MS43 Extracellular Proteins and Cellular Adhesion

Chairpersons: Adrian Goldman, Sthanam Narayana

MS43.27.1

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Integrins, Focal Adhesions and all That

Robert C. Liddington, *The Burnham Institute 10901 North Torrey Pines Road La Jolla, CA 92037, USA*. E-mail: rlidding@burnham.org

The integrin family of cell adhesion molecules provide a mechanical link between the extracellular matrix (ECM) and the cytoskeleton, and form the nuclei of structural and signaling complexes that regulate cell migration, proliferation and survival, typically in concert with receptors for soluble ligands. Integrins are initially activated by intracellular ("inside-out") signals; following ligation to the ECM, "outside-in" signals lead to reorganization of the cytoskeleton and activation of intracellular signal transduction pathways. Recent studies have demonstrated a critical role for the cytoskeletal protein talin, which binds to the integrin β subunit cytoplasmic tail, disrupting αβ tail association and promoting a conformational change in the extracellular domains that leads to enhanced affinity for ECM proteins, and the subsequent clustering of integrins on the cell surface. So what activates talin? Recent evidence points to a prominent role for the enzyme, phosphatidylinositol phosphate kinase type 1-γ (PIPKIγ), which forms complexes with both Src and talin at focal adhesions. PIPKIγ synthesizes the lipid phosphatidylinositol 4,5 bisphosphate (PtdIns(4,5)P_2), a key activator of proteins involved in focal adhesion assembly, including talin and vinculin. Our latest structural studies on integrin, PIPKIγ, talin and vinculin and their complexes, will be discussed in this context.

Keywords: cell adhesion, integrins, intracellurar signals

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The Chaperone-usher Pathway of Pilus Biogenesis: Structural Basis of the Assembly Process and of Host Recognition

Gabriel Waksman, *Institute of Structural Molecular Biology. UCL/Birkbeck, Malet Street, London WC1 7HX, UK.* E-mail: g.waksman@mail.cryst.bbk.ac.uk

Pili are cell surface organelles and essential virulence factors responsible for host recognition by gram-negative bacterial pathogens. Type P and 1 pili of uropathogenic *Escherichia coli* target bacteria to the kidney and bladder, respectively, through their specific interaction with host surface receptors. They are assembled by the chaperone-usher pathway of pilus biogenesis, which involves a chaperone which ferries pilus subunits through the periplasm and an usher which forms the site of assembly at the outer-membrane. Structural biology work in my lab has characterized the interactions between chaperone and subunits and shown that pilus subunits have truncated Ig fold where the 7th strand is entirely missing. The chaperone "donates" one of its strands to complement the truncated Ig fold of the subunits. At the usher, the donated strand is substituted with the N-terminus extension of the subunit coming next in the assembly process. This substitution process is termed "donor-strand exchange". Recent biochemical work has provided details of the donor-strand exchange reaction and shown that it proceeds via a zippering mechanism. Finally, the interactions of the pilus with the host receptor have been characterized. This work provides fundamental insight into the first event in a bacterial infection i.e. host recognition/attachment and provides the basis for designing novel antibiotics targeting specifically virulence factors.

Keywords: pilus biogenesis, host recognition, bacterial infection

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Structural Relays in Adhesion Signaling

Tina Izard, *Department of Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN, USA.* E-mail: tina.izard@stjude.org

The formation of adherens junctions or focal adhesions relies on the interactions of the cytoskeletal proteins talin or α-actinin with vinculin, which binds to actin. Vinculin contains a head (Vh1) domain that interacts in an intramolecular fashion with its tail (Vt) domain, and this interaction clasps vinculin in its inactive state [1]. The signal(s) that disrupt the Vh1-Vt interaction to activate vinculin were unknown. Surprisingly, our crystal structures of full-length, inactive vinculin [1], and of the vinculin:talin [2,3] and vinculin:α-actinin [4] complexes, and our biochemical and biological studies, have revealed that talin and α-actinin trigger vinculin activation. Specifically, talin's and α-actinin's vinculin binding sites (VBSs) activate vinculin by displacing Vt from a distance, by provoking a totally new alteration in protein structure coined helical bundle conversion [2]. Strikingly, our structure of α-actinin's VBS (αVBS) in complex with vinculin established that this VBS must first unravel to bind and activate vinculin. αVBS then binds to vinculin's Vh1 domain in an inverted orientation compared to talin's VBSs, and provokes unique changes in the conformation of full-length vinculin, opening up far-distant regions in the molecule [4]. Collectively, these findings suggest that adhesion signaling involves a chain reaction of structural signals that is triggered by α-actinin and talin, which then activate vinculin.

