The Accuracy of Seryl-tRNA Synthesis

Ivana Weygand-Durasevic[1,2]*, Ita Gruic-Sovulj[1,2], Sanda Rocak[1,2] and Irena Landeka[1,2]

[1]Department of Chemistry, Faculty of Science, University of Zagreb, HR-10000 Zagreb, Croatia

[2]Rudjer Bošković Institute, 10000 Zagreb, Croatia

Summary

The high level of translational fidelity is ensured by various types of quality control mechanisms, which are adapted to prevent or correct naturally occurring mistakes. Accurate aminoacyl-tRNA synthesis is mostly dependent on the specificity of the aminoacyl--tRNA synthetases (aaRS), *i.e.* their ability to choose among competing structurally similar substrates.

Our studies have revealed that accurate seryl-tRNA synthesis in yeast and plants is accomplished *via* tRNA-assisted optimization of amino acid binding to the active site of seryl-tRNA synthetase (SerRS). Based on our recent kinetic data, a mechanism is proposed by which transient protein : RNA complex activates the cognate amino acid more efficiently and more specifically than the apoenzyme alone. This may proceed *via* a tRNA-induced conformational change in the enzyme's active site. The influence of tRNA[Ser], on the activation of serine by SerRS variants mutated in the active site, is much less pronounced. Although SerRS misactivates structurally similar threonine *in vitro*, the formation of such erroneous threonyl-adenylate is reduced in the presence of nonchargeable tRNA[Ser] analog. Thus, the sequence-specific tRNA : SerRS interactions enhance the accuracy of amino acid recognition.

Another type of quality control mechanism in tRNA serylation is assumed to be based on the complex formation between SerRS and a nonsynthetase protein. Using *in vivo* interaction screen, yeast peroxin Pex21p was identified as SerRS interacting protein. This was confirmed by an *in vitro* binding assay. Kinetic experiments performed in the presence of Pex21p revealed that this peroxin acts as an activator of seryl-tRNA synthetase in the aminoacylation reaction.

Key words: aminoacyl-tRNA synthetase, tRNA-dependent amino acid recognition, amino acid selection, protein-protein interactions, activation of aminoacylation

Introduction

A cell places a high priority on ensuring that translation produces proteins that accurately reflect the corresponding genetic information (*1*). Still, translation is less accurate than other processes in transmition of genetic information. This inaccuracy is due mostly to erroneous selection of aminoacyl-tRNAs by the ribosomes, but the aminoacyl-tRNA synthetases (aaRS) are also responsible for the occurrence of translational mistakes. These enzymes catalyze the esterification of tRNAs with their cognate amino acids with relatively high specificity (*2*). The error in the selection of correct amino acids is in the range of 10^{-4}–10^{-5} *in vitro*. The selectivity toward tRNA is even greater: the frequency of errors involving noncognate tRNA aminoacylation is in most cases 10^{-6} or

* Corresponding author; Phone: +385-1-4561-197; Fax: +385-1-4561-177; E-mail: weygand@rudjer.irb.hr

lower (*3*). The accuracy of these processes is enhanced *in vivo*, since the quality control mechanisms exist, which can further prevent or correct the errors.
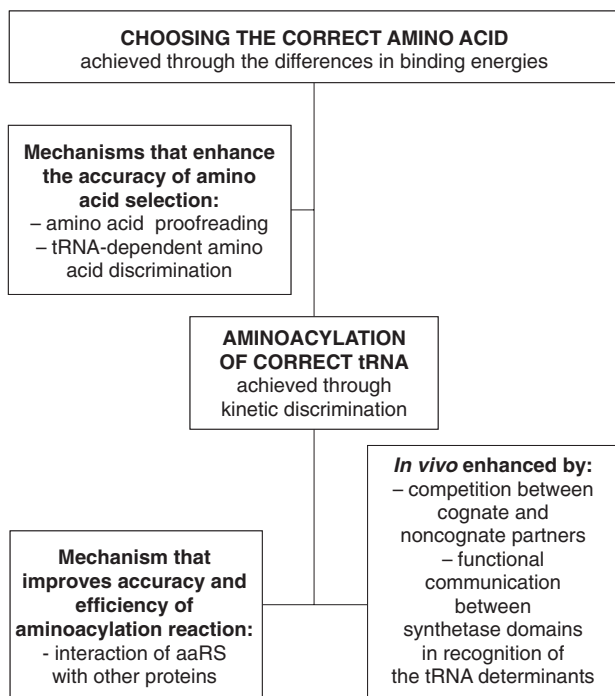

