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Coexpression of Eukaryotic tRNA^{Ser} and Yeast Seryl-tRNA Synthetase Leads to Functional Amber Suppression in *Escherichia coli*

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In order to gain insight into the conservation of determinants for tRNA identity between organisms, *Schizosaccharomyces pombe* and human amber suppressor serine tRNA genes have been examined for functional expression in *Escherichia coli*. The primary transcripts, which originated from *E. coli* plasmid promoters, were processed into mature tRNAs, but they were poorly aminoacylated in *E. coli* and thus were nonfunctional as suppressors in vivo. However, coexpression of cloned *Saccharomyces cerevisiae* seryl-tRNA synthetase led to efficient suppression in *E. coli*. This shows that some, but not all, determinants specifying the tRNA^{Ser} identity are conserved in evolution.

The amino acid specificities of tRNAs are determined by a set of nucleotides that are important for interaction with the cognate aminoacyl-tRNA synthetases (28, 31). Recent studies have implemented two approaches: in vitro aminoacylation of tRNA variants generated from synthetic tRNA genes by T7 RNA polymerase and in vivo amber suppression with mutated tRNA or synthetase genes (12, 22, 28, 31, 36). However, less is known about the conservation of determinants for tRNA identities between organisms. Early in vitro studies analyzed the interaction between aminoacyl-tRNA synthetases and tRNAs from different species. The results vary from complete incompatibility to full charging (6, 17, 33, 38, 39).

The ability to introduce and express tRNA or synthetase genes in another organism has led to major advances in the identification of sequence elements important in recognition between corresponding but heterologous partners (7, 37). Functional expression of prokaryotic tRNA genes in eukaryotes requires the signals for recognition by RNA polymerase III, which are often fortuitously found in bacterial tRNA gene sequences (7, 21). Some eukaryotic tRNA genes which do not contain introns can be expressed in *Escherichia coli* from the vectors that supply a prokaryotic promoter (37), or, alternatively, synthetic genes can be used (15). Heterologous suppression of nonsense mutations is widely used in the study of biological functions of mammalian tRNAs (10, 14, 16, 20). Recently, nonsense suppressor tRNAs that function when heterologously expressed in plant cells have been constructed (9). There are several lines of evidence indicating that heterologously expressed tRNAs may also be misaminoacylated with noncognate amino acids (8, 21, 37), which makes them useful substrates for studies concerning the specificity of synthetase-tRNA recognition.

We have recently shown that the *Saccharomyces cerevisiae* SerRS gene (*SES1*) expressed in *E. coli* complements the temperature-sensitive mutations in the prokaryotic host (46). Together with previously shown in vitro cross-charging between yeast and *E. coli* systems (6), this is evidence that at least some serine identity elements are shared between two organisms. Here we address the question of conservation of serine identity elements by studying the recognition by host and *S. cerevisiae* seryl-tRNA synthetases (SerRS) of two eukaryotic serine tRNAs expressed in *E. coli*.

MATERIALS AND METHODS

Strains and plasmids. *E. coli* strains and plasmids are listed in Table 1. Strain JR104 was constructed by P1 transduction (23) of XACΔ14 [*ara argE*(*UAG*) *rpoB gyrA Δlac pro*/F′ *lacI-Z proAB*$^+$], the donor strain (24), and KL451 [*metB glyV55* Δ(*tonB-trpAB*)/F′ *trpA221*(Am)] (26), the recipient. Met$^+$ transductants were tested for the Arg$^-$ phenotype, and one Arg$^-$ isolate was selected. The construction of the plasmids carrying the *E. coli serS* or yeast *SES1* gene was described previously (11, 47). The *Schizosaccharomyces pombe* amber suppressor tRNA gene (19) contained in a 190-bp *Rsa*I-*Sal*I fragment was cloned between the *Eco*RV and *Sal*I sites of pBR322 (a gift of G. Krupp). It was excised as a *Hin*dIII-*Sal*I fragment and recloned into pACYC184 and pBluescriptSK distal to the *tet* and *lac* promoters, generating plasmids pACYC*supSI* and pSK*supSI*, respectively. *supSH*, the gene for a human amber suppressor tRNA^{Ser} (2), contained in a 900-bp *Sau*3A fragment was cloned into the *Bam*HI site of pUC12 (a gift of J. Sedivy). The 520-bp *Dra*I-*Sal*I fragment was excised and recloned between the *Eco*RV and *Sal*I sites of pACYC184 behind the *tet* promoter, giving a plasmid named pACYC-*supSH*. The entire gene was cut out as a *Hin*dIII-*Sal*I fragment and cloned into pBluescriptSK distal to the *lac* promoter, generating plasmid pSK*supSH*.

