C-terminal truncation of yeast SerRS is toxic for *Saccharomyces cerevisiae* due to altered mechanism of substrate recognition

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Abstract Like all other eukaryal cytosolic seryl-tRNA synthetase (SerRS) enzymes, *Saccharomyces cerevisiae* SerRS contains a C-terminal extension not found in the enzymes of eubacterial and archaeal origin. Overexpression of C-terminally truncated SerRS lacking the 20-amino acid appended domain (SerRSC20) is toxic to *S. cerevisiae* possibly because of altered substrate recognition. Compared to wild-type SerRS the truncated enzyme displays impaired tRNA-dependent serine recognition and is less stable. This suggests that the C-terminal peptide is important for the formation or maintenance of the enzyme structure optimal for substrate binding and catalysis.

Key words: Aminoacyl-tRNA synthetase; tRNA recognition; Toxicity; tRNA-dependent amino acid recognition; Protein degradation

1. Introduction

Aminoacyl-tRNA synthetases (aaRS) catalyze the highly specific attachment of amino acids to their cognate tRNAs by a two-step mechanism. For most aaRS, the activation of the amino acid substrate by ATP to form an enzyme-bound aminoacyl-adenylate intermediate (step I) precedes tRNA binding and transfer of amino acid to the 3′ end of the tRNA (step II). Seryl-tRNA synthetases, class II homodimeric enzymes, can activate serine without the presence of tRNA [1,2]. Evidence from crystallographic studies of class II synthetases shows that the adenylate moiety is stably bound in the active site, lying beneath the CCA end of tRNA [3–6]. This raises the possibility that the formation of the adenylate intermediate influences tRNA binding and aminoacylation [3,5,6], or that tRNA binding enhances the accuracy of amino acid substrate selection. The second of these possibilities, tRNA-dependent amino acid recognition, has been described previously for several class I and class II synthetases [7,8], including yeast SerRS [9]. To understand the mechanism by which this occurs, we have recently generated a number of SerRS mutants with altered motif 2 loop residues and showed that these mutations affect tRNA-dependent amino acid recognition, possibly by interfering with the flexibility of the motif 2 loop [9].

In contrast to their prokaryotic counterparts, all so far identified eukaryotic seryl-tRNA synthetases (Fig. 1) contain C-terminal extensions abundant in basic amino acids [10]. In eukaryotes, extra appended domains occur frequently in aminoacyl-tRNA synthetases, but their natural role in the cell remains unclear. While the existence of a multi-synthetase complex in yeast is controversial [11,12], for some tRNA synthetases in higher eukaryotes an extra domain is believed to be important for formation of multisynthetase complexes [11,13,14]. The extensions may be adapted for specialized RNA-related functions as recently proposed for yeast methionyl- [15] and glutaminyl-tRNA synthetases [16] or may be involved in stabilizing the enzyme structure and thus important for optimization of substrate binding, as recently shown for yeast SerRS [9]. Here we present evidence that the C-terminal peptide is also required for the tRNA-dependent serine recognition displayed by yeast SerRS. The deletion of this domain leads to toxicity and protein degradation upon overproduction of the truncated SerRSC20 mutant in *Saccharomyces cerevisiae* possibly due to the altered mechanism of substrate recognition.

2. Materials and methods

2.1. Plasmids and strains

The original plasmids pCJ11*SES1* and pCJ11*SES1C20*, containing the yeast *SES1* structural gene and its truncated form, respectively, have been described [10]. These were used for transformation of *S. cerevisiae* strains WCG4a (*MAT*α *ura3 leu-3,112 his-11,15*), WCG4-11a (*MAT*α *ura3 leu-3,112 his-11,15 pre1-1*) and WCG4-11/22a (*MAT*α *ura3 leu-3,112 his-11,15 pre1-1 pre2-2*) [17] in order to study the stability of the expressed proteins in vivo.

2.2. Protein purification

Wild-type SerRS was purified from yeast as described [9,10]. In order to improve separation from endogenous SerRS, the purification scheme for SerRSC20, which has decreased affinity for cation exchangers, was modified as follows: pooled MonoQ fractions containing SerRSC20 were loaded onto a MonoS column in 30 mM potassium phosphate, pH 6.0 and the truncated enzyme was eluted with a gradient of 0–100 mM potassium phosphate, pH 7.2. The formation of heterodimers, which eluted between SerRSC20 and SerRS, was also detected in the chromatographic profile.

