Reconstituting actin-microtubule crosstalk

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Symposium: Cytoskeletal Cross Talk

1425-Symp
Regulation of actin and microtubules by TDP-43 and profilin
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TDP-43 is a classic DNA/RNA binding protein important for the normal function of numerous cellular activities that directly and indirectly impinge on the actin and microtubule cytoskeletons. When TDP-43 levels are reduced, neurons display phenotypes consistent with the loss of actin-microtubule crosstalk functions including aberrant morphology, fewer dendritic branches, and diminished area of growth cones. TDP-43 is direct binding partner of profilin (k_D = 115.2 nM), which also regulates actin and microtubule dynamics, and variants of either protein are considered causative agents in several neurodegenerative diseases. First, we explored whether TDP-43 directly interacts with the actin or microtubules cytoskeletons and whether TDP-43 could mediate cytoskeletal crosstalk by forming phase-separated biomolecular condensates. We used TIRF microscopy assays to measure the impact of purified TDP-43 on actin and microtubule assembly. TDP-43 bound and sequestered both actin and tubulin (k_D = 22.7 nM). inhibiting the assembly of either polymer. We also explored how interactions between profilin and TDP-43 modulated the dynamics of either cytoskeleton or phase separation. The addition of profilin increased TDP-43 condensate formation (number and size), further inhibited actin assembly, but stabilized microtubule assembly. To investigate this in a more physiological context, we imaged the cytoskeleton and TDP-43 in Neuroblastoma-2a cells. Cells lacking profilin displayed less robust actin and microtubule arrays compared to endogenous or rescue controls. In addition, TDP-43 is mislocalized to the cytoplasm, mirroring the disease state of amyotrophic lateral sclerosis (ALS). Rescue experiments expressing wild type profilin on plasmids restored actin and microtubule arrays and the nuclear localization of TDP-43, whereas profilin plasmids harboring ALS-related mutations did not. Thus, profilin and TDP-43 are important regulators of actin and microtubule dynamics in cells and in the context of ALS.

1426-Symp
F-actin architectures differentially constrain myosin thick filament motion
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Active stresses are generated and transmitted throughout diverse F-actin architectures within the cell cytoskeleton, and drive essential behaviors of the cell, from cell division to migration. However, while the impact of F-actin architecture on the transmission of stress is well studied, the role of architecture on the ab initio generation of stresses remains less understood. Here, we assemble F-actin networks in vitro, whose architectures are varied from branched to bundled through F-actin nucleation via Arp2/3 and the formin mDia1. Within these architectures, we track the motions of embedded myosin thick filaments and connect them to the extent of F-actin network deformation. While mDia1-nucleated networks facilitate the accumulation of stress and drive contractility through enhanced actomyosin sliding, branched networks prevent stress accumulation through the inhibited processivity of thick filaments. The reduction in processivity is due to a decrease in translational and rotational motions constrained by the local density and geometry of F-actin.

1427-Symp
Elucidating mechanisms of cytoskeletal ensemble synergy using optical tweezers
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Development of cytoskeletal reconstitution assays that reflect a physiologically relevant environment has been a challenge for the biophysics field. In particular, optical trapping approaches to investigate motor protein dynamics have typically consisted of a reductionist geometry of a single motor and single filament. These assays not only do not capture the structural hierarchy in which motors with crosslinking ability function, but they also cannot capture the emergent mechanics that develop from ensembles of cytoskeletal proteins. It is becoming increasingly clear that the sum of protein single molecule properties may not always equal the resulting mechanics of the overall ensemble. Thus, to probe the molecular determinants of force generation, communication, and synergy of hierarchical cytoskeletal mechanics with high resolution, an optical trapping approach was developed that probes multiple motors and filaments in an ensemble. Using this method, we interrogate actomyosin mechanics to elucidate myosin II ensemble synergy and force regulation within a bundled actin assembly.

1428-Symp
Reconstituting actin-microtubule crosstalk
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The actin and microtubule cytoskeletons form active networks in the cell that play vital roles in cellular processes such as cell division and motility. Crosstalk between these two cytoskeletal systems is mediated by cross-linking proteins with affinity for both types of filaments. I will provide an overview of in vitro experiments in which we study how different types of actin-microtubule cross-linking proteins may lead to filament bundling, guidance of microtubule growth and even active transport of actin filaments by growing microtubule ends.

Symposium: Ion Channels on Drugs

1429-Symp
Potassium channel openers targeting different sites of the channel
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Voltage-gated ion channels play essential roles in generating and shaping the electrical excitability of nerve and heart cells. Mutations in these ion channels can cause diseases, which can be treated with pharmaceuticals targeting specific ion channels. While many pharmaceuticals cause their effects by blocking the ion-conducting pore to prevent the ion current, only a few exert their therapeutic effect by opening (or activating) the channel. While they see limited use as pharmaceuticals at present, recent research has identified many different types of compounds that can open ion channels. Here, I will present data from three classes of compounds capable of opening voltage-gated potassium (Kv) channels. (1) The first class includes resin acids and polyunsaturated fatty acids. These negatively charged compounds have close interactions with the channel's positively charged voltage sensor, opening the channel by pulling the sensor to an activated position. This interaction occurs at the interface between the phospholipid bilayer and the channel protein. (2) Biaryl sulfonamide acids can access the voltage sensor through the extracellular space, allowing them to activate the Kv1-like Shaker channel. (3) Warfarine-like tautomers activate Kv1.5 channels via close interactions with the linker connecting the voltage sensor and the channel's ion conducting pore domain. Characterizing different binding sites and compounds that use them on different ion channels will hopefully lead to better treatments for diseases caused by altered excitability.

1430-Symp
A nanobody toolkit for the regulation of K2P channel function
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Two-Pore Domain (K2P) K^+ channels act as important regulators of the membrane potential in both excitable and non-excitable cells. Their dysfunction is implicated in a range of disorders and they represent attractive therapeutic targets. In particular, drugs that modulate TREK K2P channels may be useful for the treatment of certain forms of pain, depression and migraine. In a previous study, we demonstrated that a class of small molecule negatively-charged activators target a common gating mechanism within the selectivity filter of K2P channels to increase channel activity. Although some of these drugs are highly effective, structural conservation within the inner pore and filter gating mechanism of many related K^+ channels means that target specificity is difficult to achieve. We now show that a class of biologics based on V_{HH} domains (nanobodies), can achieve far greater selectivity as both activators and inhibitors of TREK-2 channel function and can differentiate between individual members of this family. Structures of TREK-2 in complex with these nanobodies also provides important new insights into their mechanism of action and highlight a new toolkit for the dissection of K2P channel function.

1431-Symp
Nicotinic acetylcholine receptor structural pharmacology and gating transitions
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