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The *Schizosaccharomyces pombe sup3-i* suppressor recognizes ochre, but not amber codons *in vitro* and *in vivo*

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The inefficient suppressor *sup3-i* of the fission yeast *Schizosaccharomyces pombe* is an ochre suppressor. *Sup3-i* was derived from the efficient serine inserting UGA suppressor *sup3-e*. The cloning and sequencing of the *sup3-i* gene indicate that the suppressor is different from the parent *sup3-e* by a C→T substitution in the sequence coding for the middle position of the anticodon. *In vitro* translation assays supplemented with purified *sup3-i* tRNA and programmed with *Xenopus* globin mRNAs lead to the accumulation of a read-through product in response to UAA termination signals, but not in response to UGA termination codons. Transformation of *Saccharomyces cerevisiae* nonsense mutant strains with plasmid DNA carrying the *S. pombe sup3-i* gene, led to ochre, but not amber or UGA suppression *in vivo*.
Key words: Schizosaccharomyces pombe/tRNA/serine/suppression/ochre

Introduction

Nonsense suppression mediated by tRNAs results in the incorporation of amino acids in a growing polypeptide chain at codons that normally signal the termination of translation (for a review, see Steege and Söll, 1979). With one exception to date (Hirsh, 1971), the anticodons of the suppressor tRNAs are complementary to the termination codons (UAA, UAG and UGA) that they suppress (Capecchi *et al.*, 1975; Gesteland *et al.*, 1976; Kohli *et al.*, 1979). In eukaryotic organisms this type of informational suppression has been studied mainly in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*.

In the fission yeast *S. pombe,* UGA nonsense suppression has been characterized in great detail, both genetically and biochemically (Kohli *et al.*, 1980); e.g., the tRNA sequence as well as the gene sequence of the suppressor *sup3-e* are available (Rafalski *et al.*, 1979; Hottinger *et al.*, 1982). Evidence for ochre (UAA) suppression and ochre suppressors is scant and much of this information is based on indirect genetic data. In this study we have characterized the presumed ochre suppressor *sup3-i*. The gene was isolated and characterized *in vitro* and *in vivo* and the suppressor properties of the tRNA were determined.

The suppressor *sup3-i* is an allelic form of *sup3-e* and was derived from the efficient UGA suppressor *sup3-e* by u.v. mutagenesis presumably by a single base change (Hawthorne and Leupold, 1974). In contrast to *sup3-e*, the *sup3-i* allele was referred to as being inefficient, because of its inability to completely suppress the accumulation of a red colony pigmentation of certain *ade7* nonsense mutations. The patterns of suppression of *sup3-e* and *sup3-i* are distinct; *sup3-i* suppresses a different series of nonsense mutants from *sup3-e*. It was therefore classified as an ochre suppressor (Hofer *et al.*, 1979).

Results

Gene isolation

First the *sup3-i* strain *ade7-413* was tested for the presence of the active suppressor. Then the allelism of the putative *sup3-i* with *sup3-e* was determined by constructing strains *h⁻sup3-e ade7-413 leu3-155* and *h⁺sup3-i ade7-413 leu3-155* and crossing the two with each other. The absence of double prototrophic progeny spores (*ade7⁺leu3⁺*) indicated that the suppressors in the cross were allelic.

Total DNA from the *sup3-i ade7-413* strain was isolated as described in Materials and methods. A clone bank of *Hind*III fragments was constructed in pTR262. This vector allows positive selection on tetracycline-containing media, as only plasmids containing an insert in the unique *Hind*III site of the vector allow expression of the *tet* gene (Roberts *et al.*, 1980). DNA from this clone bank was used to transform the *Escherichia coli* strain BJ5183 to tetracycline resistance.

