MS04 P01

Structural and functional studies of the probiotic organism *Lactobacillus salivarius* Mario Bumann[a], Heinz Gut[a], F. Fang[b], Paul O'Toole[b], & Martin A. Walsh[a]
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Keywords: Probiotics, Bacterial Adherence, Structural proteomics

Probiotics are live bacterial strains in foods which upon ingestion in certain amounts are beneficial to health beyond inherent basic nutrition. They have been shown to have a positive effect in the prevention and treatment of specific gastrointestinal disorders and in counteracting gut barrier dysfunction associated with inflammation and infection. Accordingly, specific probiotic strains have been shown to prevent diarrhea, shorten the duration of diarrheal episodes, and alleviate inflammatory responses. Other studies have shown that probiotics may alleviate lactose intolerance; have a positive influence on the intestinal flora of the host; competitively exclude pathogens; possess anti-colon cancer effects; reduce the clinical manifestations of atopic dermatitis, Crohn's disease, constipation, candidiasis, and urinary tract infections [1]. The mechanisms behind favourable clinical outcome from the use of probiotics are still largely unknown. As a first step, understanding the basis of gastric survival and colonization is being pursued at both the structural and functional level for the probiotic bacterium *Lactobacillus salivarius* that colonizes the human gastrointestinal tract.
An important trait for potential probiotic strains is their ability to adhere to the intestinal mucosa. The adherence of microorganisms to host tissues is often presented by surface proteins like fibronectin.
Here, we will present results from the structural analyses of the fibronectin binding protein of *L. salivarius*. Moreover, these studies are being complemented by work on a fibronectin binding protein from the pathogen *Streptococcus pneumoniae*. The sequence identity of these proteins is 44%. This protein plays a direct role in the pathogenesis of pneumococcal infections and has been identified as a potential vaccine candidate.

[1] Mercenier A, Pavan S, Pot B: Probiotics as biotherapeutic agents: present knowledge and future prospects. *Current pharmaceutical design* 2003, 9(2):175-191.

MS04 P02

Bone Morphogenetic Protein Type II Receptor Structure in Two Crystal Forms. Sue Cutfield, John Cutfield, Peter Mace. *Biochemistry Department, University of Otago, Dunedin, New Zealand.*.
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Keywords: fertility, SAD, ligand-binding

BMPRII is a type II TGF-ß serine threonine kinase receptor integral to the bone morphogenetic protein (BMP) signalling pathway. It is known to bind BMP and growth differentiation factor (GDF) ligands, and has overlapping ligand specificity with the activin type II receptor, ActRII. BMP signalling is important in many growth and development pathways as well as in mammalian reproduction.
Crystals of the human BMPRII ectodomain were grown in two different forms from the same crystallization conditions. The structures retain the basic three-finger toxin fold of other TGF- ß receptor ectodomains, and share the main hydrophobic patch used by ActRII to bind various ligands. However, they present different conformations of the A-loop at the periphery of the proposed ligand-binding interface, as a result of Cys94 'switching' between two rotamers. Evidence is presented that the two crystal forms represent ligand bound and free conformations of BMPRII.

MS04 P03

Interaction of SRP54 GTPase and SRP RNA in the free signal recognition particle Tobias Hainzl, Shenghua Huang & A. Elisabeth Sauer-Eriksson *Umeå Center for Molecular Pathogenesis, Umeå University, SE-901 87 Umeå, Sweden.* E-mail: tobias.hainzl@ucmp.umu.se

Keywords: ribonucleoproteins, protein-RNA crystal structures, RNA-protein interactions

The signal recognition particle (SRP) is a ubiquitous protein-RNA complex which targets proteins to cellular membranes for insertion or secretion. A key-role in SRP-mediated protein targeting has the conserved core consisting of the S domain of SRP RNA and the multi-domain protein SRP54. The SRP54 M domain anchors SRP54 on the SRP RNA and recognizes signal sequences of nascent polypeptide chains while the SRP54 GTPase domain, comprising the N and G domains, binds to the SRP receptor. In free SRP a direct interaction of SRP RNA with the GTPase domain has been proposed, but has never been structurally verified. Here we present the crystal structure at 2.5 Å resolution of the SRP54-SRP19-SRP RNA complex of *Methanococcus jannaschii* SRP. The structure shows that the SRP54 GTPase domain interacts with the SRP RNA in a domain arrangement in which the GTPase domain is spatially well separated from the signal peptide binding site. Given the association of both the N and G domains with SRP RNA, the restricted SRP54 inter-domain communications in free SRP suggest a regulatory function for SRP RNA. The assembly of SRP is a hierarchical process where SRP19 binding to SRP RNA precedes SRP54 binding. The previously solved structures of the SRP RNA of *M. jannaschii* in its free form and bound to SRP19 together with the present structure disclose the structural changes in SRP RNA which ultimately lead to high affinity binding of SRP54.