2.3. Preparation of $tRNA^{Ser}$

The native $tRNA^{Ser}$ was isolated from total brewer's yeast tRNA by serylation of serine isoacceptors, followed by derivatization of $tRNA^{Ser}$ by naphthoxylation and purification by chromatography on a benzoylated DEAE-cellulose column as described [18]. After deacylation, purified $tRNA^{Ser}$ accepted 1.3 nmol of serine/A_{260} unit of tRNA. The synthetic gene $tRNA^{Ser}_2$ was constructed distal to the T7 RNA polymerase promoter. After the run-off transcription of the *Bst*NI-digested DNA template [19,20], tRNA transcript was purified by electrophoresis on 12% denaturing polyacrylamide gel. Eluted tRNA was renatured by heating for 2 min at 80°C in 10 mM Tris-HCl, pH 7.2, 2 mM $MgCl_2$ and slow cooling. The specific activity of in vitro transcribed $tRNA^{Ser}_2$ was 1.1 nmol/A_{260} unit.

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2.4. Determination of kinetic parameters

The extent of adenylate formation catalyzed by the purified enzymes was assessed by pyrophosphate exchange at 30°C [33]. The reaction conditions were as in [9]. Typical concentrations of the substrate studied (serine or ATP) were $0.1–10\times K_m$, while the other substrate was kept saturated (serine at 5 mM, Mg-ATP at 2 mM (molar ratio 1:1)). The concentration of enzymes was 50 nM. Aminoacylation was performed at 30°C as described previously [10] in reaction mixtures containing 50 mM Tris-HCl, pH 7.5, 15 mM $MgCl_2$, 4 mM dithiothreitol. For all kinetic assays, the concentration of the substrate studied ([^{14}C]serine, Mg-ATP, or $tRNA^{Ser}$) were varied from 0.1 to $10\times K_m$. Saturating concentrations of other substrates were: 5 mM ATP, 10 μM $tRNA^{Ser}$ (or corresponding amount of unfractionated brewer's yeast tRNA which contained approximately 6% of serine isoacceptors), and 1 mM ^{14}C-labeled serine. Due to the very low affinity of SerRSC20 for $tRNA^{Ser}$ transcript, we were not able to determine the kinetic parameters for serine in the presence of this tRNA. Enzyme concentrations were 100 nM.

2.5. Western blot analysis

Protein extracts were prepared from WCG4a or WCG4a-11/22 cells producing wild-type SerRS (pCJ11*SES1*) or the C-terminal truncated form SerRCS20 (pCJ11*SESC20*). 5×10^6 cells were resuspended in lysis buffer with glass beads and vortexed for 20 s six times followed by centrifugation at $17\,000\times g$ for 10 min [10]. Approximately 10 μl of the supernatants was used for Western blot analysis. The procedure for Western blot analysis was performed according to [21], except that proteins were visualized by chemiluminescence (Lumiglo, Kirkegaard and Perry).

3. Results and discussion

3.1. The structure of seryl-tRNA synthetases and C-terminal extensions in eukaryotic enzymes

The amino acid sequence alignment of seryl-tRNA synthetases reveals the presence of prominent, highly basic C-terminal extensions in all cytosolic SerRS enzymes of Eukarya, which are absent from eubacterial and archaeal enzymes (Fig. 1). The function of this domain has been investigated only in *S. cerevisiae*. We have previously shown that a truncated yeast *SES1* gene, lacking the 60 base pairs that encode a 20-amino acid C-terminal extension, is able to complement a yeast *SES1* null allele strain [10]. Thus, the C-terminal extension in SerRS seems to be dispensable for the viability of the cell. However, removal of the C-terminal peptide affects both the stability of the enzyme and its affinity for the substrates in the aminoacylation reaction, indicating its importance in maintaining the overall structure of SerRS.

Two eubacterial seryl-tRNA synthetases from *Escherichia coli* and *Thermus thermophilus* have been crystallized in complexes with different substrates [4,22] and subjected to biochemical analyses in order to identify the domains important for tRNA and amino acid binding [23–25]. Our recent data have shown close functional resemblance between yeast SerRS

