starvation. The most marked outcome of this response is an immediate accumulation of the effector nucleotides, guanosine tetra- and pentaphosphate (ppGpp and pppGpp).The RelA protein of Escherichia coli is a (p)ppGpp synthetase that is activated by amino acid starvation. Here, we use single molecule tracking method (sptPALM) to investigate the RelA protein association and dissociation behavior before and after the stringent response. In contrast to the earlier work in which RelA was found to diffuse like ribosomes in normal growth conditions and to diffuse freely following the stringent response, we find RelA diffusion under both conditions to be heterogeneous. And during the stringent response, RelA diffuses more slowly than in the normal growth condition. In addition, the fraction of the slow diffusion population increases. This indicates that during the stringent response, RelA tends to bind to ribosomes more often compared to the normal growth condition, which suggests that ribosomes need to be "on" ribosomes to synthesize (p)ppGpp.

2339-Pos Board B31

The COP9 Signalosome: Activity and Regulation

Melissa Birol, Aude Echalier, Christian Dumas, Andre Padilla, Yinshan Yang, Francois Hoh.
Center of Structural Biochemistry, Montpellier, France.

The COP9 (Constitutive photomorphogenesis 9) signalosome (CSN) is a multiprotein complex exhibiting isopeptidase catalytic activity to regulate E3-cullin RING ubiquitin ligases (CRLs) and shares structural and functional similarities with the proteasome lid. The CSN removes an ubiquitin-like protein, Nedd8 (cullin-neural precursor cell expressed developmentally downregulated gene 8), from the cullin subunits of CRLs. Part of the ubiquitin-proteasome pathway, the CSN is implicated in diverse cellular functions, ranging from cell cycles and circadian rhythm to immunity. Interestingly, the CSN has a potentially critical link to various cancers. This isopeptidase catalytic activity, carried by the subunit 5 (CSN5, also known as Jab1), an MPN (Mpr1/Pad1 N-terminal) domain-containing protein corresponds to the hydrolysis of the isopeptide bond between Nedd8 and the cullin subunit. CSN5 incorporation within the CSN complex enables isopeptidase activity, whereas it remains inactive in isolation. Having elucidated CSN5 crystal structure, biochemical and in silico investigations furthered understanding in molecular regulation of its activity and led to the identification of a potential molecular trigger transitioning CSN5 to an active isopeptidase and the design of a constitutively active CSN5 variant form. Further to that work, to address CSN5 activity within the CSN, the contributions of other CSN subunits, specifically CSN6, shown to interact with CSN5 were evaluated. Additionally focusing on the CSN5-Nedd8 interaction and the ternary complex formation with CSN6, we gathered an in-depth understanding of the system at molecular, structural and functional levels through an integrated approach that includes biochemical, structural, biophysical and computational techniques.

2340-Pos Board B32

The Dicer-TRBP Interface Structure and Implications for Strand Selection During Microrna Biogenesis

Ross C. Wilson[1], Jennifer A. Doudna[2].
[1]Molecular & Cellular Biology, UC Berkeley, Berkeley, CA, USA, [2]Molecular & Cellular Biology, HHMI, UC Berkeley, Berkeley, CA, USA.

Central to the human microRNA (miRNA) pathway of gene regulation is the RNA-induced silencing complex (RISC), which is composed of an Argonaute protein bound to a guide strand of single stranded RNA that grants specificity via complementarity to the gene target. The assembly of this ribonucleoprotein depends on the RISC-loading complex (RLC), which comprises the endoribonuclease Dicer, a dsRNA binding protein such as TRBP, and an Argonaute protein.

We currently lack mechanistic details regarding the role of TRBP and its homologs in the process of RISC loading. TRBP is known to increase the stability of Dicer and promote interactions between the three protein components of the RLC. An additional hypothesized role for TRBP is to act as a chaperone that shuttles an RNA duplex from Dicer to Argonaute, potentially taking part in the process of strand selection that determines which arm of the double stranded RNA (dsRNA) will be loaded into the RISC to become the guide strand and thus which genes will be targeted.

TRBP contains two canonical dsRNA binding domains (dsRBDs) known to bind dsRNA tightly, and a third non-canonical dsRBD that binds instead to Dicer. We used NMR spectroscopy to demonstrate that these three domains do not interact with each other. Using X-ray crystallography, we have determined the structure of the Dicer-TRBP interface, revealing where and how TRBP is anchored in the context of the RLC. Subsequent cellular experiments disrupted this Dicer-TRBP interaction to examine the effects of an absent TRBP on the miRNA processing pathway. Together these results shed light on TRBP's role in the poorly understood processes of RISC-loading and strand selection.

