manner and inhibits the ability of endothelin to produce phosphorylation of MyBP-C. These results suggest that phosphorylation of cMyBP-C may be a molecular component of the vascular endothelial cell - cardiac myocyte cross-talk. Coupled with already published work, the results also suggest that cMyBP-C phosphorylation may contribute to the regulation of the turnover of myofibrillar proteins.

2859-Pos

Comparative Effects of the Proline-Alanine Rich Regions of Human and Murine Cardiac Myosin Binding Protein-C

Justin F. Shaffer[1,2], Peony Wong[2], Kristina L. Bezold[2], Samantha P. Harris[2].
[1]University of Washington, Seattle, WA, USA, [2]University of California, Davis, Davis, CA, USA.

The N-terminus of cMyBP-C can activate actomyosin interactions in the absence of Ca^{2+}, but it is unclear which sequences mediate the activating effects. Herron et al. (Circ Res, 98:1290-8, 2006) found that the Pro-Ala rich region (P-A) of human cMyBP-C could activate tension in the absence of Ca^{2+}, whereas Razumova et al. (J Gen Physiol, 132:575-85, 2008) found that murine C1 and M domains activated tension. The different results might be explained by isoform differences, especially in P-A which is only 46% identical between mouse and human cMyBP-C. The goal of this study was to determine if species-specific differences in P-A account for the different activating effects of murine and human cMyBP-C. Recombinant chimeric proteins containing the C0, P-A, and C1 domains (C0C1) from either human or murine cMyBP-C were engineered and their activating effects assessed using *in vitro* motility and ATPase assays. Consistent with previous observations, human C0C1 activated actomyosin interactions in the absence of Ca^{2+}, whereas murine C0C1 did not. However, substituting human P-A for murine P-A conferred activating properties to murine C0C1, whereas substituting murine P-A for human P-A depressed the activating effects of human C0C1. Activating effects of the chimera proteins were intermediate between those of murine and human C0C1, suggesting that C0 or C1 also contribute to activation properties. Further chimeric substitutions of C0 and C1 demonstrated that the human C1 domain also contributed to activation, whereas the C0 domain did not. These results suggest that the human P-A and C1 domains are sufficient to activate actomyosin interactions in the absence of Ca^{2+}, and that species-specific differences are likely to contribute to functional differences of cMyBP-C. Supported by NIH HL080367 to SPH and a NSF Graduate Research Fellowship to JFS.

2860-Pos

Single Sarcomere Imaging by Quantum Dots (Qdots) in the Heart

Takako Terui[1], Seine Shintani[2], Shin'ichi Ishiwata[2], Satoshi Kurihara[1], Norio Fukuda[1].
[1]The Jikei University School of Medicine, Tokyo, Japan, [2]Waseda University, Tokyo, Japan.

Numerous studies have been conducted in tissues and cells to elucidate the molecular mechanisms of myocardial contraction. However, because of a number of differences between in vitro and in vivo conditions, the dynamics of myocardial sarcomere contractions in living animals is not yet understood. In the present study, we developed a novel system allowing for real-time single sarcomere imaging in the living heart. Male Wistar rats were anesthetized with pentobarbital sodium, and median sternotomy was performed under artificial ventilation. Qdots were conjugated with anti-α-actinin antibody and then transfected from the surface of the epicardium of the beating heart, for visualization of the Z-discs. An electron microscopic study confirmed the presence of Qdots in and around the T-tubles and Z-discs in the myocardial cells of the left ventricular wall. Consistent with this, we observed a striated pattern of Qdots (~2 μm spacing) in the heart under fluorescence microscopy. We are now performing real-time single sarcomere imaging in the beating heart of the rat.

2861-Pos

Single Sarcomere Imaging in Cardiomyocytes with Quantum Dots (Qdots): Physiological Significance of SPOC in Cardiac Beat

Norio Fukuda[1], Takahiro Serizawa[2], Seine Shintani[2], Takako Terui[1], Shin'ichi Ishiwata[2], Satoshi Kurihara[1].
[1]The Jikei University School of Medicine, Tokyo, Japan, [2]Waseda University, Tokyo, Japan.

