PS04.06.09 tRNA AND AMINO ACID RECOGNITION BY CLASS II AMINOACYL-tRNA SYNTHETASES. Stephen Cusack, Anders Åberg, Anya Yaremchuk, Carmen Berthet, Laurence Seignovert, Reuben Leberman, Mikhail Tukalo, European Molecular Biology Laboratory (EMBL-Grenoble) - Grenoble Outstation, c/o ILL, 156X, 38042 Grenoble Cedéx 9, France

Similarities and differences in the modes of recognition of tRNAs and amino acids by their cognate aminoacyl-tRNA synthetases will be discussed with reference to crystal structures of substrate complexes of three *Thermus thermophilus* class II synthetases: seryl-, lysyl- and histidyl-tRNA synthetases.

New data at 2.8Å resolution collected at cryo temperature on the *T. thermophilus* seryl-tRNA synthetase-$tRNA^{ser}$ complex co-crystallised with a seryl-adenylate analogue, reveals how the synthetase interacts with the acceptor stem of the cognate $tRNA^{ser}$. A novel hydrophobic interaction between Phe-262 in the motif 2 loop and bases U68 and C69 in the major groove of the tRNA discriminates pyrimidines from purines in these positions. The motif 2 loop is observed in two distinct conformations depending on which step of the aminoacylation reaction is in progress.

The crystal structure at 2.75Å resolution of *T. thermophilus* lysyl-tRNA synthetase complexed with *E. coli* $tRNA^{lys}$ reveals how the anti-codon 34-UUU-36 (where in this tRNA, the 34-U is modified to mnm^5s^2U) is specifically recognised by an N-terminal domain. The mode of recognition is rather similar to that observed in the closely related aspartyl-tRNA synthetase which however recognises the anti-codon 34-GUC-36.

The crystal structure of *T. thermophilus* histidyl-tRNA synthetase co-crystallised with histidine, determined by multiple isomorphous replacement at 2.7Å resolution, will be described.

Progress on the structure determination of the *T.thermophilus* prolyl- and asparaginyl-tRNA synthetases will be presented.

PS04.06.10 STRUCTURES OF TRYPTOPHANYL-TRNA SYNTHETASE-LIGAND COMPLEXES Xin Huang and Charles W. Carter, Jr., Department of Biochemistry and Biophysics, CB 7260 University of North Carolina at Chapel Hill, Chapel Hill, NC 27514 USA

The crucial role of aminoacyl-tRNA synthetases (aaRSs) in maintaining the fidelity of the genetic code has motivated intense study of the sources of their specificity for cognate amino acids and tRNAs. Conclusions of the structural basis for coupling of specificity and catalysis of aaRSs can best be drawn by examining a series of different complexes involving the same enzyme. *Bacillus stearothermophilus* Tryptophanyl-tRNA synthetase (TrpRS) provides such a series. The success of the first structure in this series, that of TrpRS complexed with tryptophanyl-5'AMP has inspired us to study the structures of the complexes with other ligands such as Tryptophan (substrate) and 5'-O-[N-(L-tryptophanyl)sulfamoyl]adenosine (a stable analog of the tryptophanyl adenylate intermediate). We have synthesized 5'-O-[N-(L-tryptophanyl)sulfamoyl]adenosine and inhibition measurements showed that it is a strong inhibitor with K_i in the nanomolar range. Co-crystallization of this analog with TrpRS is underway. Translation and rotation searches are being carried out on the data from monoclinic crystals (space group $P2_1$) grown in the presence of tryptophan . Contrary to previous expectation (Carter, et al., 1990, *Acta Cryst.* **A46:**57-68), our initial results revealed that what we previously interpreted as a non-crystallographic three-fold axis is actually a three-fold screw axis. We hope to make progress in solving these two structures.

(This work supported by NIH GM48519-02)

PS04.06.11 2.9 Å CRYSTAL STRUCTURE OF LIGAND-FREE TRYPTOPHANYL-TRNA SYNTHETASE Valentin Ilyin, Charles W. Carter, Jr., Evon Winborne, Brenda Temple, and GenPei Li* Department of Biochemistry and Biophysics, CB 7260 University of North Carolina at Chapel Hill, Chapel Hill, NC 27514 USA *and* *Department of Physical Chemistry, Beijing University, Beijing, Peoples Republic of China

Ligand-free *Bacillus stearothermophilus* Tryptophanyl-tRNA synthetase (TrpRS) crystallizes either as triclinic (P1) or monoclinic (C2) forms. Systematic response surface analysis of the crystal growth conditions has revealed an optimum at about 42°C, consistent with the thermophilic origin of the enzyme. Unit cell dimensions and 32 noncrystallographic symmetry in both asymmetric units are very similar to those previously observed for monoclinic (P21) crystals of crystals grown in the presence of tryptophan. We have placed the known structure using AMORE, and shown that each asymmetric unit contains either one or two units of three enzyme dimers characterized by 3_1 screw symmetry. Thus, all three crystal forms are closely related to the primitive space group $P3_121$, previously observed at 18 Å resolution in the $P2_1$ crystals, and thought to belong to space group P321 (Carter, et al., 1990, *Acta Cryst.* **A46:**57-68).

We are using isomorphous replacement with selenomethionine-substituted TrpRS (Doublié, et al., 1994, *Acta Cryst* **A50:**164-182), together with non-crystallographic symmetry averaging and maximum entropy solvent flattening to supplement phases from positioned fragments of the known model for structure determination. Conformational differences between the different TrpRS structures, including a detailed analysis of the composition and properties of nonpolar nuclei and microclusters by the method of Ilyin (1994 *Prot. Eng.* **7:**1189-1198) will be described.

(This work supported by NIH GM48519-02)

PS04.06.12 CRYSTALLOGRAPHIC STRUCTURES OF RIBOSOMAL PROTEINS AND ELONGATION FACTOR G. Anders Liljas[1], Maria Garber[2], Arnthor Avarsson[1], Salam Al-Karadaghi[1], Natalia Davidova[2], Irina Eliseikina[2], Natalia Fomenkova[2], Olga Gryaznova[2], Natalia Nevskaya[2], Stanislav Nikonov[2], Alexey Nikulin[2], Johan Unge[1], Julia Zheltonosova[2], [1]Molecular Biophysics, Chemical Center, University of Lund, Box 124, S-221 00 Lund, Sweden; [2]Department of Structure and Function of the Ribosome, Institute of Protein Research, Russian Academy of Sciences, 142292 Pushchino, Moscow Region, Russia

The crystallographic structures of a number of ribosomal proteins have been determined, most recently L1 and L22. A majority of the ribosomal proteins have the split β-α-β fold also observed in RNA/DNA binding proteins. L1 has two domains. The first domain contains both the N- and the C-terminus and has the split β-α-β fold. The topology of the second domain is a minimal version of the "Rossmann fold" with only four parallel b-strands.

The structure of elongation factor G has a remarkable similarity to the ternary complex of EF-Tu.GTP-tRNA with direct implications for the function of EF-G. The observation that ribosomal proteins have similarities to each other and to other RNA binding proteins is also valid for EF-G where domains IV, V and possibly also III have structures similar to ribosomal proteins. The N-terminal domains G and II seem to be present in all ribosome binding GTPases. They probably constitute part of the surface that interacts with a region on the ribosome to which all those factors bind. The conformational changes of the protein are of significant functional relevance. They are found not only in the switch region, but also in neighbouring parts of the structure such as the C-helix of the G-domain at the interface between different domains. Mutations of this region with interesting phenotypes have been found not only for EF-G but also in EF-Tu.