Fig. 1. Recognition and specificity in aminoacylation reaction. Selection of proper amino acid and tRNA by aminoacyl-tRNA synthetases are crucial steps in maintaining the fidelity of translation

Basic rules that govern the recognition and specificity in aminoacylation reaction are illustrated in Fig. 1. Precision in the choice of amino acid by the cognate aaRS depends on natural chemical diversity of these compounds in the cell. The differences in amino acid side chains are often sufficient to allow their specific binding. The essential principle in achieving high specificity is the rejection of smaller substrates with similar side chain chemistry or isosteric amino acids. Aminoacyl-tRNA synthetases specific for amino acids, which have structurally similar analogs, often possess editing activity. They can correct erroneously activated amino acids and misacylated tRNAs (*4–7*). However, during the editing process, some of the correct products are also destroyed (*8*). This makes corrective pathways energetically very costly, and they are disfavored in the cell. Alternatively, the quality of aminoacyl-tRNA synthesis in the cell can be improved by the mechanisms that enhance the accuracy of substrate discrimination and consequently prevent errors. The examples are metalloenzymes, threonyl- and cysteinyl-tRNA synthetases. In both cases, improved amino acid selection is based on the fact that only the binding of cognate (or chemically very similar amino acid) to the active site changes the coordination geometry of zinc and prevents the interference of other substrates (*9–11*). In seryl-tRNA synthesis, prevention of errors can be achieved by cognate tRNA:

synthetase complex formation (*12*). On the other hand, the selection of the correct tRNA is assumed to occur as a result of preferential reaction kinetics for cognate protein-RNA complexes. This means that the accuracy of this process is enhanced by the stabilization of the transition state for charging and the existence of the antideterminants in certain tRNAs that prevent interaction with noncognate aaRSs. The specificity and efficiency of aminoacylation can be further increased by the interaction of aaRS with nonsynthetase proteins (*13,14*).

Results and Discussion

The accuracy of seryl-tRNA formation

In general, the accuracy of seryl-tRNA synthesis is determined mostly by the interaction of a serine -OH group with the enzymes' active site (*15*) and the specific recognition between the long N-terminal coiled coil (contained in all SerRSs except those from animal mitochondria) and the long variable arm of tRNA^{Ser} (*16,17*). Other structural elements of cognate tRNAs, as the sequence of the acceptor stem and the overall macromolecular shape, further define the serylation specificity in various organisms (*18–20*). Due to our interest in evolution of tRNA determinants, the differences in the recognition of substrates, and most recently in quality control mechanisms, we have been studying SerRS enzymes from several sources in our laboratory for many years. Special emphasis was given to the comparison of seryl--tRNA formation in different cellular compartments.

All seryl-tRNA synthetases, except those functioning in animal mitochondria and methanogenic archaea, have rather conserved primary structures. Without exception, SerRSs are homodimers, with two active sites for seryl-adenylate formation (*21,22*). The most conserved regions involve so called signature motifs: motif 1, which is important for dimerization, and motifs 2 and 3, which make up the active site and are involved in binding of all three substrates: ATP, serine and the 3'-end of tRNA. Schematic representation of conserved domains in eukaryotic seryl-tRNA synthetases is given in Fig. 2. Organellar enzymes have N-terminal import sequences, while all eukaryotic cytosolic enzymes have very basic C-terminal extensions (*22*). These C-terminal peptides are dispensable for cell viability but are involved in substrate recognition and overall stabilization of SerRS structure (*23*). According to structure modeling, the C-terminal extension of yeast SerRS flips back and interacts with the active site (Fig. 2). This interaction may be either direct or possibly bridged by another SerRS-interacting protein. Although we have accumulated some experimental evidence in support of this model (see below), final conclusion must await further structural studies, since none of the eukaryotic seryl--tRNA synthetases have yet been crystallized. In order to test structural and functional similarity between seryl-tRNA synthetases from various organisms, we have performed mutational analysis of yeast SerRS and analyzed the mutants *in vivo* and *in vitro*. Our experiments revealed that yeast SerRS structurally resembles its bacterial counterparts in the active site (*24*).
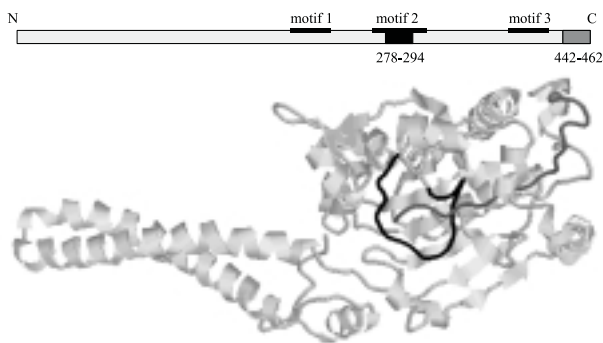
Fig. 2. Seryl-tRNA synthetase (SerRS) from yeast. (A): Schematic representation of domain arrangement in SerRS protein. Class conserved motif 1 is a part of the dimer interface, while motifs 2 and 3 interact with the substrates (ATP, amino acid and 3′ end of tRNA). Motif 2 loop, which is a part of active site, is shown in black. C-terminal extension characteristic of eukaryotic cytosolic enzymes is shown in dark gray. Numbers define amino acids comprised in indicated regions. (B): Model of yeast SerRS subunit. The model is based upon coordinates of SerRS from *T. thermophilus* (1ses.pdb). The position of the motif 2 loop (black) and the C-terminal extension (dark gray) of yeast enzyme are shown in the structural context.

