(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.

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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

motif, while the C-terminal domain shows a typical restriction endonuclease fold. By structural comparison and mutational analysis we showed that the active site of SdaI is located at the C-terminal domain and exhibits a new variation of the canonical PD...(D/E)XK active site motif. Mutational analysis of the residues from the predicted recognition helix of the wHTH motif suggests that SdaI determinants of sequence specificity are clustered at the N-terminal domain. The modular architecture of SdaI, wherein one domain mediates DNA binding while the other domain is predicted to catalyze hydrolysis, distinguishes SdaI from the previously characterized restriction enzymes interacting with symmetric recognition sequences.

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MS04 P08

C1-inhibitor structure reveals a novel mechanism of heparin potentiation Veronika Harmat[a,b], László Beinrohr[c] , József Dobó[c], Zsolt Lőrincz[c], Péter Gál[c], Gábor Náray-Szabó[a,b], Péter Závodszky[c] *[a]Structural Biology and Chemistry Laboratory, Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary. [b]Protein Modelling Group, Eötvös Loránd University, Hungarian Academy of Sciences, Budapest, Hungary. [c]Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest,Hungary.* E-mail: harmatv@ludens.elte.hu

Keywords: immune regulation, serine-protease inhibitors, electrostatic interactions

C1-inhibitor (C1-inh), a member of the serpin family of serine-protease inhibitors, is the major downregulator of inflammatory processes in blood plasma. Genetic deficiency of C1-inh results in hereditary angioedema, a dominantly inheritable, potentially lethal disease. Anti-inflammatory activity of heparin a naturally occurring glycosaminoglycan is realized through increasing inhibitory activity of C1-inh.
Activities of many serpins are modulated by ligand binding. Heparin enhances the inhibitory effect of antithrombin by "bridging" the two proteins. Allostery also plays an important role in the heparin activation of antithrombin. Polyanion potentiation of protein C inhibitor against protein C by the "co-occupation" mechanism is also based on migration of proteins along the heparin chain. These mechanisms fail to explain the effect of heparin on C1-inh activity.
We crystallized the serpin domain of C1-inh using the hanging drop method. Collection of diffraction data was carried out at 100 K on beamline X11 of EMBL-outstation at DESY, Hamburg, Germany. The structure was determined using molecular replacement and refined to 2.35 Å resolution.
The structure represents a novel latent serpin form with 7-stranded sheet A and a truncated disordered N-terminal domain, which explains functional consequences of the numerous naturally occurring mutations. Unique conformation of the C-terminal tail indicates its unexpected role as a barrier in the active-latent transition.
On the basis of surface charge pattern, heparin affinity measurements and docking of a heparin disaccharide, a heparin binding site is proposed in the contact area of the serpin-proteinase encounter complex. We show how polyanions change selectivity of C1-inh by a novel "sandwich" mechanism, explaining earlier reaction kinetic and mutagenesis data. These results provide the explanation of heparin's antiinflammatory activity and may help improving therapeutic C1-inh preparations in treatment of common inflammatory diseases, such as organ transplant rejection and heart attack.

MS04 P09

Crystal structures of flax rust avirulence proteins AvrL567-A and AvrL567–D. Gregor Guncar[a,b,1,2], Ching-I A. Wang[a,1], Jade K. Forwood[a,b], Trazel Teh[a], Ann-Maree Catanzariti[d], Gregory J. Lawrence[c], Horst Joachim Schirra[b], Peter A. Anderson[e], Jeffrey G. Ellis[c], Peter N. Dodds[c] and Bostjan Kobe[a,b,f]. *[a]School of Molecular and Microbial Sciences, University of Queensland(UQ), Brisbane, Australia [b]IMB, UQ, Brisbane, Australia [c]Division of Plant Industry, CSIRO, Canberra, Australia [d]Department of Plant and Microbial Biology, University of California, Berkeley, USA [e]SBS, Flinders University of South Australia, Adelaide, Australia [f]SRC for Functional and Applied Genomics, UQ, Brisbane, Australia [1]These authors contributed equally to this work. [2]On leave from Josef Stefan Institute, Ljubljana, Slovenia.*. E-mail: g.guncar@uq.edu.au

Keywords: avirulence, plant resistance, SAD

Plants have evolved a versatile multi-layered defence system to fight pathogens. The gene-for-gene model of plant disease resistance involves direct or indirect recognition of pathogen avirulence (Avr) proteins by plant resistance (R) proteins. Flax rust (*Melampsora lini*) AvrL567 avirulence proteins and the corresponding flax (*Linum usitatissimum*) L5, L6 and L7 resistance proteins interact directly. We determined the three-dimensional structures of two members of the AvrL567 family, AvrL567-A and AvrL567-D. The structure of AvrL567-A was solved by single-wavelength anomalous dispersion (SAD) technique, using the dataset collected on the conventional in-house Cu X-ray generator, taking advantage of a bound Co^{2+} ion. The structure of AvrL567-D was solved by molecular replacement, using the structure of AvrL567-A as a search model. The structures of both proteins are very similar and reveal a β-sandwich fold with no close known structural homologues. The polymorphic residues in the AvrL567 family map to the surface of the protein and polymorphisms in residues associated with recognition differences lead to significant changes in surface chemical properties. Analysis of single amino acid substitutions in AvrL567 proteins confirm the role of individual residues in conferring differences in recognition and suggest that recognition specificity of the L proteins for the AvrL567 family members results from the cumulative effects of multiple amino acid contacts. The structure also provides insights into possible pathogen-associated functions of AvrL567 proteins, with nucleic acid binding activity demonstrated *in vitro*, suggesting a possible role in influencing host gene expression during infection. Our studies provide some of the first structural information on avirulence proteins that