NAC domain, the one of A. thaliana ANAC019 (Ernst et al, 2004, EMBO Rep, 5:297-303), revealing a novel dimeric transcription factor fold. The structure is now the basis for mutagenesis studies to identify the DNA-binding mode (Olsen et al, 2005, Plant Science 169: 785-797) Structure determination of a new crystal form of this NAC domain shows that the dimer is likely to have some flexibility, but the solution structure as determined by small angle X-ray scattering is in good agreement with the original crystal structure. Based on ongoing crystallographic, small angle scattering and mutagenesis studies, our current model for NAC proteins binding to DNA will be presented.

Keywords: transcription factor, NAC protein, structure

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Structure of the topoisomerase IV from *S. pneumoniae* with a DNA target and quinolone drug

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Eighteen percent of all clinical bacterial infections are now treated with quinolone based antibiotics [1], which target the decatenating enzyme topoisomerase IV (a Class II topo) in gram-positive bacteria such as *S. pneumoniae*. Topoisomerase IV consists of both ParE and ParC domains. We have crystallised the complex of ParC55 (55 kDa) and ParE (30kDa) with a 32 base-pair DNA target and quinolone drug. Crystals were grown in space group $P3_2$ with cell dimensions a=b=118.30 Å, c=177.90 Å α=90°,β=90°,γ=120° both by conventional hanging drop vapour diffusion in 24-well limbro plates and by sitting drop in 96 well MRC crystallisation plates. The structure has been solved by molecular replacement (CNS) using as search models our ParC55 structure [2] and a ParE domain homology modelled on the basis of the structure of the TOPRIM domain of the yeast type IIa [3]. The DNA has been positioned from difference Fourier maps following refinement using CNS. The veracity of the model was confirmed by the ability of this phase set to determine the correct Pt sites for a K_2PtCl_4 heavy atom derivative. Subsequently multiwavelength anomalous diffraction data were collected at the SOLEIL synchrotron about the Pt absorption edge in order to calculate a MAD map. We should like to thank the beam-line personnel headed by K. McAuley at the DIAMOND synchrotron, Chilton, Oxford for their help in collecting native and fixed Pt edge synchrotron data on station IO3.
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Keywords: protein-DNA, quinolone, S. pneumoniae

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RNA splicing related proteins; Crystal structure of RNA 3'-terminal phosphate cyclase

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RNA 3'-terminal phosphate cyclase (Rtc) is an enzyme related to RNA splicing to convert the 3'-terminal hydroxyl group of truncated RNA to 2',3'-cyclic phosphate which is required just before its ligation. This reaction may occur in the following two steps: (i) Rtc + ATP → Rtc-AMP + PPi and (ii) RNA-N3'p + Rtc-AMP → RNA-N>p + Rtc + AMP. To reveal the reaction mechanism, Rtc overexpressed in *E.coli* was crystallized in the following states, Rtc, Rtc-AMP, Rtc+Mn and Rtc+ATP, and their structures have been determined at 2.25, 2.25, 3.2 and 2.4 Å resolutions, respectively. Rtc is a single protein folded into two domains, the large domain being composed of three $\beta\alpha\beta\alpha\beta\beta$ motifs arranged by psudo three-fold symmetry, and the small domain being formed with a $\beta\alpha\beta\beta\beta\alpha$ motif inserted into the third motif of the large domain. The overall structures of other derivatives are almost the same as that of Rtc. At the catalytic site of Rtc-AMP, the α-phosphate group of AMP is covalently bound to the Nε atom of His307, and the adenine moiety of AMP is stacked between the side chains of Pro126 and His283. The two hydroxyl groups at 2' and 3' positions of the ribose are bound to the side chain of Asp286 through hydrogen bonds. These structure features suggest the following reaction scheme. A Mg^{2+} ion bound to Glu10 induces conformational changes of the α and β phosphate groups of ATP which is trapped by Arg17 and Arg39, so that the Nε atom of His307 easily attacks to the α-phosphate group to form a P-N bond in the first reaction. In the second reaction, when a truncated RNA is bound, its 3'-phosphate group might be forced to react with the phosphate group of AMP, and the activated 3'-phosphate group is attacked by the 2'-hydroxyl group to generate the 2',3'-cyclic phosphodiester.

Keywords: RNA 3'-terminal phosphate cyclase, sulfolobus tokodaii, X-ray crystal structure analysis

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Crystal structure of human DGCR8 core

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A complex of Drosha with DGCR8 (or its homolog Pasha) cleaves primary microRNA (pri-miRNA) substrates into precursor miRNA and initiates the microRNA maturation process. Drosha provides the catalytic site for this cleavage, whereas DGCR8 or Pasha provides a frame for anchoring substrate pri-miRNAs. To clarify the molecular basis underlying recognition of pri-miRNA by DGCR8 and Pasha, we determined the crystal structure of the human DGCR8 core (DGCR8S, residues 493－720). In the structure, the two double-stranded RNA－binding domains (dsRBDs) are arranged with pseudo two-fold symmetry and are tightly packed against the

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