Fig. 3. Comparison of K_m values for serine in aminoacylation reaction (black bars) and amino acid activation (gray bars). Higher affinity for serine in the reaction performed in the presence of tRNA reveals conformational readjustment of amino acid binding site in SerRS : tRNA complex. Mutations in the motif 2 loop (SerRSmut4, Gly291Val; SerRSmut5, Glu281Asp, Gly291-Ala) as well as the truncation of C-terminal extension (SerRSC20) impair tRNA-dependent serine recognition.

Substrate recognition by yeast SerRS

Yeast dimeric SerRS binds two tRNA^{Ser} molecules with very different affinities (*12*). Interestingly, the binding is anticooperative and currently we are not able to predict the biological significance of this unusual mode of macromolecular interaction. However, cognate tRNA is not only the substrate, but it also mediates in serine recognition. In the presence of tRNA, K_m value for serine is decreased for an order of magnitude, indicating that sequence specific tRNA:SerRS interactions optimize the binding of serine. This is the consequence of tRNA--induced conformational change in the enzyme's active site. Two structural features of SerRS are crucial for tRNA--dependent serine recognition: the flexibility of motif 2 loop and the presence of C-terminal peptide (Fig. 3). According to structure modeling, alteration of glycines in the conserved motif 2 loop may cause the formation of more rigid conformation (*17*). Kinetic analysis of mutated yeast SerRS variants (SerRSmut4, Gly291Val; SerRSmut5, Glu281Asp, Gly291Ala) revealed that the replacement of conserved glycines disables mutated enzymes to recognize serine in tRNA-assisted manner. Consequently, these mutants complemented *Saccharomyces cerevisiae* null allele strain only weakly.

In order to get better insight into the mechanism of tRNA-dependent serine recognition, we have generated nonchargeable, 3′-truncated tRNA analog (tRNA$^{Ser}_{CC}$) and used it as potential conformational modulator in seryl-adenylate synthesis. According to our kinetic studies, the addition of equimolar amount of cognate, homologous tRNA^{Ser} per dimeric yeast SerRS was capable of inducing the complete rearrangement of the amino acid binding site, as judged by almost equal affinities for serine in tRNA-assisted seryl-adenylate formation and aminoacylation reaction. Molar surplus of tRNA did not significantly affect serine binding properties.
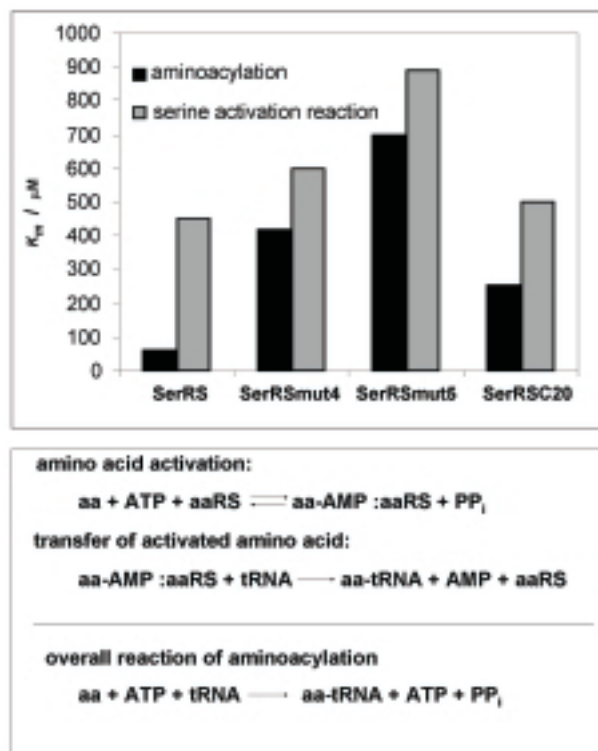
This may suggest that the conformational change occurs concomitantly at both subunits, since two seryl-adenylates were formed per mol of SerRS (*22*). Heterologous (bacterial) tRNA^{Ser}, which shares only some identity elements with yeast tRNA^{Ser} isoacceptors, is a less potent conformational modulator, as shown by kinetic analysis (Fig. 4). It is in good agreement with our previous results (*25*) that bacterial tRNA^{Ser} is a very poor substrate for yeast SerRS, both *in vivo* and *in vitro*.

