Symposium: Biological Insights from Systems Approaches to Protein Networks

44-Symp
Functional Dissection of Dynamic Molecular Networks in Innate Immunity
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Innate immune processes are very much centered on the management of molecular interactions. A variety of activities demand it: Detection of pathogens and danger signals, distinction of self- from non-self molecules, signaling to initiate both transcriptional and non-transcriptional responses, neutralization of pathogens, resolution of infection and inflammation. In the dynamic course of innate immune responses, one can roughly distinguish between a first alarm-calling phase leading to the secretion of type I interferons and pro-inflammatory cytokines and a response and execution phase during which pathogens are neutralized, presented to cells of the adaptive immune system, and the alarm is switched off. Using affinity proteomics coupled to mass spectrometry, bioinformatics, pathogen infections, RNAi and gene inactivation in mice for selected cases, we have step-wise mapped the cellular proteins involved in these processes and monitored interactions among themselves as well as with perturbing proteins from invading pathogens. We have also used pathogen-associated molecular patterns as affinity baits to map the cell's recognition machinery. Overall, we have obtained an overview of the dynamic assembly, disassembly and regulation of several protein complexes essential for innate immunity, such as the IFIT, TLR and NLRPs complexes as well as on the logic that a variety of different pathogens use to overturn cellular processes to their own advantage. Comprehensive and time-resolved maps of molecular interactions under defined inflammatory and infectious conditions in the future will help obtained a more detailed understanding of the innate immunity programs. From a biochemical and systems-biological point of view it is already clear that subtle calibration of accurate molecular interactions is of uttermost importance for the cell to safeguard its homeostasis in face of perilous environmental challenges such as pathogen infections.

45-Symp
Steps Towards a Modular Theory of Disease
Edward M. Marcotte, Kriston K. McGary, Hye Ji Cha, Tae Joo Park, John O. Woods, Martin Blom, Michelle Byrom, Andrew Ellington, John B. Wallingford.
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Systems biology has shown great promise in providing a better understanding of human disease, and in identifying new disease targets. Nonetheless, it remains extraordinarily difficult to identify causal genes in most genetic diseases, in particular highly polygenic disorders, such as, for example, coronary artery disease, diabetes, and autism, for which current approaches are most limited. I'll discuss our progress on this problem, which builds upon the tendency for many genetic disorders to relate to disfunction of specific biological modules, such as protein complexes or pathways. Our strategies include computational and experimental methods for mapping disease-relevant protein complexes, and an unusual computational approach for identifying candidate genes for diseases, based on identifying suprising disease models, including a yeast model for angiogenesis defects. These studies reveal functionally coherent, evolutionarily conserved gene networks–many predating the plant-animal divergence–capable of identifying candidate disease genes.

Symposium: Biophysics of Membrane Fusion and Fission

46-Symp
Dynamin-Catalyzed Membrane Fission
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Dynamin, best studied for its role in clathrin-mediated endocytosis, is the prototypical member of a family of multi-domain GTPases involved in fission and remodeling of multiple organelles. Recent studies have shown that dynamin alone can catalyze fission of membrane tubules and vesicle formation from planar lipid templates, albeit inefficiently. We have recently proposed a two-stage model for dynamin-catalyzed fission (1). In stage one, mechanochemical activities of assembled dynamin helices induce localized curvature stress. In stage two the tightly packed lipid-interacting pleckstrin homology domains create a catalytic center that guides lipid remodeling through hemi-fission intermediates to drive membrane fission. Guided by recent X-ray crystallographic (2,3,4) and cryo-EM (4) structural studies of dynamin, we have been using site-directed fluorescent labeling of dynamin to the study nucleotide-dependent conformational changes required for dynamin-catalyzed fission. We have also identified dynamin partners that function synergistically with dynamin to catalyze membrane fission from SUPER templates. Together these studies shed new light on the mechanisms underlying dynamin-catalyzed membrane fission.
1) Frolov and Schmid, Ann. Rev. Cell and Dev. Biol. 2011. PMID: 21599493
2) Ford, M.G.L.J., S. Jenni and J. Nunnari. 2011. Nature doi. 10.1038/nature10411.
3) Faelber, K., Y.Posor, S. Gao, M. Held, Y. Roske, D. Schultz, V. Haucke, F. Nóe, O. Daumke. 2011. Naure doi: 10.1038/nature10369.
4) Chappie, J.S., J.A. Mears, S. Fung, M. Leonard, S.L. Schmid, R.A. Milligan, J.E. Hinshaw and F. Dyda. 2011. Cell 147:209-222.

47-Symp
Imaging the Nanometer-Scale Architecture of Microvesicle Release and Recapture
Justin W. Taraska.
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After exocytosis, vesicle components that are lost into the plasma membrane must be retrieved into the cell. Models of endocytosis propose that endocytic proteins sequentially assemble onto vesicle proteins destined for recapture. We are monitoring the post-fusion behavior of microvesicle proteins in living neuroendocrine cells with three forms of microscopy: multi-color total-internal reflection fluorescence (TIRF) microscopy, interferometric photo-activation localization microscopy (iPALM), and electron microscopy. With these methods, we are tracking the nanometer-scale molecular architecture of microvesicle release and recapture during calcium-triggered membrane fusion. Our results provide a topographic map for the molecular components that corral, capture, and recycle exocytic material from the cell surface in excitable cells.

48-Symp
Calcium-Dependent Turnover of the Cardiac Sarcolemma Analyzed by Non-Invasive Capacitance Recording and Optical Methods in Intact Cardiac Tissue
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We have described two types of massive Ca-activated endocytosis (MEND) in fibroblasts, HEK293 cells and cardiac myocytes (JGP, 137, Numbers 1 and 2, 2011). On the one hand, large Ca transients can promote subsequent internalization of >50% of the cell surface by mechanisms that require PIP2 synthesis. On the other hand, large Ca transients can promote internalization of >50% of the cell surface without PIP2, in the presence of high cytoplasmic Ca, when the cytoplasmic polyamine content is increased. Both MENDforms appear to internalize selectively membrane with a high content of liquid-ordered (Lo) domains, and neither MEND form uses classical endocytic proteins. To probe the physiological function of these and other endocytic mechanisms, we developed a non-invasive method to monitor capacitance in intact cardiac tissues, and we have combined this method with optical methods to monitor the disposition of transporters with respect to the sarolemma (e.g. Na/Ca exchangers via an extracellular pHluorin fusion). Using right ventricular strips from young adult mice, large-diameter (0.5 mm) pipettes are placed on the tissue surface, and transcellular voltage gradients are generated in tissue below the tip by sinusoidal voltage oscillations at frequencies of 10 to 100 kHz. Tentatively, we have identified a large membrane pool that can be inserted into the sarcolemma by cAMP- and Ca-dependent processes. These insertion processes are countered over time by large-scale retrieval processes that depend on ornithine decarboxylase activity (i.e. require synthesis of polyamines under β-adrenergic control). Further consistent with an important physiological role of MEND-related endocytic mechanisms, lysophosphatidylcholine's and other amphipaths can promote large-scale membrane retrieval in intact cardiac tissue. Advantages and potential pitfalls of the new electrophysiological methods will be discussed.

Platform: Intrinsically Disordered Proteins

49-Plat
Conformational Flexibility of Glycosylated Unstructured Peptides
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Glycosylation of proteins plays an important role in molecular recognition among proteins with implications for a variety of cellular processes. The large amount of glycan variants enables interactions of exquisite specificity. Glycoproteins often are partially or fully disordered and intrinsically