MS04.06.02 tRNA RECOGNITION BY PROCARYOTIC ASPARTYL tRNA-SYNTHETASES. D. Moras, L. Moulinier, C. Briand, S. Eiler, A. Poterzmann and J.C. Thierry. UPR 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 aminoacid substrate to the 3'OH group of the ribose of the terminal adenosine of its cognate tRNAThe 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 diferent 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 shows 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 domain of more than hundred aminoacids involved in the correct positionning of the acceptor stem. Two crystal structures of the complex between *tt*AspRS and (i) its cognate tRNA as well as (ii) *E. coli* $tRNA^{Asp}$ 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[1], Y. Goldgur[1], L.Mosyak[1], L. Reshetnikova[2], O. Lavrik[3], V. Ankilova[3], Dept. of Structural Biology, Weizmann Institute of Science, 76100 Rehovot, Israel[1], Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia[2], Novosibirsk Institute of Bioorganic Chemistry, Novosibirsk, 630090, Russia[3]

The crystal structure of phenylalanyl-tRNA synthetase from *Thermus thermophilus* (^{tt}nPheRS) has previously been determined at 2.9Å resolution (Mosyak et. al., 1995). Here we present the structure of ^{tt}PheRS complexed with cognate $tRNA^{Phe}$ also from *Thermus thermophilus*. The crystals of the complex are of the same space group, $P3_221$, and virtually the same unit cell parameters as for the native ^{tt}PheRS (Reshetnikova et al., 1993).

Surprisingly, all four subunits of heterodimeric $(\alpha\beta)_2$ ^{tt}PheRS participate in binding of the $tRNA^{Phe}$. Multisubunit interaction of this type had not been observed in synthetase tRNA complexes before and demonstrates the functional necessity for a heterodimeric organization of the molecule. The N-terminal domain of the α-subunit (1-85) which is disordered in the native ^{tt}PheRS 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 ^{tt}PheRS will be described.

1. Mosyak,L., Reshetnikova,L., Goldgur,Y., Delarue,M. and Safro,M. (1995), Nature Str. Biol., 2, 537-547.
2. Reshetnikova,L., Khodyreva,S., Lavrik, O., Ankilova,V., Frolow,F. and Safro,M. (1993), J. Mol. Biol., 231, 927-929.

MS04.06.04 TERNARY COMPLEX OF PHE-tRNA, EF-Tu AND A GTP ANALOG. Jens Nyborg, Poul Nissen, Morten Kjeldgaard, Soren Thirup, 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 5' 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.

(1) P. Nissen et al., *Science* (1995), 270, 1464.
(2) J. Czworkowski et al., *EMBO J.* (1994), 13, 3661.
(3) M. Kjeldgaard and J. Nyborg, *JMB* (1992), 223, 721.
(4) M. Kjeldgaard et al., *Structure* (1993), 1, 35.

MS04.06.05 RNA MODIFICATION BY BASE EXCHANGE: STRUCTURE OF tRNA-GUANINE TRANSGLYCOSYLASE. Christophe Romier[1], Klaus Reuter[2], Dietrich Suck[1] and Ralf Ficner[1], [1]EMBL, Meyerhostrasse 1, 69117 Heidelberg, Germany [2]Institut fur Biochemie, Universität 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 procaryotes TGT catalyzes the exchange of guanine-34 with the guanine precursor 7-aminomiethyl-7deazaguanine ($preQ_1$).

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 (100°K). 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 occuring 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%.

The structure consists of an unexpected, irregular $(\beta/\alpha)_8$-barrel with a tightly attached C-terminal zinc-containing subdomain. The packing of the subdomain to the barrel is mediated by an α-helix, located close to the C-terminus, which displaces the eighth helix of the barrel. This structure represents the first example of a (β/α)-barrel protein interacting specifically with a nucleic-acid.

We also solved the structure of TGT in complex with $preQ_1$ which suggests a binding mode for tRNA where the phosphate backbone is recognized by the zinc subdomain, and the $U_{33}G_{34}U_{35}$ sequence by the barrel. This model for tRNA binding is consistent with a base exchange mechanism involving a covalent tRNA-enzyme intermediate.

MS04.06.06 PyrR, A BIFUNCTIONAL RNA-BINDING TRANSCRIPTIONAL ATTENUATION PROTEIN AND URACIL PHOSPHORIBOSYLTRANSFERASE Diana R. Tomchick and Janet L. Smith, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907; Robert J. Turner and Robert L. Switzer, Department of Biochemistry, University of Illinois, Urbana, IL 61801

Bacillus subtilis PyrR regulates the transcription of the pyrimidine nucleotide biosynthetic operon (*pyr*) via an attenuation mechanism, in response to exogenous pyrimidine levels. UMP-bound PyrR effects regulation at three antiterminator/terminator points in the *pyr* mRNA by binding to the 5' end of an antiterminator stem-loop structure, which permits the formation of the downstream factor-independent transcription terminator stem-loop. Transcription is thus converted from a readthrough mode with expression of the downstream biosynthetic genes, to a termination mode with reduced expression of the downstream genes. An approximately 50-nucleotide conserved sequence has been identified as the PyrR binding site to the *pyr* mRNA. While PyrR displays no recognized RNA-binding sequence motif, it contains a short sequence that is characteristic of PRPP binding enzymes, and it is a uracil phosphoribosyltransferase. The relevance of this enzymatic activity to transcriptional attenuation is as yet unknown.

