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RNA recognition mechanism of the Human Immunodeficiency Virus Type-2 Nucleocapsid Protein

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The retroviral nucleocapsid (NC) protein is a multifunctional protein essential for RNA genome packaging and viral infectivity. The NC protein, NCp8, of the HIV-2 is a 49 amino acid peptide containing two zinc fingers (ZFs) connected by seven amino acid residues called the "basic amino acid cluster." It has been shown that the N-terminal ZF flanked by the basic amino acid cluster is the minimal active domain for the specific binding to viral RNA.

The 3D structure of NCp8-f1, a 29 amino acid peptide that includes the minimal active domain of NCp8, revealed that the conformation of the cluster was stabilized by a H-bond between Asn^{11} in the 1st ZF and Arg^{27} in the cluster. Moreover, the 3D structure of NCp8-f1/N11A, in which an Ala is substituted for Asn^{11}, and NCp8-f2, a 27 amino acid peptide that includes the 2nd ZF and the cluster, were determined. In addition, the binding activity of NCp8 with the viral RNA SL3 in HIV-2 was measured, and the activity was compared with those obtained for other NCp8-derived and mutant peptides.

From these experiments, we demonstrated that the four basic amino acid residues in the N-terminus and the cluster were necessary for binding to SL3. Furthermore, the cluster's flexible orientation, which is controlled by the H-bond, appears to be a structural basis for NCp8 to exist as a multi-functional protein.

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Reaction mechanism of bacterial Glu-$tRNA^{Gln}$-dependent amidotransferase

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Gln-$tRNA^{Gln}$ is synthesized by two different pathways in nature. Most bacteria employ an indirect pathway to produce Gln-$tRNA^{Gln}$ by Glu-$tRNA^{Gln}$ amidotransferase (Glu-AdT) in a mis-acylated Glu-$tRNA^{Gln}$-dependent manner. Bacterial Glu-AdTs are heterotrimeric proteins encoded by the *gatC*, *gatA*, and *gatB* genes. GatCAB converts Glu-$tRNA^{Gln}$ into Gln-$tRNA^{Gln}$ by activating Glu-$tRNA^{Gln}$ into γ-phosphoryl-Glu-$tRNA^{Gln}$, which is subsequently transamidated into Gln-$tRNA^{Gln}$ using ammonia generated by hydrolysis of glutamine. Notably, the glutaminase and transamidase reactions are tightly coupled and dependent on the binding of Glu-$tRNA^{Gln}$ to GatCAB.

In this study, we describe the crystal structures of intact GatCAB complex, in the apo form, and in the complexes with glutamine, Mn^{2+} and ADP. The GatCAB/Mn^{2+} complex structure revealed a pair of Mn cations in the active site of GatB. The primary Mn^{2+}-binding site is identical to the Mg^{2+} site in the native crystal. The second transient binding site is located adjacent to the primary Mn^{2+}-binding site. These results strongly suggested that GatB uses a two-metal-ion mechanism of catalysis, which is reminiscent of the mechanism employed by glutamine synthetases. Furthermore, we constructed a series of $tRNA^{Gln}$ mutants based on the differences between $tRNA^{Gln}$ and $tRNA^{Glu}$, and investigated the tRNA-binding activity. Mutation analysis showed that the first U-A base pair and the D-loop of $tRNA^{Gln}$ serve as identity elements essential for discrimination by GatCAB.

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Structure of Archaeal Translation Initiation Factor aIF2βγ complex: Implication of the β subunit in GTPase Activation

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The fundamental steps in translation initiation are binding of initiator Met-$tRNA_i$ to a ribosomal small subunit, and positioning of them on the start codon of an mRNA. Archaeal/eukaryotic initiation factor 2 (a/eIF2), consisting of α, β, and γ subunits, plays pivotal roles in these steps, as it delivers Met-$tRNA_i$ to a ribosomal small subunit as a ternary complex with GTP and facilitates start site selection through a hydrolysis of GTP. Despite the distinct functions of the individual subunits have been demonstrated by many studies, it is still unclear how they cooperate to exert integrated functions, such as GTP hydrolysis and subsequent Met-$tRNA_i$ dissociation.Here, we present the structures of aIF2βγ homodimeric complex in apo and GDP forms, which show that the N-terminal two third of the β subunit interacts with the G domain of the γ subunit, but is distant from domains 2 and 3, to which the α subunit and Met-$tRNA_i$ bind. Comparison with the uncomplexed structure showed the significant conformational differences between two forms of the β subunit, particularly in the C-terminal zinc-binding domain, which was suggested previously to be involved in intrinsic GTP hydrolysis. Furthermore, Switch 1 region in the γ subunit is moved away from the nucleotide through the interaction with the conservative R87 in the β subunit. As Switch 1 region is responsible for GTP and Met-$tRNA_i$ binding, these results implicate that conformational change of the β subunit facilitates intrinsic GTP hydrolysis by sequestering Switch 1 region to the off state.

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Crystal structure of *Pyrococcus horikoshii* arginyl-tRNA synthetase complexed with $tRNA^{Arg}$ and ATP analogue

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Aminoacyl-tRNA synthetase (aaRS) catalyzes the esterification of specific amino acid to the 3'-terminal adenosine of tRNA. The proper connection between amino acid and tRNA is based on the ability of the strict recognition of 20 kinds of aaRS. The aaRSs have been classified into two classes on the basis of conserved sequences and characteristic structure motifs. Arginyl-tRNA synthetase (ArgRS) is classified into Class Ia aaRS. In this study, we determined the structure of *P. horikoshii* ArgRS complexed with $tRNA^{Arg}$, and ATP analogue AMPPNP, to elucidate the mechanism of the identity element recognition and the difference of the anticodon binding region in helix-bundle domain among ArgRS and other Class Ia aaRSs.The recombinant *P. horikoshii* ArgRS protein was co-crystallized with $tRNA^{Arg}$ transcript and AMPPNP by the hanging-drop vapour-diffusion method using PEG4000 and ammonium sulfate as precipitants. The data set diffracting to 2.0 A resolution was collected at 100 K using synchrotron radiation at the SPring-8 beamline BL41XU (Harima, Japan). The ArgRS crystals belong to the monoclinic space group $P2_1$, with unit-cell parameters a=76.27, b=60.00, c=110.66 Å, α=γ=90.00, β=106.94°. *P. horikoshii* ArgRS/$tRNA^{Arg}$ structure was determined by molecular replacement using *Thermus thermophilus* ArgRS as a search model. We will discuss the difference of identity element recognition through the structural comparisons of *P. horikoshii* ArgRS complexed with $tRNA^{Arg}$ and yeast ArgRS complexed with $tRNA^{Arg}$.

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