Cardiac sarcomeres exhibit spontaneous oscillations (SPOC) over a broad range of intermediate activating conditions, namely, at pCa ~6.0 (Ca-SPOC), or at the coexistence of MgADP and Pi under the relaxing condition (ADP-SPOC). We have reported that the period of sarcomeric oscillations in skinned myocardium correlates with that of resting heart rate in various animal species [BBRC, 343, 1146-1152 (2006)]. In the present study, we analyzed sarcomeric oscillations in isolated single cardiomyocytes of the rat, by using Qdots conjugated with anti-α-actinin antibody for clear visualization of the Z-lines. First, we measured the period and amplitude of ADP- and Ca-SPOC at various sarcomere lengths (SLs) in skinned cardiomyocytes, and found that the amplitude of oscillations was inversely related to SL. We also conducted a SL measurement in intact cardiomyocytes at various stimulation frequencies, after transfection of Qdots into the cells. At low frequencies (e.g., 1 Hz), the shortening and relengthening of the sarcomere during a contraction cycle simply reflected the changes in $[Ca^{2+}]_i$. However, an increase in stimulation frequency to the physiological level (~5 Hz) caused a phase shift of shortening and relengthening due to enhancement of the relengthening speed, resulting in the waveform being similar to what was observed during SPOC in skinned myocytes. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres may contribute to the regulation of cardiac beat *in vivo*.

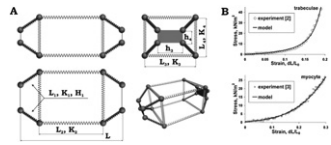
2862-Pos

Modeling of Viscoelastic Properties of Isolated Myocardial Tissue Samples at Different Levels: Cardiomyocytes and Trabeculae

Leonid Smoluk, Yuri Protsenko.
Institue of Immunology and Physiology, Ekaterinburg, Russian Federation.

Viscoelastic properties of myocardium play an important role in a heart function. They determine the extent of filling of the heart, its subsequent stroke volume and contraction velocity. We present here the 3D model consisting of elastic springs and linear damping elements on basis of our earlier model [1] (Fig. 1A). Due to changes of geometry the model manifests nonlinear viscoelastic behavior in response to longitudinal stretch. Depending on set of input parameters, the model allows to describe quantitatively nonlinear viscoelastic behavior of both single cardiomyocytes and multicellular samples like trabeculae (Fig. 1B). Model volume stability is an essential condition for model parameter selection because in vivo a volume of cardiomyocytes is virtually unchanged. It is significant that viscoelastic parameters of structural elements of the model remain constant all over the range of investigated strains.

Thus, we can describe main viscoelastic properties of myocardial tissue at different organization levels within the basis of the simple mechanical model.



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2863-Pos

Radial Force and Lattice Spacing with Multi-Spring Crossbridge Models

C. David Williams, Michael Regnier, Thomas L. Daniel.
University of Washington, Seattle, WA, USA.

Previous spatially explicit models have used crossbridges consisting of single springs aligned to the axis of the thick and thin filaments. Such one-spring models cannot account for effects of lattice spacing or radial forces generated during axial shortening. We develop crossbridge models with multiple springs to examine how different mechanisms of crossbridge deformations affect radial forces, longitudinal forces, and the effects of changes in lattice spacing. A four-spring crossbridge (4sXB) treats the S2 region and light chain domain (LCD) as linear springs, linked to the thick filament and each other by torsional springs. Changing the rest angle of the S2/LCD linking spring models force generation via a power stroke. A two-spring crossbridge (2sXB) replicates the desired abilities of the 4sXB and is less computationally expensive. Unlike the 4sXB, the length and angle of the 2sXB's springs can be determined for any head position without iterative techniques. Both the 4sXB and the 2sXB use three state kinetics that, at resting lattice spacing, are similar to previous work, easing comparison to previous studies. In contrast to single spring crossbridges, the kinetics of the 4sXB and 2sXB change with lattice spacing. Notably, the axial offset (distance between the thick filament attachment site and myosin-binding site) at which the powerstroke becomes likely to occur varies by more than 5nm over physiological lattice spacings. Both the 4sXB and the 2sXB measure the axial and radial forces generated by during production of axial force. In a typical post-powerstroke position at resting lattice spacing, the axial forces exerted by the 4sXB and the 2sXB differ by approximately 10% while the radial forces are more divergent (differing by as much as 20%), making the choice of crossbridge a critical concern in measurements of radial force. HL65497 (MR), EB001650 (CDW).

