am} product retains some wild-type function

Occasionally spores carrying *SUP70-1^{am}* grew into visible colonies, whereas spores carrying *SUP70-2^{oc}* or *Δsup70::LEU2$^+$* never grew beyond the 50 cell stage of colony development. This finding prompted a more complete investigation of growth conditions in which the temperature of spore incubation was varied.

Spores from isogenic diploid strains carrying *SUP70-2^{oc}* (IEY198), *Δsup70::LEU2$^+$* (IEY122) and *SUP70-1^{am}* (IEY83) (Table I) were tested for growth at several different temperatures (Table V). Spores carrying *SUP70-2^{oc}* or *Δsup70::LEU2$^+$* failed to form visible colonies at any temperature tested (Figure 1A,B). However, spores carrying *SUP70-1^{am}* consistently formed visible colonies at 18°C but rarely at 30°C (Figure 1A,B). Since growth of *SUP70-1^{am}*

Table V. Effect of temperature of SUP70 suppression

Strain	*SUP70* allele	*SUP70-1^{am}* plasmid	Growth temperature	
			18°C	30°C
IEY83	*SUP70-1^{am}*	none	+	−
IEY198	*SUP70-2^{oc}*	none	−	−
IEY122	*Δsup70^{+}::LEU2^{+}*	none	−	−
IEY122	*Δsup70^{+}::LEU2^{+}*	pIE45	+	−
IEY122	*Δsup70^{+}::LEU2^{+}*	pIE46	+	−

pIE45 is a single copy CEN plasmid; pIE46 is a multicopy 2μ plasmid (see Table II). '−' indicates limited growth and formation of small micro-colonies. '+' indicates normal growth and colony formation.

Table VI. Growth of *SUP70* mutant strains that overexpress glutamine $tRNA_{CAA}$

Gln-$tRNA_{CAA}$ gene	Growth phenotype at 30°C			
Plasmid	Strain *SUP70* allele:	IEY83 *SUP70-1^{am}*	IEY198 *SUP70-2^{oc}*	IEY122 *Δsup70^{+}::LEU2^{+}*
pIE32		−	−	−
pIE30		+	+	+
None		−	−	−

pIE32 is a single copy CEN plasmid; pIE30 is a multi-copy 2μ plasmid (see Table II). '−' indicates limited growth and formation of small micro-colonies. '+' indicates normal growth and colony formation.

spores at 18°C most likely results from the ability of the suppressor tRNA to read 5′-CAG-3′ glutamine codons in addition to amber 5′-UAG-3′ codons, our results suggest that the suppressor tRNA retains some wild-type function. By virtue of the known anticodon sequence of the suppresor tRNA, recognition of 5′-CAG-3′ codons must occur in the presence of an A/C mismatch at the first codon position.

We used the temperature-sensitive phenotype of *SUP70-1^{am}* to re-examine the cause of growth limitation in strains carrying mutations in the *sup70* gene. We first transformed strain IEY122 (Table I), which carries *Δsup70::LEU2^{+}*, with single- or multi-copy plasmids that carry *SUP70-1^{am}* (pIE45 and pIE46; see Table II). In the construction of *Δsup70::LEU^{+}* (Materials and methods), 1.1 kb of DNA was deleted, whereas the *SUP70-1^{am}* allele in plasmid pIE45 and pIE46 is flanked by only 98 5′ and 160 3′ nucleotides that are devoid of significant open reading frames. These plasmids therefore provide the *SUP70-1^{am}* gene function in a *Δsup70::LEU2^{+}* background but do not include the bulk of flanking sequences deleted in the null allele.

Spores from IEY122 containing either pIE45 or pIE46 grew at 18°C, indicating that the *SUP70-1^{am}* allele suppressed the growth limitation that is normally associated with the *Δsup70::LEU2^{+}* allele (Table V). Since growth at 18°C occurs in the absence of *sup70* flanking sequences, the growth limitation associated with suppressor and null mutations is probably due to loss of *sup70^{+}* function rather than loss of some other unidentified function located in the flanking sequences.

