Symposium: Biophysics of RNA Processing: Degradation, Splicing, DEAD Box Proteins

1684-Symp
Insights into Helicase Evolution from the Specificity and Mechanism of a Dead-Box Protein
Anna L. Mallam, David J. Sidote, Alan M. Lambowitz.
Molecular Biosciences and Chemsitry, University of Texas at Austin, Austin, TX, USA.
How helicase families with a conserved catalytic 'helicase core' evolved to function on varied RNA and DNA substrates by diverse mechanisms remains unclear. Here, we used the helicase core of Mss116, a DEAD-box protein that utilizes ATP to locally unwind dsRNA, to investigate helicase specificity and mechanism. Previously, we found that the two RecA-like domains of the helicase core of Mss116 are in an extended 'open state' in the absence of substrates and recognize ATP and duplex RNA in a modular manner. Upon formation of a compact 'closed state' containing an ATPase active site, conserved motifs in the first domain promote the nonprocessive unwinding of short duplex substrates bound to the second domain by excluding one RNA strand and bending the other. In the present work, we define the molecular basis for the specificity of DEAD-box proteins. However, we also find that Mss116 has ambiguous substrate unwinding properties and interacts with a variety of NTPs and nucleic acids. The efficiency of unwinding correlates with the stability of the closed-state helicase core, a complex with nucleotide and nucleic acid that forms as duplexes are unwound. Crystal structures reveal that core stability is modulated by family-specific interactions that favor certain substrates. This suggests how present-day helicases diversified from an ancestral core with broad specificity by retaining core closure as a common catalytic mechanism while optimizing substrate-binding interactions for different cellular functions.

1685-Symp
Single-Molecule Imaging of Pre-mRNA Splicing
Sanjay Tyagi.
Public Health Research Institute, Rutgers University, Newark, NJ, USA.
We have developed a single-molecule RNA imaging method to study the intracellular sites of splicing. In this approach introns and exons in natural genes are probed in situ with distinctively labeled sets of about 50 oligonucleotide probes. The attachment of so many probes renders each target molecule so intensely fluorescent that it becomes visible as a bright diffraction-limited spot. Spots that fluoresce in just one color are either free introns or spliced mRNAs, and those that are visible in both colors correspond to pre-mRNA molecules. Our imaging studies confirm that constitutively spliced introns are removed at the gene locus during transcription. However, during alternative splicing events regulated by RNA binding proteins Sex lethal (in fruit flies) and polypyrimidine tract binding protein (in HeLa cells), which ensure that only one splice form is produced in a particular cell type, splicing gets uncoupled from transcription and occurs after the release of pre-mRNA from the gene locus. Similar uncoupling occurrs when intronic polypyrimidine tracts are masked within hairpins. Live cell imaging of the post-transcriptional splicing events suggests that they occur at the periphery of nuclear speckles where many splicing factors and poly adenylated transcripts are sequestered. Recent genome-wide studies reveal that a fraction of alternative splicing events likely occur via this route.

1686-Symp
Auxiliary Factors and RNA Substrates Regulate Dead-Box Protein Activity by Modulation of the Dead-Box Protein Conformational Cycle
Ulf Harms, Alexandra Z. Andreou, Airat Gubaev, **Dagmar Klostermeier**.
Physical Chemistry, University of Munster, Munster, Germany.
DEAD-box proteins unwind RNA duplexes at the expense of ATP hydrolysis in virtually all processes involving RNA. During unwinding, they alternate between open and closed conformations, and the transition to the closed conformation has previously been linked to duplex destabilization. The eukaryotic translation initiation factor 4A (eIF4A) is a DEAD-box helicase that is thought to resolve secondary structure elements from the 5′-UTR of mRNAs to enable ribosome scanning. Its RNA-stimulated ATPase and ATP-dependent helicase activities are enhanced by other translation initiation factors, but the underlying mechanisms are unclear. eIF4A can adopt three different conformations, an open state in the absence of ligands, a half-open state stabilized by eIF4G, and a closed state populated in the presence of eIF4G and eIF4B. In single molecule FRET experiments on donor/acceptor-labeled eIF4A, we have dissected the effect of eIF4B and eIF4G on RNA-dependent ATPase- and RNA helicase activities, and on the eIF4A conformational cycle in the context of different unwinding substrates. We show that eIF4B and eIF4G, as well as different structures in the 5′-UTR, modulate the energy landscape underlying the eIF4A conformational cycle by changing the energetic differences and the energy barriers between functionally relevant conformational states, leading to changes in rate constants for inter-conversion and in equilibrium distributions. Our results reveal on a molecular level how translation initiation factors synergistically stimulate the eIF4A helicase activity in the mRNA scanning process, and show that the eIF4A conformational cycle is central for the multi-layered regulation of eIF4A activity, and for its role as a regulatory hub in translation initiation.

