the C-terminus of VDAC1 is exposed to the cytosolic side, we expect to lose the His tag upon DEVD cleavage. As result only the HA can be detected at mitochondrial level. Conversely if the C-terminus is oriented towards the IMS, the DEVD is not cleavable and both tags will be detected in mitochondria. Our results by confocal microscopy and appropriate controls with other membrane-oriented constructs showed that this strategy is able to define the sidedness of the VDAC pore.


2772-Plat

Deciphering the Interaction of FLAP and SLO

Ramakrishnan B. Kumar1, Hans Hebert2, Caroline Jegerschold1,1
1Karolinska Institutet, Huddinge, Sweden, 2KTH, Huddinge, Sweden.

Leukotrienes are pro-inflammatory lipid mediators involved in chronic inflammatory
diseases like asthma and atherosclerosis. Different enzymes and proteins are involved in the leukotriene biosynthesis pathway that stems from oxygenation of arachidonic acid (AA) by SLO (5-lipoxygenase). FLAP (5-lipoxygenase activating protein) is an integral membrane protein, belonging to the MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione Metabolism) family. Till now, there is no prominent evidence for any biochemical or enzymatic activity for FLAP, except involvement as an “activator” in leukotriene biosynthesis. The hypothesis is that, on increase in intracellular calcium concentration, SLO moves from the cytosol to the nuclear membrane and localizes near FLAP. The AA is then transferred from the nuclear membrane to SLO by homo-trimeric FLAP. Then SLO converts the AA to leukotrien A4. This interaction between FLAP and SLO is ambiguous due to intricate mechanisms which occur at the interface of the nuclear membrane and also between an enzyme and an integral membrane protein with no documented function. To understand the function and involvement of FLAP in leukotriene biosynthesis in vitro, we employ soluble phospholipid bilayers called “Nanodiscs” which mimic a membrane environment. The nanodisc will act as a stable platform for structural and functional characterization of the interaction between SLO and FLAP. After initial studies of the Ca2+ dependent recruitment of SLO to empty nanodiscs as well as the reconstitution of FLAP into nanodiscs, the entire complex 5LO-FLAP-nanodisc can be targeted. We present results of our preliminary studies of the Ca2+ induced recruitment of 5LO to empty nanodiscs as well as the reconstitution of FLAP and also between an enzyme and an integral membrane protein with no documented function. To understand the function and involvement of FLAP in leukotriene biosynthesis in vitro, we employ soluble phospholipid bilayers called “Nanodiscs” which mimic a membrane environment. The nanodisc will act as a stable platform for structural and functional characterization of the interaction between SLO and FLAP.

540a Wednesday, February 6, 2013

2773-Plat

Membrane Anchoring Amplifies Osmosensing by the Inner Membrane Receptor EnvZ

Ganesh Anandi1,2, Jeremy Wang1, Linda Kenney1,3,1
1Department of Biological Sciences, National University of Singapore, Singapore, Singapore, 2Mechanobiology Institute, National University of Singapore, Singapore, Singapore, 3Department of Microbiology and Immunology, University of Illinois-Chicago, Chicago, IL, USA.

A fundamental question in signalling biology is how do receptors transmit the effects of extracellular stimuli across membranes? Transmembrane segments that connect a receptor extracellular domain to its intracellular domain are often considered to be critical elements for transmitting these signals. The dynamics of receptor signalling is not clearly understood, as there are few biophysical probes to directly monitor transmembrane regions. Amide Deuterium Exchange Mass Spectrometry (HDXMS) provides an excellent method to describe the conformational dynamics of membrane bound proteins. Our recently published studies with the cytoplasmic domain of the bacterial inner membrane osmosensor kinase EnvZ (EnvZc) have revealed the molecular basis for osmosensing through modulation of helix-coil transitions in a critical four helix bundle subdomain (Wang, LC, Morgan, L, Godakumbura, P, Kenney, LJ and Anand, GS, EMBO J. 31(11):2648-59, 2012). This study highlighted the importance of local folding-unfolding equilibria in the functioning of EnvZ signaling. Molecular level was that a cytoplasmic deletion fragment of EnvZ lacking the transmembrane helices was able to fully rescue the osmosensing function in a strain lacking the envZ gene. This immediately raised the question as to what is the function of membrane anchoring? Our preliminary results indicate that the helix-coil transitions and associated local unfolding we observed in EnvZc also occur in transmembrane helix regions in EnvZ. Conformational dynamics of full-length EnvZ embedded within nanodiscs indicate that the osmosensing core of EnvZ shows greater deuterium exchange compared to EnvZc, suggesting a regulatory role of the membrane in modulating the autokinase activity of EnvZ. Our results reveal that membrane anchoring is responsible for ‘damping down’ the helix-coil transitions and likely increases the sensitivity range for the receptor. These results are broadly relevant to all receptors. Supported by Mechanobiology RCE and VA-110BX000372 to LJK.