Gln-$tRNA_{CAA}$ over-expression relieves SUP70-mediated growth limitation

The extent of growth limitation observed in haploid strains carrying mutant glutamine $tRNA_{CAG}$ alleles (*SUP70-1^{am}*, *SUP70-2^{oc}* or *Δsup70::LEU2^{+}*) depends on the copy number of wild-type genes encoding the isoacceptor glutamine $tRNA_{CAA}$. This was demonstrated by first subcloning a glutamine $tRNA_{CAA}$ gene from plasmid pGT20 (Tschumper and Carbon, 1982) into a centromere-containing vector to create plasmid pIE32 and into a 2μ vector to create plasmid pIE30 (Table II). These plasmids were then transformed into the diploid strains IEY83, IEY198 and IEY122, which carry the *SUP70-1^{am}*, *SUP70-2^{oc}* or *Δsup70::LEU2^{+}* alleles in heterozygous condition over *sup70^{+}*, respectively (Table I). The diploids were sporulated and tetrads were analyzed (Table VI; Figure 1).

Tetrads from pIE32-mediated transformants of strains IEY83, IEY198 and IEY122 each consisted of two spores that grew normally and two spores that formed micro-colonies. Identical results were observed from each of the corresponding untransformed diploid strains. These results indicate that one extra copy of glutamine $tRNA_{CAA}$ has no effect on the growth limitation imposed by mutations in the *sup70* gene. However, among tetrads derived from pIE30-mediated transformants of strains IEY83, IEY198 and IEY122, those tetrads that retained the plasmid each contained four spores that formed colonies of normal size (Figure 1C). This result indicates that multiple, additional copies of a glutamine $tRNA_{CAA}$ gene carried on a 2μ plasmid alleviate the growth limitation imposed by *SUP70* suppressor mutations or by a deletion of the *sup70^{+}* gene. This suggests that under these conditions glutamine $tRNA_{CAA}$ can read 5′-CAG-3′ glutamine codons by the equivalent of prokaryotic U/G wobble, which usually does not occur in yeast.

Discussion

SUP70 mutations define a new class of yeast nonsense suppressor

This study describes a new class of yeast nonsense suppressor that affects glutamine $tRNA_{CAG}$, the product of the *sup70* gene. To our knowledge, the *SUP70-1^{am}* amber suppressor is the first yeast suppressor in which the mutation causing

suppression is located at position 36. Suppressor mutations at this position in *E.coli* are very inefficient, possibly because they disrupt the extended anticodon arm (Yarus *et al.*, 1986; Raftery and Yarus, 1987). Despite the location of the mutation, the yeast *SUP70-1*^{am} suppressor is relatively efficient as judged by its ability to suppress all amber mutations that were tested.

Although it should be possible to derive amber and ochre nonsense suppressors by single-base mutation from eight different tRNA families, including glutamine, prior studies in yeast (Sherman, 1982) have yielded nonsense suppressors affecting only three tRNA families, tyrosine, leucine and serine. Failure to identify suppressors affecting the other tRNAs might be due to the potentially negative effects of such suppressors on growth. This appears to be the case for glutamine tRNA. The *SUP70* ochre and amber suppressors described in this study both inhibit growth in haploid strains to such an extent that they would not have been recovered in selections that employ haploid strains.

Relationship between suppression and growth inhibition

Nonsense suppressors have the potential to inhibit growth in several different ways. Growth could be inhibited by over-suppression, which occurs when a suppressor tRNA recognizes a normal termination codon efficiently enough to produce potentially lethal read-through products. Growth could also be inhibited by a different mechanism if the suppressor tRNA is unable to carry out the function of the corresponding wild-type tRNA. If loss of wild-type function depletes the cell of an essential tRNA, then the inability to translate a given codon would be lethal. Finally, some suppressor mutations that should have been identified but have never been found might block one or more steps in the synthesis of the tRNA, thereby preventing expression of the suppressor phenotype.

We examined the phenotype of a *sup70* deletion in order to determine whether growth inhibition associated with the *SUP70* suppressors was due to over-suppression or to loss of wild-type function. Growth was inhibited in haploid strains carrying the deletion to the same extent as in strains carrying a suppressor. Assuming that no functions other than *sup70* are altered in strains carrying the deletion, which we believe to be the case, our results suggest that growth inhibition is caused by depletion of glutamine $tRNA_{CAG}$ in strains carrying the *sup70* deletion. In strains carrying an ochre or amber suppressor mutation, growth inhibition is most likely caused by loss of the ability to read 5′-CAG-3′ codons.

