**133-Symp**
**Mapping the Phosphoinositide Landscape in Mammalian Cells**
**Tamas Balla**.
Section on Molecular Signal Transduction, Program for Developmental Neuroscience, NICHD, NIH, Bethesda, MD, USA.
Polyphosphoinositides (PPIs) are universal lipid regulators that control a great variety of cellular function serving as docking sites and conformational switches for a large number of signaling proteins associated with cellular membranes. PPIs are produced from phosphatidylinositol (PtdIns) that also is a structural lipid of biological membranes. PtdIns synthesis is believed to occur primarily in the ER, although it is debated whether it also happens at the plasma membrane (PM). Considerable progress has been made to determine the localization and dynamic changes in PPIs both in live and fixed cells, yet little has been done to map the distribution and possible functional diversity of the PtdIns pools in mammalian cells. Here we devised a strategy to address this question by using a PtdIns specific PLC enzyme isolated from Listeria monocytogenes together with a highly sensitive diacylglycerol sensor to determine the distribution and also to alter the level of PtdIns in living cells. Our studies reveal that a significant metabolically highly active PtdIns pool exists associated with tiny mobile structures within the cytoplasm in addition to the known ER and PM PtdIns pools. We will show our most recent data on the consequences of PtdIns depletion within the various PtdIns pools and on PPI production and will also discuss principles of PPI modulation of multiple effectors.

**134-Symp**
**Activation of TRP Channels by Phosphoinositide Depletion and Protons in *Drosophila* Photoreceptors**
**Roger C. Hardie**.
University of Cambridge, Cambridge, United Kingdom.
Phototransduction in *Drosophila* microvillar photoreceptors is mediated via a G-protein coupled phospholipase C (PLCβ4 encoded by the *norpA* gene), resulting in the activation of two classes of $Ca^{2+}$ and cation permeable channels, TRP and TRPL. Representative of the TRPC subfamily, these are the founding members of the TRP ion channel superfamily, but exactly how the channels are activated is unresolved. PLC's obvious action is to hydrolyze $PIP_2$ generating DAG and $InsP_3$ whilst simultaneously reducing $PIP_2$, the concentration of which can be accurately controlled using genetically encoded $PIP_2$ biosensors to monitor levels. The fact that hydrolysis of $PIP_2$ by PLC also releases a proton is seldom recognized and has neither been measured in vivo, nor implicated previously in a signaling context. We have now shown that light induces a rapid (< 10 ms) acidification originating in the microvilli which is eliminated in *norpA* mutants lacking PLC. Furthermore, following depletion of $PIP_2$ and other phosphoinositides by a variety of experimental manipulations, both TRP and TRPL channels become remarkably sensitive to rapid and reversible activation by lipophilic protonophores such as 2-4 dinitrophenol, whilst heterologously expressed TRPL channels can be directly activated by acidification of the cytosolic surface of inside-out patches. These results indicate that a combination of phosphoinositide depletion and cytosolic acidification is sufficient to activate the light-sensitive channels. Together with the demonstration of light-induced, PLC dependent acidification, this suggests that TRPC channels in *Drosophila* photoreceptors may be activated in a combinatorial fashion by PLC's dual action of phosphoinositide depletion and proton release.

