nents of individual tryptophan or clusters of tryptophan residues located close to each other. Also, we have created an algorithm for the structural analysis of the tryptophan environment in 3D atomic structures of proteins from Protein Data Bank (PDB). The successful design of the methods of spectral and structural analysis opened an opportunity for establishing a relationship between the spectral and structural properties of a protein. We have integrated the developed software modules, introduced a new program for the assignment of tryptophan residues to spectral-structural classes, and created a web-based toolkit PFAST: Protein Fluorescence and Structural Toolkit. PFAST contains 3 modules:

1. FCAT is a fluorescence-correlation analysis tool, which decomposes protein fluorescence spectra to reveal the spectral components of individual tryptophan residues or groups of tryptophan residues located close to each other, and assigns spectral components to one of five previously established spectral-structural classes.
2. SCAT is a structural-correlation analysis tool for the calculation of the structural parameters of the environment of tryptophan residues from the atomic structures of the proteins from the PDB, and for the assignment of tryptophan residues to one of five spectral-structural classes.
3. The last module is a PFAST database that contains protein fluorescence and structural data obtained from results of the FCAT and SCAT analyses.

Symposium 11: Collective Motor Dynamics in Cell Division

969-Symp Light microscopy of kinetochore protein architecture and mechanisms achieving spindle bipolarity

Ted Salmon

Biology, University of North Carolina, Chapel Hill, NC, USA.

The kinetochore is a protein assembly that links centromeric DNA and the plus ends of spindle microtubules. More than 70 different proteins within 12 or more protein complexes are now known to contribute to the core linkage and many are highly conserved from yeast to man. Their architecture within the kinetochore is poorly understood because the kinetochore core is only about 100 nm thick, about half the Abbe limit of resolution for light microscopy, and because it is has been very difficult to obtain molecular specificity at the higher resolution provided by electron microscopy. We have developed a light microscopy method for obtaining the relative distance between two different color fluorescent probes that label different protein domains at kinetochores. This method called "kinetochore-speckle high resolution co-localization" (K-SHREC) has an accuracy near 10 nm when applied to metaphase cells when sister kinetochores become aligned in opposite directions by pulling forces toward opposite poles. A 3-D Gaussian fitting algoithum is used to find the centroid coordinates of objective point spread function (PSF) images of red and green fluorescently labeled protein domains . The difference, Delta, between centroids is computed after correcting for chromatic aberrations. We are building-up the protein architecture of human and budding yeast kinetochores by K-SHREC measurements relative to the special CENP-A histone within centromeric nucleosomes at the inner kinetochore and the microtubule attachment site at the N-terminus of the Ndc80 complex within the outer kinetochore.

Spindle bipolarity is critical for achieving accurate chromosome segregation. Micromanipulation of meiotic spindles and asters assembled within Xenopus egg extracts has revealed multiple ways that the microtubule motor cytoplasmic dynein functions in achieving spindle bipolarity. Supported by NIH GM 24364.

970-Symp Large-Scale Coordination of Actin Polymerization Forces

Julie Theriot[1], Kinneret Keren[1], Zachary Pincus[1], Greg Allen[1], Erin Barnhart[1], Cyrus Wilson[1], Catherine Lacayo[1], Alex Mogilner[2]

[1] *Stanford University Sch Med, Stanford, CA, USA*
[2] *Univ California Davis, Davis, CA, USA.*

Many complex cellular processes, from mitosis to cell motility, depend on the ability of the cytoskeleton to generate force, either through polymerization and depolymerization of actin or microtubules or through the action of motor proteins. Large-scale cell behavior relies on coordination of cytoskeletal forces with other mechanical elements including cell-substrate adhesions and membrane tension. We have examined the mechanisms of cellular force coordination in the crawling motility of fish epidermal keratocytes. These simple-shaped cells are notoriously well-coordinated, and are able to maintain essentially constant cell shape, speed and directional persistence during rapid motility. Nevertheless, individual keratocytes demonstrate a variety of shapes and speeds. We performed detailed quantitative and correlative analysis of cell morphology and movement on a large number of cells, and used this extensive data set as a basis for the development of a mathematical model that relates the molecular-level dynamics of actin network treadmilling and the forces imposed on this network by the cell membrane to the large-scale geometry of the cell. Our model is able to quantitatively explain the main features of keratocyte shapes, and predict the relationship between cell geometry and speed. Surprisingly, we do not find a significant role for the contractile activity of myosin II in cell body translocation or in trailing edge retraction; instead, the major contribution of myosin II activity in these cells appears to be in assisting actin network disassembly. The dynamic coupling between membrane tension, cytoskeletal assembly/disassembly dynamics, and motor protein activity that we have documented in keratocyte motility is likely to contribute to other large-scale actin-dependent cell shape changes including cytokinesis.

971-Symp Non-equilibrium Dynamics of the Cytoskeleton

Fred C. MacKintosh

Free University, Amsterdam, Amsterdam, The Netherlands.

