that SilB C-terminal domain could function as a metallochaperone to the SilABC system.

**1291-Pos**

**Crystal Structure and Metal-Binding Specificity of ZneB, the Periplasmic Adaptor Protein of A Heavy Metal Resistance System from Cupriavidus Metallidurans CH34**

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In Gram-negative bacteria, tripartite efflux systems are involved in the transport of a broad range of toxic compounds such as drugs or heavy metals out of the cell. These protein complexes span the entire bacterial cell envelope, and are composed of an inner membrane transporter belonging to the resistance nodulation cell division (RND) family, an outer membrane protein member of the Outer Membrane Factor (OMF) family, and a periplasmic adaptor protein, member of the Membrane Fusion Protein (MFP) family. The periplasmic adaptor plays an important role in the recruitment of the two integral membrane partners and for the assembly of a functional transport complex. It has been proposed that this component could also contribute to the binding of the substrate. We report here on the characterization of ZneB, the MFP of a Heavy Metal Efflux-RND system from Cupriavidus metallidurans CH34. Using mass spectrometry, we have demonstrated that ZneB has a high specificity for zinc binding with a metal stoichiometry of 1:1 to the protein. The protein was crystallized in the presence of zinc and the apo- and metallated-forms were detected in the same asymmetric unit. The involvement of two histidine and a glutamate residues in the metal ion coordination site was confirmed by site-directed mutagenesis. The comparison of apo- and Zn-bound conformations based on the crystal structures and on data obtained in solution reveals important conformational changes upon zinc binding, suggesting an active role of the MFP in the efflux mechanism. The characterization at the molecular level of the efflux system proteins and the comparison with their counterparts in homologous RND-type transport systems involved in multidrug resistance will allow a better understanding of the resistance mechanisms.

**1292-Pos**

**Crystal Structure of the N-terminal Region of Brain Spectrin Reveals A Helical Junction Region and A Stable Structural Domain**

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The crystal structure of a recombinant protein consisting of the first 147 residues of brain α spectrin was solved to 2.3 Å. The N-terminal region consists of the partial domain (Helix C') and the anti-parallel, triple helical coiled-coil first structural domain (helices A1, B1, and C1). The data revealed that each asymmetric unit contained two crystallographically independent structures (1 and 2). The crystal structure of the first structural domain resembled that of the first structural domain of erythroid α-spectrin, determined before by solution NMR studies, with some specific differences, especially at the N-terminal region, including Helix C' and the region connecting Helix C' with the first structural domain (the junction region). The first ten residues are in a disordered conformation, followed by Helix C' with an apparent, flexible bend. The junction region exhibits a helical conformation in contrast with an unstructured junction region in erythroid α spectrin. A special feature that has not been reported in other spectrin domains is the long and flexible A1B1 loop of 13 residues. This loop is likely the recognition site for interaction with other proteins. Hydrogen bonds and hydrogen bond networks were identified in the first structural domain and compared with those in erythroid α-spectrin. We suggest that these hydrogen bonds might contribute toward the stability of brain and erythroid spectrin.

**1293-Pos**

**An Overlapping Kinase and Phosphatase Docking Site Regulates Activity of the Retinoblastoma Protein**

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Insights into the molecular mechanisms that regulate the phosphorylation state and corresponding activity of the retinoblastoma tumor suppressor protein (Rb) are fundamental to understanding the control of cell proliferation. While much focus has been placed upon regulation of Cyclin-dependent kinase (Cdk) activity towards Rb, less is known about Rb dephosphorylation catalyzed by the major Rb phosphatase, protein phosphatase-1 (PP1). Using x-ray crystallography, we have determined the crystal structure of a PP1:Rb peptide complex to 3.2Å that reveals an overlapping kinase and phosphatase docking site. Kinetic assays show that Cdk and PP1 docking to Rb are mutually exclusive and that this docking site is required for efficient dephosphorylation, as well as phosphorylation of Rb. Cell cycle arrest assays demonstrate that the ability of PP1 to compete with Cdks is sufficient to retain Rb activity and block cell cycle advancement. These results establish a novel mechanism for the regulation of Rb phosphorylation state in which kinase and phosphatase compete for substrate docking.

