(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