Suppression assays. The suppression of amber mutations in strains JR104 and XAC-A24 was tested by plating *E. coli* transformed with the plasmids carrying the genes for eukaryotic amber suppressor tRNAs, and where indicated with the genes for synthetases on a compatible plasmid, on selective plates. M9 glucose minimal plates were used for testing

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TABLE 1. Strains and plasmids

Strain or plasmid	Genotype, description, source, and/or reference
E. coli	
XAC-A24	F′ *ara argE*(*UAG*) *rpoB gyrA* Δ*lac pro*/F′ *lacI-Z proAB*⁺
JR104	F′ *trpA*(*UAG*)*211*/*glyV55* Δ(*tonB-trpAB*) *argE*(*UAG*) *rpoB*
Plasmids carrying synthetase genes	
pBR*serS*	2.0-kb *Eco*RI-*Hin*dIII fragment carrying *E. coli serS* cloned in pBR322 (11)
pBR*SES1*	3.6-kb *Eco*RI fragment carrying *S. cerevisiae SES1* with ≈1 kb of its upstream sequence cloned in pBR322 (47)
pUC19*SES1*	3.0-kb *Sal*I-*Bam*HI fragment from pBR*SES1* cloned in pUC19 behind the *lac* promoter; includes 350 bp of yeast *SES1* upstream sequence (46)
pUC8*SES1*(+)	1.4-kb *Bam*HI fragment containing the yeast *SES1* structural gene inserted in frame into the *lacZ* coding region of pUC8 (46)
pUC8*SES1*(−)	Same as above, but transcriptional orientation opposite to that of *lacZ* (46)
pET3*SES1*	1.4-kb *Bam*HI fragment containing the yeast *SES1* structural gene cloned into pET3 distal to T7 promoter (46)
Plasmids carrying suppressor tRNA genes	
pACYC*supSI*	190-bp *Rsa*I-*Sal*I fragment carrying the *S. pombe supSI* gene (19) cloned between *Eco*RV and *Sal*I sites of pACYC, distal to *tet* promoter (this work)
pSK*supSI*	346-bp *Hin*dIII-*Sal*I fragment from pACYC*supSI* cloned into pBluescriptSK behind *lac* promoter (this work)
pACYC*supSH*	520-bp *Dra*I-*Sal*I fragment carrying human *supSH* gene (2) cloned between *Eco*RV and *Sal*I sites of pACYC, distal to *tet* promoter (this work)
pSK*supSH*	678-bp *Hin*dIII-*Sal*I fragment from pACYC*supSH* cloned into pBluescriptSK behind *lac* promoter (this work)

argE(*UAG*) or *trpA*(*UAG*) suppression, while M9 lactose minimal plates were used for *lacI*(*UAG*)-*lacZ*. Plates were supplemented with ampicillin (100 μg/ml), chloramphenicol (50 μg/ml), arginine (40 μg/ml), and tryptophan (40 μg/ml) as required and were incubated at 30°C. Suppression of *argE* (*UAG*) and *lacI*(*UAG*)-*lacZ* is not dependent on the nature of the inserted amino acid (24), while the amber mutation at the position 211 in *trpA* can be suppressed with only a limited set of amino acids, including serine (26).

For assaying β-galactosidase activity, pACYC clones of tRNA genes, together with the yeast *SES1* or *E. coli serS* gene on a compatible plasmid, were transformed into *E. coli* XAC-A24. β-Galactosidase activity was determined according to the method of Miller (23). Suppression is defined as 100% from the β-galactosidase activity of strain XACΔ14, which contains a *lacI-Z* fusion, with no amber mutation in *lacI* (24).

