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Insights into mRNA Recognition from a PIWI–siRNA Complex

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RNA interference (RNAi) and related RNA silencing phenomena use short antisense guide RNA molecules to repress expression of target genes. Argonaute proteins, containing N-terminal PAZ domains and C-terminal PIWI domains, are core components of these mechanisms. We present the native crystal structure[1] of a PIWI protein from Archaeoglobus fulgidus (AfPiwi) and also in complex with an siRNA-like duplex[2], that mimics the 5' end of a guide RNA strand bound to an overhanging target mRNA.

The structures reveal a highly conserved and stable metal-binding site that anchors the 5' nucleotide of the guide RNA. The first base pair of the duplex is unwound, separating the 5' nucleotide of the guide from the complementary nucleotide on the target strand, which exits with the 3' overhang through a short channel. The remaining base-paired nucleotides assume an A-form helix, accommodated within a channel in the PIWI domain, which can be extended to place the scissile phosphate of the target strand adjacent to the putative slicer catalytic site. This study provides insights into mechanisms of target mRNA recognition and cleavage by an Argonaute-siRNA guide complex.

[1] Parker J. S., Roe S. M., Barford D., *Embo J.*, 2004, **23**, 4727-37. [2] Parker J. S., Roe S. M., Barford D., *Nature*, 2005, *in press*.

Keywords: RNA-protein complexes, siRNA, protein crystallography

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Crystal Structure of *Sulfolobus tokodaii* Aspartyl-tRNA Synthetase

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In the translation system, aspartyl-tRNA synthetase (AspRS) catalyzes aspartylation of the cognate tRNA through the two steps of reactions. At first, it strictly recognizes aspartic acid to produce aspartyl-AMP from ATP in the presence of Mg^{2+}. Then it is bound to the corresponding $tRNA^{Asp}$, and transfers the aspartic acid to the 3' terminus of the tRNA. Two types of AspRS are known; dinscriminating and non-discriminating. The former is bound only $tRNA^{Asp}$, while the latter is bound both $tRNA^{Asp}$ and $tRNA^{Asn}$. Thermoacidophilic archaea *Sulfolobus tokodaii* (*St*) belongs to the non-discriminating type, missing AsnRS gene. The crystal structure of AspRS from *St* has been determined at 2.3 Å resolution. *St*-AspRS is a dimeric enzyme consisting of two identical subunits. This is the first AspRS structure from crenarchaea. Each subunit is composed of the catalytic, the anticodon-binding and the hinge domains. Structural comparison with those of the three published AspRS of different sources shows that the 3rd residue (C36) of the $tRNA^{Asp}$ anticodon is specifically recognized by the main chain amide group of discriminating AspRS, suggesting taht C36 is the essential identity of $tRNA^{Asp}$. However, in the case of non-discriminating AspRS, the contacting residue is replaced with proline, which has no amide group. Furthermore the Pro residue in question is put away from the 3rd residue of the anticodon so that the corresponding 3rd residue (U36) of $tRNA^{Asn}$ is also acceptable in this site. This situation is conserved in every non-discriminating AspRS.

Keywords: aspartyl-tRNA synthetase, *Sulfolobus tokodaii*, X-ray crystal structure analysis

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Structure and Function of Unusual Archaeal Serly-tRNA Synthetases

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Aminoacyl-tRNA synthetase establish the genetic code by attaching particular amino acid to the 3' ends of tRNAs bearing the cognate anticodons. Sequence comparisons reveal two major types of seryl-tRNA synthetases (SerRS): a canonical form found in most organisms and a divergent type of SerRS found only in certain methanogenic archaea. On the sequence level, these atypic, methanogenic enzymes are characterized by alterations in two regions involved in tRNA binding. First, the N-terminal module of atypical SerRS has no detectable sequence homology to the N-terminal tRNA recognition domain of canonical SerRS, which is composed of an extended a-helical coiled coil and is shorter in size. Second, a sequence region referred to as the motif 2-loop in canonical SerRS, where it is involved in major groove recognition of approaching tRNA, is shortened significantly in atypical SerRS. Together, these differences imply a distinct, yet unknown, mechanism of tRNA recognition in methanogenic SerRSs. Besides, motif 2-loop is a part of the catalytic site what raises questions how these SerRSs determine the amino acid specificity.

To analyse this mechanism at atomic level we have determined X-ray crystal structure of atypical SerRS. Crystals diffracted well beyond 2.1Å resolution. The structure revealed a novel and unique RNA-binding fold of N-terminal domain in methanogenic SerRSs. The co-crystal structures of the enzyme in complex with the (pseudo-) substrate serine and AMPNP and a stable thio-analogue of seryl adenylate allowed an understanding of the catalytic mechanism and the structural basis of amino acid specificity of the unique non-canonical SerRS family.

Keywords: seryl-tRNA synthetases , RNA-binding domain, amino acid specificity

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Crystallographic and Functional Studies of Nip7 a Conserved Protein Involved in pre-rRNA Processing

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Ribosome biogenesis requires the coordinate function of a large number of factors including endo- and exonucleases, RNA helicases, rRNA modifying enzymes and snoRNPs. Nip7p function was inferred from studies using *Saccharomyces cerevisiae* conditional strains. These studies revealed that Nip7p is required for 27S pre-rRNA processing and 60S ribosome biogenesis. In addition, Nip7p interacts with Rrp43p, a component of the exosome complex, and with the nucleolar proteins Nop8p and Nop53p. Highly conserved Nip7p homologues are found in all eukaryotes and putative homologues are also found in *Archaea*. The C-terminal half of the protein contains a conserved domain (named PUA, after pseudo-uridine archeosine synthetase). This domain is found in several other RNA modifying enzymes. Functional analysis by means of primer extension revealed that both Nip7p and Rrp43p deficiency leads to similar defects in pre-rRNA processing. For structural studies, we have cloned the *Pyrococcus abyssi* Nip7p homologue (PaNip7) and produced the recombinant protein in *E. coli*. Following induction, PaNip7 was purified and submitted to crystallization trials. X-ray diffraction data were collected using synchrotron radiation from native crystals and an iodide derivative. PaNip7 crystal structure was solved using the SIRAS method and refined at 1.8 Å resolution. 3D structure shows a two alpha-beta domain protein. Structural and functional analysis will be presented.

Keywords: Nip7p, 3D structure, rRNA processing