955-Sym
Gravitational Stabilization of an RNA/Protein Droplet Emulsion by a Nuclear Actin Network
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Actin plays a structural role in the cytoplasm. However, actin takes on new functions and structures in the nucleus that are poorly understood. The nuclei of the large oocytes of the frog X. laevis specifically import actin to reach high concentrations; however, it remains unclear what, if any, structural role this actin plays. Here, we use microrheological and confocal imaging techniques to probe the local architecture and mechanics of the nucleus. Our data show that actin forms a weak network that spatially organizes the nucleus by kinetically stabilizing embedded liquid-like RNA/protein bodies which are important for cell growth. In actin-disrupted nuclei, this RNA/protein droplet emulsion is destabilized, and undergoes rapid gravitational sedimentation and fusion. This suggests that gravitational stabilization of subcellular structures is an important mechanical requirement in large cells.

956-Sym
Mechanical Architecture of Chromosome Segregation
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During cell division, each daughter cell must inherit exactly one copy of the chromosome. Errors can lead to cell death or cancer in somatic cells, and development disorders in the germline. How does the cell generate mechanical force to move its chromosomes and segregate them? While we know nearly all the molecules essential for chromosome segregation, we do not understand the underlying mechanical principles. Two macromolecular machines are responsible for chromosome segregation: the μ-scale spindle moves chromosomes through its dynamic microtubules, and the 100nm-scale kinetochore anchors chromosomes to spindle microtubules. How do these two machines accurately and robustly segregate chromosomes, and how do their μm-scale constituents work together to generate μm-scale movements? First, I will first describe our efforts to understand how microtubule forces move kinetochores, and how kinetochores can hold on to growing and shrinking microtubules. We measure the deformation of moving kinetochores in live cells with sub-diffraction imaging, and from this data infer the nature and location of kinetochore-microtubule force-generating interfaces. Second, I will describe our laser ablation experiments probing the mechanical architecture of the spindle, or how the spindle bears the load of chromosome movement. We hope that these experiments will provide insight into how dynamic macromolecular machines, such as the kinetochore and spindle, can achieve functional robustness and accuracy.

Platform: Cardiac Muscle II

957-Sym
Dephosphorylated Cardiac Myosin-Binding Protein C (cMyBP-C) Activates Native Cardiac Thin Filaments within the C-Zone of Native Cardiac Thick Filaments
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cMyBP-C is a potent modulator of actomyosin motion generation within two distinct ~350nm regions (C-zones) flanking the myosin thick filament bare zone. cMyBP-C’s modulatory capacity is tunable by phosphorylation of 4 serines within the cMyBP-C motif. To determine cMyBP-C’s phosphorylation-dependent impact on calcium activation of fully regulated actin thin filaments (i.e. containing troponin and tropomyosin), we observed rhodamine-phalloidin labeled native thin filament shards (~250nm) moving over native thick filaments isolated from mouse ventricular tissue (Previs et al., 2012). For these experiments, the state of cMyBP-C phosphorylation in native thick filaments was either ~64% on each of the 4 serines upon isolation (wild-type) or reduced to ~22% by lambda-phosphatase treatment. Native thin filament movement was observed by TIRF microscopy at 25mM KCl, 100μM ATP, 20℃. At pC5, thin filaments landed on wild-type thick filaments and moved for 402 ± 21nm at an initial fast velocity of 2.2 ± 0.1μm/s before slowing abruptly to 0.9 ± 0.1μm/s for 311 ± 19nm within the C-zone. Similar trajectories were observed on phosphatase-treated thick filaments (fast velocity: 2.0 ± 0.1 μm/s, 392 ± 16μm/s slow velocity: 0.8 ± 0.1μm/s, 347 ± 15μm/s within C-C0). At pC7, little to no thin filament motion was observed on wild-type thick filaments but surprisingly, on phosphatase-treated thick filaments, a single slow phase of velocity (0.6 ± 0.1μm/s) with a run length (397 ± 137nm) similar to the C-zone length was observed. We conclude that cMyBP-C plays two mechanistic roles within the thick filament C-zone in vivo: 1) allowing for cMyBP-C phosphorylation-dependent activation of the thin filaments at resting calcium levels; 2) providing an internal load that governs the speed of thin filaments once the muscle is calcium activated.