Detection of expressed tRNAs by Northern (RNA) blot hybridization. *S. pombe* and human amber suppressor tRNA^Ser were expressed in strain XAC-A24 from pSK*supSI* and pSK*supSH*, respectively. The transformants were grown at 30°C in M9 glucose minimal medium, supplemented with arginine and ampicillin, to mid-logarithmic phase and induced by addition of IPTG (isopropyl-β-D-thiogalactopyranoside) (3 mM). After the cultures were shaken for an additional 2 h, the cells were harvested and total tRNA was isolated at pH 7.4 at room temperature, as previously described (41). For Northern analysis, tRNA was fractionated by electrophoresis on a denaturing 8% polyacrylamide gel, electrophoretically transferred to a GeneScreen Plus membrane (NEN Research Products), and hybridized with 5′-^{32}P-oligodeoxyribonucleotides (20-mers) complementary to a region of the variable and anticodon arm of *S. pombe* tRNA^Ser (nucleotides 31 to 47C) and human tRNA^Ser (nucleotides 29 to 47A) (42). Prehybridization and hybridization were performed overnight at 42°C in a solution containing 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrolidone, 1 mM ATP, 10 mM EDTA (pH 8.0), 15 mM sodium phosphate (pH 6.7), 1 mM sodium pyrophosphate, and 0.2 mg of poly(A) per ml. Two final washes were performed in 6× SSC–0.05% sodium dodecyl sulfate at 42°C for 10 min each.

Determination of in vitro aminoacylation levels. tRNA was prepared from XAC-A24 transformants as described above. Aminoacylation with purified yeast SerRS (46) was performed under conditions used for the *S. cerevisiae* cognate system (47), except the concentration of total tRNA was 5 to 25 μM and that of the enzyme was 0.16 μM.

Separation of in vivo-aminoacylated and uncharged tRNAs. *S. pombe* amber suppressor tRNA was isolated from *E. coli* JR104 transformed with pACYC vector carrying the tRNA gene and compatible plasmids with synthetase genes. Transformants were grown under selective conditions in M9 glucose medium supplemented with ampicillin, chloramphenicol, and, when necessary, arginine and tryptophan. tRNA isolation was performed at 4°C and pH 4.5 (45). The charged tRNA was electrophoresed on acidic 6.5% polyacrylamide–8 M urea gels at 4°C for 24 h at ≈12 V/cm. The portion of the gel around the xylene cyanol dye, which contained the tRNA of interest, was electroblotted onto a Nytran membrane prior to hybridization with 5′-^{32}P-labeled oligodeoxyribonucleotide probes (1×10^6 to 2×10^6 cpm/ml). The hybridization and posthybridization washes were as described above.

RESULTS

Rationale and system design. We wanted to test in vivo the interaction between eukaryotic tRNA^Ser species and *E. coli* or yeast SerRS. The expression of eukaryotic synthetases (32, 46) and tRNAs (15, 37) in *E. coli* has the advantage of testing for the recognition between heterologous partners in the presence of a full complement of aminoacyl-tRNA synthetases and tRNAs, i.e., in an environment sensitive to the competition effects and their contribution to identity. An easy way to monitor the proper utilization of eukaryotic tRNAs in prokaryotic protein synthesis is by suppression of nonsense mutations. Serine-specific tRNAs are especially suitable to be used in such experiments, since in contrast to many other tRNAs (18, 34), the anticodon is not presumed to be a recognition element for

interaction with the cognate synthetase, and consequently the requirement for its alteration does not change the tRNA identity (28, 29, 35).

Plasmid constructs to express in *E. coli* different amounts of eukaryotic tRNAs and *S. cerevisiae* or *E. coli* SerRS were designed (Table 1). These plasmids carry the *supSI* or *supSH* gene, which encode, respectively, *S. pombe* and human $tRNA^{Ser}$ which had been converted to amber suppressors in vitro (2, 19). These tRNAs were efficient suppressors in the homologous system (2, 19). The *S. cerevisiae* amber suppressor $tRNA^{Ser}$ gene *SUP-RL1* was not included in this study, since its coding region is interrupted by an intron (30), which precludes proper processing of the tRNA precursors in *E. coli*. The *supSI* and *supSH* genes lack the 3′-CCA sequence. However, it is known that in the process of tRNA biosynthesis, *E. coli* tRNA nucleotidyl transferase can add CCA to such transcripts to allow functional expression of eukaryotic tRNAs in *E. coli* (3). The tRNA genes, cloned behind plasmid promoters, were introduced into two *E. coli* tester strains (JR104 and XAC-A24). Each strain carries two amber markers which can be suppressed by serine insertion (see Materials and Methods). When transformation with two plasmids was required, tRNA genes were on plasmid pACYC184 while synthetase genes were carried on pBR-derived plasmids compatible with the p15A origin of the tRNA plasmid.