Approximately 30 000 colonies were screened for the presence of the *sup3-i* gene using *in situ* hybridization of bacterial colonies (Pearson *et al.*, in preparation). Nine colonies gave a positive hybridization signal with the ^{32}P-labelled 1.0-kb *Hind*III/*Bam*HI *sup3-e* fragment used as a probe. DNA prepared from these colonies was analyzed by restriction endonuclease treatment with *Hind*III and other restriction endonucleases that cut the *sup3-e* gene, and hence are diagnostic for the presence of the desired *sup3-i Hind*III fragment. Two of the nine colonies that were picked carried a plasmid with a 2.35-kb *Hind*III insert. This insert gave the desired restriction digest pattern of *sup3-e* when cut with *Eco*RI and *Kpn*I. For further study DNA was prepared from one of these colonies.

Gene sequence

The 2.35-kb *Hind*III fragment was analyzed and a restriction map of a 1.9-kb *Hind*III/*Eco*RI subfragment containing the tRNA genes was established (Figure 1). The *Hind*III/*Kpn* fragment of 492 bp length (see Figure 1) was sequenced.

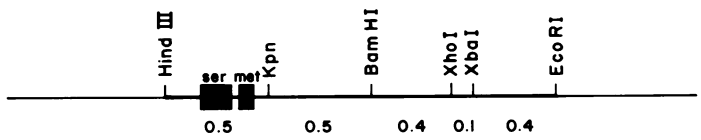

Fig. 1. Restriction map of the 1.9-kb *Hind*III/*Eco*RI *S. pombe* DNA fragment containing the *sup3-i* gene. The location of the tRNA genes is indicated by the solid boxes. The numbers are distances (in kbp).

Schizosaccharomyces pombe sup3-i Gene

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      10         20         30         40         50         60         70         80         90        100
AAGCTTGTAGTTTTAGGAGTTTACTTTGATAATAAAAAAAATATATTCTAACACCAAAAACAATATTTTCTTAACATAAAGCAATGCTTTTGTGAAATCA
TTCGAACATCAAAATCCTCAAATGAAACTATTATTTTTTTTATATAAGATTGTGGTTTTTGTTATAAAAGAATTGTATTTCGTTACGAAAACACTTTAGT

      110        120        130        140        150        160        170        180        190
ACCATTTGAGCATTGGAAACTTCCTATCAAAAATTACATAGAAATTTTTGATTTTGGCTATATAAATTACATTTCATGATAAATGTACAGCTAAAGTCAC
TGGTAAACTCGTAACCTTTGAAGGATAGTTTTTAATGTATCTTTAAAAACTAAAACCGATATATTTAATGTAAAGTACTATTTACATGTCGATTTCAGTG

      210        220        230        240        250        260        270        280        290
TATGTCCGAGTGGTTAAGGAGTTAGACTTTAATCCTGTATTCTAGTCATCTAATGGGCTTTGCCCGCGCAGGTTCAAATCCTGCTGGTGACGGTATTTGT
ATACAGGCTCACCAATTCCTCAATCTGAAATTAGGACATAAGATCAGTAGATTACCCGAAACGGGCGCGTCCAAGTTTAGGACGACCACTGCCATAAACA

      310        320        330        340        350        360        370        380        390        400
GCGCGGTAGGAGAGTGGAACTCCGACGGGCTCATAACCCGTAGGTCCCAGGATCGAAACCTGGCCGCGCAACTCTTTTTTTGCTTGTTTTTAAAATAACT
CGCGCCATCCTCTCACCTTGAGGCTGCCCGAGTATTGGGCATCCAGGGTCCTAGCTTTGGACCGGCGCGTTGAGAAAAAAACGAACAAAAATTTTATTGA

      410        420        430        440        450        460        470        480        490
TTTTATTAAGTATTGGTGTAGCGTTTTGATGAATGAGAAATGTTTTGTTCTTATATAAATATGTAGATAGGGACGTCCTTGGTGCTATTGGTACC-3'
AAAATAATTCATAACCACATCGCAAAACTACTTACTCTTTACAAAACAAGAATATATTTATACATCTATCCCTGCAGGAACCACGATAACCATGG-5'
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SER→ MET→

Fig. 2. Nucleotide sequence of the *Hind*III/*Kpn* fragment containing the tRNA genes (boxed). Their transcription direction is indicated by arrows. Nucleotides 233–247 (dotted) represent the intervening sequence in the *sup3-i* tRNA gene.

