**PS04.10.14 YEAST CYTOSOLIC CYCLOPHILIN A AS ANALOGY OF HUMAN ISOFORM.** Michiko Konno, Sawako Fujioka, Akiko Kashima, Ochanomizu University, Department of Chemistry, Faculty of Science, Otsuka, bunkyo-ku, Tokyo 112 Japan

Prokaryotes to eukaryotes have ubiquitously and abundantly cyclophilin (CyP) soluble in the cytoplasm; CyPA and membrane-bound CyP; CyPB. While CyPB is seen to be homolog to ninaA, biological role of CyPA is still poorly understood. Even though yeast strains lacking either or both CyPs have been reported to grow normally, CyPA should be involved in some important machinery for life cycle of alive cell. Close comparison of structrural natures between yeast CyPA here determined and human CyPA (hCyPA) previously reported (1) is made to confirm functional identity of these two.

Crystals belong to *triclinic*, space group *P1* with lattice constants of $a$=44.45(2), $b$=53.11(2), $c$=32.018(6) Å, $\alpha$=84.92(2), $\beta$=95.22(4), $\gamma$=108.56(4)°, Z=2, Dobs=1.21gcm$^{-3}$, Vm=2.07Å$^3$Da$^{-1}$, Vsol=40.6%. Intensity data were collected using Sakabe camera at BL6A2 of Photon Factory in Tsukuba. The structure of yCyPA was solved by molecular replacement method using hCyPA as a search model (XPLOR program). Refined model of two CyPA molecules and 126 water molecules gave Rfactor of 18.5% for 17931 reflections (F>2$\sigma$) in the region of 5.0 to 1.9 Å resolution. All C$\alpha$ atoms of two CyPAs are well superimposed with a r.m.s. deviation of 0.23 Å.

yCyPA also has $\beta$-barrel structure composed of two orthogonal four stranded antiparallel $\beta$-sheets flanking two $\alpha$-helixes at the top and bottom as in hCyPA and E. *coli* CyPA (2). The predicted peptide binding site is identified to be located in the cleft on the upper $\beta$-sheet. In yCyPA the same residues take the same arrangement as those essential to recognition and binding of peptide-substrates in hCyPA. The hydrophobic pocket, by which peptide-substrates are recognized, is formed by side chains of five non-polar residues; Phe58, Met59, Trp119, Leu120 and His124 with Phe111 at bottom; and Arg53 and Asn100 are positioned on the opposite side of the pocket such that the backbone of the peptide containing a proline can be hydrogen bonded by these two residues to make the proline *cis*-form.

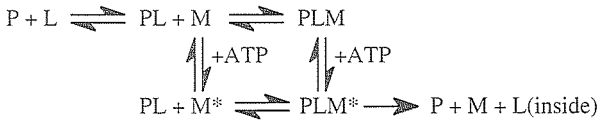
(1) H. Ke, *J. Mol.Biol.* (1992) **228**, 539-550.
(2) M. Konno *et al.*, *J. Mol.Biol.* (1996) **256**, 897-908.

**PS04.10.15 CRYSTAL STRUCTURE OF A PHOSPHATASE RESISTANT MUTANT OF SPORULATION RESPONSE REGULATOR SPO0F FROM BACILLUS SUBTILIS.** Madhusudan, John M. Whiteley, James A. Hoch, James Zapf, Nguyen H. Xuong* and Kottayil I. Varughese*, The Scripps Research Institute, La Jolla, CA 92037 and *University of California at San Diego, La Jolla, CA 920930359

Spo0F is an aspartyl pocket containing phosphotransferase in the signaling pathway controlling sporulation in *Bacillus subtilis*. It belongs to the superfamily of bacterial response regulatory proteins, which are activated upon phosphorylation of an invariant aspartate residue in a divalent cation dependent reaction by cognate histidine kineses. We have determined the crystal structure of a Rap phosphatase resistant mutant, Spo0F Y13S, at 1.9 Å. The structure was solved by single isomorphous replacement and anomalous I scattering techniques. The overall structural fold is ($\beta/\alpha$)5 and contains a central $\beta$-sheet. The active-site of the molecule is formed by three aspartates and a lysine at the carboxyl end of the $\beta$-sheet and it accommodates a calcium ion. The structural analysis reveals that the overall topology and metal binding coordination at the active-site were similar to the chemotaxis response regulator CheY. Structural differences between Spo0F and CheY in the vicinity of the active-site provide insight into how similar molecular scaffolds can be adapted to perform different biological roles by alteration of a few amino acid residues. These differences may contribute to the observed stability of the phosphorylated species of Spo0F, a feature demanded by its role as a secondary messenger in the phosphorelay controlling sporulation.

**PS04.10.16 A DOMINANT-NEGATIVE MUTANT OF *E. COLI* MALTOSE-BINDING PROTEIN: X-RAY STRUCTURES AND GENERAL LESSONS.** Sherry L. Mowbray and Brian H. Shilton, Department of Molecular Biology, Swedish Agricultural University, S-751 24 Uppsala, Sweden

Studies of a dominant-negative mutant of *E. coli* maltose-binding protein shed new light on the mechanism of binding protein-dependent transport systems. X-ray structures and small-angle X-ray scattering studies of open, ligand-free forms of the mutant show that the unliganded protein is essentially identical with the wild type protein, while only a small region of the protein located between the two structural domains is altered in the closed, ligand-bound form. The physiological effects of the mutant can be explained by the kinetic scheme:




where P, L and M are binding protein, ligand, and membrane permease complex, with M* representing the activated form necessary to transport. It is proposed that wild type binding protein promotes the M to M* transition through binding more tightly to M*, in the same way that enzymes function by binding more tightly to transition states. In the mutant, the binding to M* is weaker, and though binding to M can occur, the transition to M* is in that case not favored. This proposal explains the basic function of the transport system, as well as a number of previously puzzling results. The structures of both binding protein and membrane permease are likely to be more open in the activated complex, a suggestion supported by the observation that multiple forms are possible within the primarily "closed" and "open populations in solution.

**PS04.10.17 CELL CYCLE PROTEINS.** M.E. Noble*, J.A. Endicott*, N.R. Brown*, E. Garman*, R. Dzivenu*, A. Lawrie*, L.N. Johnson*, P. Nurse, and R.T. Hunt+.*Laboratory of Molecular biophysics, South Parks Road, Oxford, U.K., +ICRF, Clare Hall Labs., South Mimms, Herts, U.K., = ICRF, Lincoln's Inn Fields, London, U.K

Progress through the cell cycle is regulated by the "Cyclin Dependent Kinases" (CDKs). Different CDKs are activated at appropriate times by association with cognate "cyclins", and by phosphorylation of a conserved threonine. CDKs may also be found in complex with other proteins, such as p13$^{suc1}$ and the CDK inhibitors. Our aim is to study proteins of the cell cycle, and to understand the structural principles behind their actions and interactions. This study has produced the crystal structures of p13$^{suc1}$, and of an active 260 residue fragment of cyclin A (cyclin A3)[2].

Although p13$^{suc1}$ is essential for successful passage through the cell cycle, its mode of action is not known. Our structure of p13$^{suc1}$ reveals a compact, principally beta-sheet fold. It differs from the structure of a human homologue of p13$^{suc1}$, and from another structure of p13$^{suc1}$ itself [3], where details of the protein purification and crystallisation were different. These proteins formed dimers, in which beta strands from distinct monomers interlace to form a beta-sheet like that observed in our structure.