We are currently producing mutants to probe the function of p130Cas.

Cyclin A is first observed late in the G1 phase of the cell cycle, and its abundance increases throughout S and G2. It forms complexes with either CDK2 or CDK2. Our structure of a genetically truncated form of cyclin A (residues 171-452) consists of two strikingly similar helical domains, despite low sequence homology. Comparison with the structure of a complex between a fragment of cyclin A and CDK2 showed that in contrast to the major structural changes of CDK2, cyclin remains essentially unaltered when complexed. Thus, cyclin acts as a template, complimentary to the basally active conformation of CDK2 in the complexed structure.

Our poster will describe details of the results mentioned above, as well as the results of on-going work to further characterise these proteins.


PS04.10.18 CRYSTALLOGRAPHIC AND BIOCHEMICAL ANALYSIS OF AN INHIBITOR OF APOPTOSIS, BCL-2. Jason W. O'Neill and Kam Zhang, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA

Programmed cell death (apoptosis) plays an important role in animal development and homeostasis of cell numbers. The primary regulator of human apoptosis is Bcl-2, a novel regulator of human apoptosis is Bcl-2, a novel protein identified in chickens which strongly inhibits programmed cell death. Bcl-2 acts as a template, complimentary to the basally active conformation of CDK2 in the complexed structure.

PS04.10.20 CRYSTALLOGRAPHIC ANALYSIS OF A FRAGMENT FROM CHICKEN TENASCIN CONTAINING TWO FIBRONECTIN-TYPE III DOMAINS. Klaus Piontek, Daniel Bisig, Peter Weber, Lloyd Vaughan, Kaspar Winterhalter, Laboratory of Biochemistry, Swiss Federal Institute of Technology (ETH), CH-8092 Zürich, Switzerland.

Tenasin-C is an extracellular glycoprotein composed of six identical polypeptide chains which are linked together at their N-terminal ends to form a hexabrachion structure. Each polypeptide comprises a series of 13.5 tandem repeats homologous to epidermal growth factor followed by 8-13 fibronectin type III (TNfn)-like domains and finally one fibrinogen-like domain at the C-terminus. Alternative splicing of TNfn domains between the domains 5 and 6 leads to the three major isoforms found in chicken. In the smallest isoform (190 kDa) these two domains are adjacent to each other. Tenasin-C isoforms are differently expressed in development and in response to malignancy, leading to the expectation of isoform-specific functions. TNfn5 binds heparin and other uridrnia acid-containing glycosaminoglycans, presumably via a conserved heparin binding motif. Furthermore, TNfn5 binds to the neural cell adhesion molecule contactin/F11, an interaction which is inhibitable by heparin. To explore these interactions in greater detail we have prepared a fusion protein containing domains 5 and 6 and attempt now the crystal structure determination of this 20 kDa protein.

TNfn56 crystallizes from 27% PEG2000, 100mM Na-acetate, pH 4.5 at RT in a monoclinic (P2_1, a=47.7Å, b=54.9Å, c=97.0Å, β=104.4°) and a orthorhombic form using synchrotron radiation (DESY/Germany) and an orthorhombic (P2_2_2_1, a=45.3Å, b=71.9Å, c=58.6Å, V=2.2.6 Å³/da, 1 mol/u.) form. Both crystal forms diffract to about 2.6 Å resolution. The majority of the monoclinic crystals is twinned and the diffraction limit of this form frequently lies only between 3.2 and 4.0 Å. Furthermore, the formation of the orthorhombic form can not be directed. Despite the sparse availability of suitable single crystals we have collected a data set monocrystals to 2.6 Å resolution using synchrotron radiation (DESY/Germany) and a data set to 2.8 Å resolution on a conventional X-ray source. Presently we attempt the structure determination with the molecular replacement technique and, in parallel, with isomorphous replacement.

PS04.10.21 CURRENT PROGRESS ON CRYSTALLOGRAPHIC COMPLEXES OF TRANSCLUDIN. Susan M. Redford, Heidi Hamm†, Joseph Noel. Structural Biology Lab, The Salk Institute, La Jolla, CA 92037, University of Illinois College of Medicine, Chicago, IL 60680

The opportunity to understand the structure and function of a G protein signal transduction cascade at the atomic level is the underlying goal of this research. The current studies employ peptides that mimic rhodopsin’s functionally essential cytoplasmic face in complex with G_{αγ} to probe the structural consequences of receptor/G-protein binding. Recently the heterotrimer structure, G_{αγ}, has been resolved at 2 Å resolution. In these studies, a chimeric G_{αγ} crystal, which has been E. Coli expressed, is substituted for G_{αγ}. G_{αγ} crystals are then grown in complex with peptides which mimic rhodopsin’s cytoplasmic loops 2 and 3.

Signaling cascades provide mechanisms to receive, transmit and amplify cellular messages, and thus are critical pathways for mounting a cellular response to an extracellular stimulus. Heterotrimeric G-proteins have evolved as ubiquitous solutions for signal transduction and amplification throughout many cells, and are involved in processes such as vision, olfaction, hormone signaling, and responses to cytokines. The α subunit has been studied extensively biochemically, as it contains the GTPase function,