2341-Pos Board B33

The Assembly of ASB9 with Ubiquitin Degradation Substrate CKB

Jonathan Parnell.
Chemistry, University of California, San Diego, San Diego, CA, USA.

Ankyrin repeat and SOCS box containing protein 9 (ASB9) belongs to the largest family (ASB family) of SOCS box containing proteins. The ASB family acts as the substrate recognition subunits of ElonginBC-Cullin-SOCS-box (ECS) type E3 ubiquitin ligase complexes. There are 18 members of the ASB family, with ASB9 being the most characterized. ASB9 has an N-terminal ankyrin repeat domain (ARD) and a C-terminal SOCS box. The ASB9-ARD has been implicated in binding to creatine kinase (CK) isoforms and the SOCS box in complex with Elongin B/C and Cullin 5 to ubiquitinate and target CK for degradation. However, our knowledge is limited regarding the interaction of ASB9 with CKs. We have performed analytical ultracentrifugation (AUC) and small angle X-ray scattering (SAXS) experiments on various N-terminal truncations of ASB9 (1-252, 19-252, and 35-252), CKs and their complexes. Our results suggest that ASB9 and CKB/CKM form a 1:1 complex dependent upon the presence of the disordered ASB9 N-terminus. Surprisingly, binding of ASB9-ARD to CK appears to break up the dimers of CK into monomers. This monomerization pathway could possibly explain the loss of enzymatic activity of CK upon binding ASB9. Further characterization of this complex requires a thorough understanding of the molecular details of this interaction. Crystal structures of both CKs and ASB9 have been published, but a recent docking attempt to model the complex was not very successful. Therefore, we are using HDXMS data to determine the minimal region of ASB9 that binds to CK and attempting to determine the crystal structure of the complex between ASB9 and CK. The data reported here will be of use for the future characterization of other ASB family proteins with their substrates.

2342-Pos Board B34

Kinetics of Interactions between LOV Domains from Chlamydomonas Reinhardtii

Carey K. Johnson[1], Kathrin Magerl[2], Katee Wyant[1], Ashley McDade[1], Will Newhart[1], David C. Arnett[3], Bernhard Dick[2].
[1]Chemistry, University of Kansas, Lawrence, KS, USA, [2]Chemistry, University of Regensburg, Regensburg, Germany, [3]Chemistry, Northwestern College, Orange City, IA, USA.

Kinetics of Interactions between LOV domains from *Chlamydomonas reinhardtii*

Carey K. Johnson, Kathrin Magerl, Katee Wyant, Ashley McDade, Will Newhart, David C. Arnett, and Bernhard Dick.

We have investigated the kinetics of interactions between Light-Oxygen-Voltage (LOV) domains of the light-sensing protein phototropin from *Chlamydomonas reinhardtii*. The photochemical response of phototropin to blue light involves adduct formation between a flavin mononucleotide cofactor and a nearby Cys residue. Interactions between LOV domains, either domains LOV1 and LOV2 within the same phototropin, or between LOV domains from different phototropin molecules, are thought to play a role in the subsequent activation of the phototropin kinase domain. We studied the kinetics of exchange of LOV domains between complexes (dimers or higher order oligomers) by stop-flow FRET measurements. A biphasic response with time constants from tens to hundreds of seconds was sensitive to prior exposure of LOV domains to blue light. LOV-LOV interactions have also been detected at the single-molecule level by TIRF imaging, providing further information about the interaction kinetics.

2343-Pos Board B35

Towards the Dynamical Origin of the Oligomeric Plasticity of RNA-Associated Sm Assemblies

Charles E. McAnany, Cameron Mura, Berk Ekmekci.
Chemistry, University of Virginia, Charlottesville, VA, USA.

Sm proteins are a family of ancient, structurally-conserved RNA-binding proteins. Bacterial Sm proteins self-assemble as homo-hexamers with broad RNA processing function; eukaryotic Sm proteins form hetero-heptamers that act in specific RNA-associated pathways. Archaeal Sm proteins have been found to assemble into hexamers, heptamers, and octamers. This degree of oligomeric plasticity is uniquely puzzling, given the 3D structural conservation of Sm monomers. We are exploring this unusual heterogeneity starting at the monomer level, using molecular dynamics simulations to characterize the conformational properties of Sm subunits and then rings, with the goal of elucidating the origin of this oligomeric plasticity. An Sm paralog from the thermophilic archaeon Archaeoglobus fulgidus is known to alter its oligomeric state based on solution pH and the presence of RNA, making it a particularly interesting system for our atomistic simulations. Here we report the first steps in