Networks of filamentous proteins play a crucial role in cell mechanics. These *cytoskeletal* networks, incorporating various cross-linking and other associated proteins largely determine the (visco)

elastic response of cells. In the cell, these networks are far from equilibrium: their mechanical properties can reflect internal active force generation by molecular motors. We develop a simple three-component *in vitro* model system consisting of myosin II, actin filaments, and cross-linkers [1]. By measuring the dynamics and mechanical properties, we quantify the effects of non-equilibrium stresses arising from motor activity. We show theoretically and experimentally how this motor activity can result in a 100-fold stiffening of the cytoskeleton. We present a quantitative theoretical model connecting the large-scale mechanical properties of this active gel to molecular force generation [2]. Based in part on this theoretical model, we investigate and explain the large shape fluctuations observed for microtubules *in vivo* [3]. Although microtubule bending is suppressed by the surrounding elastic cytoskeleton, large motor-induced forces cause significant bending fluctuations on short length scales, which are then frozen-in by the surrounding matrix. These lateral bending fluctuations naturally result in wandering of the orientation of the microtubule tip, and an apparent persistent random walk of the microtubule, with a small non-equilibrium persistence length approximately 100 times smaller than that resulting from thermal fluctuations alone. Thus, large non-thermal forces govern the growth of microtubules and can explain the highly curved shapes observed in the microtubule cytoskeleton of living cells.

References

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972-Symp Architectural Dynamics of the Meiotic Spindle Revealed by Single-Fluorophore Imaging

Tarun Kapoor

Rockefeller University, New York, NY, USA.

Bipolarity of the meiotic spindle, required for proper chromosome segregation, is maintained throughout cell division despite rapid microtubule turnover. How this is achieved has remained mysterious as determining the organization of individual spindle microtubules has been difficult. Here, we develop single fluorophore speckle imaging to examine microtubule organization in the vertebrate meiotic spindle. We find that the mean length of microtubules is ~40% of spindle length. Long and short filaments distribute randomly throughout the spindle and those in close proximity can move in the same direction with highly heterogeneous velocities. The ratio between microtubule and spindle lengths remains unchanged as spindles elongate upon dynein/dynactin inhibition. However, maintaining this ratio depends on proper kinesin-5 function. Our data suggest that force transmission within the spindle must be understood in terms of crosslinking dynamics of a tiled array of individual filaments, most of which do not span the distance from the pole to the metaphase plate.

Symposium 12: Non-Conducting Functions of Ion Channels

973-Symp Mutations in ion channels and their auxiliary subunits can lead to neurological or cardiovascular diseases

Lori L. Isom

University Michigan, Ann Arbor, MI, USA.

Mutations in ion channels and their auxiliary subunits can lead to neurological or cardiovascular diseases. In recent years it has become apparent that ion channels are part of large, multi-protein complexes, comprising not only the channel pore and its auxiliary subunits, but also components of the cytoskeleton, regulatory kinases and phosphatases, trafficking proteins, extracellular matrix proteins, and possibly even other ion channels. Sodium channel beta subunits do not form the ion-conducting pore, but are multifunctional proteins that play critical roles in modulation of channel function, regulation of channel expression levels at the plasma membrane, cell adhesion, neurite outgrowth, and transcription. Beta subunits signal through multiple pathways on multiple time scales in a tissue-specific, and possibly even subcellular domain-specific, manner . This feature makes sodium channels unique among the superfamily of voltage- and ligand-gated ion channels. In vitro evidence suggests that sodium channel beta subunits serve as critical communication links between adjacent cells, the extracellular environment, and intracellular signaling mechanisms, possibly including other ion channels. We propose that disruption of any member of a sodium channel signaling complex in vivo has the potential to disrupt channel function, resulting in paroxysmal disease, such as epilepsy or cardiac arrhythmia. In addition, because beta subunits can function as cell adhesion molecules in the absence of the ion conducting pore, mutations in beta subunit genes may result in defects in axon guidance or cell-cell communication. Understanding the molecular composition of individual sodium channel signaling complexes in excitable cells, as well as the conducting and non-conducting functions of the beta subunits may yield important insights into the molecular basis of inherited disease.

974-Symp Role of an Ion Channel Regulatory Protein Complex in Neuronal Physiology

Irwin Levitan

University Pennsylvania Sch Med, Philadelphia, PA, USA.

Many ion channels are intimately associated with one or more auxiliary proteins that participate in the regulation of channel activity. While the molecular details of ion channel regulatory protein complexes have been widely studied, their physiological roles remain poorly understood. We have taken advantage of Drosophila genetics to explore the role of the Slowpoke channel binding protein Slob in the modulation of large conductance calcium-dependent potassium channel activity in vivo. Slob has been shown previously to exhibit protein kinase activity in vitro, although its physiological substrates have not yet been identified. Patch