Platform: Protein-Nucleic Acid Interactions II

2774-Plat

PICH: A DNA Translocase Essential for Resolving Anaphase Bridged DNA

Andreas Biebricher1, Seiki Hiramatsu,3, Jacqueline Enzlin1, Erwin Peterman2, Ian Hickson3, Gis J.L. Wuite1.
1VU University Amsterdam, Amsterdam, Netherlands, 2University of Oxford, Oxford, United Kingdom, 3University of Copenhagen, Copenhagen, Denmark.

PICH (PKL1-Interacting Checkpoint Helicase) is a recently identified member of the RAD54 subgroup of SNF2 family proteins. PICH localizes to so-called ultra-fine anaphase bridges (UFBs) in mitosis alongside a multi-protein complex of DNA repair proteins including BLM, the Bloom’s syndrome gene product. Very little is known about the function of PICH or how it is recruited selectively to UFBs. Nevertheless, depletion of PICH results in genomic instability, including an elevated frequency of sister chromatid exchanges, micronuclei and loss of heterozygosity. Using a combination of microfluidics, single-molecule fluorescence microscopy and optical tweezers, we have defined the properties of PICH in an in vitro model of an anaphase bridge. We show that PICH binds with a remarkably high affinity to dsDNA and displays ATP-dependent dsDNA translocase activity. The application of stretching forces to the DNA, which mimics the effects of the mitotic spindle on a UFB, enhances the binding of PICH to dsDNA, but also serves to diminish stretching-induced DNA melting. Based on our findings, we suggest that PICH plays several roles in the development and processing of UFBs: (i) to recognize and bind to dsDNA exposed by the mitotic spindle force-induced unwrapping of nucleosomes, (ii) to help expel exclusively these unwrapped nucleosomes, (iii) to stabilize stretched dsDNA, and (iv) to recruit the DNA repair machinery required for UFB resolution in anaphase.

2775-Plat

Computational Study of RNA Translocation in a Hexameric Helicase

Wen Ma, Klaus Schulten.

Beckman Institute, University of Illinois, Urbana-Champaign, Urbana, IL, USA.

Hexameric helicases are ATP-driven molecular motors that participate in important genetic processes. A particularly interesting helicase is E. coli transcription termination factor Rho, which translocates towards the 3'-end of nascent transcript. It is still an open question of how the ATP hydrolysis cycle is coupled to RNA translocation in Rho. We present results from all-atom molecular dynamics simulations that studied the conformation transitions and the corresponding energy landscape in the hydrolysis cycle based on the available crystal structure of Rho (Nathan D. Thomsen and James M. Berger. 2009, Cell, 139:523-534). We define collective variables involving the conformations of key residues at the monomer-monomer interface in different ATP binding states. The simulations reveal how interface conformational changes propagate around the circular helicase and regulate RNA translocation along the central channel in a collaborative manner. Monomers change their relative positions along the translocation direction based on the ATP binding states. The suggested allosteric inter-monomer communication in Rho is also revealed by network analysis based on cross-correlation of protein motion. Lys326 in each monomer is crucial in ratcheting the RNA and its movement is coupled to the ATP binding state. Arg269 participates directly in linking monomer-monomer communication with the ATP binding state. The simulations further demonstrate the influence of different RNA sequences (poly(U) and poly(C)). Our study, which elucidates the structure-function relationship in Rho, can be extended to other hexameric helicase systems, such as E1 and DnAB, whose crystal structures in complex with substrates are available.

2776-Plat

DNA Scanning Mechanism of a Translocating Motor Protein

Carolina Carrusco1, Neville Gilhooly1, Mark S. Dillingham1, Fernando Moreno-Herrero1,2
1Centro Nacional de Biotecnología, Madrid, Spain, 2University of Bristol, Bristol, United Kingdom.

Unpaired DNA breaks can lead to genomic instability or cell death. For repair by the ubiquitous homologous recombination pathway, broken ends are first processed to produce a 3'-ssDNA overhang. In Bacillus subtilis, this reaction