1687-Symp
Molecular Mechanisms of Viral RNA Detection: RIG-I and MDA5
Sun Hur.
Biological Chemistry and Molecular Pharmacology, Harvard University, Boston, MA, USA.
The ability to distinguish ''self'' from ''non-self'' is fundamental to proper functioning of both the innate and adaptive immune systems. Several pattern recognition receptors (PRR) in the innate immune system are responsible for the initial detection of foreign molecules associated with pathogens such as bacterial cell wall components or viral nucleic acids. My laboratory has been interested in understanding the molecular mechanisms of one such family of receptors, RIG-I and MDA5, which recognize viral RNAs during infection and elicit the type I interferon response against a broad range of viruses. These receptors share the common domain architecture, including the DExD/H motif helicase domain. In this talk, I will present our recent discoveries on the structural and biochemical mechanisms by which these receptors differentially utilize the helicase domain to recognize their cognate viral RNAs and activate the signaling pathway.

Symposium: Molecules of Memory: Glutamate Receptor Channels

1688-Symp
Conformational Changes Underlying Glutamate Receptor Gating
Mark Mayer.
LCMN, NIH, Bethesda, MD, USA.
The gating mechanism of glutamate receptor ion channels involves conformational changes triggered by the binding of glutamate that open the ion channel, followed within milliseconds by nearly complete desensitization. In a collaboration with the Subramaniam Lab at NCI, cryo-EM structures of full length AMPA (GluA2) receptors, stabilized in the active state by allosteric modulators, reveal concerted conformational changes in the ligand binding domain that produce a vertical contraction accompanied by a corkscrew like rotation that leads to expansion of the linkers leading to the M3 helix bundle; in the desensitized state the ATD dimer pairs adopts multiple conformations with evidence for substantial reorganization of the ligand binding domain tetramer. By contrast, for the GluK2 kainate receptor the ATD dimer assembly remains intact in the desensitized state, while the LBD rearranges into a 4-fold symmetric structure in which the linkers leading to the M3 helix bundle adopt a contracted conformation that is however different from the closed state. Measurements of ATD tetramer association by analytical ultracentrifugation reveal a 1000-fold high higher affinity for GluK2 versus GluA2 that underlies the different behavior of the ATD in the desensitized state, movements of which however are secondary to and play no major role in desensitization.

1689-Symp
AMPA Receptor Structure, Function, and Dynamics
Robert E. Oswald, Ahmed H. Ahmed, Christopher P. Ptak, Madeline Martinez.
Molecular Medicine, Cornell University, Ithaca, NY, USA.
AMPA receptors (GluAs) are essential neuronal ligand-gated ion channels involved in learning and memory. The dimeric conformation of the GluA ligand-binding domain is involved in the coupling of agonist binding to channel gating. We have used NMR, crystallography, ITC, and single channel recording to study the mechanism of action of antagonists and partial agonists on the GluA2 receptor. Antagonists form stable but open cleft binding sites with little dynamics, with binding driven largely by enthalpy. On the other hand, considerable dynamics are observed in the binding site in the presence of partial agonists, whose binding, in most cases, has a large entropic component. Allosteric modulators bind to a large surface that is formed by the dimer interface of two ligand-binding domains in the resting and channel activated states. This binding prevents the dissociation of the dimer interface and inhibits desensitization of the receptor. The desensitized conformation is disrupted along the dimer interface; however, little is known about the dynamic equilibrium between the