As judged by the comparison of the specificity constants, SerRS:tRNA complex activates cognate amino acid more efficiently and more specifically than the apoenzyme alone (Fig. 5).

Misactivation of threonine

Seryl- and threonyl-tRNA synthetases are evolutionary and structurally related enzymes which recognize amino acids with beta-hydroxyl group. The hydroxyl groups of both amino acids make important contribution to specificity of recognition by their respective aminoacyl-tRNA synthetases (*9,15*). Thus, slight misactivation of threonine by yeast SerRS is not surprising. However, the formation of erroneous threonyl-adenylate is reduced in the presence of inactivated tRNA^{Ser}, indicating that the sequence-specific tRNA:SerRS interactions enhance the accuracy of amino acid recognition. Misacti-
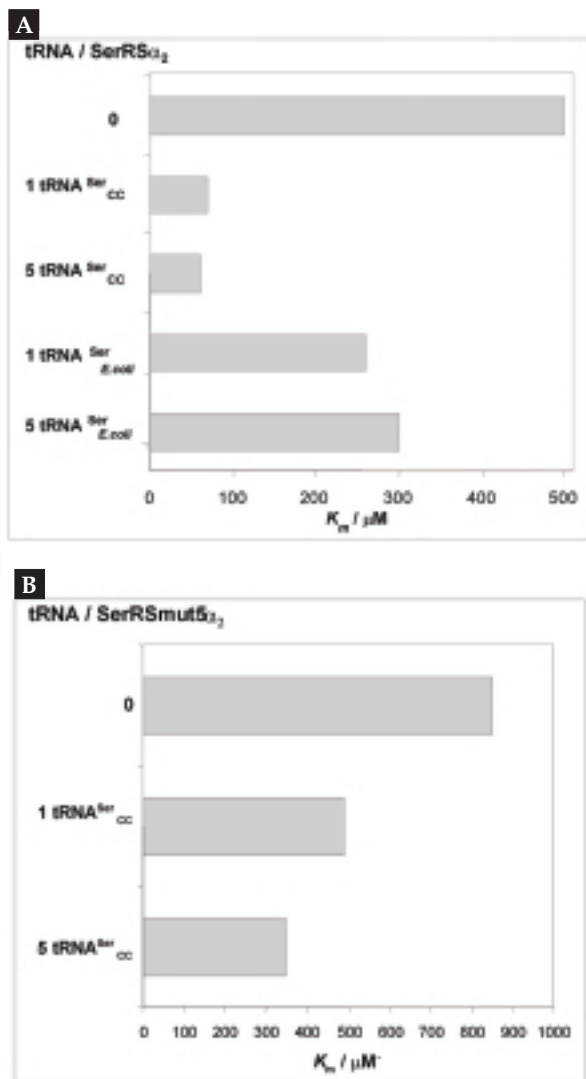
A

tRNA / SerRSα₂

B

tRNA / SerRSmut5α₂

Fig. 4. Binding of nonchargeable tRNAs^{Ser} increases the SerRS affinity for serine in the activation reaction. K_m values for serine were determined in the amino acid activation reaction in the absence (tRNA/SerRS was 0) and presence (tRNA/SerRS was 1 and 5) of nonchargeable tRNAs^{Ser}. (A): tRNA/SerRS $_2$ represents molar ratio of tRNA and SerRS dimer. (B): tRNA/SerRSmut5 $_2$ represents molar ratio of tRNA and SerRSmut5 (Glu281Asp, Gly291Ala) dimer.

Fig. 5. SerRS:tRNA^{Ser} complex is kinetically optimized species for serine activation. Specificity constants (k_{cat}/K_m) were evaluated from K_m and k_{cat} values determined in the amino acid activation reaction in the absence (– tRNA) and presence (tRNA$^{Ser}_{CC}$ and tRNA$^{Ser}_{E.\ coli}$) of nonchargeable tRNAs$^{Ser}_{CC}$.