[1] Borgon R.A., *et al.*, *Structure*, 2004, **12**, 1189. [2] Izard T., *et al.*, *Nature*, 2004, **427**, 171. [3] Izard T., Vonrhein C., *J. Biol. Chem.*, 2004, **279**, 27667. [4] Bois P.R.J., *et al.*, *Mol. Cell. Biol.*, *in press.*

Keywords: vinculin, talin, adhesion junctions

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Complementing Pathogens or Structural Insights into Pathogen Evasion of the Complement System

Susan M. Lea, Pietro Roversi, Frank Cordes, Beverly Prosser, Steven Johnson, *Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, UK.* E-mail: susan.lea@biop.ox.ac.uk

The innate immune system is the body's first line of defense against infection acting to destroy and remove anything perceived as foreign. To cause prolonged disease a pathogen must evade this defense. We are using structural methods to study pathogen systems which act to evade innate immunity in a variety of ways (i) the complement regulator acquiring surface proteins [1] of *Borrelia burgdorferi* (ii) complement regulatory proteins secreted in the saliva of soft ticks and (iii) the type three secretion system of *Shigella flexneri* [2] used to facilitate entry of the bacterium into host cells, so hiding it from the immune system. Recent data will be presented.

[1] Cordes F., Roversi P., et al., *Nat. Struct. Molec. Biol.*, 2005, doi: 10.1038/nsmb902. [2] Cordes F., et al., *J. Biol. Chem.*, 2003, **278**, 17103-7.

Keywords: complement regulation, lyme disease, *shigella*

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Factor XI Structure reveals a Novel Receptor Mediated Activation Pathway

Jonas Emsley[a], Paul McEwan[a], Evangelos Papagrigoriou[a], Peter Walsh[b], [a]*Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, Nottingham, NG72RD, UK.* [b]*Sol Sherry Thrombosis Research Center, Temple University School of Medicine, Philadelphia, PA, USA.* E-mail: jonas.emsley@nottingham.ac.uk

Factor XI (FXI) is an essential component to normal blood hemostasis and inherited deficiency is associated with excessive bleeding complications after surgery or trauma. FXI functions to cleave factor IX within the intrinsic coagulation pathway. The FXI protease is activated through a unique mechanism of binding to the leucine rich repeat receptor Glycoprotein Ib (GpIb) on the surface of platelets. It is then cleaved by thrombin also bound to a different region of the GpIb receptor.

We have crystallised the intact recombinant FXI zymogen and determined the structure to 3Å resolution. Each FXI monomer has four homologous subunits called apple domains (designated A1, A2, A3, and A4, from the N terminus) which mediate protein-protein interactions. At the C-terminus there is a serine protease with a typical catalytic triad. The structure reveals a remarkable "flying saucer" quaternary arrangement with the four apple domains forming a ring

and the serine protease domain positioned on top. The structure of the individual apple domains is represented by a novel topological motif. The FXI structure combined with our previous structural analysis of the Glycoprotein Ib receptor domain[1] allows us to construct a model of the activating ternary complex formed with thrombin.

[1] Uff et al., *J. Biol. Chem.*,2002, **277**, 35657 - 35663.
Keywords: receptor binding, coagulation, protease

MS43.27.6
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Structural Studies of the CLD from Aggrecan
Anna Lundell[1], Anders Aspberg[2], Derek Logan[1], [1]*Dept. Molecular Biophysics, Lund University.* [2]*Dept Connective Tissue Biology, Lund University.* E-mail: anna.lundell@mbfys.lu.se

Aggrecan is an important protein in the extracellular matrix (ECM) in the cartilage and its function is to organize the forming of the hyaluronan-lectican complexes in the ECM. Aggrecan consists of three globular domains and a central region of an elongated glycosaminoglycans-carrying region. The N-terminal domain (G1) binds to hyaluronan and the C-terminal domain (G3) has been shown to be involved in the binding to two types of ligands: sulphated glycolipids on the cell surface and dimeric/multimeric ECM proteins, e.g. fibulin-2 and tenascin-R. The CLD within the G3 domain of aggrecan has been observed to make a tight protein-protein interaction (K_D 12nM) with fibronectin type III repeats (FnIII) 3-5 from tenascin-R (TN3-5). Interestingly it has been shown that, though the complex is totally dependent on Ca^{2+}, as would be expected of a CLD, the interaction does not depend on carbohydrate. This is one of only a few direct protein-protein interactions of CLDs involving Ca^{2+}.

