The spliceosome is a highly dynamic macromolecular machinery that undergoes several structural and compositional rearrangements during its assembly and disassembly, respectively, its activation, as well as during the pre-mRNA splicing reaction. All these steps proceed in a highly ordered and strictly controlled manner with most of them driven by ATP. At least eight different ATPases, which all belong to the family of DExD/H-box helicases, are specifically involved in defined steps of the splicing cycle. These DExD/H-box proteins are thought not only to unwind dsRNA, but they might also act as RNPase disrupting protein-RNA complexes, which is an important process to achieve splicing and regeneration of the sliceosome. Besides the conserved helicase (DExD/H) core domain, the spliceosomal DExD/H-box proteins contain additional N- or C-terminal domains, which are important for the assembly and interaction with other proteins of the spliceosome. However, molecular details regarding their exact function and specificity are mostly unknown. We have recently determined the crystal structure of the catalytic domain of hPrp28 showing that the two halves of the catalytic domain are displaced with respect to each other and therefore no productive catalytic centre is formed. In contrast to other DExD/H-proteins the N-terminal half of the catalytic domain doesn't bind ATP, raising the question on interacting and activating partners in the spliceosome. Additionaly we have solved the first structure of a helicase-associated C-terminal domain that is found in the spliceosomal DEAD/H-box proteins Prp22, Prp2, Prp16, and Prp43. Interestingly, this domain was predicted to consist of two domains, but the crystal structure clearly demonstrates that it folds into one functional domain.

Keywords: RNA splicing, ATPases, domain structure

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Molecular basis for recognition of cognate tRNA by tyrosyl-tRNA synthetase from three kingdoms

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Aminoacyl-tRNA synthetases (aaRSs) play a central role in the assembly of amino acids into polypeptide chains. The 20 aaRSs are divided into two classes of 10 enzymes each. Tyrosyl-tRNA synthetase (TyrRS) is a class I enzyme, but is unusual in that it is a functional dimer. The specific aminoacylation of tRNA by TyrRSs relies on the identity determinants (the anticodon bases, the C1-G72 base pair, and the discriminator base A73) in the cognate $tRNA^{Tyr}$s. We have determined the crystal structure of *Saccharomyces cerevisiae* TyrRS (SceTyrRS) complexed with a Tyr-AMP analogue and the native $tRNA^{Tyr}$(G-Psi-A) at 2.4 Å resolution [1]. Structural information for TyrRS-$tRNA^{Tyr}$ complexes is now full-line for three kingdoms. Because the archaeal/eukaryotic TyrRSs-$tRNA^{Tyr}$s pairs do not cross-react with their bacterial counterparts, the recognition modes of the identity determinants by the archaeal/eukaryotic TyrRSs were expected to be similar to each other but different from that by the bacterial TyrRSs. Interestingly, however, the $tRNA^{Tyr}$ recognition modes of SceTyrRS have both similarities and differences compared to those in the archaeal TyrRS: the recognition of the C1-G72 base pair by SceTyrRS is similar to that by the archaeal TyrRS, whereas the recognition of the A73 by SceTyrRS is different from that by the archaeal TyrRS but similar to that by the bacterial TyrRS. Thus the lack of cross-reactivity between archaeal/eukaryotic and bacterial TyrRS-$tRNA^{Tyr}$ pairs most probably lies in the different sequence of the last base pair of the acceptor stem (C1-G72 versus G1-C72) of $tRNA^{Tyr}$. On the other hand, the recognition mode of Tyr-AMP is conserved among the TyrRSs from the three kingdoms.

[1] Tsunoda *et al*., *Nucleic Acids Res*. **35**, 4289-4300 (2007).

Keywords: aaRS, TyrRS, tRNA complex

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High resolution structure of bacterial GatCAB reveals the C-tail domain structure in GatB

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Majority of prokaryotes and organelles lack GlnRS which synthesizes Gln-tRNAGln by attaching Gln to tRNA directly. These organisms utilize a tRNA-dependent amidotransferase (AdT) to synthesize Gln-tRNAGln by an amidation of Glu mischarged on tRNAGln. Likewise, in prokaryotes lacking AsnRS, Asn-tRNAAsn is formed from Asp-tRNAAsn by a similar pathway. The both amidation are catalyzed by the heterotrimeric AdT consisted of GatC, GatA and GatB (GatCAB). Bacterial GatCAB recognizes both tRNAs with two identity elements. The positive determinant is the first U1-A72 base pair to discriminate tRNAGln and tRNAAsn from all of tRNAs. The size of D-loop of tRNAGln and tRNAAsn is the negative determinant to eliminate tRNAGlu and tRNAAsp. The structural and biochemical analyses of GatCAB indicated that the first U1-A72 base pair is recognized by the cradle domain of GatB, and the C-tail domain of GatB is essential for recognition of the D-loop of tRNAGln. However, in previous studies, the C-tail domain was disordered in GatCAB structure at 2.3 Å resolution. Recently, we obtained high quality crystals of GatCAB by adding MPD in the crystallization condition, and determined GatCAB structure at 1.9 Å resolution. The high resolution structure revealed that the C-tail domain forms four helices-bundle constructed by a hydrophobic core including L472 of GatB, which is a prerequisite for stabilization of the C-tail domain structure. Furthermore, with combining the structural and biochemical analysis of GatCAB and the multiple sequence alignment of GatB, we proposed that two loops of GatB would play important role for recognition of tRNAGln identity elements.

Keywords: aminoacyl-tRNA synthetases, tRNA, RNA-protein interactions

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Structural basis for dsRNA recognition by nonstructural protein 1 of influenza A virus

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