***S. pombe* and human amber suppressor serine tRNAs expressed in *E. coli* are not efficiently recognized by *E. coli* aminoacyl-tRNA synthetases.** To test for the function of the eukaryotic tRNAs in *E. coli*, we introduced the plasmids carrying the tRNA genes into our tester strains. No suppression was obtained when the tRNAs were expressed from pACYC plasmids, while the suppression was very poor when expression was from pBluescript constructs (Fig. 1). Recognition in *E. coli* of the yeast or human $tRNA^{Ser}$ may be affected by competition with *E. coli* $tRNA^{Ser}$, which may bind *E. coli* SerRS more efficiently. Therefore, we overproduced *E. coli* SerRS by cotransformation with the pBR*serS* plasmid. However, no effect on suppression in vivo was observed (Fig. 1), indicating that the eukaryotic tRNAs were poorly recognized by the bacterial synthetase. Since the lack of suppression may also be due to defects in tRNA biosynthesis, total cellular RNA was isolated from strain XAC-A24 transformed with the tRNA gene plasmid or with the parent vector alone and subjected to Northern analysis. Filters were probed with synthetic oligonucleotides complementary to the tRNA of interest. Bands migrating closely with pure *E. coli* serine tRNA were detected in extracts of cells which carry tRNA plasmids (Fig. 2, lanes b, c, e, and f) but not in extracts of cells which carry the empty vector (Fig. 2, lanes a and d). The results demonstrate that the genes for the *S. pombe* and human amber suppressor $tRNA^{Ser}$ are transcribed and processed in *E. coli*. The expression of both genes from pBluescript was very much lower than expression of *E. coli* tRNA genes, as judged from the same Northern blot probed with an oligonucleotide complementary to *E. coli* $tRNA^{Glu}$. The processing of the *supSI* transcript to mature size is very efficient, as unprocessed precursor cannot be detected on the Northern blot (Fig. 2, lane c), even when the gel is overloaded with RNA (600 μg per slot). However, as determined by scanning of the autoradiogram, about 30% of the human tRNA precursor stays in the unprocessed form and gives rise to a more slowly migrating band that hybridizes to a human tRNA-specific oligonucleotide probe (Fig. 2, lanes e and f).

***S. cerevisiae* SerRS expressed in *E. coli* charges *S. pombe* and human amber suppressor $tRNA^{Ser}$ in *E. coli*.** If the eukaryotic tRNAs are not properly charged in *E. coli*, suppressor activity is impaired. Overexpression of eukaryotic SerRS may then improve charging and consequently suppression. To test this hypothesis, we cotransformed the tester strains with plasmids encoding yeast SerRS and the eukaryotic suppressor tRNAs. The recognition of both *S. pombe* and human $tRNA^{Ser}$ by the eukaryotic yeast synthetase in vivo was reflected in suppression of *E. coli* amber mutations (Fig. 1), showing at the same time proper utilization of heterologous tRNAs in prokaryotic protein synthesis.

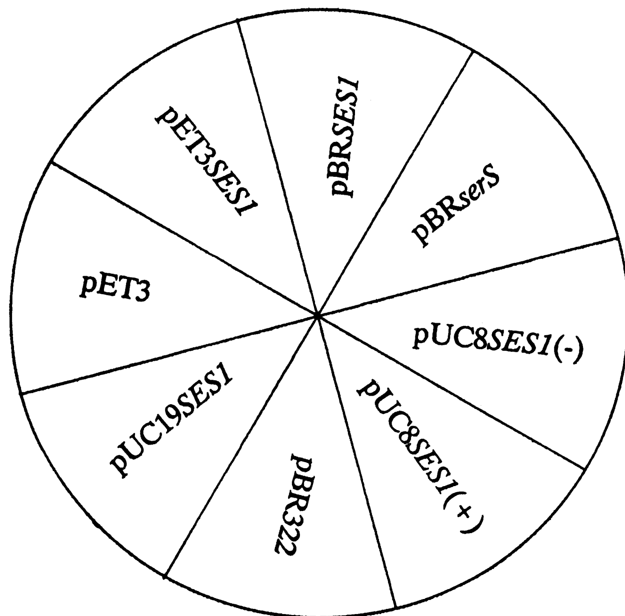
Suppression levels were quantitated by assaying β-galactosidase activity produced in strain XAC-A24 from the suppressed *lacZ* gene by the eukaryotic Ser-$tRNA^{Ser}$ species, in either the presence or absence of coexpressed yeast SerRS (Table 2). The results confirm the requirement for the eukaryotic synthetase to give significant suppression. The suppressor efficiencies of eukaryotic tRNAs charged by the yeast SerRS in *E. coli* are one-third of the activity obtained when suppressing the same amber mutation with *E. coli* $tRNA^{Ser}$. This suggests that the extent of charging of the *S. pombe* and human $tRNA^{Ser}$ by yeast SerRS in *E. coli* is less than the level of *E. coli* Ser-tRNA present in *E. coli*.

