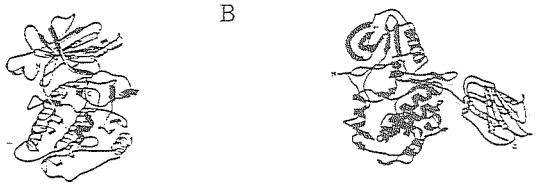
twitchin (B) containing the autoinhibited kinase domain and a C-terminal Ig-like domain shows that the Ig-like domain extends from the kinase domain opposite to the active site and exposes possible myosin interacting surfaces. Our studies of the regulation and domain architecture of giant protein kinases jointly with an analysis of autoinhibitory sequences of other autoinhibited kinases point to possible common and diverse features of the intrasteric regulatory mechanisms.

A B



MS04.10.07 CRYSTAL STRUCTURE OF A HISTIDINE KINASE DOMAIN OF THE ANAEROBIC SENSOR PROTEIN ARCB FROM *E. COLI*. Toshio Hakoshima[1], Masato Kato[1], Toshiyuki Shimizu[1], Kazuya Ishige[2], and Takeshi Mizuno[2]. [1]Department of Molecular Biology, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama, Ikoma, Nara 630-01, Japan and; [2]School of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464, Japan

The *Escherichia coli* ArcB protein is the anaerobic sensor and a member of the large family of the so-called two-component signal transduction proteins. ArcB comprising 778 amino acid residues possesses three phosphorylation sites (His-292, Asp-576, His-717). These multi-phosphorylation sites were suggested to make a phosphotransfer circuit that is crucial for the mechanism underlying signal transduction through ArcB. The C-terminal histidine kinase domain is composed of 125 amino acid residues, in which the active His-717 is located. Here, three-dimensional structure of this domain has been determined at 2.06Å resolution. The crystal belong to the space group $P2_12_12_1$ with unit cell dimensions, a=30.56Å, b=34.93Å, c=1 10.78Å. The structure was solved by MIR methods and was refined. The current model has an R-factor of 23%. The kinase domain of ArcB is found to form an all-α structure consisting of six helices (H1 to H6); 75% of its residues locates on the α helices. The molecules has a kidney-like shape with dimensions, 30Å by 30Å by 45Å. Two helices H3 and H6 are 7- and 8-turns long, respectively, and are bent at the centers of the helices. The helices H4 and H5 form a four-helix bundle subdomain together with the C-terminal half of the helix H3 and the N-terminal half of the helix H6. The active residue, His-64 (corresponding to His-717 in the intact ArcB), locates at the surface of the helix H4 and is surrounded with Glu61, Lys-65 and Lys-67 from the same helix and with Gln-83 and Gln-86 from the helix H5. This active site lies in the internal curvature of the kidney-shaped molecule. The helices H1 and H2 form another subdomain with the N-terminal half of the helix H3 and the C-terminal half of the helix H6.

MS04.10.08 STRUCTURE OF THE PHOSPHORYLATED FORM OF THE CDK2:CYCLIN-A COMPLEX. Philip D. Jeffrey, Alicia A. Russo, Nikola P. Pavletich, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA

Progression through the eukaryotic cell cycle is controlled by the cyclin-dependent kinases (CDKs), which are in turn under the control of multiple levels of regulation. The isolated CDK is inactive, becoming partially activated upon binding of the corresponding cyclin subunit. Full activation of the kinase complex is achieved upon phosphorylation at a site on the CDK subunit (Thr 160 in CDK2).

We have previously determined the structure of a CDK2:CyclinA complex in its unphosphorylated state at 2.3 Angstrom resolution (ref. 1). Comparison of the CDK2 subunit with the structure of the uncomplexed CDK2 (ref. 2) revealed extensive conformational changes in two regions (the PSTAIRE helix and activation loop). In contrast, comparison of the cyclinA subunit with the uncomplexed cyclinA structure (ref. 3) did not indicate any conformational change on complex formation.

Subsequently, we have crystallized the fully active complex containing CDK2 that was phosphorylated at Thr 160. The structure of this complex reveals further conformational changes caused by phosphorylation. The contribution of these changes to the full activation of the kinase will be discussed.