**135-Symp**
**Regulation of Voltage-Gated Calcium Channel Activity by Palmitoylation: A Fatty Acid Tale**
**Ann R. Rittenhouse**.
University of Massachusetts Medical School, Worcester, MA, USA.
The phospholipid phosphatidylinositol 4,5-bisphospate ($PIP_2$) interacts with voltage-gated $Ca^{2+}$ channels to facilitate their opening. Conversely, inhibition of channel activity following activation of $G_q$-protein coupled receptors is associated with $PIP_2$ breakdown. $PIP_2$'s freed fatty acid tails appear to remain associated with channels, stabilizing closed conformations. My lab is testing a model where an accessory β-subunit $Ca_V\beta2a$ acts as a phospholipid mimic resistant to metabolism; its two palmitoyl groups bind to the channel at a site normally occupied by $PIP_2$'s two fatty acid tails. To test this model, we have concentrated on examining N-type $Ca^{2+}$ channel modulation in a recombinant system where $Ca_V2.2$ is coexpressed with $\alpha_2\delta$-1 and one of four $Ca_V\beta$ subunits. $M_1$ muscarinic or neurokinin-1 receptor stimulation inhibits N-current from $Ca_V\beta1b$-, $Ca_V\beta3$-, or $Ca_V\beta4$-containing channels, but enhances N-current from $Ca_V\beta2a$-containing channels. Exogenously applied arachidonic acid produces the same pattern of modulation. Further studies with mutated, chimeric $Ca_V\beta$ subunits and free palmitic acid revealed palmitoylation of $Ca_V\beta2a$ is essential for loss of inhibition. Loss of inhibition appears independent of kinetic changes that occur with different channel complexes. In contrast, channel mutations that reorient $Ca_V\beta2a$'s relative position to $Ca_V2.2$ rescue inhibition suggesting that in these experiments, the palmitoyl groups become sufficiently displaced that endogenously released arachidonic acid can once again bind to the inhibitory site. These findings suggest a new dynamic function for palmitoylation and predict that other doubly palmitoylated proteins reach up into the membrane with their lipid fingers to interact with and change the functioning of transmembrane proteins.
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## SYMPOSIUM 6: Allostery and Ligand Control of Function

**136-Symp**
**Allostery in an Ensemble**
**Vincent J. Hilser**.
Johns Hopkins University, Baltimore, MD, USA.
Over the past 50 years of structural biology research, three incontrovertible, and as yet not fully reconciled, aspects of protein behavior have emerged: First, protein molecules manifest a broad range of seemingly complex functions, ranging from catalysis to allostery to signal transduction. Second, protein molecules undergo conformational fluctuations, and in some cases these fluctuations are thermodynamically well-represented as local order/disorder transitions throughout the molecule. Third, many proteins and protein domains are natively disordered (i.e. intrinsically unstable), and may be unfolded in one or more functionally relevant states. Here we develop a general description of proteins fluctuations, which allows us to unify these observations and interpret them in the context of the functional demand to propagate the effects of binding to other sites in the molecule. We show; 1) that conformational fluctuations are in fact required for propagating binding effects, 2) that the relevant equilibrium constants for fluctuations must be poised to respond to functionally-relevant perturbations, and 3) that the behavior of multi-domain proteins, where one or more domains is natively unfolded, are driven by the same functional requirements and can be explained by the same thermodynamic principles that govern fluctuations in folded, yet conformationally dynamic proteins.

**137-Symp**
**Ligand-Regulated Protein/Protein Interactions: A Versatile Way to Build an Environmental Sensor**
**Kevin H. Gardner**.
University of Texas Southwestern Medical Center, Dallas, TX, USA.
Many biological processes are regulated by environmental cues via control of protein conformation, leading to functional changes. Commonly, this is mediated by sensory protein domains that bind both protein targets and biochemical cofactors, using the cofactors to trigger allosteric changes in the sensory domain. Notably, related domains can integrate different input stimuli and effector activities in various proteins.
To understand the mechanisms used by these sensory domains in diverse settings, we have combined solution NMR spectroscopy with other biophysical and biochemical approaches to compare the properties of related domains. Here, I will present some of our results of the Per-ARNT-Sim (PAS) domains, a group of small (~130 aa) modules that control both intra- and intermolecular protein/protein interactions. These domains also often bind internal cofactors, letting us examine stimulus/effector linkage in these systems.
As examples of these studies, I will discuss are photosensory PAS domains, where in situ laser irradiation during NMR experiments allows us to trigger a light-induced covalent bond formation within these proteins. This event generates conformational changes that disrupt inhibitory protein/protein interactions, activating downstream effectors. Combining experimental and computational approaches, we can quantitatively describe how alterations in protein/cofactor interactions perturb the structural and functional activation of these proteins.
In parallel, our studies on PAS domains in the hypoxia response system have provided a foundation for understanding how these domains participate in heterodimeric transcription factors. Interestingly, these domains share many common features with the light-regulated systems, including ligand binding and alternative protein conformations, despite having completely different biological contexts. Taken together, these data identify common facets of PAS-based signaling and lay the foundation for artificial control of these systems in the future.