Fig 6. Nonchargeable tRNA^{Ser} decreases the misactivation of threonine by yeast SerRS. K_m (Thr)/ K_m (Ser) ratios were calculated from K_m values for theronine and serine determined in the amino acid activation reaction in the absence (– tRNA) and presence (+ tRNA) of nonchargeable tRNA$^{Ser}_{CC}$.

vation of threonine by mutant SerRS (SerRSmut5, Glu-281Asp, Gly291Ala) is more pronounced than with wild type enzyme, showing that mutated aaRS has more relaxed specificity. It seems that mutants are partly deprived of quality control mechanisms, as a consequence of less flexible motif 2 loop. Although the determination of K_m value for threonine was not very precise because of its low misactivation (which is about 0.4 %), it is evident that the ratio of K_m values for threonine and serine is very different when measured without and with tRNA^{Ser}. In the absence of tRNA it is estimated to be only about eight (K_m for threonine is about 4 mM *vs.* K_m for serine which is 0.5 mM). This ratio is raised for an order of magnitude in the presence of tRNA$^{Ser}_{CC}$, due to a profound effect of tRNA on serine affinity and almost negligible influence on threonine binding (Fig. 6).
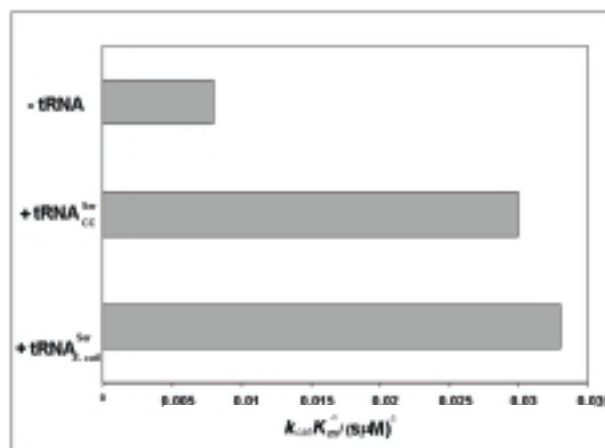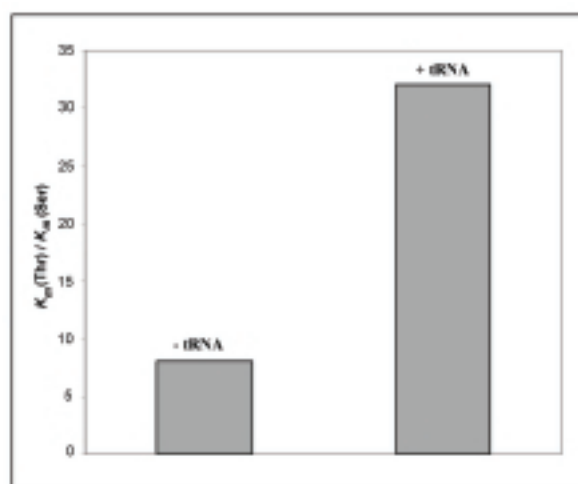
tRNA-dependent discrimination of amino acids as a quality control mechanism in serylation

Consistent with the crystallographic data on *Thermus thermophilus* SerRS (*17*) and our biochemical studies (*12*), serylation *in vivo* is achieved by a tRNA^{Ser} : SerRS complex as a macromolecular catalyst that discriminates amino acids with high specificity. The initial interaction between the long extra arm of tRNA^{Ser} and N-terminal SerRS coiled-coil positions the 3′-end of cognate tRNA into the active site of another subunit. Either tRNA : SerRS docking interactions or the interaction of the tRNA acceptor end with motif 2 loop residues induce the conformational change in the active site and increase the affinity for serine. Conformational flexibility of exposed motif 2 loop seems to be important for this structural re-

adjustment. We suggest that SerRS misacylation errors are diminished by the described mechanism, making the editing function unnecessary. So far, tRNA-dependent amino acid discrimination has been proven for yeast cytosolic enzyme and maize mitochondrial SerRS (12,24,26), but we assume that the same mode of serine recognition could be universally employed by all seryl-tRNA synthetases.

Does yeast SerRS need protein cofactor(s) to improve the specificity of tRNA recognition?

