C-terminal helix. The H2 helix in each dsRBD is important for recognition of pri-miRNA substrates. This structure, together with fluorescent resonance energy transfer and mutational analyses, suggests that the DGCR8 core recognizes pri-miRNA in two possible orientations. We propose a model for DGCR8's recognition of pri-miRNA.

Keywords: microRNA, DGCR8, dsRBDs

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Crystal structure of the avian reovirus inner capsid protein sigmaA

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Avian reovirus, an important avian pathogen, expresses eight structural and four non-structural proteins. The structural sigmaA protein is a major component of the inner capsid, clamping together lambdaA building blocks. SigmaA has also been implicated in the resistance of avian reovirus to the antiviral action of interferon by strongly binding double-stranded RNA, and thus inhibiting activation of the double-stranded RNA-dependent protein kinase (PKR) in the host cell cytoplasm. We have solved the structure of bacterially expressed sigmaA and refined it using data to 2.3 Å resolution. Twelve sigmaA molecules are present in the *P*1 unit cell, arranged as two short double helical hexamers. A positively charged patch is apparent on the surface of sigmaA on the inside of this helix and mutation of either of two key arginine residues (Arg155 and Arg273) within this patch abolishes double-stranded RNA binding. The structural data provides evidence for a proposed mode for the cooperative binding of sigmaA to double-stranded RNA.

Keywords: protein crystallography, site-directed mutagenesis, double-stranded RNA binding

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Structural basis for recruitment of replicative DNA polymerase to PCNA

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Proliferating cell nuclear antigen (PCNA) is responsible for processivity of DNA polymerase. The crystal structures of Pyrococcus furiosus DNA polymerase (PfuPol) and that in complex with Pfu monomeric PCNA allowed us to construct a convincing model of the polymease-PCNA ring interaction, without causing steric hindrance with the PCNA ring. Electron microscopic analysis confirmed that this complex structure in fact exists in solution, exhibiting one of multiple functional polymerase configurations. Together with supportive data from mutational analyses, it is concluded that the novel interaction is formed between a long stretched loop of PCNA and the Thumb-2 domain of the polymerase, in addition to the authentic PCNA-polymerase recognition site (PIP box). This second recognition site appears to pull the Thumb-2 domain, thereby opening the central cleft of PfuPol so as to allow the accommodation of the substrate DNA. In comparison with the previously reported structures of polymerases complexed with DNA, the present structures suggest that the second recognition site play a crucial role in switching between the polymerase and exonuclease modes through stabilizing solely the polymerase mode.

Keywords: DNA polymerases and replication proteins, DNA replication, protein complex structure

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Mechanistic insight into isopentenylation of the anticodon of tRNA via a channel

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Post-transcriptional chemical modifications of ribonucleotides in the anticodon loop of tRNAs serve as essential elements to define the decoding property in the genetic code translation. In both eubacteria and eukaryote, tRNAs that read the codon beginning with uridine usually possess a modified adenosine base: N^6-isopentenyl-adenosine (i^6A), at the position 37 adjacent to the tRNA anticodon. An enzyme responsible for i^6A formation in tRNA is known as tRNA isopentenyltransferase (tRNA-IPT) which catalyze the alkylation of electron-rich acceptors by the hydrophobic moiety of allylic isoprenoid pyrophosphate. Interestingly, i^6A nucleotides liberated by degradation of cellular tRNAs stimulate biosynthesis of cytokinins, which are central regulators of cell division and differentiation in plants. Moreover, multiple reports recently highlighted the potential role of tRNA-IPT and i^6A as a general suppressor for human cancers. Despite intensive mutational as well as structural studies, the precise mechanisms for tRNA-recognition and i^6A formation by tRNA-IPT have remained elusive. Here we unveil those problems by determining the crystal structure of *Escherichia coli* tRNA-IPT: MiaA, complexed with an intact substrate tRNA, to a resolution of 2.5 A. MiaA is composed of two globular domains clamping the tRNA anticodon helix on its both sides. The anticodon loop is drastically distorted and protrudes a modified A37 base into the entrance of a reaction channel traversing through the catalytic domain. Soaking crystals with a prenyl-donor analogue, dimethylallyl *S*-thiodiphosphate (DMASPP) reveals the detailed reaction mechanism taking place in the middle of the channel. We will also discuss on the molecular evolution of tRNA-IPT from both the structural and catalytic respects.

Keywords: tRNA, RNA-protein complexes, X-ray crystallography of RNA