**PS04.16.11 CRYSTAL STRUCTURE OF THE YEAST CELL-CYCLE CONTROL PROTEIN, P13suc1, IN A STRAND-EXCHANGED DIMER.** N. Khazanovich[1], K. S. Bateman[1], M. Chernaia[1], M. Michalak[2], M. N. G. James[1], [1]MRC Group in Protein Structure and Function, [2]Cardiovascular Disease Research Group, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada TOG 2H7

P13suc1 from *S. pombe* is a member of the CDC28 kinase specific (CKS) class of cell-cycle control proteins, that includes CKS1 from *S. cerevisiae* and the human homologues CksHs1 and CksHs2. P13suc1 participates in the regulation of p34cdc2, a cyclin-dependent kinase controlling the $G_1$-S and the $G_2$-M transitions of the cell cycle. The CKS proteins are believed to exert their regulatory activity by binding to the kinase, in which case their function may be governed by their conformation or oligomerization state. Analysis of various assemblies of the CKS proteins, as found in different crystal forms, should help to clarify their role in cell cycle control. Previously determined X-ray structures of p13suc1, CksHs1 and CksHs2 show that these proteins share a common fold but adopt different oligomeric states. P13suc1 and CksHs1 were solved as monomers [1,2]. In addition, CksHs2 and pl3suc1 were observed in assemblies of strand-exchanged dimers [3,4].

We report the X-ray crystal structure of pl3suc1 to 1.95 Å resolution in space group $C222_1$. It is present in the crystals as a strand-exchanged dimer. The overall monomeric fold is preserved in each lobe of the dimer but a single ß-strand (Ile94 to Asp102) is exchanged between the central ß-sheets of each molecule.

Strand exchange, which has been observed for p13suc1 in two different space groups, and for CksHs2, is now confirmed to bc an intrinsic feature of the CKS family. A switch between levels of assembly may serve to coordinate the function of the CKS proteins in cell cycle control.

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**PS04.16.12 STRUCTURAL COMPARISON OF TWO HIGHLY HOMOLOGOUS THERMOPHILIC BACTERIAL ALCOHOL DEHYDROGENASES.** Yakov Korkhin, Felix Frolow, Oren Bogin, Moshe Peretz, A. Joseph Kalb(Gilboa) and Yigal Burstein, Faculty of Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel.

The NADP-dependent alcohol dehydrogenases from *Thermoanaerobium brockii* (TBAD) and *Clostridium beijerinckii* (CBAD) have 75% sequence identity and yet they differ by 26°C in their inactivation temperatures ($T_{1/2}^{60min.}$= 93°C for TBAD and $T_{1/2}^{60min.}$= 67°C for CBAD). The structures of TBAD and CBAD in the holo-enzyme form have been solved and refined to a resolution of 2.5Å and 2.05Å respectively. The overall three-dimensional structures are highly homologous with $RMSD_{C\alpha}$= 0.6Å. and $RMSD_{all\ atoms}$= 1.0Å. Structural differences between the two enzymes are discussed in terms of their role in thermostabilization. Based on the 3D-structures, certain point mutations have been engineered and their consequences for enzyme stability have been assessed.

**PS04.16.13 STRUCTURE OF CALCIUM-FREE MANNOSE-BINDING PROTEIN** Kenneth K.-S. Ng, Shaun S. Snyder, William I. Weis, Department of Structural Biology, Stanford University, Stanford, CA 94305-5400

Calcium ions play key roles in the structures and biological activities of the carbohydrate-binding proteins known as C-type lectins. In the liver asialoglycoprotein receptor, for example, the reversible conformational change that accompanies calcium ion binding and release is integral to the protein's role as an endocytic carrier. Crystal structures of the calcium-bound form of several C-type lectins have previously been determined. We now report the structure of the calcium-free form of the carbohydrate-recognition domain of rat liver mannose-binding protein (MBP-C) and the one-calcium form of rat serum mannose-binding protein (MBP-A). The structures were solved by molecular replacement using the calcium-bound structures of MBP-C and MBP-A as search models. The loops which are involved in calcium-binding in the native state adopt different conformations when calcium ions are not present. For MBP-C, the four copies of the protein in the asymmetric unit reveal an additional range of loop conformations. Structural changes correlate with the kinetics of calcium-dependent changes in intrinsic tryptophan fluorescence.

**PS04.16.14 WHY IS *BACILLUS LICHENIFORMIS* ALPHA-AMYLASE SO THERMOSTABLE?** A. Shaw and R.Bott. Genencor International Inc., 180 Kimball Way, South San Francisco, CA94080.

*Bacillus licheniformis* is a mesophilic organism that secretes an alpha-amylase which is hyperthermostable. It is used industrially for starch liquefaction, during which the enzyme and substrate are steam jetted at 105°C or greater, with retention of 80% of its activity. This α-amylase requires calcium for activity and stability. It has been crystallized in an orthorhombic form with two molecules/asymmetric unit.

To begin to understand why a protein from a mesophile is hyperthermostable, the crystal structure of the enzyme with calcium bound has been determined, and refinement completed to 1.9 angstroms resolution.

We shall present a comparison of the *Bacillus* alpha-amylase structure with known non-thermostable alpha-amylase structures, and discuss the factors responsible for the remarkable stability of the *Bacillus* enzyme.

**PS04.16.15 ADAPTATION TO EXTREME ENVIRONMENTS: INSIGHTS FROM HALOPHILIC FERREDOXIN AND ACIDOPHILIC RUSTICYANIN.** Menachem Shoham and Dong Zhao, Case Western Reserve University School of Medicine, Department of Biochemistry, Cleveland, Ohio 44106-4935.

What causes a protein to withstand extremes in pH or to remain soluble in saturated salt? Some insights can be gained by comparing the crystal structures of extremophilic proteins with those of other members of the same family.

The crystal structure of a halophilic 2Fe-2S ferredoxin from *Haloarcula marismortui* suggests two mechanisms for keeping this protein soluble and active at the saturated salt solution prevailing in the cytosol of this archaebacterium: 1. *halophilic substitution* of polar uncharged surface residues by aspartic and glutamic acid; 2. *halophilic addition* of an extra domain consisting of two amphipathic helices and intervening loops. This domain is inserted in between the two antiparallel β strands 1 and 2 instead of the β hairpin found at this position in plant-type 2Fe-2S ferredoxins. The surface of this domain is entirely made up of 15 carboxylates. Glutamic and aspartic acid are known to be the best water-binding