If *sup70* mutations deplete the intracellular pool of tRNA that reads 5′-CAG-3′ codons, then most or all of this tRNA may be encoded by *sup70*. Additional genes coding for tRNAs capable of reading 5′-CAG-3′ glutamine codons might exist, but they probably contribute insufficient amounts of product to support normal growth. Since yeast tyrosine tRNA genes with identical coding regions are differentially transcribed (Sherman, 1982), perhaps glutamine $tRNA_{CAG}$ genes other than *sup70*, if they exist, suffer from relatively low rates of transcription. Alternatively, the products of such genes could be transcribed efficiently but might differ structurally from the *sup70*$^{+}$ gene product and might read 5′-CAG-3′ codons relatively inefficiently.

We were unable to directly determine the number of redundant glutamine $tRNA_{CAG}$ gene copies due to cross-hybridization between the genes for glutamine $tRNA_{CAG}$ and glutamine $tRNA_{CAA}$. There are at least eight genes encoding the two isoacceptors combined, but attempts to distinguish the two gene families were unsuccessful. 5′-CAG-3′ codons are among a class of rare codons in yeast that are generally read by the products of low-copy or single-copy tRNA genes (Olson *et al.*, 1981; Bennetzen and Hall, 1982; Ikemura, 1982; Guthrie and Abelson, 1982). We therefore anticipate that the majority of the eight or more glutamine tRNA genes will turn out to encode glutamine $tRNA_{CAA}$.

The *sup70* gene maps on chromosome 13 whereas a gene encoding glutamine $tRNA_{CAG}$ was previously reported to map on chromosome 11 (Calderon *et al.*, 1984). This would suggest the existence of at least two glutamine $tRNA_{CAG}$ genes if not for a comparison of DNA sequences showing that both are derived from the same locus. We believe that the mapping strategy used by Calderon *et al.* to place the gene on chromosome 11, which relied on site-directed plasmid integration, led to an erroneous map position. Since repeated sequences associated with transposable elements reside in the vicinity of the *sup70* gene (Edelman, 1987), linearization of the plasmid within these repeated sequences could have resulted in recombination at chromosomal sites unlinked to the endogenous gene. Since we showed in our experiments that the site of integration was linked to a *SUP70* suppressor mutation and since DNA sequence analysis indicates that the two cloned genes are synonymous, we conclude that both of the cloned genes correspond to a single locus on chromosome 13. According to this interpretation, the existence of only one glutamine $tRNA_{CAG}$ gene has been documented.

We can explain the relationship between suppression and growth inhibition if we assume that the *sup70* mutations cause depletion of tRNA capable of reading 5′-CAG-3′ codons. Given this assumption, the suppressor mutations would be expected to exhibit dominant gain of function (suppression) concomitant with recessive loss of wild-type function (inability to read 5′-CAG-3′). Furthermore, a single copy of the *sup70*$^{+}$ gene introduced into a strain carrying a *SUP70* suppressor should relieve growth inhibition without affecting suppression. Our analyses confirm that these expectations are met. We would also expect the same degree of growth inhibition in strains carrying a *sup70* deletion versus a *SUP70* suppressor, assuming that the suppressor causes complete loss of the ability to read 5′-CAG-3′ codons. This expectation is met when strains are grown at 30°C. However, at 18°C the *SUP70-1*^{am} suppressor permits some growth. As discussed further below, we interpret this to mean that the amber suppressor tRNA retains some wild-type function at this temperature.

The *REV1-1* mutation described in this study suppresses growth inhibition not only in strains carrying a *SUP70* suppressor but also in a strain carrying a *sup70* deletion. It therefore alleviates the requirement for *sup70* function during growth. *REV1-1* may cause the production of an altered tRNA capable of reading 5′-CAG-3′ codons in cells depleted of the normal *sup70* gene product. For example, the *REV1-1* mutation might alter one of the genes encoding glutamine $tRNA_{CAA}$, allowing it to read 5′-CAG-3′ instead of or in addition to 5′-CAA-3′. Alternatively, *REV1-1* could change the decoding specificity of a tRNA allowing it to read 5′-CAG-3′ codons through a change in base modification

or it could confer improved transcription of duplicate *sup70* gene copies, if they exist, that might otherwise be transcribed at a low level.