	Organisms	C-termini of aligned SerRS sequences	SerRS length
Bacteria	*E. coli*	LRPYMNG--LEYIG----------	430
	H. influenzae	LRPYMGG--LDVIGK----------	429
	C. burnettii	LKSYMGG--VDYF----------	423
	M. genitalium	LKKYLDF--DTIK----------	417
	M. pneumoniae	LLKYLDF--DKITKPK----------	420
	M. tuberculosis	LVPFVGVEVLEPVA----------	419
	T. thermophilus	LIPYMGKEVLEPCG----------	421
	Synechocystis sp.	VLQDFLKR--DYL----------	430
	B. subtilis	LRPYMGN--REVMKP----------	425
	S. aureus	LVPFMGG--KTQISKPVK----------	428
	S. cerevisiae, mitochondrial	LREFMNG--QRYI----------	446
	Z. mays, mitochondrial	LRPYMGG--LELLSPKFK----------	489
Archaea	*H. marismortui*	LRPYMGG--QEVIEGSEKIGESAVGAGEKE----------	460
	M. jannaschii	IKKLPEV--PQLITWPKKDE----------	521
	M. maripaludis	VGKLPEI--PKLITWP----------	514
	M. thermoautotrophicum	VEGVVEK--GRIITWPRQD----------	513
	A. fulgidus	LRKYLEP--IESAPKDFIMPAKSQ----------	446
	P. horikoshii	LWKYTGF--KEIVPVEKKERCCAT----------	455
Eukarya	*S. cerevisiae*	LRKYIPGE-PEFLPFVNELPKNSTSSKDKKKKN----------	462
	S. pombe	LQPYMGG--KTFLPFTKELPKNSTSKKGKN----------	450
	A. thaliana	LQPFMGG--ETFLPFKAKPVVADTKGKKSKA----------	451
	Z. mays	LQPYMGG--IEFLPFKQPLDVKQASDSKSNKSKSKGNAA----------	457
	C. elegans	IQQWMPENYRTFIPFVKPAPIDEDAKKATGKK----------	487
	H. sapiens	LKEFMPPGLQELIPFVKPAPIEQEPSKKQKKQHEGSKKKAAARDVTLENRLQNMEVTDA	514

Fig. 1. Detail of multiple sequence alignment of seryl-tRNA synthetases. Only the C-terminal parts of the proteins are shown. The organisms with corresponding accession numbers are as follows: *Escherichia coli* (P09156), *Haemophilus influenzae* (P43833), *Coxiella burnettii* (P39919), *Mycoplasma genitalium* (P47251), *Mycoplasma pneumoniae* (P75107), *Mycobacterium tuberculosis* (EMBL Z83864), *Thermus thermophilus* (P34945), *Synechocystis* sp. (P73201), *Bacillus subtilis* (P37464), *Staphyloccocus aureus* (P95689), *Saccharomyces cerevisiae*, putative mitochondrial (P38705), *Zea mays*, mitochondrial (EMBL Y13053), *Haloarcula marismortui* (EMBL X91007), *Methanococcus jannaschii* (58477), *Methanococcus maripaludis* (O30520), *Methanobacterium thermoautotrophicum* (O27914, O30521), *Archaeoglobus fulgidus* (O28244), *Pyrococcus horikoshii* (TrEMBL O58441), *Saccharomyces cerevisiae* (P07284), *Shizosaccharomyces pombe* (EMBL Z97210), *Arabidopsis thaliana* (Q39230), *Zea mays* (GenBank AJ007665), *Caenorhabditis elegans* (EMBL Z68882), *Homo sapiens* P49591). The accession numbers are from SwissProt, except where indicated. Cytosolic SerRS enzymes of Eukarya have prominent, highly basic C-terminal extensions absent from eubacterial and archaeal enzymes. Numbers at the right margin denote total number of amino acid residues in the proteins. Multiple alignment of protein sequences was performed using CLUSTAL_X [40].

active site residues and analogously positioned amino acids in their eubacterial counterparts [9]. Thus, the structures of eubacterial SerRS-tRNA^{Ser} co-crystals are useful models for rationalizing yeast SerRS structure. Based on the eubacterial crystallographic [4,22] as well as biochemical and genetic data from various organisms [2], tRNA^{Ser} recognition may be in general attributed to interactions between the synthetase and tRNA at both the acceptor stem and the long variable arm. The long variable arm constitutes the principal determinant for recognition of SerRS in all investigated organisms [2]. Its interaction with the N-terminal coiled-coil of one SerRS subunit, brings the 3′ end of tRNA into the active site of another subunit [4]. The recognition of the acceptor end seems to be species-specific [2]. The positioning of the 3′ end of tRNA into the active site of the enzyme induces a conformational change in a motif 2 loop, both in *E. coli* and in yeast [5,9]. We wondered whether the C-terminal peptide of yeast SerRS was involved in this process. In a model structure of yeast SerRS (not shown), based upon coordinates of SerRS from *T. thermophilus* (*1ses*.pdb), the C-terminal extension of the yeast enzyme appears to flip back and interact with the active site, as predicted according to the thermodynamically most stable conformation.

