Symposium 5: RNAs Large and Small

1027-Symp
Principles of RNA Tertiary Structural Organization Revealed by Group II Intron Crystal Structures
Anna Pyle.
Yale Univ, New Haven, CT, USA.
No Abstract.

1028-Symp
Structural Insights into Metabolite-Sensing Messenger RNAs
Robert Batey.
Univeristy of Colorado, Boulder, Boulder, CO, USA.
Riboswitches are structured elements found in the 5'-untranslated regions of mRNAs that directly bind a small molecule to regulate gene expression in a cis-fashion. These riboregulatory elements typically consist of two domains: an aptamer domain that binds a cellular metabolite and an expression platform that directs expression of the mRNA. Our laboratory is primarily focused upon understanding the three-dimensional structures of these mRNA elements in complex with their effector ligand and how this binding event leads to regulatory control. We have solved the X-ray crystal structures of the aptamer domains of a number of riboswitches in complex with their cognate ligands, each revealing a complex tertiary architecture that scaffolds the ligand-binding pocket. In each case, the RNA directly or indirectly senses almost all of the functional groups of the ligand, resulting in high binding specificity. Complementing these structures, we have used biochemical methods to probe the nature of the unliganded form of the aptamer domains, illuminating aspects of their ligand-dependent folding. These studies reveal that regulation is achieved through a series of ligand-induced tertiary structural changes in the RNA that serve to stabilize a helix that forms part of a secondary structural switch with the expression platform.

1029-Symp
Biophysical Analysis of Gene Regulation Pathways Controlled by Bacterial Non-Coding RNAs
Nilshad Salim, Dandan Li, Martha Faner, **Andrew L. Feig.**
Wayne State Univ, Detroit, MI, USA.
Small non-coding RNAs are transcribed in bacteria in response to a variety of cellular stimuli. These sRNAs often work in conjunction with the RNA binding protein Hfq to regulate gene expression, control stress response pathways, and modulate virulence. Using a combination of biophysics and genetics we have deconstructed these pathways and developed an integrated model for the molecular handoffs that control each step in the regulatory cycle. We will present our work on DsrA and OxyS, sRNAs associated with cold shock and oxidative stress respectively, as well as the mRNAs they regulate and the proteins they bind. While much of the work in this area has focused on post-transcriptional regulation of gene expression, our data lead us to propose a new model for these pathways that requires both co-transcriptional and post-transcriptional processes inducing structural rearrangements at both the RNA and DNA levels in response to these environmental stimuli.

1030-Symp
Translation Factor Control of Ribosome Dynamics During Protein Synthesis
Ruben L. Gonzalez Jr..
Columbia University, New York, NY, USA.
Conformational rearrangements of the ribosome, its transfer RNA (tRNA) substrates, and its associated translation factors are hypothesized to play important mechanistic roles throughout all stages of protein synthesis. In order to determine how the structural dynamics of the translational machinery couple to the molecular mechanisms underlying protein synthesis, we are using single-molecule Förster resonance energy transfer (smFRET) to directly characterize these dynamics and their regulation during translation. Here we report a series of distinct smFRET signals which have allowed us to directly characterize the intrinsic conformational dynamics of a highly-mobile ribosomal domain, the L1 stalk, as well as the coupling between L1 stalk and tRNA dynamics, throughout protein synthesis. Our smFRET data reveal that the translating ribosome exists in a dynamic equilibrium between two global conformational states, global state 1 (GS1) and global state 2 (GS2), and that transitions between GS1 and GS2 involve coupled movements of the L1 stalk and the ribosome-bound tRNAs that are accompanied by a rotation of the small ribosomal subunit relative to the large subunit. Most importantly, we find that translation factors involved in the elongation, termination, and ribosome recycling stages of protein synthesis specifically recognize GS1 or GS2 and precisely regulate the rates of transitions between GS1 and GS2 as part of their mechanisms of action. Thus, our results support the view that specific regulation of the global state of the ribosome is a fundamental characteristic of all translation factors and a unifying theme throughout protein synthesis.

Symposium 6: The Proton Gets Channeled

1031-Symp
The Hv1 Voltage-Gated Proton Channel: Are Two Pores Better than One?
Francesco Tombola.
Univ California, Irvine, Irvine, CA, USA.
Voltage-gated proton channels serve different functions in a variety of excitable and non-excitable cells, such as acid extrusion from neurons, muscles, and epithelial cells, and regulation of the NADPH oxidase in phagocytic cells. The first -and so far only- cloned voltage-gated proton channel, Hv1 (also known as VSOP), belongs to the family of proteins containing voltage-sensing domains (VSDs). In all other known channels of this family, the function of ion conduction is performed by a pore domain permeable to sodium, potassium or calcium ions. In Hv1, there is no pore domain, and the function of proton conduction is performed by the VSD itself. The Hv1 protein was recently shown to form dimers, in which each subunit has its own proton pore and gate, and the channel was found to function also when dimerization was prevented. This posed an interesting problem. If the two subunits of Hv1 can work separately, why do they form dimers? Do they gain any new functional feature by dimerization? Here, I will describe our attempts to answer these questions, focusing on the study of cooperativity between Hv1 subunits. I will also review our current understanding of the structural organization of Hv1 channels, and discuss the relationship between the gating of Hv1 and the gating of ion-conducting pores recently discovered in the VSDs of mutant voltage-gated potassium and sodium channels.

1032-Symp
Voltage-Dependent Activation and Proton Permeation in Hv1
I. Scott Ramsey.
Virginia Commonwealth Univ, Richmond, VA, USA.
The hallmark biophysical features of voltage-gated proton conductances (G_{vH+}) include exquisite H^+ selectivity and activation by both voltage and the transmembrane pH gradient. Heterologous expression of the voltage sensor domain (VSD) protein Hv1 is sufficient to reconstitute G_{vH+}, and the *Hvcn1* gene that encodes Hv1 is required for G_{vH+} and murine phagocytic leukocytes. Hv1 is therefore likely to represent the long-sought molecular correlate of G_{vH+}. In contrast to tetrameric voltage-dependent cation channels, each VSD subunit forms a distinct H^+ permeation pathway in dimeric Hv1 channels. Rather than mediating transmembrane ion flux by dehydrating permeant ions in a pore domain selectivity filter, Hv1is likely to achieve rapid proton-selective transmembrane H^+ transfer by a Grötthuss-type hydrogen-bonded chain (HBC) mechanism. In order to investigate the mechanism of proton permeation in Hv1, we performed charge-neutralizing mutagenesis of candidate H^+-titratable residues that we expected would be required for formation of the HBC. Whereas mutations of conserved acidic and basic residues in Hv1 dramatically affected both voltage- and pH gradient-dependent activation, we were unable to identify any single neutralizing mutation that was sufficient to abrogate expressed H^+ currents. Our data therefore suggest that H^+-selective permeation in Hv1 is likely to require H^+ transfer in a water wire rather than by side-chain titration. We interpret our mutagenesis data in the context of an Hv1 open-state homology model and describe a hypothetical mechanism for H^+ permeation in Hv1.

1033-Symp
Structure and Mechanism of Influenza Proton Channels
James J. Chou[1], Rafal M. Pielak[1], Jason R. Schnell[2], Junfeng Wang[1].
[1]Harvard Med School, Boston, MA, USA, [2]University of Oxford, Oxford, United Kingdom.
pH-activated, proton selective channels are required during viral entry and replication of influenza viruses. We determined the high resolution structures of