The structural studies will give us a first insight how lecticans use the CLD domain to interact with different ECM proteins. This is the first structure showing a calcium-dependent protein-protein interaction involving a C-type lectin domain that is not mediated by a carbohydrate. We have also solved the CLD of aggrecan in a unbound state, giving clues of the importance of the calcium ions.
Keywords: protein-protein interaction, calcium-binding protein, complex structure

MS44 Nucleic Acids - Transcription, Translation and Repair
Chairpersons: Stephen Neidle, Eric Westhof

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Eukaryotic Translesion Synthesis DNA Polymerases: Structure and Function
Aneel K. Aggarwal[1], Deepak T. Nair[1], Jose Trincao[1], Sacha N. Uljon[1], Robert E. Johnson[2], Carlos R. Escalante[1], Thomas A. Edwards[1], Satya Prakash[2] , Louise Prakash[2], [1]*Structural Biology Program, Department of Physiology and Biophysics, Mount Sinai School of Medicine, Box 1677, 1425 Madison Avenue, New York, NY 10029.* [2]*Sealy Center for Molecular Science, University of Texas Medical Branch, 6.014 Medical Research Building, 11th & Mechanic Streets, Galveston, TX 77555.* E-mail: aggarwal@inka.mssm.edu

Cellular DNA is continually damaged by external and internal agents, and both eukaryotes and prokaryotes possess DNA polymerases that can replicate through DNA lesions. Humans have four such (Y-family) polymerases – Polκ, Polι, Polη, and Rev1 – each with a unique DNA damage bypass and fidelity profile. Polη, for example, is unique in its ability to replicate through UV-induced cyclobutane pyrimidine dimers (CPDs), while Polκ is inefficient at replicating through a T-T dimer but can readily extend from mispaired termini. Polι is perhaps the most unusual with varied efficiencies and fidelities opposite different template bases. I will present our structural work on these eukaryotic DNA repair polymerases, with an emphasis on the basis of their specialization in lesion bypass.
Keywords: DNA-polymerase, DNA-repair, replication

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Mechanism for *de novo* RNA Synthesis by T7 RNA Polymerase
Whitney Yin, Wm Dexter Kennedy, *Department of Chemistry and Biochemistry, Institute of Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712, USA.* E-mail: xtalmd@mali.utexas.edu

DNA-directed RNA polymerases (RNAP) are distinguished by their ability to initiate *de novo* synthesis of polymer, RNA, from a promoter without the requirement for a 3'OH primer-terminus. The RNAP from bacteriophage T7 requires no accessory factors for RNA synthesis during the initiation phase of transcription. T7 RNAP is unique, however, in its requirement for *de novo* synthesis to start with the incorporation of two GTPs at the beginning of transcription.

We examined the structural and kinetic basis for *de novo* synthesis of RNA by using a series of novel DNA constructs which varied the template DNA initiation sequence and the incoming NTP analogues. Nine structures of T7 RNAP with promoter DNA and/or an incoming pair of NTPs were determined to 2.2 to 3.2 Å resolution and are *the first structural examples of de novo RNA synthesis by an RNA polymerase*. Different promoter-template DNA constructs are bound to the enzyme in virtually identical conformations. The two initiating NTPs are accommodated in the enzyme by changes to both the geometry of the active site and a novel bend in template DNA. The incoming NTPs are recognized by novel enzyme and template DNA interactions. Active site residues make a large contribution to the recognition of the initiating NTPs in addition to the specific DNA template base-pair interactions. Pre-steady state kinetic measurements support the idea that discrimination of initiating nucleotide by the enzyme plays a greater role than template specification of nucleotide selection for *de novo* synthesis of RNA by T7 RNA polymerase.
Keywords: transcription initiation, polymerase, nucleotide selection

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Structural Basis for RNA-regulated Gene Expression
Jennifer A. Doudna, Ian J. MacRae, Kaihong Zhou, *Department of Molecular Cell Biology, University of California at Berkeley, Berkeley, CA. U.S.A.* E-mail: doudna@berkeley.edu

RNA molecules have been discovered at the heart of several central aspects of gene expression, from protein biosynthesis by the ribosome to the targeting of new proteins to the correct intracellular locale to RNA interference (RNAi). Understanding how these RNA-mediated processes work will illuminate central aspects of modern cell biology and also provide important clues to the possibly fundamental role of RNA in the evolution of life. I will describe our efforts to understand the structural basis for RNA function, highlighting recent discoveries about the recognition and cleavage of double-stranded RNA in the early steps of the RNAi pathway.
Keywords: RNA structure, RNA, gene expression

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Structural Basis for Specific Recognition of the UsnRNP m_3G-cap by Snurportin1
Ralf Ficner[a], Anja Strasser[a], Achim Dickmanns[a], Reinhard Lührmann[b], [a]*Institute for Microbiology and Genetics, University Goettingen, Germany.* [b]*Max-Planck-Institute for Biophysical Chemistry, Goettingen, Germany.* E-mail: rficner@gwdg.de

The small nuclear ribonucleoprotein particles (snRNPs) are the major components of the splicing machinery that removes introns from pre-mRNA. In metazoans, the snRNP biogenesis is an ordered process requiring both nuclear and cytoplasmic phases. After transcription, the snRNAs U1, U2, U4, and U5 are exported into the cytoplasm, where the assembly with seven Sm proteins occurs and the snRNA 5′-cap nucleotide is modified from a 7-methyl-guanosine (m^7G-) to a 2,2,7-trimethyl-guanosine (m_3G-) cap. The hyper-methylated m_3G-cap represents one of the two nuclear localisation