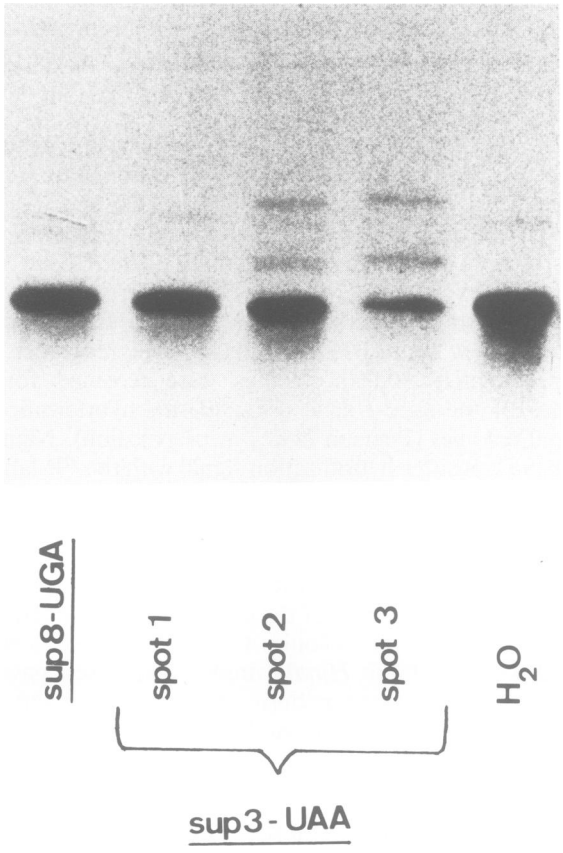

Fig. 3. *In vitro* suppression by serine tRNAs isolated from strain *sup3-i*. 0.5 μg of the three serine tRNAs separated by gel electrophoresis (spots 1–3) were added to the 25 μl translation assays with *X. laevis* globin mRNA. The translation products were separated on a denaturing polyacrylamide gel. The fluorograph of the gel is shown. Elongated polypeptides are observed only upon addition of spot 2 and spot 3 tRNAs. As controls assays were performed with opal suppressor tRNA *(sup8-UGA)* and without added tRNA (H_2O). For further explanations see text.

Analysis of the DNA sequence revealed that the only difference between the *sup3-e* sequence (Hottinger *et al.*, 1982) and the *sup3-i* sequence was a C→T transition in the position coding for the middle base of the anticodon. This change enables the *sup3-i* tRNA anticodon to pair with the UAA ochre termination codon.

Purification of sup3-i tRNA and in vitro suppression.

Transfer RNA was isolated from the strain *ade7-413 sup3-i* and fractionated by column chromatography. The fractions were assayed for suppressor activity in a wheat germ translation system programmed with rabbit globin mRNA as template (Kohli *et al.*, 1979). β-Globin is terminated by a UGA termination codon and can be elongated by addition of UGA suppressor tRNA. On denaturing polyacrylamide gels the elongated polypeptide can be separated from normal globin. α-Globin mRNA carries a UAA termination codon. Thus the α-globin protein can be elongated with help of UAA suppressor tRNA to yield a readthrough product which is elongated by 22 amino acids and also separable on gels.

The purification of *sup3-i* tRNA was achieved essentially as previously described for *sup3-e* tRNA (Kohli *et al.*, 1979; Rafalski *et al.*, 1979). 370 mg of unfractionated tRNA were chromatographed on benzoylated DEAE-cellulose. The fractions active in suppression were pooled and applied to a Sepharose-4B column. The third purification step consisted of RPC-5 chromatography. The fractions giving the highest suppressor activity were combined (200 μg) and subjected to two-dimensional gel electrophoresis. Upon staining of the gel, three major tRNA spots were recognized. These three RNAs were extracted from the gel and subjected to further analysis.

All three tRNAs turned out to be chargeable with the amino acid serine. They accepted between 500 and 700 pmol serine per A_{260} unit of RNA. According to earlier experience (Rafalski *et al.*, 1979) this is characteristic for pure tRNAs obtained by this procedure, although the measured values are only 30–45% of the ones expected. Obviously the three serine tRNA species, which separated on the gel, correspond to different isoacceptors that were not resolved by the previous column chromatography (see Discussion). Addition of these three tRNAs to the *in vitro* protein synthesis system described above, revealed that mainly spot 3 (but also spot 2 to a lesser extent) contains suppressor activity.