3.2. SerRSC20 overproduction is toxic for S. cerevisiae

It has previously been shown that the C-terminal extension of SerRS strongly influences the stability of the protein [10]. In contrast to the wild-type SerRS, attempts to overproduce the mutant form of SerRS lacking the C-terminal fragment (SerRSC20) were unsuccessful, although the mRNA levels of *SES1* and *SES1C20* were comparable [10]. To further examine the in vivo stability of SerRS and SerRSC20, Western blot analysis was performed with cell extracts prepared from wild-type strain WCG4 [17] overexpressing SerRS (pCJ11*SES1*) or the mutant form SerRSC20 (pCJ11*SES1*C20). The intracellular levels of the two proteins were analyzed after growth in selective media containing galactose (plasmid-borne *SES1* inducing conditions) for 16 and 24 h (Fig. 2A). The level of full-length SerRS seems to decrease slightly after prolonged growth in inducing media, indicating a minor level of degradation of the wild-type protein. The intracellular level of SerRSC20 after 16 h of induction, on the other hand, is significantly lower than that of the wild-type SerRS. After 24 h only small amounts of SerRSC20 are observed suggesting rapid degradation of the mutant SerRS protein in the cells. Whereas it seems that the cells tolerate overproduction of wild-type SerRS, these data show that SerRS lacking the C-terminal extension, SerRSC20, is unstable and is subjected to degradation.

In order to examine whether degradation of SerRSC20 can be prevented the strain WCG4-11/22a was used, which contains single mutations in two genes encoding components of the proteasome (*pre1-1 pre2-2*) [17,26]. The strain was transformed with plasmids pCJ11*SES1* and pCJ11*SES1*C20 and cell extracts for Western blot analysis were prepared after growth for 24 and 36 h in selective medium containing galactose (Fig. 2B). In the proteasome-deficient background the full-length form of SerRS seems to be completely stabilized, since no visible degradation occurred between 24 and 39 h of induction. The SerRSC20 protein, however, is visible only after 24 h of induction. After 39 h the truncated protein is no longer present in its original size (Fig. 2B). In conclusion,

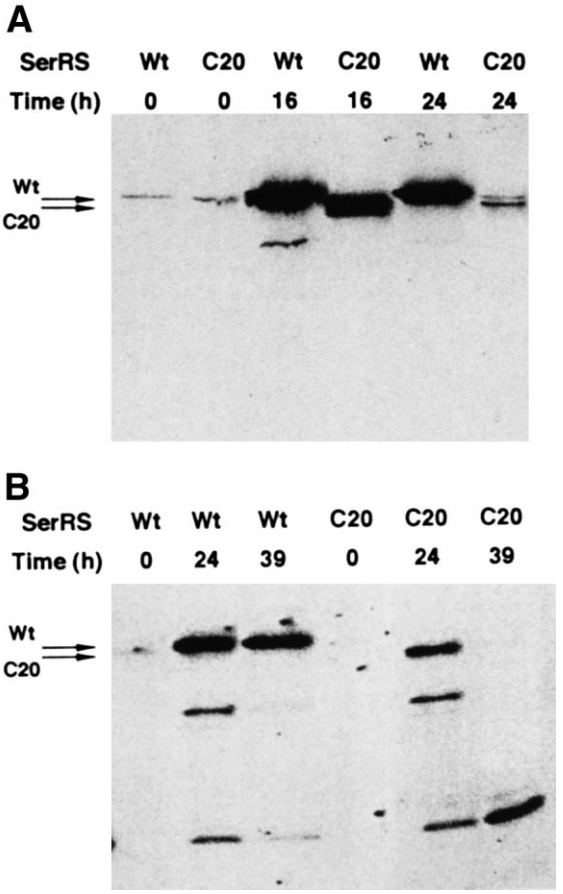

Fig. 2. Western blot analysis of SerRS and SerRSC20. WCG4a (A) or WCG4-11/22a (*pre1-1 pre2-2*) (B) cells producing wild-type SerRS (pCJ11*SES1*) or the C-terminal truncated form SerRCS20 (pCJ11*SESC20*) were used for Western blotting. Cells were grown at 30°C overnight in SC-Leu medium containing glucose (plasmid born *SES1* non-inducing medium). In order to obtain exponential growing cultures, 10^6 cells from the overnight culture were diluted into 5 ml SC-Leu medium containing glucose and allowed to grow for 6 h (three generations). Thereafter, 10^6 cells were diluted into 5 ml SC-Leu medium containing galactose (induces plasmid-borne *SES1*) and grown at 30°C. For cell extract preparation 5×10^6 cells were removed after 16 and 24 h (A) and 24 and 39 h (B). Also shown is the chromosomal-encoded wild-type SerRS (cultures grown under non-inducing conditions). Time: hours of induction of plasmid born *SES1*. Wt: wild-type SerRS (SerRS). C20: SerRSC20.

it seems that whereas degradation of SerRS is completely abolished, proteolysis of the truncated form of SerRS, although delayed, is still occurring. The appearance of a lower molecular weight band of the same intensity as the original SerRSC20 signal after 39 h induction may suggest that limited proteolysis has taken place rather than complete degradation of SerRS. The lower molecular weight band is also weakly present for wild-type SerRS. This potential degradation intermediate may be obtained by cleavage of SerRS molecules in a proteasome-independent manner. It seems reasonable to conclude from these data that the removal of the C-terminal extension of SerRS may not only lead to instability of the protein, but also leads to a cytotoxic effect. This interpretation is in good agreement with the observation that proteasome-deficient cells (*pre1-1* and *pre1-1 pre2-2*) transformed with pCJ11*SES1*C20 show strongly reduced growth compared to the cells producing wild-type SerRS (data not shown).