The *sup70* suppressors are not the only yeast suppressors that confer recessive lethality. Previous studies have shown that diploids heterozygous for certain serine or arginine tRNA suppressors give rise to haploid suppressor-bearing spores that fail to germinate. (Etcheverry *et al.*, 1982; Edelman, 1987). However, the recessive-lethal glutamine tRNA suppressors described in this study are unique in that suppressor-bearing haploid spores germinate and divide to the 50 cell stage before arresting growth. Germination followed by limited growth may occur because the spores contain some residual $sup70^+$ gene product derived from the heterozygous diploid that is not depleted until several generations of vegetative growth have been completed. Alternatively, tRNA encoded by a gene other than *sup70* may be capable of reading 5′-CAG-3′ codons at a low level sufficient to support germination and a few rounds of cell division but insufficient to support continuous vegetative growth. We cannot distinguish between these possibilities using currently available information.

Glutamine $tRNA_{UAG}$ reads 5′-CAG-3′ codons at 18°C

Haploid strains carrying the $SUP70\text{-}1^{am}$ amber suppressor fail to grow at 30°C but grow to some extent at 18°C. Slow growth at 18°C also occurs when $SUP70\text{-}1^{am}$ is introduced on a single-copy plasmid in a strain that is deleted for the wild-type $sup70^+$ gene. By contrast, haploid strains carrying the $SUP70\text{-}2^{oc}$ ochre suppressor or the *sup70* deletion fail to grow at any temperature. Overall, these results suggest that the amber suppressor glutamine $tRNA_{UAG}$ reads 5′-CAG-3′ codons at a level sufficient to support some growth at 18°C but probably with reduced efficiency compared with the wild-type glutamine $tRNA_{CAG}$. To our knowledge, $SUP70\text{-}1^{am}$ is the first yeast nonsense suppressor that has been shown to retain some wild-type decoding capacity.

In order for the 3′-AUC-5′ anticodon of the $SUP70\text{-}1^{am}$ tRNA to interact successfully with a 5′-CAG-3′ codon, an A/C mismatch must be tolerated at the first position of the codon. The presence of this mismatch may explain why codon reading is inefficient. Other examples of successful codon reading in the presence of a first position mismatch have been reported. For example, certain *glyT* missense suppressors in *E. coli* act by a similar mechanism (Murgola and Pagel, 1980; Kirsebom and Isaksson, 1985). Also, wild-type glutamine $tRNA_{CAG}$ in yeast can tolerate a U/G interaction at the first position of the codon as evidenced by its ability to read 5′-UAG-3′ amber codons at a low level (Weiss and Friedberg, 1986; Weiss *et al.*, 1987). In these exceptional cases where first position mismatches or U/G interactions are tolerated, we suggest that codon reading may occur by a mechanism resembling the two-out-of-three reading system proposed for degenerate codons in yeast mitochondria (Lagerkvist, 1978; Bonitz *et al.*, 1980).

5′-CAG-3′ codons are translated when glutamine $tRNA_{CAA}$ is over-expressed

When a gene encoding glutamine $tRNA_{CAA}$ is introduced on a multi-copy plasmid into strains carrying a *SUP70* suppressor or a *sup70* deletion, growth inhibition is partially suppressed. We interpret this result to mean that over-expression of glutamine $tRNA_{CAA}$ promotes translation of 5′-CAG-3′ codons in strains depleted of glutamine $tRNA_{CAG}$. When over-expressed, glutamine $tRNA_{CAA}$, whieh contains a 3′-GUU-5′ anticodon, apparently can read 5′-CAG-3′ codons using a mechanism that is formally equivalent to prokaryotic-like, third position U/G wobble. By contrast, the $SUP70\text{-}2^{oc}$ suppressor tRNA, which contains a 3′-AUU-5′ anticodon, fails to read amber codons by wobble and thus behaves in a fashion more typical of yeast tRNAs (Guthrie and Abelson, 1982).

To our knowledge, the 5′-CAG-3′ codon-reading ability of glutamine $tRNA_{CAA}$ is the first example of third position U/G wobble in yeast. This unprecedented behavior for a eukaryotic tRNA might be explained if suppression of growth inhibition was the consequence of a gain of function that occurred only in cells over-expressing glutamine $tRNA_{CAA}$. Gain of function could result if a portion of the over-expressed tRNA is under-modified and as a result acquires the ability to read 5′-CAG-3′ codons. Gain of function due to under-modification seems plausible since it has been proposed that modification of U at the first anticodon position of several yeast tRNAs may prevent U/G wobble (Guthrie and Abelson, 1982). However at present we cannot further assess the validity of this model because the nature and location of base modifications in yeast glutamine $tRNA_{CAA}$ have not been analyzed.