Because the two rabbit globin mRNAs carry different termination codons, the experiments described so far do not allow a differentiation between UAA and UGA suppressor

Table I. List of strains

Strain		Genotype	Reference, source
Escherichia coli			
HB101		*r_B^- m_B^- F⁻pro⁻ gal⁻ str^R recA⁻*	Boyer and Roulland-Dussoix (1969)
BJ5183		*F⁻ recBC⁻ sbcB endoI gal⁻ met⁻ str^R thi⁻ bio⁻ hsd*	Losson and Lacroute (1983)
Strain	**Mating type**	**Pertinent genotype**	**Reference, source**
S. cerevisiae			
F2	α	*ade2-1*	G.R.Fink
S664	α	*his4-385 met8-1 leu2-1*	S.Roeder
S670	α	*ade1-UGA arg4-17 his3-MM1 inos1⁻ inos4⁻ leu2-2 lys1-1 can^r*	S.Roeder
398	a	*arg4-17 lys1-1 met8-1 trp1-1*	S.Henry
YH-D5	α	*his4-260 leu2-2 trp1-1 can^r*	Hottinger *et al.* (1982)
YH-D9	α	*his4-166 leu2-2 trp1-1 can^r*	Hottinger *et al.* (1982)
YH-E1	a	*ade2-1 arg4-17 met8-1 trp1-1*	This work
YH-I4	a	*ade1-UGA arg4-17 leu2-2 lys1-1 trp1-1 can^r*	This work
YH-I7	α	*arg4-17 his4-385 leu2-1 met8-1 trp1-1*	This work
Genotype	**Mutant codon**	**Other remarks**	**Reference**
S. pombe			
972		Wild-type, mating type h⁻	U.Leupold
975		Wild-type, mating type h⁺	U.Leupold
ade1-40	UGA		Hawthorne and Leupold (1974)
ade6-704	UGA		Hawthorne and Leupold (1974)
ade7-C3		Frameshift allele	U.Leupold
ade7-84	UGA		Hawthorne and Leupold (1974)
ade7-413	presumed UAA		Thuriaux *et al.* (1974)
			Hofer *et al.* (1979)
arg1-230	presumed UAA		Thuriaux *et al.* (1975)
glu1-57	UGA		U.Leupold
leu3-155	UGA		U.Leupold
sup3-i ade7-413			Hofer *et al.* (1979)
sup3-e ade6-704			Hofer *et al.* (1979)

activity. This could have been achieved by the separation of α- and β-globin mRNAs and their individual use for *in vitro* suppression as was done before (Kohli *et al.*, 1979). We chose an alternative approach that consists of the use of unfractionated globin mRNAs from *Xenopus laevis*. It has been demonstrated by nucleotide sequencing that the major adult globin mRNAs have UAA termination codons (Kay *et al.*, 1980; Knöchel *et al.*, 1983). Thus *Xenopus* globin mRNAs were translated with added *sup3-i* tRNAs (spots 1–3) or, as controls, *sup8-e* tRNA or no added tRNA. The result of this experiment is shown in Figure 3. Again tRNAs from spot 3 and spot 2 were active in this specific assay for UAA suppression and gave 50% and 30% readthrough, respectively (percentage of radioactivity in the two higher mol. wt. protein bands). No elongated globins were observed with UGA suppressor tRNA and in the control without added tRNA. The finding that some suppressor activity is found for spot 2 and spot 3 tRNA is best explained as the result of cross-contamination. The two spots were not fully separated on the gel (see also Discussion).