3.3. tRNA binding increases thermal stability of SerRS

Full-length SerRS and the SerRSC20 truncation mutant were purified to electrophoretic homogeneity and characterized in vitro. The involvement of the C-terminal peptide in the stabilization of the overall enzyme structure was demonstrated by comparison of the thermal stability of the full-length and truncated SerRS enzymes. In accordance with our previous observation [10], SerRSC20 was heat-inactivated much faster than the wild-type enzyme but the inactivation was more efficiently prevented by tRNA binding (Fig. 3). This finding supports our assumption that proteolysis in vivo is due to folding of the mutant protein into a less stable conformation, which may exhibit an altered mechanism of substrate recognition (see below).

3.4. Kinetic parameters of SerRSC20

In order to examine the impact of truncation on substrate recognition and catalysis, the kinetic parameters for wild-type and mutant SerRS were determined independently in the amino acid activation and aminoacylation reactions. We were primarily interested in determining whether the recently observed mechanism of tRNA-dependent serine recognition employed by the full-length SerRS [9] is affected by the removal of the C-terminal peptide. The described mode of substrate selection strongly depends on the flexibility of the motif 2 loop, which undergoes a conformational change possibly induced by positioning of the 3′ end of tRNA into the active site [5,9]. We reasoned therefore that alteration of the active site structure induced by the lack of interaction with the C-terminal peptide might influence substrate recognition.

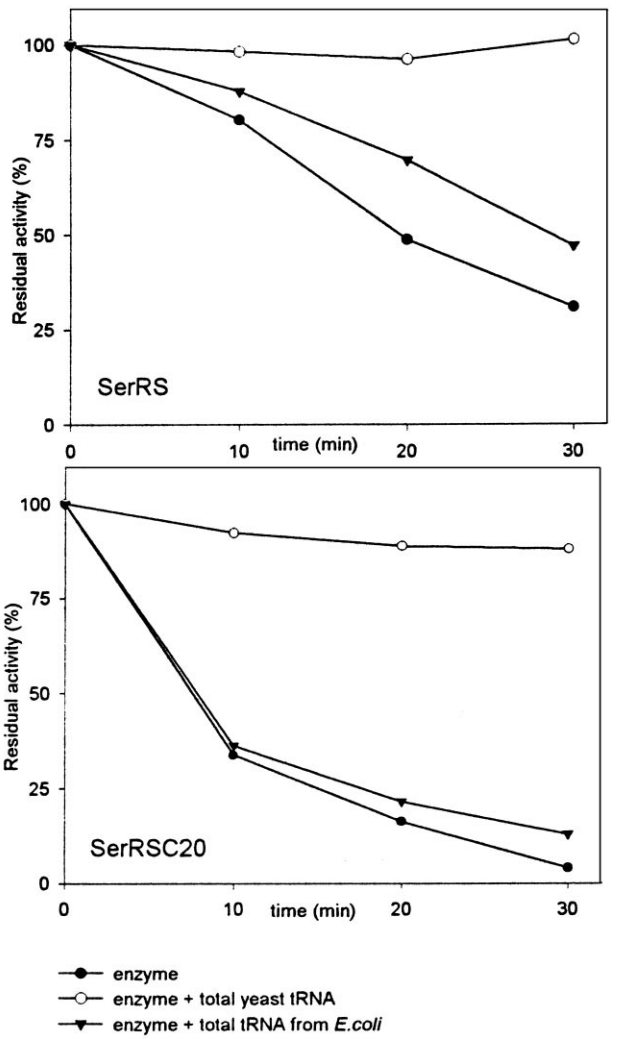

Fig. 3. tRNA binding greatly increases thermal stability of the enzyme. Enzymes were incubated at 42°C in 50 mM Tris-HCl pH 7.5, 2 mM DTT, 5% glycerol with or without tRNA in excess. At indicated times aliquots were withdrawn and assayed for aminoacylation activity.