This was borne out by the determination of the amount of charged tRNA by acid gel electrophoresis, which allows the separation of in vivo charged *S. pombe* amber suppressor $tRNA^{Ser}$ isolated from transformed *E. coli* cells (Fig. 3). About twice as much aminoacylated tRNA was isolated from JR104 cells transformed with pET3*SES1* (Fig. 3, lane b) as from the cells lacking the *SES1* gene (Fig. 3, lanes c and d). Regardless of the extent of *E. coli* SerRS overexpression, the majority of *S. pombe* $tRNA^{Ser}$ was isolated from *E. coli* in uncharged form (Fig. 3, lanes c and d). Therefore, the absence of suppression in vivo is probably not caused by insufficient expression of eukaryotic suppressor tRNAs but is the consequence of poor recognition by prokaryotic synthetases. A similar result was obtained for human $tRNA^{Ser}$ (data not shown). In vitro aminoacylation of *S. pombe* and human $tRNA^{Ser}$ by *S. cerevisiae* SerRS confirmed the reduced acylation in the heterologous cases. Total RNA extracted from the overproducing *E. coli* strains was aminoacylated with purified *E. coli* GluRS and *S. cerevisiae* SerRS. GluRS charged approximately the same fraction of tRNA in samples enriched with eukaryotic tRNAs as in the control sample prepared from an *E. coli* strain transformed with the empty vector (Table 3). Increased aminoacylation levels were detected in samples containing overexpressed *S. pombe* and human $tRNA^{Ser}$ when yeast SerRS was used in the charging assay (Table 3).

DISCUSSION

tRNA biosynthesis in heterologous systems. The expression of eukaryotic tRNA genes in *E. coli* requires transcription, addition of the 3-terminal CCA sequence, and biosynthesis of specific modified nucleosides for production of mature tRNAs. The finding of functional amber suppressors derived from *S. pombe* and human serine tRNAs demonstrates heterologous expression. This was not unexpected, as a yeast ochre suppressor $tRNA^{Tyr}$ gave rise to functional suppression in *E. coli* in vivo (37). While most of the modified nucleosides found in eukaryotic $tRNA^{Ser}$ species are also found in different *E. coli* tRNA species, 5-methylcytidine and *N*-2,2-dimethylguanosine do not occur in *E. coli*. Therefore, the human or *S. pombe* $tRNA^{Ser}$ species formed in *E. coli* will lack these modifications. However, nucleotide modification is generally not essential for aminoacylation (31), although there are some synthetases which require particular modifications for proper recognition (25, 44).

strain	XAC-A24		JR104		XAC-A24		JR104	
marker tested	$argE_{am}$	$lacI_{am}/lacZ$	$argE_{am}$	$trpA_{am}$	$argE_{am}$	$lacI_{am}/lacZ$	$argE_{am}$	$trpA_{am}$
serS construct	tRNA suppressor							
	pACYC*supSI*				pACYC*supSH*			
pBR322	–	–	–	–	–	–	–	–
pBR*serS*	–	–	–	–	–	–	–	–
pBR*SES1*	–	–	–	–	–	–	–	–
pUC19*SES1*	+	–	+	++	±	–	±	–+
pUC8*SES1*(+)	±	–	±	±	–	–	–	–
pUC8*SES1*(–)	–	–	–	–	–	–	–	–
pET3*SES1*	+++	+++	+++	+++	++	++	++	++
	tRNA suppressor							
	pSK*supSI*				pSK*supSH*			
	±	–	±	–	±	–	±	–