As an alternative to gain of function, suppression of growth inhibition might result from enhancement of an intrinsic function in cells that express glutamine $tRNA_{CAA}$ at a normal level. For example, in normal cells glutamine $tRNA_{CAA}$ might translate 5′-CAG-3′ codons inefficiently or the tRNA might function in this manner only in cells depleted of the competing glutamine $tRNA_{CAG}$. In either case, the potential ability of glutamine $tRNA_{CAA}$ to promote translation of 5′-CAG-3′ codons at a low level could explain why haploid spores depleted of glutamine $tRNA_{CAG}$ germinate and divide several times before growth arrest whereas haploid spores depleted of certain other yeast tRNAs fail to germinate altogether (Etcheverry *et al.*, 1982; Edelman, 1987).

In a previous study it was shown that wild-type glutamine $tRNA_{CAA}$ can translate 5′-UAA-3′ ochre codons when the tRNA is over-expressed (Pure *et al.*, 1985). This occurs in the presence of a G/U interaction at the first codon position, which is formally equivalent to first position wobble. As discussed above, over-expressed wild-type glutamine $tRNA_{CAA}$ can also read 5′-CAG-3′ codons by third position wobble or by a mechanism that has an equivalent outcome. However, glutamine $tRNA_{CAA}$ apparently cannot wobble at both positions simultaneously in yeast, since no 5′-UAG-3′ amber suppression was observed when the tRNA was over-expressed.

By contrast, glutamine $tRNA_{CAA}$ (anticodon 3′-GUU_m-5′) in mouse cells infected with Mo-MuLV virus can apparently read a viral amber codon and promote read-through in a manner that appears to require simultaneous first and third position wobble (Philipson *et al.*, 1978; Varmus, 1985; Yoshinaka *et al.*, 1985; Kuchino *et al.*, 1987). We have not resolved why the yeast and mouse-viral systems differ in terms of glutamine $tRNA_{CAA}$ decoding behavior.

Potential for expressing ciliate protozoan genes in yeast

Certain ciliated protozoa deviate from the universal genetic code in that 5′-UAG-3′ and 5′-UAA-3′ termination codons are translated as glutamine (Caron and Meyer, 1985; Fox, 1985; Helfenbein, 1985; Horowitz and Gorovsky, 1985; Prat *et al.*, 1986; Preer *et al.*, 1985; Cupples and Perlman, 1986; Grivell, 1986). These organisms have evolved tRNAs with anticodons complementary to UAG and UAA stop codons and can therefore read the stop codons using standard anticodon:codon interactions (Kuchino *et al.*, 1985; Hanyu *et al.*, 1986). Not surprisingly, ciliate mRNAs are poorly translated in conventional *in vitro* translation systems (Hruby *et al.*, 1977; David and Smith, 1981; Rinaldy *et al.*, 1981; Preer *et al.*, 1981). 5′-UAG-3′ and 5′-UAA-3′ codons found in the coding regions of sequenced genes from several ciliates have been proposed to be the specific *in vitro* translation blocking sites (Meyer *et al.*, 1984; Caron and Meyer, 1985; Preer *et al.*, 1985).

Due to the unusual nature of the ciliate genetic code, it has been difficult to clone ciliate genes using conventional expression systems. However, it should be possible to express ciliate genes in yeast by designing an appropriate host that can translate UAG and UAA codons as glutamine, thereby allowing for the production of a full length product identical to that found in the ciliate from which a particular gene was derived. An expression library could be constructed in which ciliate cDNAs are inserted into a plasmid shuttle vector adjacent to a suitable yeast promoter. Provided that the suppressor tRNAs are charged with glutamine, normal cilitate products should be produced in a yeast host carrying plasmid library DNAs and the relatively efficient amber and ochre glutamine tRNA nonsense suppressors. The growth defects caused by these suppressors can be alleviated by the inclusion of the *REV1-1* mutation or a multi-copy glutamine $tRNA_{CAA}$ gene, either of which suppresses the growth defect by restoring efficient decoding of normal 5′-CAG-3′ glutamine codons. In theory, it should be possible to identify any ciliate gene for which antibodies are available by detecting expression of the corresponding gene product in yeast.