As shown in Table 1, there was no difference in the kinetic parameters for SerRSC20 and SerRS in the amino acid activation step assayed by ATP-PP_i exchange. This indicates the existence of similar active site conformations in the two proteins prior to tRNA binding. Furthermore, the enzymes were analyzed for misactivation of several non-cognate amino acids. No such activity was observed. In accordance with our previous results [9] the presence of $tRNA^{Ser}$ enhances the affinity of SerRS for serine (Table 1). The differences in the affinities of SerRS for serine, in the absence (ATP-PP_i exchange) and presence (aminoacylation) of $tRNA^{Ser}$ prepared by transcription in vitro confirms the capability of unmodified tRNA to modulate the binding of serine. The full-length SerRS charges $tRNA^{Ser}$ transcript with only slightly reduced specificity constant (2.7-fold) compared to the native $tRNA^{Ser}$ (1.7-fold higher K_m and 1.7-fold lower k_{cat}), indicating that nucleotide modifications make only a small contribution to recognition by yeast seryl-tRNA synthetase, as already shown by Himeno et al. [20,27]. SerRSC20, however, exhibits two important differences in the kinetic parameters for aminoacylation: it binds native $tRNA^{Ser}$ with a 2-fold decreased K_m value while its affinity for $tRNA^{Ser}$ transcript was decreased 7-fold in comparison with SerRS. The differences in affinities for native tRNA displayed by the mutant and wild-type enzyme were even higher when crude yeast tRNA was taken as a substrate, indicating that in vivo toxicity of SerRSC20 was not due to inhibition of the charging of $tRNA^{Ser}$ by non-cognate tRNAs. However, the higher affinity of C-terminally truncated SerRSC20 for its cognate tRNA could be a potential cause of the toxic behavior of the protein when overproduced in *S. cerevisiae* (see below). The unmodified $tRNA^{Ser}$ is a very poor substrate for SerRSC20 in comparison with the full-length enzyme. The specificity constant for aminoacylation of $tRNA^{Ser}$ transcript with SerRSC20 was reduced 20-fold, with a significant effect on K_m for tRNA which was increased 7-fold. Since recognition by wild-type SerRS does not require modified $tRNA^{Ser}$ (Table 1), it is difficult to predict whether the lack of the modification themselves, or the specific conformation of the tRNA molecule, which may be altered in the absence of modifications [28], lowers its affinity for truncated SerRS. It seems that the wild-type enzyme is not sensitive to subtle changes in tRNA structure, since the main contribution to the apparent affinity of SerRS for $tRNA^{Ser}$ comes from the interaction between the long variable arm and the N-terminal domain of SerRS. In contrast to *E. coli* SerRS [29], the yeast enzyme does not use the global structural features of its cognate tRNA for discrimination from other type 2 tRNA species [27]. However, if $tRNA^{Ser}$ transcript has a slightly relaxed conformation, then the positioning of its 3′ end in the active site of SerRS could be more flexible. This flexibility may be accommodated by properly structured full-length SerRS, but not by truncated SerRSC20,

Table 1
Kinetic parameters of wild-type and truncated SerRS

Enzyme	Substrate	K_m (mol/l)	k_{cat} (s^{-1})	Rel. k_{cat}/K_m
ATP-PP_i exchange				
Serine	SerRS	4.8×10^{-4}	2.2	1
Serine	SerRSC20	5.0×10^{-4}	2.0	0.87
SerRS	ATP	4.0×10^{-4}	2.6	1
SerRSC20	ATP	4.0×10^{-4}	2.1	0.80
Aminoacylation reaction				
SerRS	total yeast tRNA	1.20×10^{-7}	0.55	1
SerRSC20	total yeast tRNA	5.00×10^{-8}	0.46	2.0
SerRS	serine	6.25×10^{-5}	0.50	1
SerRSC20	serine	2.52×10^{-4}	0.46	0.23
SerRS	native $tRNA^{Ser}$	9.70×10^{-8}	0.45	1
SerRSC20	native $tRNA^{Ser}$	5.10×10^{-8}	0.39	1.6
SerRS	serine	6.50×10^{-5}	0.58	1
SerRSC20	serine	2.81×10^{-4}	0.48	0.19
SerRS	$tRNA^{Ser}$ transcript	1.61×10^{-7}	0.27	1
SerRSC20	$tRNA^{Ser}$ transcript	1.12×10^{-6}	0.09	0.05
SerRS	serine	6.70×10^{-5}	0.59	
SerRSC20	serine	n.d.	n.d.	

explaining the differences in the affinities of the two enzyme forms for the transcript. Based on these experiments, we would like to propose that the main role of the C-terminal peptide in yeast SerRS is to ensure the optimal conformation of the active site for substrate recognition by guiding proper positioning of the 3′ end of tRNA into the active site. Consistent with such an idea is our finding (Table 1) that truncation of the C-terminal extension in SerRS interferes with tRNA-dependent serine recognition. Binding of the cognate tRNA enhances the affinity of wild-type SerRS for serine, while this mechanism is impaired in the mutant lacking the C-terminal domain, as shown by comparable K_m values for serine determined during seryl-adenylate formation and in the aminoacylation reaction. Enhanced accuracy of substrate selection, via tRNA-dependent serine recognition, may especially help the discrimination from $tRNA^{Leu}$, the other type 2 tRNA in *S. cerevisiae*. As recently shown by Himeno et al. [27], only one nucleotide insertion to the long variable arm confers an efficient serine acceptor activity upon $tRNA^{Leu}$ in vitro.