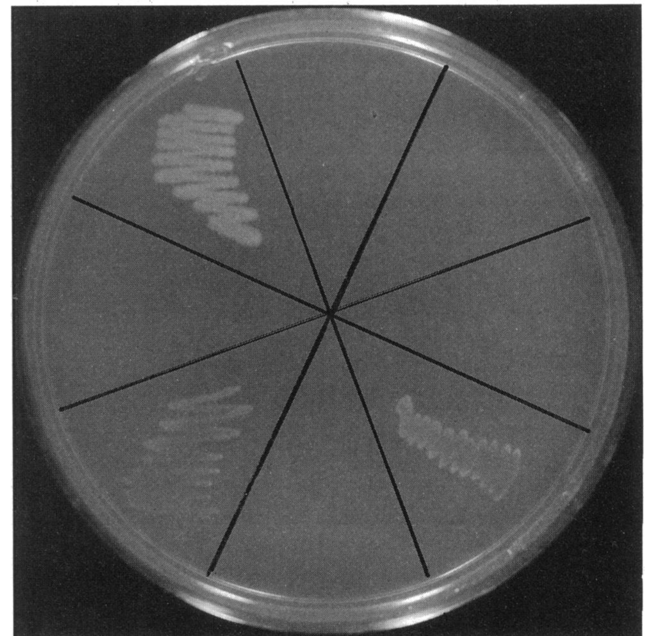


FIG. 1. Suppression of *E. coli* amber mutations by *S. pombe* and human $tRNA^{Ser}$. *E. coli* strains were transformed with vectors carrying the genes for *S. pombe* or human amber suppressor $tRNA^{Ser}$ and, as indicated, with the *S. cerevisiae SES1* or *E. coli serS* gene on a compatible plasmid. The suppression was checked by streaking the transformants on selected plates [M9 glucose for *argE*(*UAG*) or *trpE*(*UAG*) and M9 lactose for *lacI*(*UAG*)-*lacZ*] supplemented with required antibiotics and amino acids. The degree of suppression is indicated by very good growth (+++), good growth (++), weak growth (+), or very weak growth (±) at 30°C. The plate with restreaked JR104 transformants, shown on the lower right, was incubated at 30°C for 20 h. The description of the plasmids used for transformation is provided on the lower left. Their relevant characteristics are described in Table 1.

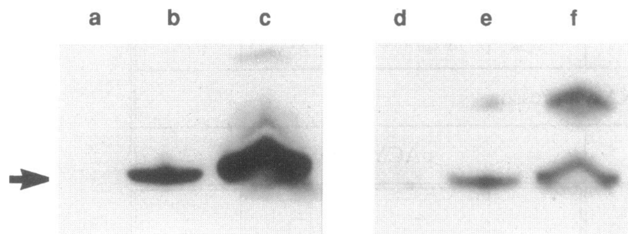


FIG. 2. Northern blot analysis of total tRNA isolated from *E. coli* transformed with plasmids carrying *S. pombe* or human amber suppressor $tRNA^{Ser}$. The position of pure *E. coli* $tRNA^{Ser}$ is indicated by the arrow. Total RNA, isolated from strain XAC-A24 transformed with empty pSK vector (lanes a and d; 200 μg), pSK*supSI* (lanes b [120 μg] and c [600 μg]), or pSK*supSH* (lanes e [120 μg] and f [600 μg]) was fractionated on an 8 M urea–8% polyacrylamide gel, electrophoretically transferred to a nylon membrane, and probed with ^{32}P-end-labeled deoxyoligoribonucleotides complementary to a segment of *S. pombe* (lanes a, b, and c) or human (lanes d, e, and f) $tRNA^{Ser}$.

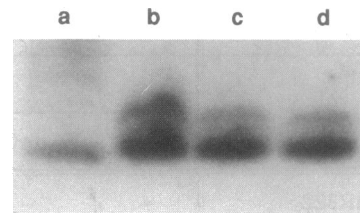


FIG. 3. Separation of charged and uncharged *S. pombe* $tRNA^{Ser}$ isolated from *E. coli*. Strain JR104 carrying the *S. pombe* amber suppressor $tRNA^{Ser}$ gene on plasmid pACYC was cotransformed with pET3*SES1* (lane b), empty pET3 (lane c), or pBR*serS* (lane d). Total RNA (100 μg) was separated by electrophoresis on an acidic denaturing 6.5% polyacrylamide gel at 4°C, as described in Materials and Methods. Deacylated total *E. coli* RNA, containing expressed *S. pombe* $tRNA^{Ser}$, was run in lane a.

Misacylation. Acylation of tRNA with a noncognate amino acid has been well documented in several heterologous systems (8, 17, 21). Our suppression assay may not detect mischarging in *E. coli* of *S. pombe* or human $tRNA^{Ser}$ with an amino acid other than serine, since the amber suppression of *trpA* at position 211 depends on insertion of several other amino acids besides serine (26). However, competition by the efficiently charging yeast SerRS for the eukaryotic tRNAs would certainly minimize any misacylation.