Aminoacyl-tRNA synthetases in general do not require protein cofactors for activity and specificity of substrate recognition. Instead, idiosyncratic domains are attached or inserted in the conserved class defining catalytic core (27–29), and are responsible for binding and recognition of cognate tRNAs (2,21). However, in higher eukaryotes, these enzymes are known to participate in complex formation with other synthetases and nonsynthetase proteins (30–36). Although in yeast such large complexes have not been found, smaller complex containing two class I synthetases, MetRS and GluRS, and protein Arc1p were identified (13,37,38), confirming the existence of an evolutionary intermediate toward higher-order organization of aaRSs. Arc1p is the first example of a protein that is not itself an enzyme but is required for the efficient aminoacylation in eukaryotic cells. Arc1p interacts with a subset of tRNAs, but its binding of tRNA^{Ser} isoacceptors was shown to be negligible (13).

Several lines of evidence from our laboratory suggest that yeast SerRS may interact with another cellular protein. The enzyme comprises positively charged C-terminal extension, whose truncation leads to toxicity *in vivo*, due to altered substrate recognition (23). However, truncated SerRS has even somewhat higher affinity for native tRNA *in vitro*, indicating that C-terminal peptide is not a tRNA binding domain. tRNA-dependent serine recognition is impaired after truncation of C-terminal peptide, suggesting that it either stabilizes the proper conformation of the active site, or directly leads the 3'-end of tRNA into the active site. We reasoned that the stabilization of the SerRS active site could also be mediated *via* the interaction between the C-terminal peptide and another cellular protein.

In order to find out whether SerRS interacts specifically with other cellular components of *S. cerevisiae*, we screened yeast cDNA library using the two-hybrid system with SerRS as a bait. Five positives were selected and sequenced. We identified yeast peroxin Pex21p as SerRS interacting protein. The interaction was confirmed *in vitro* using GST pull-down. Pex21p is a protein of 288 amino acid residues with calculated molecular weight of 33045 Da. It is a member of yeast peroxin family and is engaged in peroxisomal biogenesis (39). Together with Pex18p (which shares 23 % of overall identity), Pex21p is required for peroxisomal localization of Pex7, and therefore is important for protein targeting *via* the peroxisomal targeting signal 2 pathway (PTS2). To reveal functional significance of Pex21p-SerRS interaction, we purified Pex21p and showed that Pex21p increases the efficiency of aminoacylation by SerRS *in vitro* (Fig. 7; Table 1). This unusual interaction reflects another func-

tion of the peroxin, related to the activity of serylation, since Pex21p acts as an activator of SerRS. The interaction between SerRS and Pex21p does not affect the affinity for tRNA, showing that different mechanisms are employed by Pex21p and Arc1p in enhancing the aminoacylation efficiency and specificity.
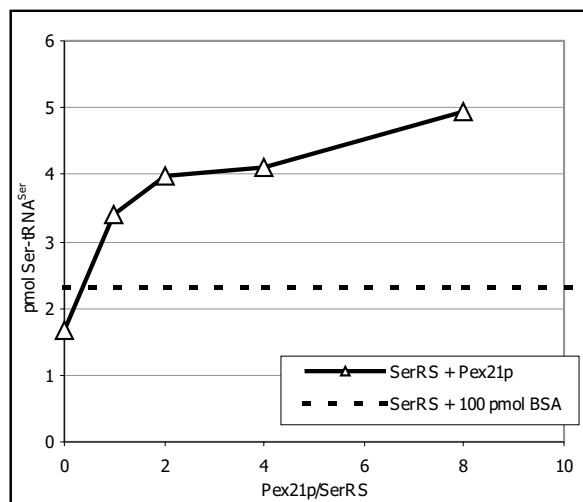
Outlook

Fig. 7. Pex21p activates yeast seryl-tRNA synthetase. Kinetic analysis of SerRS aminoacylation reaction at different concentrations of Pex21p. Amounts of seryl-tRNA^{Ser} formed in aminoacylation were plotted against the molar ratio of Pex21p and SerRS.

