MS04.06.02 tRNA RECOGNITION BY PROCARYOTIC ASPARTYL tRNA-SYNTHETASES. D. Moras, L. Moulignier, C. Briand, S. Eiller, A. Peterkowitz and J.C. Thierry. UPF de Biologie Structurale, IGBMC, 1 rue Laurent Fries, BP 163, 67404 Illkirch Cedex, France.

Aspartyl tRNA-synthetase (AspRS) like all members of class II synthetases attaches its amino acid substrate to the 3'OH group of the ribose of the terminal adenosine of its cognate tRNA. The structural analysis done with the yeast system, corroborated by mutagenesis experiments, established the details of the mechanism of the two steps reaction.

The crystal structure of the homologous complex of E.coli reveals a different relative position of the terminal adenosine in the catalytic pocket. On the other hand the crystal structure of the inactive heterologous complex between the E.coli enzyme and yeast tRNAAsp assembles two different conformations of the bound tRNA. One is non productive with the CCA end away from the active site. The other one is very reminiscent of that observed in the yeast homologous complex. The main differences between eucaryotic and procaryotic aspRSs are associated with an insertion in the acceptor stem. Two crystal structures of the complex between ttAspRS and (i) its cognate tRNA as well as (ii) E. coli tRNAAsp have been solved. In both cases, the tRNA terminal acceptor end is not located in the active site pocket. Taken together, these observations shed new light on the reaction mechanism and the procaryotic-eucaryotic systems separation.

MS04.06.03 THE CRYSTAL STRUCTURE OF PHENYLALANYL-tRNA SYNTHETASE FROM THERMUS THERMOPHILUS COMPLEXED WITH COGNATE tRNA^Phe. M. Safro, Y. Goldgur1, L. Mosyak1, L. Reshetnikova2, O. Lavrik3, V. Ankilova3, Dept. of Structural Biology, Weizmann Institute of Science, 76100 Rehovot, Israel; 2, Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia; 3, Novosibirsk Institute of Bioorganic Chemistry, Novosibirsk, 630090, Russia.

The crystal structure of phenylalanyl-tRNA synthetase from Thermus thermophilus (ttPheRS) has previously been determined at 2.9 Å resolution (Mosyak et al., 1995). Here we present the structure of ttPheRS complexed with cognate tRNA^Phe also from Thermus thermophilus. The crystals of the complex are of the same space group, P2_12_1, and virtually the same unit cell parameters as for the native ttPheRS (Reshetnikova et al., 1995). Surprisingly, all four subunits of heterodimeric (ttPheRS)2 ttPheRS participate in binding of the tRNA^Phe. Multisubunit interaction of this type had not been observed in native tRNA complexes before and demonstrates the functional necessity for a heterodimeric organisation of the molecule. The N-terminal domain of the α-subunit (1-85) which is disordered in the native ttPheRS crystals is stabilized by interactions with tRNA^Phe and becomes visible. The structure of the complex provides an answer to the question, which of the two possible candidates actually recognizes the anticodon: it is the C-terminal domain of the β-subunit possessing the known RNA-binding fold. The conformational changes of the bound tRNA^Phe and numerous contacts with ttPheRS will be described.


MS04.06.04 TERNARY COMPLEX OF PHE-tRNA, EF-Tu AND A GTP ANALOG. Jens Nyborg, Poul Nissen, Morten Kjeldgaard, Soren Thrup, Galina Polekhina and Brian F.C. Clark, Dept. of Molecular and Structural Biology, University of Aarhus, Langelandsgade 140, DK-8000 Aarhus C, Denmark; Ludmila Reshetnikova, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov Str., 117984 Moscow, Russia.

The structure of the ternary complex of yeast Phe-tRNA, T. aquaticus EF-Tu and the GTP analog GDPNP has been determined to a resolution of 2.7 Å (1). When the model of this complex is compared to the structure of EF-G:GDP (2) an unexpected macromolecular mimicry is observed. The biological implications of this mimicry will be discussed.

Elongation factors EF-Tu and EF-G catalyze the elongation step of prokaryotic protein biosynthesis. Their actions are controlled by GTP. EF-Tu:GTP forms a ternary complex with all aminoacylated tRNAs, protects the amino acid ester bond and assists in placing the cognate aminoacylated tRNA into the ribosomal A site. Inactive EF-Tu:GDP is released from the ribosome. A peptide bond is formed between the aminoacylated tRNA and the peptide on peptidyl tRNA in the P site. EF-G:GTP translocates the newly formed peptidyl tRNA into the P site and at the same time advances the mRNA one codon.

Structures of E. coli EF-Tu:GDP (3) and T. aquaticus (4) EF-Tu:GDPNP have been determined earlier. They reveal a large conformational rearrangement of domains of EF-Tu upon activation. The structure of the ternary complex of EF-Tu shows that the tRNA is recognized in three areas. One side of the T-stem helix is bound to domain 3 of EF-Tu. The 3' phosphate is recognized by conserved residues at the interface of all three domains. The 3' terminal A base is bound at a depression on domain 2 while the amino acid is found in a pocket between domains 1 and 2.


MS04.06.05 RNA MODIFICATION BY BASE EXCHANGE: STRUCTURE OF tRNA-GUANINE TRANSGLYCOSYLASE. Christophe Romier1, Klaus Reuter2, Dietrich Suck1 and Ralf Nissen1, 1EMBL, Meyerbestrasse 1, 69117 Heidelberg, Germany; 2Institut fur Biochemie, Universitat Erlangen, Fahrstr. 17, 91054 Erlangen, Germany.

tRNA-guanine transglycosylases (TGT) are enzymes modifying the anticodon of tRNAs specific for Asn, Asp, His and Tyr, leading to the replacement of guanine-34 at the wobble position by the hypermodified base guanine. In prokaryotes TGT catalyzes the exchange of guanine-34 with the guanine precursor 7-aminoimidlyl-7-deaza guanine (preQ1).

TGT from Zymomonas mobilis, a 43 kDa protein, was expressed in E. coli, purified by crystallization, and further recrystallized for X-ray diffraction studies. The sensitivity of the crystals to X-rays prompted us to carry out the search for heavy atom derivatives under cryogenic conditions (100K). Crystals belong to space group C2 with unit cell dimensions a=90.9Å, b=64.9Å, c=71.2Å, and β=96.6°.

The structure was solved by means of multiple isomorphous replacement including anomalous scattering (MIRAS) using six independent derivatives. Due to non-isomorphism problems occurring even between native crystals, most of the derivatives data were useful only to low resolution. The final model, refined at 1.85 Å resolution, includes 372 residues out of 386, 1 zinc ion, and 203 water molecules, and has a R-factor of 19.0% and a R-free of 21.4%.