In vivo suppression

The pattern of suppression of *sup3-e* and its derivative allele *sup3-i* have been determined previously in *S. pombe* (Hawthorne and Leupold, 1974; Thuriaux *et al.*, 1975). At the time of the determination the nonsense mutations recognized by each class of suppressor had not yet been unambiguously identified. The nonsense alleles and the strains given in Table I were used to construct strains to yield the updated pattern of suppression given in Table II. The *ade6* and *ade7* auxotrophs of *S. pombe* accumulate a red pigment in media with limiting amounts of adenine (Egel *et al.*,

Table II. Pattern of suppression of *sup3-e* and *sup3-i* in *S. pombe* and in *S. cerevisiae*

S. pombe	*sup3-e*	*sup3-i*	*S. cerevisiae*	*sup3-e*	*sup3-i*
UGA alleles			UGA alleles		
ade1-40	+	−	*ade1-UGA*	+	−
ade6-704	+	−	*his4-166*	+	−
ade7-84	+	−	*his4-260*	+	−
glu1-57	+	−	*leu2-2*	+	−
leu3-155	+	−			
UAA alleles			UAA alleles		
ade7-413	−	+[a]	*ade2-1*	−	+[a]
arg1-230	−	+	*arg4-17*	−	+
			leu2-1	−	+
			lys1-1	−	+
			UAG alleles		
			his4-385	−	−
			met8-1	−	−
			trp1-1	−	−

[a] *sup3-i* inefficiently suppresses the accumulation of red colony pigmentation on media with limiting amounts of adenine or lacking adenine.

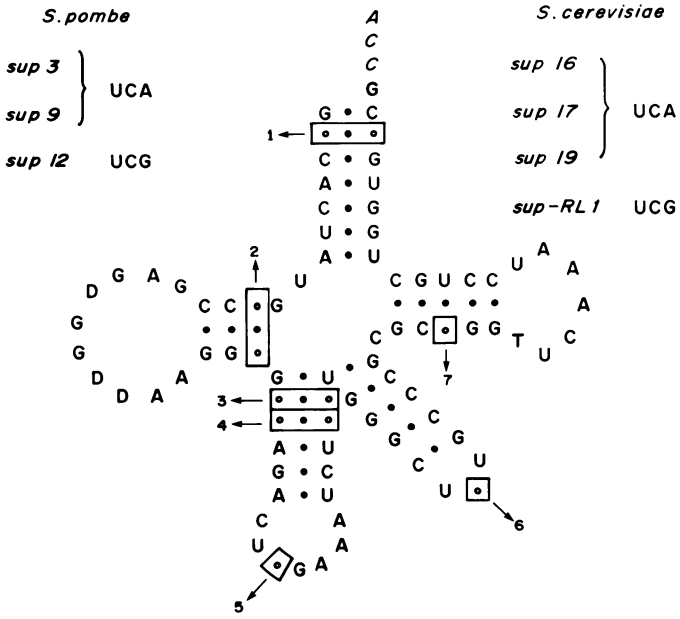

Fig. 4. The common secondary structure of all the minor serine tRNAs of *S. cerevisiae* and *S. pombe*. The solid bars indicate base pair differences; the solid dots single base differences. **(1)** G·U, *S. cerevisiae*; U·A, *S. pombe*. **(2)** G·C, *S. cerevisiae*; U·A, *S. pombe*. **(3)** A·U, *S. cerevisiae*; U·A, *S. pombe*. **(4)** C·G, $tRNA^{Ser}_{UCA}$ of *S. cerevisiae*; G·C in $tRNA^{Ser}_{UCG}$ of of *S. cerevisiae*; U·A, *S. pombe*. **(5)** C, $tRNA^{Ser}_{UCG}$ of both yeasts. **(6)** U, *sup3*$^+$ of *S. pombe*; C in all other minor serine tRNAs of the two yeasts. **(7)** U, *S. cerevisiae*; A, *S. pombe*.

1980). The degree of suppression of this pigmentation in suppressible alleles has been used as a qualitative measure of the efficiency of suppression. Based on this qualitative measure, *sup3-e* efficiently suppresses the *ade6* and *ade7* UGA alleles given in Table II, whereas *sup3-i* only inefficiently suppresses *ade7-413*, resulting in a red colony color.