1. Jeffrey, P. D., Russo, A. R. and Pavletich, N. P (1995) *Nature* 376, 313-320.
2. DeBondt, H. L., Rosenblatt, J., Jancaraik, J., Jones, H. D., Morgan, D. O. and Kim, S.-H. (1993) *Nature* 363, 595-602.
3. Brown, N. R., Noble, M. E. M., Endicott, J. A., Garman, E. F., Wakatsuki, S., Mitchell, E., Rasmussen, B., Hunt, T. and Johnson, L. N. (1995) *Structure* 3, 1235-1347

MS04.10.09 HORMONE BINDING TO THE THYROID HORMONE RECEPTOR. R. Fletterick, R. Wagner, J. Baxter, A. Shiau, J. Apriletti. University of California, San Francisco, Dept. of Biochemistry/Biophysics, San Francisco, CA 94143-0448, USA

This receptor is a central metabolic regulator and is a member of the family of nuclear receptors that regulate transcription of responsive genes. The structures of two isoforms α and β were determined to understand the mechanism of recognition of hormone and the changing relationship of the receptor to its receptor, repressor and activator partners. The ligand binding domain (LBD) of the receptor comprises 12 helices and employs the hormone as a component of the hydrophobic core of the domain. The structures of the LBD with hormone and hormone analogs suggest means for precise recognition. The LBD can form dimers with homologs of the receptor or homodimers. The structures of two different homodimers have been determined to learn whether the means of association is used in transcriptional control.

PS04.10.10 CRYSTAL STRUCTURE OF mTNF. K.J. Baeyens, P. Brouckaert*, A. Raeymaekers*, W. Fiers*, C.J. De Ranter & H.L. De Bondt, Laboratory for Analytical Chemistry and Medicinal Physicochemistry, K.U.Leuven, Leuven, Belgium; *Laboratory of Molecular Biology, VIB and U. of Ghent, Belgium

TNF is a trimer consisting of 17 kDa subunits. The 3D-structure of human (h) TNF at 2.6 Å resolution has been reported. TNF interacts with two types of receptor, TNF-R55 and TNF-R75. Cellular signaling occurs by triggering one or the other, or both receptors, depending on the cell type and conditions. hTNF in the mouse only interacts with TNF-R55, and hence is a specific ligand for the latter. Remarkably, in the normal mouse, hTNF is 50-fold less toxic as compared to murine (m) TNF, indicating that the

interaction of mTNF with TNF-R75 contributes in a specific way to the systemic toxicity. In order to investigate in more detail these receptor-specific interactions, we have established the 3D-structure of mTNF. The latter was expressed in soluble form in E. coli, and purified by established procedures. Crystals suitable for X-ray studies were produced by the hanging drop vapor diffusion technique at 5 °C using methoxypolyethylene glycol 2000 and isopropanol as precipitants. The crystals belong to the space group P1 with unit cell dimensions a = 49.45 Å, b = 49.92 Å, c = 52.32 Å, α= 114.63°, β = 104.15°, γ= 90.44° and one trimer in the asymmetric unit. Crystals were stable to X-rays and diffracted beyond 2.8 Å. Cooling the crystals during data collection was necessary, since the crystals dissolved at room temperature.

The 3D-structure of mTNF was solved by molecular replacement using hTNF as a model. The structure provides insights into the difference in murine receptor specificity between hTNF and mTNF. Also, a firm basis is now avaliable to explain the structure/function relationship of receptor-specific mTNF muteins.

PS04.10.11 CRYSTAL STRUCTURE OF CheR, BACTERIAL CHEMOTAXIS RECEPTOR METHYLTRANSFERASE. Snezana Djordjevic and Ann Stock. Center for Advanced Biotechnology and Medicine, Department of Biochemistry, UMDNJ, Piscataway, New Jersey 08854, and Howard Hughes Medical Institute.

CheR is a methyltransferase which catalyzes the *S*-adenosyl-L-methionine-dependent transfer of methyl groups to specific glutamate side chains on a set of membrane chemoreceptor proteins that mediate bacterial chemotaxis. Methylation/demethylation of the receptors is involved in cellular adaptation to the concentrations of the chemoeffectors. An increase in attractant concentration in the medium results in an increase in the level of receptor methylation whereas a negative signal, such as removal of an attractant or an increase in repellent concentration is followed by a net decrease in receptor methylation.

