Acta Cryst. (2002). A**58** (Supplement), C6

A STRUCTURAL VIEW OF PROTEIN METHYLATION

X. Cheng J. Horton S. Khan X. Cheng
Emory University School of Medicine Biochemistry Dept. Rollins Research Building, Suite G239 1510 Clifton Road ATLANTA GEORGIA 30322 USA

Protein methylation on arginine and lysine residues is emerging to be an important post-translational modification in eukaryotes. The methylation affects interaction of target protein with other molecules, regulating cellular processes such as RNA processing and/or transport, chromatin structure and gene transcription. Protein arginine (R) methyltransferases (PRMT) have up to 8 members in human. PRMT1 is the predominant PRMT in mammal, accounting for 85% of the cellular PRMT activity and is essential for early postimplantation development in mouse. Here we describe the crystal structures of rat PRMT1 in complex with peptides containing single or three arginines, respectively. The results reveal a two-domain structure - an AdoMet-binding domain and a barrel-like domain - with the active site pocket located in between the two domains. Mutagenesis studies confirmed that two glutamic acids in the active site are essential for enzymatic activity and the dimerization of PRMT is essential for methyl donor AdoMet binding. Three peptide-binding channels are identified. The multiple peptide binding channels are probably important for binding of PRMT1 substrates that often contain multiple arginines in RGG or RXR contexts.

Protein lysine (K) methyltransferases (PKMT) belong to a rapidly growing family of enzymes that methylates specific lysines on histone tails. Histone methylation is an integral part of the 'histone code' that regulates the transcriptional accessibility of chromatin. We will present the first structure of a PKMT, discuss the role of conserved sequence motifs within the PKMT, particularly the SET domain that is widely spread among many proteins associated with chromatin function.

Keywords: PROTEIN METHYLTRANSFERASES, ARGININE METHYLATION, LYSINE METHYLATION

Acta Cryst. (2002). A**58** (Supplement), C6

STRUCTURE OF THE PLATELET GLYCOPROTEIN IBa N-TERMINAL DOMAIN REVEALS AN UNMASKING MECHANISM FOR RECEPTOR ACTIVATION

E. Jonas[1] S Uff[1] J Clemetson[2] K Clemetson[2] T Harrison[1]
[1]Department of Biochemistry, University of Leicester, Leicester LE1 7RH, United Kingdom [2]Theodor Kocher Institute, University of Berne, Freiestrasse 1, CH-3012 Berne, Switzerland

Glycoprotein Ib (GPIb) is a platelet receptor with a critical role in mediating the arrest of platelets at sites of vascular damage. GPIb binds to the A1 domain of von Willebrand factor (vWF) at high blood shear initiating platelet adhesion and contributing to the formation of a thrombus. GPIb contains binding sites for vWF, thrombin, P-selectin and Mac-1 in its extracellular N-terminal domain and for filamin and 14-3-3z in its cytoplasmic domain. Mutations in GPIb result in the congenital bleeding disorders, Bernard Soulier syndrome (BSS) and Platelet-type von Willebrand's disease (Pt-vWD). The interaction between GPIb and vWF is regulated and they do not normally interact under static conditions. To investigate the molecular basis of GPIb regulation and ligand binding we have determined the structure of the N-terminal domain of the GPIba chain. This structure is the first determined from the cell adhesion/signaling class of leucine rich repeat (LRR) proteins. The fold consists of an N-terminal b-hairpin, eight leucine-rich repeats, a disulphide bonded loop and a C-terminal anionic region. Negatively charged binding surfaces on the LRR concave face and anionic region indicate a two step kinetic binding, which can be regulated by an unmasking mechanism involving conformational change of a key loop. The structure also reveals a novel LRR motif in the form of an M shaped arrangement of three tandem b-turns

Keywords: RECEPTOR PLATELET GLYCOPROTEIN

Acta Cryst. (2002). A**58** (Supplement), C6

STRUCTURE OF DENGUE VIRUS: IMPLICATIONS FOR FLAVIVIRUS ORGANIZATION, MATURATION, AND FUSION

M. Rossmann[1] R. Kuhn[1] W. Zhang[1] S. Pletnev[1] J. Corver[2] E. Lenches[2] C. Jones[1] S. Mukhopadhyay[1] P. Chipman[1] E. Strauss[2] T. Baker[1] J. Strauss[2]
[1]Purdue University Biological Sciences 1392 Lilly Hall of Life Sciences WEST LAFAYETTE IN 47907-1392 USA [2]California Institute of Technology

The first structure of a flavivirus has been determined by using a combination of cryo-electron microscopy and fitting of the known structure of glycoprotein E into the electron density map. The virus core, within a lipid bilayer, has a less ordered structure than the external, icosahedral scaffold of 90 glycoprotein E dimers. The three E monomers per icosahedral asymmetric unit do not have quasi-equivalent symmetric environments. Difference maps indicate the location of the small membrane protein M relative to the overlaying scaffold of E dimers. The structure suggests that flaviviruses, and by analogy also α-viruses, employ a fusion mechanism in which the distal β-barrels of domain II of the glycoprotein E are inserted into the cellular membrane.

Keywords: DENGUE VIRUS, CRYOEM, FUSION

Acta Cryst. (2002). A**58** (Supplement), C6

UNCOATING OF HUMAN RHINOVIRUS 2

E.A. Hewat[1] E. Neumann[1] D. Blass[2]
[1]Institut de Biologie Structurale Jean-Pierre Ebel, 41 rue Jules Horowitz, 38027 Grenoble, France [2]Institute of Medical Biochemistry, University of Vienna, Vienna Biocenter (VBC), Dr. Bohr Gasse 9/3, A-1030 Vienna, Austria

Delivery of the rhinovirus genome into the cytoplasm of the cell for virus reproduction involves a co-operative structural modification of the viral capsid. We have studied this phenomenon for human rhinovirus serotype 2 (HRV2). The structure of the empty capsid has been determined to better than 1.5nm resolution by cryo-electron microscopy and the atomic structure of native HRV2 used to examine the conformational changes of the capsid and its constituent viral proteins VP1, VP2, VP3 and VP4. The transformation from full to empty capsid involves an overall 4% expansion of the radius. Both proteins around the 5-fold axis, VP1 and the N-terminus of VP3, make an iris type of movement to open up a 10Å diameter channel to allow exit of the RNA genome. The small VP4 and the 59 residues of the N-terminus of VP1 are not represented inside the capsid. There is evidence that the N-terminus of VP1 exits the capsid at the pseudo-3-fold axis at the junction of VPs 1, 2 and 3. A quite remarkable and unexpected modification occurs at the 2-fold axis where the N-terminal loop of VP2 bends inwards. This as yet undocumented movement of VP2 is probably associated with the detachment and expulsion of the RNA rather than the formation of an opening in the capsid for passage of RNA or protein.

Keywords: CRYO-ELECTRON MICROSCOPY UNCOATING HRV2