The effect of the *sup3-i* gene on defined amber and ochre nonsense mutants *in vivo*, was tested in *S. cerevisiae* nonsense mutant strains. Furthermore the patterns of suppression of *sup3-e* and *sup3-i* in *S. cerevisiae* were compared. The suppressor genes were cloned in the yeast vectors YEp13 *(sup3-e)*, YRp17 *(sup3-e, sup3-i)* and YRp101 *(sup3-i)* to test suppression of the different nonsense mutants of *S. cerevisiae* used in this study.

The *S. cerevisiae* strains YH-D5, -D9, -EI, -I4 and -I7 (Table I) were transformed with the suppressor carrying plasmids. Initially after transformation, colonies were grown on media selecting only for the vector (TRP$^+$ selection for YRp17, LEU$^+$ selection for YEp13 and YRp101). After growing the colonies, they were replica plated onto different selective media to test for suppression. The replica plates were incubated at 30°C for 5–6 days and then scored. The results of the transformations are shown in Table II.

The *sup3-i* gene leads to suppression of all the ochre mutants tested: the amber and UGA mutants, however, were not recognized by *sup3-i*. The efficiency of suppression of the ochre alleles appears to be low; e.g., the suppression of the *leu2-2* allele (strain YH-D5) by *sup3-e* leads to vigorous growth on media lacking leucine, whereas suppression of *leu2-1* (strain YH-I7) by *sup3-i* leads to weak growth even after prolonged incubation. In both cases the suppressor gene had been cloned in YRp17. The *ade2-1* allele in strain YH-I4, furthermore, also appears to be only incompletely suppressed by *sup3-i* judging by the pink colony pigmentation of the transformants (red on selective media plates, pink on complete media plates). Treatment of the strains to be transformed according to Singh *et al.* (1979) to eliminate the (possibly present) Psi-element, which strongly affects the suppression efficiency of active suppressors (Ono *et al.*, 1981; Tuite *et al.*, 1983) did not affect the outcome of the experiments.

Discussion

Sup3-i is an inefficient ochre suppressor allele

The DNA sequence determination of the *sup3-i* gene and the *in vivo* and *in vitro* suppression assays identified *sup3-i* as an ochre-specific suppressor. The *sup3-i* allele was derived by u.v. mutagenesis from the wild-type in two mutational steps of the site coding for the middle base of the anticodon as depicted in Figure 4. The DNA sequence TTA corresponding to the anticodon of the suppressor tRNA is complementary to the ochre termination codon UAA. The ochre suppressor is functional and active both in *S. pombe* and in the foreign host *S. cerevisiae*. An interesting point is that suppression appears to be inefficient in both of the above mentioned organisms. In *S. pombe* all of the *ade7* ochre alleles are inefficiently suppressed by *sup3-i* (Hawthorne and Leupold, 1974). The low level of suppression observed in *S. cerevisiae* was very likely due to the absence of the Psi-element in the strains we used. This genetic element has a strong influence on suppression, especially ochre suppression, in *S. cerevisiae* (Cox, 1965; Tuite *et al.*, 1983). Suppression of ochre alleles was shown to be 10-fold more efficient in Psi$^+$ strains than in *Psi*$^-$ strains (Liebman and Sherman, 1979). In our case, the presence of *sup3-i* on a multicopy vector in the *S. cerevisiae* transformants probably explains why all the ochre alleles tested were suppressed, albeit some rather weakly.

Sup3-i suppresses ochre but not amber codons

Our data on *in vivo* and *in vitro* suppression show that *sup3-i* tRNA does not read UGA termination codons. More interesting was the question whether the suppressor would recognize amber codons, because it is well known that bacterial ochre suppressors read both UAA and UAG triplets (Steege and Söll, 1979). In contrast *S. cerevisiae* ochre suppressors do not recognize UAG termination codons (Capecchi *et al.*, 1975; Gesteland *et al.*, 1976; Ono *et al.*, 1981).