The methyltransferase from *Salmonella typhimurium*, has been over-expressed in *E. coli* and purified to homogeneity. The enzyme was crystallized in the presence of the product of the methylation reaction, *S*-adenosyl-L-homocysteine. Multiple rounds of microseeding were required to obtain crystals suitable for X-ray diffraction studies. The crystal lattice belongs to a monoclinic space group $P2_1$ with unit cell dimensions of a = 55.0 Å, b = 48.0 Å, c = 63.2 Å and β = 112.3°. There is one monomer of 283 residues per asymmetric unit and Matthew's value is 2.4 $Å^3$/Da. An X-ray diffraction native data set to 2 Å, with an R_{sym} of 4.9 %, has been collected on an R-axis II image plate detector. Initial phases were obtained to 3 Å using several mercurial and one platinum derivative crystals which gave an overall figure of merit of 0.71. An MIR electron density map was subjected to density modification procedure. This map contained interpretable elements of secondary structure and enabled us to build a partial model. Subsequent electron density maps were calculated by combining MIR and partial model phases. CheR is a mixed α/β protein with two distinct domains. We have identified electron density corresponding to *S*-adenosyl-L-homocysteine associated with the larger domain.

PS04.10.12 CRYSTAL STRUCTURE OF THE SH2 DOMAIN OF THE PROTEIN SHC: pH-DEPENDENT SELF-ASSOCIATION. Klaus Fütterer, Stéphane Réty, Richard A. Grucza, Gabriel Waksman, Washington University School of Medicine, Department of Biochemistry and Molecular Biophysics, Saint Louis, MO 63110, USA.

The Src Homologous and Collagen-like (SHC) protein plays an essential role in signal transduction pathways leading to the activation of the protein Ras. SHC contains a Src Homology 2 (SH2) domain, the function of which is to specifically recognize tyrosine phosphorylated peptide ligands. The structure of an SH2 domain can be described as a central β-sheet flanked by two α-helices (G. Waksman et al., J. Kuriyan, 1992, Nature 358: 646-653). The peptide binding surface of SH2 domains in general consists of two binding pockets on either side of the central β-sheet. While the binding pocket for the phosphotyrosine is conserved, its counterpart accommodating the third amino acid C-terminal to the phosphotyrosine varies in shape and depth between different SH2 domains, reflecting specificity for different peptide ligands.

This contribution presents the crystal structure of the SH2 domain of SHC determined by multiple isomorphous replacement at a resolution of 2.5 Å (S. Réty et al., G. Waksman, 1996, Protein Science, in press). The SH2 domain of SHC reveals a fold similar to other SH2 domains. The peptide binding surface of SHC-SH2 resembles that of the SH2 domain of Src (G. Waksman et al., J.Kuriyan, 1993, Cell 72: 779-790) in that it presents a clearly delineated pocket for the third residue C-terminal to the phosphotyrosine, but it is deeper and more distinct in SHC than in Src.

A novel feature of the SH2 domain of SHC is the observation of an intermolecular disulfide bond accompanied by an extensive dimer interface between two symmetry-related molecules. The potential dimerization of SHC-SH2 in solution was studied under reducing conditions using analytical ultracentrifugation and polyacrylamide gel electrophoresis. The results suggest that the SH2 domain of SHC dimerizes in a pH-dependent manner, where low pH conditions (~pH 4.5) are conducive to dimer formation. Dimerization of SHC may have important biological implications, possibly promoting the assembly of large heteromultimeric signaling complexes.

PS04.10.13 STRUCTURE AND IMPORTANCE OF THE DIMERIZATION DOMAIN IN ELONGATION FACTOR TS FROM *THERMOS THERMOPHILUS*. Youxing Jiang*, Steffen Nock†, Martina Nesper†, Mathias Sprinzl†, Paul B. Sigler#‡, *The Department of Chemistry, #The Department of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale Univ., CT 06511, USA, †Laboratorium fur Biochemie, Universitat Bayreuth, ‡To whom correspondence should be addressed

Elongation factor Ts (EF-Ts) functions as a nucleotide exchange factor which catalyze the GDP/GTP exchange in elongation factor Tu. *Thermus thermophilus* elongation factor Ts forms a homodimer in solution. The crystal structure of the EFTs dimerization domain has been solved and refined to a resolution of 1.7 Å. This high resolution structure gives a clear picture of the dimerization interactions of EFTs. The three-stranded anti-parallel β-sheet of each subunit comes together to form a β-sandwich held together by a disulfide bond between the Cysl90 of each subunit and by the hydrophobic core constructed by Ile60, His62, Ile64, Gly71, Leu73 and Phel92. Based on the structure, two mutants, C190A and L73D, were designed to disrupt the dimer interface. The activity analyses show that monomeric EF-Ts loses the ability to catalyze exchange of GDP/GTP in EF-Tu which indicates that *Thermus thermophilus* EF-Ts functions as a homodimer.