Table 1. The influence of Pex21p on SerRS kinetics

SerRS	$\dfrac{k_{cat}}{s^{-1}}$	$\dfrac{K_m \text{ (Ser)}}{M}$	$\dfrac{K_m \text{ (tRNA)}}{M}$
−Pex21p	0.0088	62.5	0.19
+Pex21p	0.0260	20.0	0.15

We have shown that the accuracy of seryl-tRNA synthesis is enhanced by two quality control mechanisms: (*i*) tRNA-dependent amino acid recognition, (*ii*) SerRS-Pex21p interaction. The first mechanism decreases the misacylation of tRNA^{Ser} isoacceptors with structurally similar, noncognate amino acids, and the second enhances the overall aminoacylation efficiency. In both cases, the macromolecular complex acts as more efficient and more specific catalyst than the apoenzyme alone. The optimization of serine binding and consequently better discrimination against noncognate amino acids is achieved by the conformational change in the enzyme's active site, which requires flexible motif 2 loop. The mechanism used by Pex21p in affecting the serylation activity of SerRS is still unknown. The experiments related to mapping the SerRS/Pex21p interactive domains are in progress. Since the analysis of Pex21p amino acid sequence predicts a potential nuclear localization signal, also found in Arc1p, it is possible that Pex21p functions in nucleus as well, maybe as a part of an aminoacylation dependent nuclear tRNA export pathway in yeast (40–42). In this respect, it would be in-

teresting to find out whether this protein is involved in regulating SerRS subcellular distribution, as recently determined for Arc1p complexed with GluRS and MetRS (*43*).

References

1. M. Ibba, D. Söll, *Science, 286* (1999) 1893–1897.

2. M. Ibba, D. Söll, *Ann. Rev. Biochem. 69* (2000) 617–650.

3. H. Jakubowski, E. Goldman, *Microbiol. Rev. 56* (1992) 412–429.

4. L. F. Silvian, J. Wang, T. A. Steitz, *Science, 285* (1999) 1074–1077.

5. T. L. Hendrickson, T. K. Nomanbhoy, P. Schimmel, *Biochemistry, 39* (2000) 8180–8186.

6. S. Fukai, O. Nureki, S. Sekine, A. Shimada, J. Tao, D. G. Vassylyev, S. Yokoyama, *Cell, 103* (2000) 793–803.

7. R. S. Mursinna, T. L. Lincecum, S. A. Martinis, *Biochemistry, 40* (2001) 5376–5381.

8. H. Jakubowski, FASEB J. 7 (1993) 168–172.

9. R. Sankaranarayanan, A.-C. Dock-Bregeon, B. Rees, M. Bovee, J. Caillet, P. Romby, C. S. Francklyn, D. Moras, *Nature Struct. Biol. 7* (2000) 461–465.

10. K. Musier-Forsyth, P. J. Beuning, *Nature Struct. Biol. 7* (2000) 435–436.

11. K. J. Newberry, Y.-M. Hou, J. J. Perona, *EMBO J. 21* (2002) 2778–2787.

12. I. Gruic-Sovulj, I. Landeka, D. Söll, I. Weygand-Durasevic, *Eur. J. Biochem. 269* (2002) 5271–5279.

13. K. Deinert, F. Fasiolo, E. C. Hurt, G. Simos, *J. Biol. Chem. 276* (2001) 6000–6008.

14. S. Rocak, I. Landeka, I. Weygand-Durasevic, *FEMS Microbiol. Lett. 214* (2002) 101–106.

15. H. Belrhali, A. Yaremchuk, M. Tukalo, K. Larsen, C. Berthet-Colominas, R. Leberman, B. Beijer, B. Sproat, J. Als-Nielsen, G. Grubel, J.-F. Legrand, M. Lechmann, S. Cusack, *Science, 263* (1994) 1432–1436.

16. V. Biou, A. Yaremchuk, M. Tukalo, S. Cusack, *Science, 263* (1994) 1404–1410.

17. S. Cusack, A. Yaremchuk, A. M. Tukalo, *EMBO J. 15* (1996) 2834–2842.

18. H. Asahara, H. Himeno, K. Tamuru, N. Nameki, T. Hasegawa, M. Shimizu, *J. Mol. Biol. 236* (1994) 738–748.

19. M. E. Saks, J. R. Sampson, *EMBO J. 15* (1996) 2843–2849.

20. B. Lenhard, O. Orellana, M. Ibba, I. Weygand-Durasevic, *Nucleic Acids Res. 27* (1999) 721–729.

21. S. Cusack, *Current Opin. Struct. Biol. 7* (1997) 881–889.

22. I. Weygand-Durasevic, B. Lenhard, S. Filipic, D. Söll, *J. Biol. Chem. 271* (1996) 2455–2461.

23. B. Lenhard, M. Praetorius-Iba, S. Filipic, D. Söll, I. Weygand-Durasevic, *FEBS Lett. 439* (1998) 235–240.

24. B. Lenhard, S. Filipic, I. Landeka, I. Skrtic, D. Söll, I. Weygand-Durasevic, *J. Biol. Chem. 272* (1997) 1136–1141.

25. I. Weygand-Durasevic, N. Ban, D. Jahn, D. Söll, *Eur. J. Biochem. 214* (1993) 869–877.

26. J. Rokov-Plavec, S. Lesjak, I. Landeka, I. Mijakovic, I. Weygand-Durasevic, *Arch. Biochem. Biophys. 397* (2002) 40–50.

