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Supporting Information

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“On the mechanism of peptide bond formation on ribosome; synthesis of peptide bond does not require a free vicinal hydroxyl group on the terminal ribose of peptidyl-tRNA”

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General:

Taq polymerase was from Qiagen (Hilden, Germany), T7 RNA polymerase, T4 DNA ligase and restriction enzymes were from New England Biolabs (Frankfurt, Germany). p-nitrophenyl acetate and natrium periodate were from Fluka (Steinheim, Germany). Other analytical grade chemicals were obtained from Roth (Karlsruhe, Germany). [^{14}C]Leucine (52 mCi/mmol) was purchased from Amersham, Life Sciences (Freiburg, Germany). NTase from E. coli equipped with C-terminal 6x histidine tag (6.65 mg/ml, specific activity, 8872 U/mg) was overexpressed in E. coli from a plasmid provided by Dr. M. Bocchetta, Chicago[23] and purified by affinity chromatography. Seryl-tRNA synthethase was cloned and purified from E. coli cells.[24] Transcription/translation system for in vitro protein synthesis was obtained from RiNA GmbH (Berlin, Germany). E. coli overexpressed and purified release factor 1 from T. thermophilus[25] was used to immunize rabbits, following standard one-month immunization protocol (Eurogentec, Belgium). After three boosts, serum was collected, centrifuged and stored at –20°C. Polyclonal antibodies dissolved in 50 mM Tris/HCl, pH 7.5 and 10 mM $MgCl_2$ were purified by passing through Mono Q Sepharose (Pharmacia, Freiburg, Germany) followed by chromatography on EMD Tentakel SO_3^- (Merck, Darmstadt, Germany) with gradient from 0-400 mM NaCl in 50 mM Na-acetate, pH 5.0. Fractions eluted at 150 mM NaCl were collected, dialyzed and stored in 10 mM Tris/HCl, pH 7.5 in 50% glycerine at -20°C. Protein concentration was 16 mg/ml. Main band in SDS-Page corresponded to 50 and 25 kD, respectively.

Preparation of suppressor tRNA$^{Ser(CUA)}$-2´dA:

Amplification of suppressor $tRNA^{Ser(CUA)}$ gene from the plasmid pUCt19-tser-amber (RiNA GmbH, Berlin) was performed by PCR with a primer corresponding to the 3´end of the tRNA (5´TGGCGGAGAGAGGGGGATT 3`) and a primer containing the T7 promotor sequence (5´GGGAATTCTAATACGACTCACTATAGG 3`). PCR product was used as a template for T7 transcription. Transcripts were purified on a Sephadex A25 column (2 x 20 cm, Pharmacia,Freiburg, Germany) and desalted by gel permeation chromatography on a Biogel P6 (Biorad, München, Germany) column (2.5 x 50 cm) according to described procedure.[26] The $tRNA^{Ser(CUA)}$-A transcript could be aminoacylated up to 800 pmoles/A_{260} unit. It was inhomogeneous with respect to the CCA end. This inhomogeneity could be eliminated by regeneration of the CCA end by NTase[27]. Final aminoacylation capacity of $tRNA^{Ser(CUA)}$-A was 800 pmoles/A_{260} unit.

The exchange of 3´-terminal adenosine of $tRNA^{Ser(CUA)}$ for 2´deoxy adenosine was achieved by NTase in the presence of pyrophosphate.[14] Two mL reaction mixture containing 20 mM Tris-HCl (pH 8.5), 20 mM $MgCl_2$, 1 mM sodium pyrophosphate, 6 mM 2´dATP, 15 µM $tRNA^{Ser(CUA)}$, and 4000 Units NTase. The reaction mixture was incubated 4 h at 37 °C. The tRNA was isolated by phenolization and ethanol precipitation.

