

The structure consists of an unexpected, irregular (β/α)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 preQ₁ which suggests a binding mode for tRNA where the phosphate backbone is recognized by the zinc subdomain, and the U₃₃G₃₄U₃₅ 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 Sm³⁺ 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 Mg²⁺/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.

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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 AUUGUAC. 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. Birse¹, Anders Åberg¹, Kieron Brown¹, Sylvie Doublé^{1*}, Ulrike Kapp¹, Katharina Strub² and Stephen Cusack¹. ¹European Molecular Biology Laboratory (EMBL-Grenoble)-Grenoble Outstation, c/o ILL, 156X, 38042 Grenoble Cedex 9, France, ²Département 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 P4₁22/P4₃22 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.