Serine identity. Because of the lack of conservation of anticodon bases, it was suggested by early studies that the anticodon of $tRNA^{Ser}$ species is not involved in recognition by SerRS (43). Recently it was demonstrated that *E. coli* SerRS indeed does not require the anticodon but requires nine other nucleotides (Fig. 4a) as the minimal identity set for tRNA recognition, including a long variable arm of $tRNA^{Ser}$ (29). Of these nine nucleotides, seven are totally conserved in all known *E. coli* serine tRNAs, with the 3-70 position either a U-A or an A-U pair. The extra arm is a common recognition element in both yeasts and *E. coli* (1, 4, 5, 13, 29, 40). These findings suggest that in *E. coli* several regions of tRNA, located mainly in the acceptor stem, D stem, and extra arm, but not the anticodon, facilitate the recognition process by SerRS.

Our experiments show that the in vivo charging of eukaryotic $tRNA^{Ser}$ by *E. coli* SerRS is too low to effect amber suppression. This is in agreement with very low level of in vitro cross-charging observed previously (6) and suggests that the determinants for serine identity are only partially conserved among *E. coli*, yeast, and human $tRNA^{Ser}$. Possibly some of the determinants required for the recognition by *E. coli* SerRS are missing in the eukaryotic tRNAs, or some negative determinants in the yeast or human tRNA may prevent serylation by the *E. coli* synthetase. However, as seen in other cases, the *S. pombe* and human $tRNA^{Ser}$, although distant in evolution, share enough determinants for recognition to be charged by *S. cerevisiae* SerRS (Fig. 4).

An examination of Fig. 4 may shed light on the requirements for serylation by the different synthetases. Panel a shows the consensus sequence of all *E. coli* $tRNA^{Ser}$ species with exclusion of the invariant nucleotides found in all tRNAs. Panel b shows the conserved positions of all known *S. cerevisiae* serine tRNA molecules which are the natural substrates for yeast SerRS. The remaining panels represent the sequences of the *S. pombe* or human amber suppressor $tRNA^{Ser}$ species; the invariant nucleotides (found in all tRNAs) are also shown as dots. The nucleotides indicated in squares are *E. coli* identity elements conserved in *S. cerevisiae*, *S. pombe*, and human tRNAs. The circled nucleotides in panels b, c, and d denote three residues which are common in the yeast and human tRNAs and not found in *E. coli*. As has been pointed out earlier (4, 27), these nucleotides may represent identity elements for yeast SerRS and be a reason for the poor acylation of *E. coli* tRNA by this enzyme. However, it is pertinent to note that the nucleotides in diamonds are common to all *E. coli* and all *S. cerevisiae* $tRNA^{Ser}$ species as well as to the *S. pombe* and human suppressor tRNAs used in this study. One of these, A-38, is common among all serine tRNAs known to date (53 species from 30 organisms). Whether these nucleotides repre-

TABLE 2. Efficiency of suppression by serine tRNA[a]

serS construct	β-Galactosidase activity with the following tRNA suppressor:			
	None	*S. pombe*	Human	*E. coli*
Empty vector	0.7	0.9	0.7	67
pBR*serS*	0.7	0.8	0.8	ND[b]
pET3*SES1*	0.8	24.2	23.1	ND

[a] *E. coli* XAC-A24 [relevant genotype *argE*(*UAG*) *lacI*(*UAG*)-*lacZ*] was cotransformed with plasmid pACYC184 carrying different serine-specific amber suppressor tRNA genes and the *serS* or *SES1* gene on the compatible plasmid. Cultures of selected double transformants were grown at 30°C in M9 glucose medium supplemented with required antibiotics and, when necessary, with arginine. The assays of β-galactosidase activity were carried out as described by Miller (23). Values are given as percentages of that for the wild-type *lacI-lacZ* fusion strain (XACΔ14), which gives ≈260 U of β-galactosidase under the same conditions.

[b] ND, not determined.

TABLE 3. In vitro aminoacylation of *S. pombe* and human suppressor $tRNA^{Ser}$ isolated from *E. coli*[a]

tRNA construct	Relative aminoacylation level	
	E. coli GluRS	*S. cerevisiae* SerRS
pSK*supSI*	1	2.5
pSK*supSH*	1	1.8
pBluescriptSK	1	1

[a] Total tRNA was isolated from strain XAC-A24 transformed with empty pBluescriptSK vector or the vectors carrying eukaryotic tRNA genes. After deacylation, the tRNA was used in an in vitro aminoacylation assay with purified *E. coli* GluRS or *S. cerevisiae* SerRS as described in Materials and Methods. The background level was 5 pmol of serine per A_{260} unit.