**1294-Pos**

**Crystal Structures Of Yeast Mitochondrial ATPase with Uncoupling Mutations**

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Yeast mitochondrial ATP synthase is a transmembrane protein responsible for synthesis of more than 90% of ATP under aerobic conditions. The water soluble portion of ATP synthase, F1, is composed of five subunits with stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$ and has a combined molecular weight of 360 kDa. The three active sites of ATPase are formed at the interfaces between alternating α- and β-subunits. ATPase is capable of efficient ATP hydrolysis, accompanied by rotation of central stalk subunits, $\gamma\delta\epsilon$, within the $\alpha_3\beta_3$ core and is capable of ATP synthesis if central subunits are forced to rotate in the opposite direction. A number of mutations in ATP synthase have been identified that result in the uncoupling of catalytic function and proton flow across the mitochondrial membrane. These uncoupling mutations cluster at the interface between γ-subunit and $\alpha_3\beta_3$ catalytic core of ATPase. In this work, four X-ray crystal structures of ATPase with single amino acid substitutions αN67I, αF405S, βV279F, and γI270T were solved at resolutions ranging from 3.2 Å to 2.74 Å. This study will present a structural comparison of the mutant structures with the wild type structures to understand the mechanism of coupling. However, the crystal structures likely represent the ground state of catalytic reaction cycle while the mutations may result in notable distortions of enzyme structure during other stages of catalytic cycle. Additionally, the uncoupling of the ATPase may be caused by changes in the energy of interaction between the portions that are rotating in the molecular machine thereby altering transition to the higher energy states. Structural based hypotheses are presented to explain the role of these residues in the coupling of the enzyme. Supported by NIH R01GM0662223

**1295-Pos**

**Molecular Mechanism of the Peptidoglycan Hydrolysis by FlgJ, A Putative Flagellar Rod Cap Protein From Salmonella**

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The axial structure of the bacterial flagellum consists of three parts: the filament as a helical propeller; the hook as a universal joint; and the rod as a drive shaft connecting the hook and the rotor. The assembly of the axial structure, which occurs at its distal end, requires cap complexes attached to the growing end. FlgD and FliD are cap proteins necessary for hook and filament growth, respectively. For efficient penetration of the growing rod through the peptidoglycan (PG) layer, it is likely that the rod cap locally degrades PG. FlgJ is a putative rod cap protein with a PG-hydrolyzing activity (muramidase). Previous studies have shown that the N-terminal region of FlgJ interacts with the rod proteins and that the C-terminal region shows a sequence similarity to muramidase family, such as autolysin and AcmA. To understand the mechanisms of rod formation, we determined the atomic structure of a C-terminal fragment of FlgJ at 1.7 Å.

The crystal structure revealed the entire muramidase domain of FlgJ. In spite of no significant sequence similarity, the putative active site of FlgJ closely resembles that of hen egg white lysozyme (HEWL), which is a well-studied muramidase. A glutamic acid residue at position 184 in FlgJ is invariant among FlgJ family and the E184Q mutant of FlgJ shows no muramidase activity, indicating

that Glu184 is essential for its catalytic activity. We have also determined the residues involved in substrate recognition by single amino-acid substitution experiments based on the structure. These results indicate that a large conformational change of sub-domain is required to exert the muramidase activity. We will discuss a possible PG-hydrolyzing mechanism of FlgJ in flagellar assembly.

**1296-Pos**

**Structure of the Newcastle Disease Virus F Protein in the Post-Fusion Conformation**

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Newcastle disease virus (NDV) is a member of the Paramyxoviridae family. The NDV fusion (F) glycoprotein, which is responsible for merging the viral and cellular bilayers during entry. The X-ray crystal structures have been solved of F proteins in the post-fusion and the pre-fusion conformations, providing atomic level information regarding the conformational transitions accompanying fusion. However, our understanding of the similarities between different F glycoproteins in these two conformational states remains incomplete.

Here, we present the crystal structure of the secreted, uncleaved ectodomain of the NDV F protein. Previous structural analysis of a related NDV F protein was missing key elements of the functional regions of the protein, including two helical segments (HRA and HRB) that assemble into a stable six helix bundle (6HB) in the post-fusion form. We have produced the NDV F protein in pre- and post-fusion conformations, using analogous constructs that produced a pre-fusion PIV5 F structure and a post-fusion HPIV3 F structure. We demonstrate that the two NDV F proteins exhibit the pre-and post-fusion forms through EM analysis and we have solved the crystal structure of the post-fusion form of the NDV F protein. In contrast to the previously determined NDV F structure, our new crystal structure contains the 6HB at the base of the stalk region, consistent with the EM observations and the previously determined HPIV3 F structure. Global superposition of the NDV and HPIV3 structures demonstrates maximum correspondence between distal portions of the structures, with orientation or adjustments in linking domains and the extended HRA stalk. Electrostatic profiles of the NDV, HPIV3, and PIV5 F structures show elements of conserved charge distributions despite significant sequence differences in these glycoproteins, which may be important for their common functionality.