To ensure that there is no $tRNA^{Ser(CUA)}$-A left in the $tRNA^{Ser(CUA)}$-2´A preparation, periodate oxidation with 300 µM $NaIO_4$ in 50 mM NaAc (pH 6.5) was performed for 2 h at room temperature.[15] The excess of periodate was deactivated by addition of 300 µM glucose. Suppressor $tRNA^{Ser(CUA)}$-2´dA was dissolved in a buffer consisting of 20 mM NaCl, 50mM Na-acetate pH 5.2 and 10mM $MgCl_2$ and applied on a Sephadex A25 column (1 x 5 cm). The column was washed by the same buffer containing 250 mM NaCl to remove soluble nucleotides and the $tRNA^{Ser(CUA)}$-2´dA was eluted by 650 mM NaCl. The tRNA fraction was desalted on a Biogel P6 column (2 x 20 cm) and concentrated by evaporation at 20°C. $tRNA^{Ser(CUA)}$-2´dA was characterized by boronate affinity gel electrophoresis (Fig.1).[28] The electrophoresis was run at 120 V in the presence of 7 M urea. The $tRNA^{Ser(CUA)}$-2´dA could be aminoacylated up to 250 pmoles/A_{260} unit.

Cell-free transcription/translation of estrerase 2:

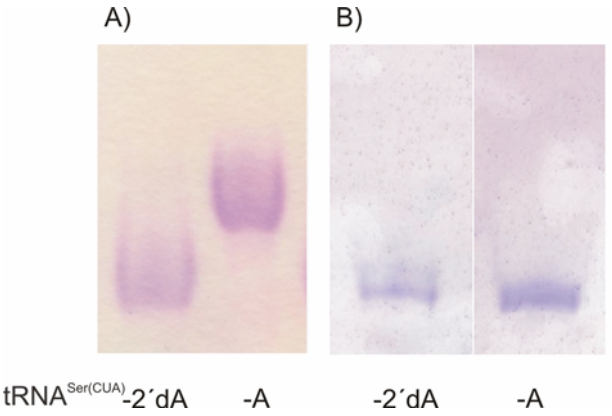
The cell-free transcription/translation was performed at 37 °C according to the supplier´s manual. For radioactive labelling of synthesized protein 0.5 mM [^{14}C]leucine (10.4 mCi/mmol) was present in the reaction mixture. The plasmid pIVEX-Est2-S155X coding for the esterase 2 from Alicyclobacillus acidocaldarius (5 nM) and 0.6 µg

polyclonal antibodies against RF1 were present in the reaction mixture.[13] Suppressor $tRNA^{Ser(CUA)}$-A and suppressor $tRNA^{Ser(CUA)}$-2´dA, respectively, were added as indicated in the figure legends presented in the article. Reaction mixtures were incubated for 30 min. Analysis of in vitro synthesized proteins was done by SDS–PAGE. The gels were stained by Coomassie blue followed by esterase activity staining.[21] Incorporation [^{14}C]leucine into in vitro synthesized proteins was visualised by radioactivity imaging. In parallel, newly protein was quantified by determination of radioactivity in 10% hot trichloroacetic acid precipitate. The esterase activity was quantitatively determined by a spectrophotometric assay.[22] For this, aliquots of 2 µl were withdrawn and mixed with 1 ml of 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM p-nitrophenyl acetate. Ester hydrolysis was measured by the production of p-nitrophenoxide monitored at 405 nm.

Supplementary material, Figure 1.

Separation of $tRNA^{Ser(CUA)}$-A and $tRNA^{Ser(CUA)}$-2´dA by affinity electrophoresis on boronate modified polyacrylamide gels. $tRNA^{Ser(CUA)}$-A (30 pmol) and $tRNA^{Ser(CUA)}$-2´dA (30 pmol), respectively, were run on 12.5% acrylamide-7 M urea gels in the presence (A) and absence (B) of 2.5% 2-acrylamidophenyl boronate. The bands were located by staining with stains all.

Supplementary material, Figure1



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