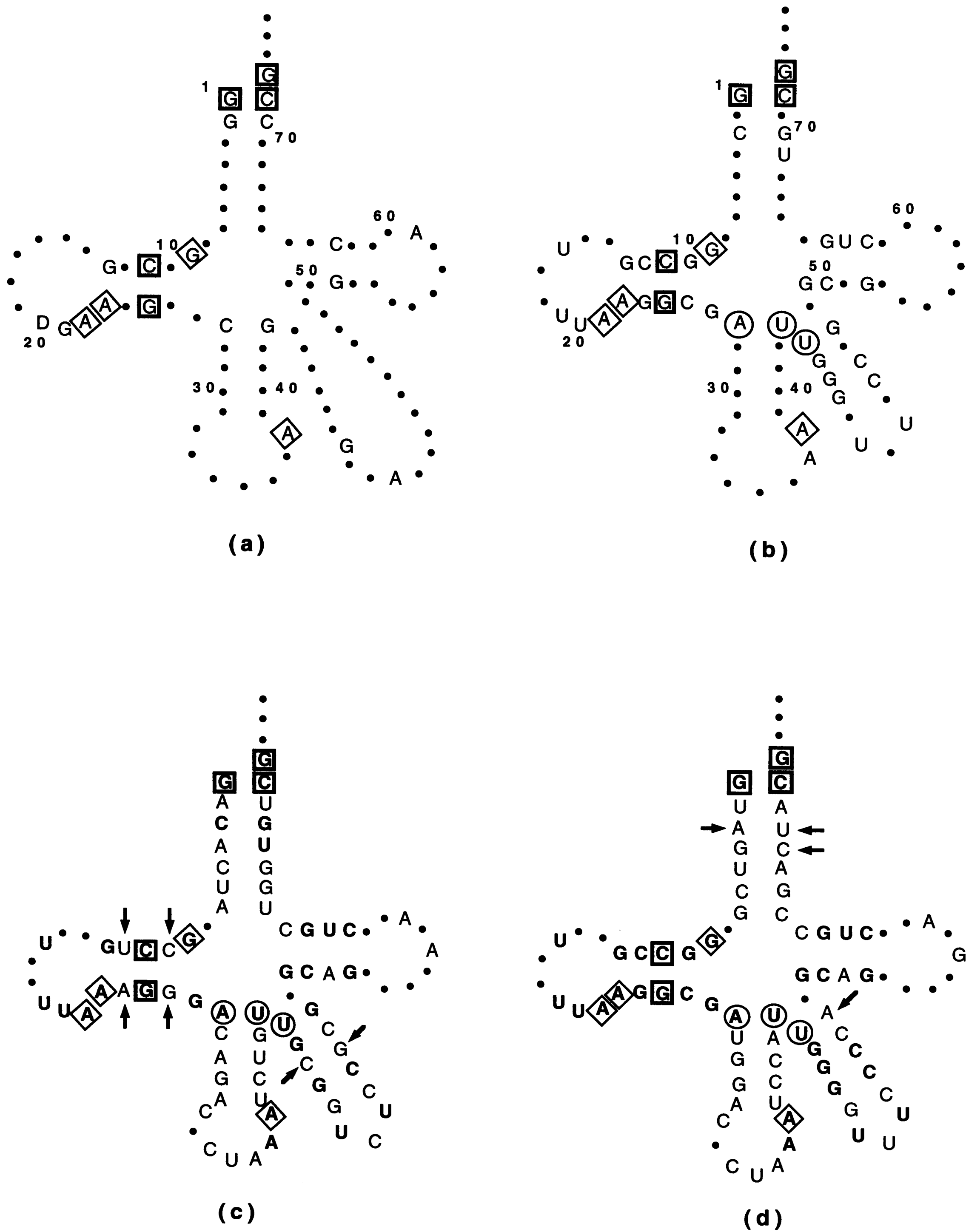


FIG. 4. Cloverleaf arrangement of serine tRNAs. (a and b) Consensus tRNA^{Ser} structures of *E. coli* and *S. cerevisiae*, respectively; (c and d) sequences of the *S. pombe* and human amber suppressor serine tRNAs, respectively. The squares indicate nucleotides which are *E. coli* identity elements conserved in *S. cerevisiae*, *S. pombe*, and human serine tRNAs. The residues common to yeast and human tRNAs and not found in *E. coli* are shown in circles. The nucleotides in diamonds represent these which are conserved in all *E. coli*, all *S. cerevisiae*, and *S. pombe* and human serine tRNAs. Arrows indicate positions which deviate from the *S. cerevisiae* tRNA^{Ser} consensus structure.

sent additional identity elements or facilitate synthetase recognition in a more general manner remains to be determined.

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