**1297-Pos**

**Structure of DNA Binding Domain of Plant Telomere Binding Proteins Represents Unique Features of Telomere Binding Protein Family**

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Telomeres, the ends of linear eukaryotic chromosomes, are composed of short repeats of G-rich sequences and play essential roles in genome stability with various telomere binding proteins. To characterize the binding mode of plant telomere DNA and telomere binding protein, we determined the structures of DNA binding domain and telomere complex of NgTRF1, atTRF and RTBP1, double strand telomere binding proteins of plants, by multidimensional NMR spectroscopy and X-ray crystallography. We have identified the DNA binding interface of the DNA binding domain of TBPs, which is composed of 4 $\alpha$-helices by means of chemical shift perturbation analysis. The complex crystal structure of $NgTRF1^{561\ 681}$ and plant telomere DNA $(TTTAGGG)_2$ have shown the molecular details of the interaction between them and we confirmed the interaction biochemically through site-directed mutagenesis. From the comparison with the structure of human telomere binding protein, we tried to show the unique features of plant telomere binding protein in the mode of telomere DNA binding as well as the similarity with the telomere binding proteins in other organisms. To our knowledge, this is the first report of the complex structure of telomere binding protein and telomere DNA in plant.

**1298-Pos**

**Structural and Functional Characterization of an Unusual SH3 Domain from the Fungal Adaptor Protein Bem1**

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Protein interactions form the basis of most biological processes and in eukaryotes are often mediated by conserved modular domains that recognize linear motifs. Among the most common protein interaction domains is the SH3 domain that generally recognizes PxxP containing peptides. SH3 domains are approximately 60 amino acids long and are composed of five beta strands. We are studying the SH3 domain from the fungal adaptor protein Bem1p that plays an important role during polarized growth and activation of MAPK signaling pathways. This SH3 domain is unusual, even though its sequence conforms to the SH3 domain consensus, it requires an extra 40 amino acids at its C-terminus for folding. Furthermore, in addition to binding PxxP containing peptides, it also binds the Cdc42p GTPase in a PxxP-independent manner. We are using in vitro binding assays and NMR spectroscopy to structurally and functionally characterize this unusual SH3 domain. Contrary to a previous report, we find that the Bem1 SH3 domain can simultaneously bind the Cdc42p GTpase and PxxP-containing peptides and that the binding of one does not affect the affinity for the other. Structural characterization by NMR shows that the extra sequence contains two alpha helices that pack tightly against the SH3 domain and thus form an integral part of the fold. Our findings provide with an example of how a common protein interaction domain can evolve to have additional atypical structural features and associated functions.

**1299-Pos**

**Structural Investigation of a Fibronectin Type III Domain Tandem from the A-band of the Titin**

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Single molecules of the giant muscle protein titin span half muscle sarcomeres, from the Z-disk to the M-band, and have key roles in sarcomere assembly and elasticity. In the A-band titin is attached to thick filaments and here the sequence shows fibronectin type III and immunoglobulin-like domains. These are mostly arranged in regular patterns of eleven domains called the large super-repeats. The large super-repeat occurs eleven times and this entire region thus forms nearly half of the titin molecule. Through interactions with myosin and C-protein, it is involved in thick filament assembly. We are determining the atomic structure, dynamical properties and the inter-domain arrangement of overlapping double and triple domain fragments of the large super-repeat by NMR spectroscopy. Ultimately, we hope to combine the data to reconstruct the overall conformation of the super-repeat. Here we investigated the A59-A60 domain tandem, which was expressed in bacteria from cDNA. The assignment of the backbone atoms was obtained using triple resonance NMR experiments. An initial structure was determined by backbone chemical shifts and homology modeling using the CS23D and Rosetta software packages. It was refined using RDC data to give realistic models for both domains. As we expected, these are both double-β-sheet sandwich structures characteristic of fibronectin type III domains. We are also carrying out relaxation measurements to probe the dynamics of the domains and their linker region.

**1300-Pos**

**Localization of the Fission Yeast U5.U2/U6 Spliceosome Subunits**

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The spliceosome is a dynamic macromolecular machine that catalyzes the excision of introns from pre-mRNA to generate protein-coding transcripts. The megadalton-sized spliceosome is composed of four small nuclear RNPs (U1, U2, U5, and U4/U6) and numerous pre-mRNA splicing factors. The formation of an active spliceosome is hypothesized to occur in a stepwise manner requiring the assembly and disassembly of large multiprotein/RNA complexes. A promising structural approach to obtain information about spliceosome complexes is single-particle cryo-electron microscopy (cryo-EM), a powerful technique that is ideal for determining the structures of large dynamic complexes at protein concentrations too low for crystallization. Formerly, our group determined structure of the fission yeast U5.U2/U6 spliceosome complex by cryo-EM. This U5.U2/U6 spliceosome complex contains the U2, U5, and U6 snRNAs, pre-mRNA splicing intermediates, U2 and U5 snRNP proteins, the Nineteen Complex (NTC), and second-step splicing factors. However, the location of these subunits in the complex was not determined. Using antibody labeling and single particle EM we are now localizing these individual subunits within the density map of the U5.U2/U6 spliceosome complex. This work now enables us to propose a structural model for U5.U2/U6 organization.

**1301-Pos**

**3D Solution Structure of the C-terminal Chromodomain of the Chloroplast Signal Recognition Particle**

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Chloroplasts use chloroplast signal recognition particle (cpSRP) pathway to import important cargo like light harvesting chlorophyll protein (LHCP).