The *sup3-i* suppressor of *S. pombe* acts specifically on ochre mutations *in vivo* in *S. pombe*. Recently two *S. pombe* mutants were identified to be amber according to genetic criteria (Thuriaux *et al.*, 1982; P.Thuriaux, personal communication). They are not suppressed by the known ochre (among them *sup3-i*) and opal suppressors but by the omnipotent suppressors *sup1, sup2* and *sup11* and two newly isolated amber specific suppressors. In addition, one of these presumptive amber codons was converted by a single mutation to an ochre mutation suppressible by *sup3-i*. Likewise, the introduction of the *sup3-i* gene into *S. cerevisiae* leads to a specific suppression of UAA mutations. None of the UAG or UGA mutations tested were suppressed.

The genetic data are corroborated by the results of the *in vitro* translation assays with purified *sup3-i* tRNA. Accumulation of readthrough products was found only when UAA codons signaled termination of translation. UGA (this work) and UAG (M.Capecchi, personal communication) are not read in the corresponding *in vitro* suppression assays.

The ochre suppressor *sup3-i* of *S. pombe* shows the same restriction to the reading of UAA termination codons as the ochre suppressor tRNAs of *S. cerevisiae*. This behavior of yeast ochre suppressors is attributed to the modification of the uridine present at the first position of the anticodon, the so-called wobble position. Recent work on the *S. cerevisiae* ochre suppressor tRNA has resulted in the identification of this modified base as mcm^5s^2U (Laten *et al.*, 1983). It has been demonstrated that $tRNA^{Glu}$ carrying this modified base is restricted to the decoding of the triplet GAA and does not recognize GAG (Yoshida *et al.*, 1971). In an approach to determine the nature of the wobble nucleoside of *sup3-i* tRNA, the gel-purified samples (spots 1 – 3, see Results) were subjected to hydrolysis and h.p.l.c. of the resulting nucleosides (Gehrke *et al.*, 1983). Unfortunately the results obtained do not allow a clear-cut interpretation (C.Gehrke and P.Agris, personal communication). Spot 3 tRNA which is the species with the highest suppressor activity does not carry mcm^5s^2U. Instead it contains the nucleoside mcm^5U as does the opal suppressor tRNA *sup3-e* (Rafalski *et al.*, 1979). In addition, the hydrolysate contains other compounds of as yet unknown chemical structure. Further work has to be done to demonstrate unambiguously the nature of the wobble nucleoside in *sup3-i* tRNA.

Similarity of the minor serine tRNAs of S. cerevisiae and S. pombe

The successful expression of the *S. pombe sup3-i* gene parallels that of the *sup3-e* gene (Hottinger *et al.*, 1982). Proper *in vivo* function requires accurate transcription of the gene and processing of the transcript as well as aminoacylation by the heterologous aminoacyl-tRNA synthetase. The suppression of *S. cerevisiae* ochre, but not amber nonsense mutants by *sup3-i* furthermore suggests that the heterologous minor serine tRNA molecules are modified in a manner allowing them to replace functionally the *S. cerevisiae* ochre serine tRNA ochre suppressors *(sup16, sup17, sup19).*

A comparison (Figure 4) of base differences between the published sequences of minor serine tRNAs (tRNA and gene sequences) of *S. cerevisiae* and *S. pombe* (Gauss and Sprinzl, 1983a, 1983b; Pearson *et al.*, in preparation), show a difference of 10 bases between $tRNA^{Ser}_{UCA}$ species and nine bases for the $tRNA^{Ser}_{UCG}$ species of the two organisms, *versus* differences of three bases or less between the minor serine tRNAs within each organism. This represents a much closer homology than that observed for many other corresponding tRNA species of *S. pombe* and *S. cerevisiae*. A study of Figure 4 reveals that most of the differences are found in the stem regions of the tRNA with maintenance of the base pairing. The loop regions (with the obvious exception of the anticodon) are completely homologous. Thus the overall secondary structure of the minor serine tRNAs in these organisms is identical. In view of this it seems likely that the tRNA modifying enzymes of *S. cerevisiae* recognize and modify the *sup3* tRNAs in such a way as to allow restriction of the wobble in the anticodon typical of *S. cerevisiae* ochre suppressors.