958-Plat
Cardiac Myosin Binding Protein-C Influences the Rates of MgADP Release and MgATP Binding Differently for the Two Cardiac Myosin Isoforms Expressed in Mouse Myocardium
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Transgenic mouse models lacking myosin binding protein-C (cMyBP-C) have been used extensively to examine the influence of cMyBP-C on myocardial performance. Mouse ventricle normally express predominately α-myosin heavy chain (α-MyHC), while cMyBP-C knockout models (~ cMyBP-C) express a mixture of α- and β-MyHC isoforms. Performance comparisons within control mice and these knockouts are complicated by differences in MyHC isoform profile. We tested whether cMyBP-C differentially affected MgATP-dependent kinetics in the two cardiac MyHC isoforms. We applied varying MgATP concentrations to chemically-skinned myocardial strips undergoing length titration analysis and were able to deduce actomyosin crossbridge MgADP release rate (kADP) and MgATP binding rate (kATP). Knockout mice treated for 10 days with L-thyroxine (vt41q) expressed 100% α-β-MyHC as did transgenic controls expressing wildtype cMyBP-C (WTt/t-T4). Values for kADP were not different between vt41q (95.7 ± 3.5 s⁻¹) and WTt/t-T4 (90.3 ± 9.6 s⁻¹); but kATP was significantly lower in the vt41q (154 ± 2 mM⁻¹ s⁻¹) compared to WTt/t-T4 (302 ± 18 mM⁻¹ s⁻¹), P < 0.01. Mice fed a diet of 6-n-propyl-2-thiouracil (PTU) expressed 100% β-MyHC. Values for kADP were significantly higher in vtPTU (24.8 ± 1.0 s⁻¹) compared to non-transgenic controls (NTGPTU, 15.6 ± 1.3 s⁻¹), P < 0.01, but kATP was not significantly different in vtPTU (77.5 ± 14.5 mM⁻¹ s⁻¹) compared to NTGPTU (99.8 ± 20.0 mM⁻¹ s⁻¹). These findings in the structural context of an intact cardiac sarcomere suggest that the presence of cMyBP-C influences MgATP-dependent kinetics differently for the two cardiac myosin isoforms. cMyBP-C does not influence MgADP release rate of α-MyHC, but effectively doubles the MgATP binding rate. In contrast, the presence of cMyBP-C reduces the MgADP release rate in the β-MyHC isoform, but does not change MgATP binding kinetics.

959-Plat
Helix Three (H3) of the M-Domain of Cardiac Myosin Binding Protein-C is not Essential for Functional Effects in Permeabilized Rat Trabeculae
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Cardiac binding protein c (cMyBP-C) is a sarcomeric protein involved in the regulation of cardiac muscle contraction. Cardiac specific phosphorylation sites in the M-domain regulate binding of cMyBP-C to myosin and actin. Immediately downstream of the phosphorylation sites is a bundle of three α-helices that are conserved across all species and isoforms of MyBP-C, however the functional significance of these helices is unknown. Helix 3 (H3) bears high homology to the inhibitory peptide of troponin I, suggesting it as a potential binding site for interactions with actin. To understand the functional significance of H3, we produced a recombinant N-terminal protein containing domains C1, M and C2 (C12C) with the H3 sequence deleted (H3KO). We then assessed effects of the mutant H3KO protein on contractile forces in permeabilized rat trabeculae and compared them to effects of the wild-type C12C. Results showed that 5μM C12C significantly increased Ca²⁺-sensitivity of force (ΔpCa50=0.29 ± 0.03), whereas 5μM H3KO had no effect (ΔpCa50=0.02 ± 0.01) and 10μM C12C only modestly increased Ca²⁺-sensitivity (ΔpCa50=0.11 ± 0.02). Similarly, whereas 10μM C12C activated force in the absence of Ca²⁺ (pCa 9.0), H3KO activated force in the absence of Ca²⁺ only at concentrations >200μM. Together, these results establish that H3 is not absolutely required for the functional effects of the N-terminal domains of cMyBP-C to either increase Ca²⁺-sensitivity of force or to activate tension in sarcomeres, but that H3 contributes to the overall efficacy of the N-termius in mediating these effects. Potentially, H3 could dock the N-terminus of cMyBP-C with actin or extend/stabilize cMyBP-C interactions with actin or other regulatory proteins. This work was supported by NIH HL080367 (SPH), NDSEG and AHA graduate fellowships (JKK), and AHA undergraduate fellowship (JKK).