3.5. Correlation between cellular toxicity and kinetic parameters

The balance between tRNA and synthetase concentrations greatly contributes to the precision of aminoacylation in vivo, as shown in various systems [30–32]. The specific tRNAs are not present in the cell in substantially larger amounts than their cognate synthetases [33]. Thus, sequestering of the cognate tRNA is likely to occur upon synthetase overproduction and cause a tRNA-dependent arrest of cell growth. Even overproduction of an inactive enzyme that retains its tRNA binding capacity could starve cells of a specific charged tRNA species [34]. On the other hand, overexpression of a mutant synthetase less able to discriminate between tRNAs may result in misacylation or enhanced, non-productive binding of non-cognate tRNAs as described for tyrosyl-tRNA synthetase [35–37].

Since overproduction of SerRSC20 was toxic to the cell under conditions where wild-type synthetase [10] and a number of its active site mutants [9] accumulated in the cell to levels up to 150-fold above the normal quantities, the truncation of the C-terminal peptide obviously affects substrate recognition and catalysis. The toxicity of SerRSC20 in vivo was not due to a defective interaction with cognate tRNA. Indeed, SerRSC20 charges $tRNA^{Ser}$ in vitro with a k_{cat}/K_m ratio 1.6 times higher than the wild-type synthetase. However, the 2-fold increased affinity of the truncated enzyme for native $tRNA^{Ser}$ seems to be a rather small effect to be solely responsible for depletion of the available $tRNA^{Ser}$ pool. It cannot be excluded, on the other hand, that the binding of non-cognate tRNAs, in addition to partly non-productive binding of $tRNA^{Ser}$, contributes to toxicity. Our experiments also suggest that the impaired ability of SerRSC20 to perform tRNA-dependent serine recognition is strongly correlated with toxic behavior. As proposed above, the C-terminal peptide is responsible for positioning the 3′ end of tRNA into the active site of the enzyme, which will then induce the conformational change in motif 2 loop required to optimize serine binding site. This may happen either by direct interaction between the C-terminal peptide of SerRS and the acceptor end of tRNA, or indirectly whereby the C20-appended domain ensures a suitable active site conformation optimal for the binding of substrates. In this context, the lost ability of the truncated enzyme to exhibit tRNA-dependent serine recognition could diminish the transfer of serine to tRNA. Our data also provide evidence that a very defined SerRS conformation is essential for tRNA recognition: although unmodified $tRNA^{Ser}$ is a good substrate for wild-type SerRS, it is poorly recognized by SerRSC20. As a result of this low affinity of the transcript for the mutated enzyme, it was not possible to determine K_m for serine in the aminoacylation reaction.

The toxic behavior of overproduced SerRSC20 was observed in yeast strains which also produce normal endogenous levels of SerRS. Thus in addition to causing an imbalance in overproducing cells due to altered interactions with substrates, the overexpression of mutant enzyme is expected to decrease the concentration of functional SerRS due to heterodimer formation. This provides a further example of dominant negative inhibition, a phenomenon whereby the function of a wild-type gene product is impaired by a coexpressed mutant variant of the same gene product [38]. Analogously, it has been shown that the overexpression of a C-terminal fragment of *E. coli* IleRS exerts an inhibitory effect on this monomeric enzyme, by interfering with its folding [39].