MS04 P04

Structure and function of Survivin-Borealin-INCENP Core Complex in mitosis A. A. Jeyaprakash[a], U. R. Klein[b], E. A. Nigg[b], E. Conti[ab] [a]*European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, D-69117 Heidelberg, Germany.* [b]*Max-Planck-Institute of Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany.*. E-mail: jeyaprak@embl.de

Keywords: protein-protein interaction, mitosis, Chromosomal Passenger Complex

Survivin, Borealin, INCENP and the kinase Aurora-B are the members of the Chromosomal Passenger Complex

(CPC). The CPC is an essential regulator of mitosis and coordinates multiple chromosomal and cytoskeletal events, such as the correction of centromere-microtubules attachment, the stabilization of the spindle and the completion of cell division. In performing these diverse functions, the complex moves from the inner centromere to the central spindle during the metaphase-anaphase transition, and finally translocates to the midbody during cytokinesis[1]. Localization is key to its function, as the CPC acts in phosphorylating multiple protein targets during mitotic progression. The three regulatory components of the complex (INCENP, Survivin and Borealin) target the CPC enzymatic activity (the kinase Aurora B) at the correct place and time during cell division, ensuring the phosphorylation of the correct set of substrates. We have determined the 1.4 Å resolution crystal structure of the regulatory core of the CPC and explored the requirements for targeting the CPC to the central spindle and midbody. Survivin, Borealin and INCENP interact as a 1:1:1 complex rather than as an oligomer as was instead expected[2]. We have engineered structure based mutants to dissect the CPC into different subcomplexes. siRNA rescue experiments with mutants reveal that the CPC functions as a single structural unit and the intertwined structural interactions of the core components lead to a functional interdependence. Association of the regulatory 'passenger' subunits creates a helical bundle, whose composite molecular surface presents conserved residues essential for central spindle and midbody localization.

[1] Vagnarelli, P., and Earnshaw, W. C., *Chromosoma*, 2004, 113, 211.
[2] Vader, G., Medema, R. H., and Lens, S. M., *J Cell Biol.*, 173, 833.

MS04 P05

Structural basis for mRNA degradation by the RnaseJ.

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Keywords : **rinonuclease**, **metallo RNA enzymes**, **SAD**

Ribonucleases J1 and J2 of B. subtilis are evolutionarily conserved endoribonucleases with functional but no sequence homology to E. coli RNase E. We have resolved the cristal structure of the T. thermophilus RNase J orthologue by the SAD method. The active site of RNase J, with two zinc ions and a uridine monophosphate (UMP) residue, is located at the interface of a metallo-ß-lactamase and a ß-CASP domain (named for metallo-ß-lactamase, CPSF, Artemis, SnmI, Pso2). This core of the enzyme is connected through a flexible linker to a small C-terminal domain. The three dimensional arrangement of the different domains and the charge distribution of sites potentially involved in substrate recognition are surprisingly similar to the recently resolved structure of E. coli RNase E.

MS04 P06

Structural analysis of SmeT, a repressor of the *S. maltophilia* multidrug efflux pump SmeDEF. Maria J. Mate-Perez[a], Alvaro Hernandez[b], Jose Luis Martinez[b], Antonio Romero[a] *(a)Centro de Investigaciones Biologicas (CSIC) Departamento de Ciencia de Proteinas, C/Ramiro de Maeztu 9, 28040 Madrid, Spain (b) Centro Nacional de Biotecnología (CSIC) Departamento de Biotecnologia Microbiana, C/ Darwin 3, Campus de Cantoblanco, 28049 Madrid. E-mail: mmate@cib.csic.es*

Keyword: protein crystallography, transcription regulation, antibiotic resistance

SmeT from *Stenotrophomonas maltophilia* is a transcriptional repressor that belongs to the TetR family of transcriptional regulators. SmeT is involved in the regulation of smeDEF, the first several multidrug resistance pump (MDR) that has been described in *S. maltophilia*. The MDR's contribute to the intrinsic antibiotic resistance phenotype displayed by this bacteria that is an opportunistic pathogen associated with several human diseases.
SmeT is a dimeric, 219 residues protein that exhibits a 22% of identity to the tetracycline repressor (TetR) and 15% to QacR of *S. aureus*. Crystals of SmeT belong to the P21 space group with unit cell a=56.7 b=58.6 c=83.2 beta=103.1 and contain a dimer in the assymetric unit. The structure was solved by SIRAS using a mercury derivative. The structure of SmeT shows 10 alfa-helices, with one small N-terminal DNA-binding domain formed by three helices that constitute a classical helix-turn-helix (HTH) motif. The rest of the structure is formed by the dimerization/drug binding domain. Although this domain of SmeT, TetR and QarC displays little sequence homology they contain a region of significant structural homology. The structure of SmeT in complex with its DNA operator will give information on the way the protein recognises the DNA and the conformational changes involved on the binding.

MS04 P07

Unexpected domain architecture of Type IIP restriction endonuclease SdaI Giedre Tamulaitiene, Saulius Grazulis, Virginijus Siksnys, *Institute of Biotechnology. Vilnius, Lithuania.* E-mail: eigie@ibt.lt

Keywords: restriction endonuclease, domain structure, DNA-binding proteins

Restriction endonucleases comprise one of the largest and most diverse families of functionally related enzymes. Based on cofactor requirements, site specificity, subunit composition, enzymatic mechanism they have been classified into four types: I, II, III and IV [1]. Type II restriction endonucleases recognize short sequences of 4-8 base pairs and cleave DNA within or close to their recognition site [1]. All Type II restriction enzymes, except BfiI [2], exhibit similar structural core harboring conserved catalytic amino acid residues which give the name "PD-(D/E)XK" for the whole family [3]. Beyond the similarities of the structural core, restriction endonucleases show little resemblance. Type IIP restriction endonuclease SdaI recognizes palindromic 8 base pairs sequence 5'-CCTGCAGG-3' and cleaves it after an A base to produce four nucleotide 3'-overhangs. We obtained crystals of apo-SdaI by vapor diffusion method and solved a crystal structure by SIRAS at 2.0 Å resolution. Unlike orthodox Type IIP enzymes, which are single domain proteins [3], the SdaI monomer is composed of two structural domains. The N-terminal domain contains a classical winged helix-turn helix (wHTH) DNA binding