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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Influenza A viruses are important human pathogens resulting in periodic pandemic threaten, while nonstructural protein 1 of influenza A virus (NS1A) shields the virus against host defense as an immunosuppressor. Mutational inactivation of the dsRNA binding activity of NS1A highly attenuates virus replication. This study investigated the structural principles of dsRNA recognition by NS1A protein. The complex crystal of NS1A RNA binding domain (NS1A RBD) with dsRNA diffracted X-rays to 1.7 Å and was in space group *C*2 with unit cell dimensions of *a*=60.707 Å, *b*=57.218 Å, and *c*=83.709 Å. The crystal structure revealed that NS1A RBD forms a dimeric six-helical fold and used a dimeric anti-parallel helices $\alpha 2/\alpha 2'$ to recognize the major groove of the dsRNA as a sequence-independent mode. The RNA helix adopted 40°bending towards the NS1A RBD at both ends of the helix to facilitate the RNA-protein interactions. The highly conserved residues within a positive patch, including R35, R37, R38, and K41 played the primary roles for dsRNA binding by hydrogen bonds and electrostatic interactions. Outside this positive patch, conserved residues, such as T5, D29, D34, S42 and T49, also contributed for dsRNA binding through hydrogen bonds directly or via water bridges. The significant conformational change of invariable residue R38 before and after NS1A RBD binding to dsRNA indicated that R38 played a key role for dsRNA binding by penetrating its side chain into dsRNA helix. The protein-RNA interactions observed from the crystal structure were further supported by the isothermal titration calorimetry assay of NS1A RBD and its mutants binding to dsRNA. Moreover, Agrobacterium co-infiltration assay suggested that arginine 38 may also play important roles for dsRNA binding *in vivo*.

Keywords: RNA-protein interactions, viral proteins, X-ray crystallography of biological macromolecules

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X-ray structure of a cysteine-less mutant galectin-1

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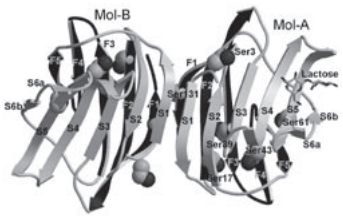
Galectin-1 (Gal-1) is a member of the beta-galactose binding animal lectin family, having a wide range of biological activity. We prepared a mutant Gal-1 (CSGal-1) where all Cys residues were replaced by Ser, because Gal-1 is susceptible to oxidation at Cys residues. To elucidate how the substitutions of amino acid residues affect the three dimensional structure of the protein, the X-ray structure of CSGal-1/lactose complex has been determined at 1.86 Å resolution. The monomer of GSGal-1 adopts a beta-sandwich structure formed by two anti-parallel beta-sheets. By dimerization of Mol-A and Mol-B, pairs of the same beta-sheets are connected to give two large anti-parallel beta-sheets, and a lactose molecule occupies the carbohydrate-bindindg site of Mol-A, as shown in a figure. The r.m.s. deviations for C-alpha atoms is 0.41 Å between CSGal-1 and the wild-type human Gal-1, and the interactions between protein and the bound lactose molecule are equivalent to each other, showing that two structures are almost identical. The substitution of six Cys residues for Ser does not affect the overall structure and the carbohydrate-binding site structure of the protein.



Keywords: X-ray structure, lectins, galactose-binding protein

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Far-red fluorescent protein mKate reveals pH-induced *cis-trans* isomerization of the chromophore

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The far-red monomeric fluorescent protein mKate (Lex = 588 nm, Lem = 635 nm), originating from wild-type red fluorescent progenitor eqFP578 (sea anemone *Entacmaea quadricolor*), is characterized by the pronounced pH dependence of fluorescence, relatively high brightness, and high photo-stability. The protein also demonstrates “kindling” phenomena- the increase of fluorescence brightness upon irradiation by excitation light. The protein has been crystallized at pH ranging from 2 to 9 in three space groups and four structures have been determined by X-ray crystallography at the resolution of 1.75 - 2.6 Å. The phenomenon of pH-dependent fluorescence of mKate has been shown to be due to reversible *cis-trans* isomerization of the chromophore phenolic ring. In the non-fluorescent state at pH 2.0, the crystal structure of mKate shows the chromophore in the *trans*- isomeric form. The weakly fluorescent state of the protein at pH 4.2 is characterized by a mixture of *trans* and cis isomers. The chromophore in a highly fluorescent state at pH 7.0/9.0 adopts the *cis* form. Three key residues, Ser143, Leu174, and Arg197, residing in the vicinity of the chromophore, have been identified as being primarily responsible for the far-red shift in the spectra. Structure-based single S158A amino acid mutation destabilizes the trans conformation of the chromophore, causing the annihilation of the kindling effect, with the concomitant increase of pH stability and brightness of the mKate_S158A variant. Analysis of the stereochemistry of the intermonomer interfaces has revealed a group of residues consisting of Val93, Arg122, Glu155, Arg157, Asp159, His169, Ile171, Asn173, Val192, Tyr194, and Val216, as being most likely responsible for the observed monomeric state of the protein in solution.

Keywords: fluorescent proteins, cis-trans isomerization, pH dependence of fluorescence

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Two threonyl-tRNA synthetases with complementary functions; Crystal structure of ThrRS-1

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