Materials and methods

Strains and plasmids

Yeast strains are listed in Table I. *E.coli* strain 6507 (HB101 *hsdR, hsdM, leu, pro, recA, pyrF74::Tn5*) was obtained from D.Botstein. *E.coli* strain JM109 [*recA1, endA1, gyrA96, thi, hsdR17, supE44, relA1,* Δ*lac-proAB,* (F′, *traD36, proAB, lacI*^{q}*ZΔM15*)] and the M13 phages mp18 and mp19 (Yanisch-Perron *et al.*, 1985) were obtained from Amersham. The plasmids used in this study are described in Table II. The plasmid cloning vectors YCp50 (Rose *et al.*, 1987), YIp5, and YEp24 (Broach, 1983) were obtained from R.Davis. EC402 (Table II) was obtained from E.Craig. Plasmid pGT20 containing glutamine $tDNA_{CAA}$ was provided by J.Carbon (Tschumper and Carbon, 1982).

Genetic methods

Media used for the growth of yeast are described by Gaber and Culbertson (1982). 5-fluoro-orotic acid (5-FOA) was used to select Ura^- cells using the method of Boeke *et al.* (1984). Media used for the growth of *E.coli* are described by Miller (1972). Yeast genetic techniques and nomenclature are described in the Cold Spring Harbor Yeast Course Manual (Sherman *et al.*, 1971). Unless otherwise indicated, all growth tests for yeast were performed at 30°C. Genetic linkage values were derived from tetrad data using the formula of Perkins (1949). *E.coli* transformation was performed using the method of Mandel and Higa (1970). Yeast transformation was performed using the method of Ito *et al.* (1983).

Spore-cell matings were performed as follows. Asci from a sporulated diploid heterozygous for a suppressor were dissected by micromanipulation on a Petri dish containing nutrient medium. One spore from each tetrad was mixed with an excess of cells from a haploid strain. After incubation for 3–4 days at 30°C, the mixture was cultured on a fresh plate and then replica plated to synthetic media appropriate for the selection of diploids heterozygous for the suppressor. In those cases where heterozygous diploids formed, it was also verified that only two of the remaining three spores of the tetrad grew and that both lacked the suppressor.

In order to assay for mitotic plasmid instability, strains harboring a plasmid were single-colony purified on nutrient medium to allow spontaneous mitotic loss or non-disjunction of the plasmid (Hieter *et al.*, 1985; Koshland *et al.*, 1985). Purified colonies were then replica plated to appropriate media to test for the absence of plasmid-linked prototrophy.

DNA methods

Yeast DNA was isolated by the method of Olson *et al.* (1979). Plasmid DNA was isolated from yeast and *E.coli* by the methods of Nasmyth and Reed (1980) and Birnboim and Doly (1979), respectively. Rapid plasmid DNA lysates from *E.coli* were prepared according to Holmes and Quigley (1981). Phage lysates and DNA were prepared as described in the *M13 Cloning and Sequencing Handbook* (Amersham).

Agarose gel-separated restriction fragments were transferred to nitrocellulose filters for hybridization by the method of Southern (1975). M13 hybridization probes were made by the second-strand synthesis method of Hu and Messing (1982). DNA sequences were determined by the method of Sanger *et al.* (1977). DNA sequences were analyzed using programs from the Genetics Computer Group, Inc. (Devereaux *et al.*, 1984) and programs written by I.Edelman.

Construction of the Δsup70::LEU2$^+$ allele

In order to construct a deletion/substitution mutation in the *sup70* gene, the *Hin*dIII–*Bam*HI fragment carrying the *sup70*$^+$ gene (Figure 2) was first subcloned into pBR322. An internal 1.1 kb *Eco*RV–*Xho*I fragment carrying *sup70* was removed from the plasmid by restriction enzyme digestion and replaced with the *LEU2*$^+$ gene on a *Hpa*I–*Sal*I fragment. The resulting plasmid, pIE35, was linearized by co-digestion with *Bam*HI and *Sal*I and used to transform strain IEY83 (Table I) to a Leu^+ phenotype using the method of one-step gene replacement (Rothstein, 1983). Among the Leu^+ transformants, some were Trp^- in phenotype as the result of replacement of the *SUP70-1*^{am} allele with the Δ*sup70*$^+$*::LEU2*$^+$ allele. One Leu^+ Trp^- transformant, designated IEY122, was further tested for correct gene replacement by genetic crosses in which the *LEU2*$^+$ gene was shown to be linked to the *sup70* locus and by Southern hybridization in which one of the two *sup70* alleles in the IEY122 diploid was shown to contain the expected 700 bp insertion carrying the *LEU2*$^+$ gene.

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