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Crystal structure of RNA aptamer in complex with human immunoglobulin G

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Aptamers are single-stranded nucleic acids that bind to molecular targets, including proteins, with high affinity and specificity. RNA aptamer can be discovered in vitro selection process on the basis on their high affinity for the targets. Recently, various applications of RNA aptamers have been introduced, not only to determine a drug target involved in disease pathology, but also to develop therapeutic agents. We have demonstrated the ability of RNA aptamer to bind to human immunoglobulin G (IgG) with high affinity and specificity. The aptamer was shown to bind to the Fc domain of human IgG, but not to other IgG's, with high affinity. The aptamer was observed to compete with protein A, but not with the Fcr receptor, for IgG binding. Here, we have determined the crystal structure of the aptamer in complex with human IgG. The electron density has clearly proved two aptamers bound to dimeric IgG. The tertiary structures of the recognition sites on the Fc domain differ significantly between human IgG and other species of IgGs. The structure also explains high specificity and affinity of the selected aptamer to human IgG

Keywords: crystallography of protein RNA, immunoglobulins, RNA-protein interactions

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Structural studies of human RIG-I in complex with double-stranded RNA

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Retinoic acid inducible gene-I (RIG-I) is a critical component of innate immune system that is responsible for the detection and elimination of invading viruses. RIG-I like Toll-like receptor 3 detects viral RNAs inside the cell and initiates downstream signaling to activate IRF-3 and NF-kB genes resulting in the production of Type-I interferon. RIG-I and related viral RNA recognition proteins such as MDA5 and LGP2 are located in the cytoplasm, while Toll-like receptor 3 resides in the endosome. RIG-I is composed of N-terminal CARD domain for signaling via the adaptor molecule IPS-1 and C-terminal helicase domain for RNA recognition. The C-terminal helicase domain recognizes viral double-stranded RNA or single-stranded RNA with 5'-triphosphate group. The helicase activity of RIG-I is required to promote conformational changes of the CARD domain so that it can interact with IPS-1. To study the mechanism of viral dsRNA recognition by RIG-I, the structure of RIG-I C-terminal domain in complex with dsRNA is being studied. Selenomethionine incorporated RIG-I was expressed using E.coli and purified for crystallization. RIG-I:dsRNA binding was studied by EMSA method to investigate the RIG-I:dsRNA binding property in vitro. The X-ray data was collected from RIG-I:dsRNA complex crystals to 2.6 Å using synchrotron radiation. Structural studies of RIG-I(helicase domain):dsRNA complex will reveal how RIG-I can recognize viral RNA and discriminate it from self RNA and show the mechanism of RNA-induced conformational changes.

Keywords: RIGI, virus, immune system

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Structural requirements for recognition and nuclear import of spliceosomal UsnRNPs

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The biogenesis of nuclear UsnRNPs requires a cytoplasmic maturation step. In the cytoplasm UsnRNAs and sm-proteins form a core UsnRNP, which is a prerequisite for dimethylation of the m7G-cap of the UsnRNA to a m3G-cap by tri-methyl-guanosine-synthase (TGS1). The nuclear import of the UsnRNPs is mediated by importinβ (Impβ) and snurportin1 (SPN1), that specifically recognises m3G-cap. In contrast to any other characterized active nuclear import the Impβ/SPN1/UsnRNP complex does not require RanGTP for the terminal release from the nuclear pore complex (NPC). We have determined the crystal structure of the human TGS1- methyltransferase domain, which consists of the canonical catalytic domain and an additional N-terminal subdomain that is strictly required for enzymatic activity. A m7GTP molecule, representing the minimal substrate, and S-adenosyl-homocysteine are bound in deep clefts of the TGS1 surface an are held in close proximity to each other. We also have solved the crystal structures of the C-terminal domain of SPN1 [1], revealing the structural basis for the specific recognition of the m3G cap, and of the N-terminal domain of SPN1bound to the nuclear import receptor Impβ [2]. The crystal structure of Impβ in complex with the N-terminal domain of SPN1 reveals that Impβ adopts an open conformation, which is unique for a functional Impβ/cargo complex and it resembles the conformation of the Impβ/RanGTP complex. As binding of RanGTP to Impβ usually triggers the release of import complexes from the NPC, we propose that by mimicking a conformation similar to Impβ /RanGTP the independent dissociation of Impβ/SPN1 from the NPC is energetically aided.

[1] Strasser et al., EMBOJ 24, 2235-2243, 2005.
[2] Wohlwend et al., JMB 374, 1129-1138, 2007.

Keywords: transport, RNA-protein interaction, methylase

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Crystallographic studies on molecular motors and switches of the spliceosome

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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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