27. J. Cavarelli, D. Moras, *FASEB J. 7* (1993) 9–86.

28. M. Delarue, *Curr. Opin. Struct. Biol. 5* (1995) 48–55.

29. G. J. Arnez, D. Moras, *Trends Biochem. Sci. 22* (1997) 211–216.

30. M. Mirande, *Prog. Nucleic Acids Res. Mol. Biol. 40* (1991) 95–142.

31. P. Kerjan, C. Cerini, M. Semeriva, M. Mirande, *Biochim. Biophys. Acta, 1199* (1994) 293–297.

32. V. V. Filonenko, M. P. Deutscher, *J. Biol. Chem. 269* (1994) 17375–17378.

33. S. B. Rho, K. H. Lee, J. W. Kim, K. Shiba, Y. J. Yo, S. Kim, *Proc. Natl. Acad. Sci. USA, 93* (1996) 10128–10133.

34. M. T. Norcum, J. A. Warrington, *Protein Sci. 7* (1998) 79–87.

35. S. B. Rho, M. J. Kim, J. S. S., W. Lee, H. Motegi, S. Kim, K. Shiba, *Proc. Natl. Acad. Sci. USA, 96* (1999) 4488–4493.

36. S. Quevillon, J.-C. Robinson, E. Berthonneau, M. Siatecka, M. Mirande, *J. Mol. Biol. 285* (1999) 183–195.

37. G. Simos, A. Segref, F. Fasiolo, K. Hellmuth, A. Shevchenko, M. Mann, E. C. Hurt, *EMBO J. 15* (1996) 5437–5448.

38. G. Simos, A. Sauer, F. Fasiolo, E. C. Hurt, *Mol. Cell, 1* (1998) 235–242.

39. P. E. Purdue, X. Yang, P. B. Lazarow, *J. Cell Biol. 143* (1998) 1859–1869.

40. G. Simos, E. Hurt, *Curr. Biol. 9* (1999) R238–241.

41. H. Grosshans, E. Hurt, G. Simos, *Genes Dev. 14* (2000) 830–840.

42. H. Grosshans, G. Simos, E. Hurt, *J. Struct. Biol. 129* (2001) 288–294.

43. K. Galani, H. Grosshans, K. Deinert, E. C. Hurt, G. Simos, *EMBO J. 20* (2001) 6889–6898.

Točnost sinteze seril-tRNA

Sažetak

Točnost biosinteze proteina nadziru različiti kontrolni mehanizmi koji sprečavaju ili ispravljaju greške u translaciji. Specifičnost aminoacil-tRNA-sintetaza (aaRS) pri izboru i kovalentnom povezivanju pripadnih aminokiselina i tRNA ključna je u ovom procesu.

Istraživanja u našem laboratoriju pokazala su da se specifičnost i učinkovitost sinteze seril-tRNA u kvascu i biljkama povećavaju tRNA-ovisnim prilagođavanjem veznog mjesta za serin u aktivnom mjestu seril-tRNA-sintetaze (SerRS). Dakle, makromolekularni kompleksi tRNA i enzima imaju bolja katalitička svojstva od apoenzima. Naši rezultati kinetike pokazuju da se vezanjem tRNA bitno mijenja konformacija veznog mjesta za serin u enzimu divljeg tipa, dok je konformacijska promjena slabija kod enzima s mutacijama u aktivnom mjestu. Iako SerRS može aktivirati i serinu sličan treonin, stvaranje treonil-adenilata smanjeno je u prisutnosti aminoacilacijski inaktivnog analoga tRNA. Time je pokazano da su interakcije između pripadne tRNA i SerRS bitne za točan izbor aminokiseline.

Djelotvornost serilacije povećava se i interakcijom SerRS s nesintetaznim proteinom, peroksinom Pex21p. Ta neočekivana interakcija uočena je prvo *in vivo*, pretragom kvaščeve biblioteke u sustavu dvaju hibrida sa SerRS kao interakcijskim proteinom. Interakcija je potvrđena *in vitro*. Kinetički eksperimenti pokazali su da Pex21p djeluje kao aktivator SerRS, što ovu neobičnu interakciju čini biološki značajnom jer povećava učinkovitost aminoaciliranja.