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References

[1] Leberman, R., Härtlein, M. and Cusack, S. (1991) Biochim. Biophys. Acta 1089, 287–298.
[2] Härtlein, M. and Cusack, S. (1995) J. Mol. Evol. 40, 519–530.
[3] Cavarelli, J., Rees, B., Ruff, M., Thierry, J.-C. and Moras, D. (1993) Nature 362, 181–184.
[4] Biou, V., Yaremchuk, A., Tukalo, M. and Cusack, S. (1994) Science 263, 1404–1410.
[5] Cusack, S., Yaremchuk, A. and Tukalo, M. (1996) EMBO J. 15, 2834–2842.
[6] Cusack, S., Yaremchuk, A. and Tukalo, M. (1996) EMBO J. 15, 6321–6334.
[7] Hong, K.-W., Ibba, M., Weygand-Durasevic, I., Thomann, H.-U. and Söll, D. (1996) EMBO J. 15, 1983–1991.
[8] Ibba, M., Hong, K.-W., Sherman, J.M., Sever, S. and Söll, D. (1996) Proc. Natl. Acad. Sci. USA 93, 6953–6958.
[9] Lenhard, B., Filipic, S., Landeka, I., Skrtic, I., Söll, D. and Weygand-Durasevic, I. (1997) J. Biol. Chem. 272, 1136–1141.
[10] Weygand-Durasevic, I., Lenhard, B., Filipic, S. and Söll, D. (1996) J. Biol. Chem. 271, 2455–2461.
[11] Mirande, M. (1991) Prog. Nucleic Acid Res. Mol. Biol. 40, 95–142.
[12] Harris, C.L. and Kolanko, C.J. (1995) Biochem. J. 309, 321–324.
[13] Kerjan, P., Cerini, C., Semeriva, M. and Mirande, M. (1994) Biochim. Biophys. Acta 1199, 293–297.
[14] Rho, S.B., Lee, K.H., Kim, J.W., Shiba, K., Yo, Y.J. and Kim, S. (1996) Proc. Natl. Acad. Sci. USA 93, 10128–10133.
[15] Simos, G., Segref, A., Fasiolo, F., Hellmuth, K., Shevchenko, A., Mann, M. and Hurt, E.C. (1996) EMBO J. 15, 5437–5448.
[16] Whelihan, E.F. and Schimmel, P. (1997) EMBO J. 16, 2968–2974.
[17] Heinemeyer, W., Gruhler, A., Mohrle, V., Mahe, Y. and Wolf, D.H. (1993) J. Biol. Chem. 268, 5115–5120.
[18] Gruic-Sovulj, I., Luedemann, H.C., Hillenkamp, F., Weygand-Durasevic, I., Kucan, P. and Peter-Katalinic, J. (1997) J. Biol. Chem. 272, 32084–32091.
[19] Sampson, J.R. and Uhlenbeck, O.C. (1988) Proc. Natl. Acad. Sci. USA 85, 1033–1037.
[20] Soma, A., Kumagai, R., Nishikawa, K. and Himeno, H. (1996) J. Mol. Biol. 263, 707–714.
[21] Ibba, M. and Hennecke, H. (1995) FEBS Lett. 364, 272–275.
[22] Price, S., Cusack, S., Borel, F., Berthet-Colominas, C. and Leberman, R. (1993) FEBS Lett. 324, 167–170.
[23] Borel, F., Vincent, C., Leberman, R. and Härtlein, M. (1994) Nucleic Acids Res. 22, 2963–2969.
[24] Vincent, C., Borel, F., Willson, J.C., Leberman, R. and Härtlein, M. (1995) Nucleic Acids Res. 23, 1113–1118.
[25] Willson, J.C., Härtlein, M. and Leberman, R. (1995) J. Bacteriol. 177, 3347–3350.
[26] Hilt, W. and Wolf, D.H. (1996) Trends Biochem. Sci. 21, 96–102.
[27] Himeno, H., Yoshida, S., Soma, A. and Nishikawa, K. (1997) J. Mol. Biol. 268, 704–711.
[28] Steinberg, S. and Cedergren, R. (1995) RNA 1, 886–891.
[29] Asahara, H., Himeno, H., Tamuru, K., Nameki, N., Hasegawa, T. and Shimizu, M. (1994) J. Mol. Biol. 236, 738–748.
[30] Rogers, M.J. and Söll, D. (1990) Prog. Nucleic Acid Res. Mol. Biol. 39, 185–208.
[31] Bedouelle, H. (1990) Biochimie 72, 589–598.
[32] Sherman, J.M., Rogers, M.J. and Söll, D. (1992) Nucleic Acids Res. 20, 2847–2852.
[33] Jakubowski, H. and Goldman, E. (1984) J. Bacteriol. 158, 769–776.
[34] Schmidt, E. and Schimmel, P.R. (1993) Proc. Natl. Acad. Sci. USA 90, 6919–6923.
[35] Bedouelle, H., Guez, V., Vidal-Cros, A. and Hermann, M. (1990) J. Bacteriol. 172, 3940–3945.
[36] Vidal-Cros, A. and Bedouelle, H. (1992) J. Mol. Biol. 223, 801–810.
[37] Bedouelle, H. and Nageotte, R. (1995) EMBO J. 14, 2945–2950.
[38] Herskowitz, I. (1987) Nature 329, 219–222.
[39] Michaelis, J.-E.A., Schimmel, P.R., Shiba, K. and Miller, W.T. (1996) Proc. Natl. Acad. Sci. USA 93, 14452–14455.
[40] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) Nucleic Acids Res. 25, 4876–4882.