Materials and methods

General

[α-^{32}P]ATP, restriction endonucleases, polynucleotide kinase, T4 DNA polymerase, T4 DNA ligase, calf alkaline phosphatase and zymolyase 60000 were obtained commercially. All enzymes were used as recommended by the manufacturer. Calf alkaline phosphatase was further purified by gel filtration (Efstratiadis *et al.*, 1977). Novozym SP234 was a gift of Dr. Knut Aunstrup of Novo Laboratories, Denmark. [γ-^{32}P]ATP was prepared by the method of Walseth and Johnson (1979).

Media

The media for growing and culturing *S. cerevisiae* and *S. pombe* have been described by Fink (1970) and Gutz *et al.* (1974), respectively. Media for *E. coli* were as described by Miller (1972).

Strains

S. cerevisiae: the strains used in these experiments are listed in Table I. The strains YH-E1 and YH-I7 were constructed using random spore procedures (Fink, 1970).

S. pombe: the strains used in these experiments were all from the stock collection of Leupold and are listed in Table I. Standard genetic methods of *S. pombe* (Kohli *et al.*, 1977) were used. Random spore procedures were employed in all the strain constructions and diagnostic crosses.

E. coli: the strains listed in Table I were used throughout these experiments, both for transformation and isolation of plasmid DNA.

DNA preparation

Plasmid DNA was amplified in the *E. coli* strains and isolated according to the method of Clewell (1972). *S. cerevisiae* DNA was prepared according to the method of Cryer *et al.* (1975). *S. pombe* DNA was isolated using the method described by Hottinger *et al.* (in preparation). Purification as well as separation of DNAs was accomplished by CsCl-ethidium bromide density gradient centrifugation.

Cloning

The following vectors were used in these experiments: pBR322 (Bolivar *et al.*, 1977), pTR262 (Roberts *et al.*, 1980), YRp101 and YRp17 (Hottinger *et al.*, 1982). A clone bank of *Hind*III fragments of *S. pombe sup3-i ade7-413* DNA in the positive selection vector pTR262 was established. 0.5 μg *Hind*III-digested, linearized pTR262 was ligated with 0.5 μg completely digested *Hind*III fragments of total DNA in a volume of 50 μl with 0.2 units of T4 DNA ligase at 10°C for 18 h. The ligation reaction was then used to transform BJ5183 to tetracycline resistance. The *sup3-i* gene-containing clones were detected by colony hybridization (Pearson *et al.*, in preparation) using a 1.0-kb *Hind*III/*Bam*HI fragment carrying the *sup3-e* gene (Hottinger *et al.*, 1982) as a probe. The probe was ^{32}P-labelled in a fill-in replacement reaction using T4 DNA polymerase (O'Farrell *et al.*, 1980). The plasmid inserts from colonies giving a positive hybridization reaction were screened for the proper restriction pattern of *sup3-e*. The 2.35-kb *Hind*III fragment containing *sup3-i* was subsequently cloned in the hybrid yeast vectors for later transformation of yeast strains.

Transformation

The procedure of Struhl *et al.* (1979) was followed with some modifications. Approximately 3 – 4 x 10^9 exponentially growing cells were resuspended in 10 ml 0.7 M sorbitol and treated with 0.1 mg Zymolyase 60000. Conditions were set such that spheroplasting lasted roughly 45 min at 30°C.

DNA sequencing

This was carried out according to Maxam-Gilbert (1980).

Transfer RNA purification and in vitro suppression

tRNA was isolated from the strain *ade7-413 sup3-i* and subsequently purified by column chromatography and polyacrylamide gel electrophoresis as described by Kohli *et al.* (1979) and Rafalski *et al.* (1979). *In vitro* protein synthesis was performed in wheat germ extracts as described by Kohli *et al.* (1979) with the exception that ^{35}S-labelled methionine (1385 Ci/mmol) was used. The resulting polypeptides were electrophoresed on slab gels of 18% acrylamide – 0.1% bisacrylamide – 0.375 M Tris-HCl, pH 8.8 – 0.2% SDS and a stacking gel of 5% acrylamide pH 6.8. The gels were dried and subjected to fluorography according to Chamberlain (1979). Globin mRNA from adult *Xenopus laevis* was isolated as described by Widmer *et al.* (1981).

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