Structures of PyrR in two oligomerization states will be presented. A dimeric form of the enzyme was obtained as a Sm^{3+} co-crystal. The structure was solved via MAD from data collected at energies around the LII edge of Sm on beamline 19 at the ESRF. Initial phasing was via the program MLPHARE and the atomic model was refined to 1.6Å. The physiologically relevant hexameric form of PyrR crystallized in the space group R32 (d_{min} of 2.3 Å) and was solved via molecular replacement. The structure of a Mg^{2+}/UMP complex of PyrR will also be presented.

An analysis of the potential RNA-binding site(s) of PyrR and mechanism of transcriptional attenuation will be presented, as well as a comparison of the enzyme to other phosphoribosyltransferase structures.

Supported by NIH grants DK-42303 to J.L. Smith and GM-47112 to R.L. Switzer, and NIH Training Grant GM-07283 to R.J. Turner.

MS04.06.07 U1A SPLICEOSOMAL PROTEIN BINDS TO TWO DIFFERENT RNA TARGETS USING THE SAME STRATEGY. Nagai, K., Oubridge, O., Price, S., Jovine, L., Avis, J. & Evans, P. R., MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

U1A spliceosomal protein binds to hairpin II of U1 small nuclear RNA and, together with other proteins, forms the U1 snRNP essential in pre-mRNA splicing. It also binds to the 3' untranslated region (3' UTR) of its pre-mRNA and controls its own production through inhibition of polyadenylation at the 3' end of mRNA. The crystal structure of U1A protein bound to a 21 nucleotide RNA hairpin, representing its binding site in U1 snRNP, was solved at 1.92Å resolution using a single isomorphous derivative. The AUUGCAC sequence within the ten nucleotide loop fits tightly into the groove on the surface of the protein and the bases of the heptanucleotide are splayed out. The bases of the heptanucleotide and the C:G base pair, which closes the loop, stack onto an aromatic protein side chain, an adjacent base or both. These bases also form an extensive hydrogen bond network with protein side chains, main chain amide and carbonyl groups. Bases of the last three nucleotide in the loop have no apparent interaction with the protein.

The 3' UTR of U1A protein mRNA folds into an evolutionarily conserved secondary structure with two internal loops containing the sequence AUUGCAC, found in hairpin II of U snRNA, and its variant AUUG*U*AC. The C:G base pair is also found at the equivalent positions in the 3'UTR binding site. The conservation of the heptanucleotide and the C:G base pair strongly suggests that they form identical protein contacts in the 3'UTR complex. Based on this assumption and the 2-fold symmetry in the 3'UTR binding site we have been able to model of the 3'UTR complex in which two molecules of U1A protein form a dimer.

U2B" protein, a component of U2 snRNP, shares a high sequence homology with U1A protein and binds to hairpin IV of U2 snRNA only when it is complexed with U2A' protein which contains the Leu-rich sequence repeats. We have been able to grow crystals of a ternary complex between U2B"/U2A' proteins and the cognate binding site and its crystallographic analysis is underway. This structure will reveal how U1A and U2B" proteins bind their cognate binding sites.

(1) Oubridge, C. et al. Nature 372, 432-438 (1994)
(2) Jovine, L. et al. Structure in press

PS04.06.08 CRYSTAL STRUCTURES OF SIGNAL RECOGNITION PARTICLE (SRP) SRP9 PROTEIN AND SRPΦ14/9 FUSION PROTEIN. Darcy E.A. Birse[1], Anders Åberg[1], Kieron Brown[1], Sylvie Doublié[1]*, Ulrike Kapp[1], Katharina Strub[2] and Stephen Cusack[1]. [1]European Molecular Biology Laboratory (EMBL-Grenoble)-Grenoble Outstation, c/o ILL, 156X, 38042 Grenoble Cedéx 9, France, [2] Département de Biologie Cellulaire, Université de Genève, Sciences III, CH-1211 Geneva 4, Switzerland, *Department of Biological Chemistry and Molecular Pharmacology, Harvard University Medical School, Boston, Mass., 02115, U.S.A

The mammalian Signal Recognition Particle (SRP) is a cytoplasmic ribonucleoprotein particle (RNP) that plays an essential role in the targeting of secretory and membrane proteins to the rough endoplasmic reticulum (RER). Targeting occurs co-translationally and translocation across the RER membrane begins before polypeptide synthesis is complete.

The mammalian SRP is an 11S cytoplasmic RNP which consists of six polypeptides (SRP9, SRP14, SRP19, SRP54, SRP68, SRP72) and a single RNA molecule.

SRP9 protein has been crystallized in the point group 321/622 with cell parameters $a=b=64.0$ Å, $c=110.5$ Å. Using synchrotron radiation on single flash-frozen crystals, a complete data set showing diffraction beyond 2.3 Å resolution with a R_{sym} on intensities of 4.5% was collected. MAD data at four wavelengths was collected on selenomethionated protein at the MAD beamline (BL19 ESRF) complete to 2.8 Å resolution with R_{sym} on intensities of 6.0%.

For structural and functional studies a fusion protein, denoted SRPΦ14/9, has been constructed which can functionally replace the SRP9/14 heterodimeric subunit in the SRP. The SRPΦ14/9 has been crystallized in the space group $P4_122/P4_322$ with cell parameters $a=b=69.7$ Å, $c=95.7$ Å. A synchrotron data set (BL19 ESRF) on a single flash-frozen crystal, complete to 2.8 Å resolution with R_{sym} on intensities of 4.4% has been collected. Selenomethionyl SRPΦ14/9 has also been crystallized for use in phase determination by MIR and/or MAD methods.