2864-Pos

Sarcomere Velocity Regulates the Cross-Bridge Cycling Rate in Cardiac Muscle: a Novel Theory for the Muscle Molecular Motor

Amir Landesberg, Moran Yadid.
Technion - Israel Institute of Technology, Haifa, Israel.

Background: The mechanisms regulating cross-bridges (XBs) cycling during stretch and shortening are controversial. We hypothesize that XB strong to weak transition (weakening) rate increases during shortening and decreases during lengthening in an identical velocity dependent manner. Our hypothesis reproduces the muscle basic properties as the force-velocity relationship and regulation of energy consumption. The study investigates this unifying hypothesis during lengthening and shortening. **Methods:** Trabeculae were isolated from rat right ventricles (n=9). Sarcomere length was measured by laser diffraction. The number of strong XB (N_{XB}) was evaluated by measuring the dynamic stiffness. Stretches (n=42) and releases (n=48) at different velocities and instants were imposed on sarcomere isometric contractions. **Results:** Faster stretches yielded larger forces. An overt identical linear correlation between force and N_{XB} development was obtained for any stretch velocity (0-2.17μm/s), implying that the force increased due to the increase in N_{XB}, whereas the unitary force per XB (F_{XB}) was constant. The stiffness development rate linearly depended on the lengthening velocity with a proportion coefficient of 6.9 ± 0.46. Shortening yielded both a decrease in N_{XB} and F_{XB}. Interestingly, the stiffness decline rate depended linearly on the shortening velocity (0-6.27μ/s) with similar proportion coefficient of 6.08 ± 2.45. When identical perturbation (lengthening or shortening) was imposed at different instants during the twitches, similar rate of change in the stiffness and force development were observed. Thus, the phenomena are not dominated by N_{XB} but relate to an inherent property of the single strong XB. **Conclusions:** The independence of XB weakening rate on the perturbation onset time and the identical dependence on the velocity during shortening and lengthening strongly support the hypothesis that XB dynamics is dominated by a single velocity dependent kinetic.

Cytoskeletal Protein Dynamics

2865-Pos

Escherichia Coli Single FtsZ Dynamic Rings: Growing, Cyclization, Opening, Reannealing and Depolimerization

Pablo Mateos-Gil[1,2], Alfonso Paez[2], Ines Hörger[2], Pedro Tarazona[2], Jesus Mingorance[3], Germán Rivas[4], Marisela Vélez[1,2].
[1]Instituto de Catalisis y Petroleoquimica (CSIC), Madrid, Spain, [2]Universidad Autónoma de Madrid, Madrid, Spain, [3]Hospital la Paz, Madrid, Spain, [4]Centro de Investigaciones Biológicas (CSIC), Madrid, Spain.

The prokaryotic homologue of tubulin FtsZ plays a major role in cell division. *In vivo* it is localized at the center of the bacteria forming a dynamic ring that constricts during division. *In vitro* it binds and hydrolyzes GTP, and polymerises in a GTP-dependent manner. The role played by the hydrolysis in filament stability is not well understood.

In this work we have studied isolated FtsZ protofilaments by AFM obtaining information about the polymerization, steady state and depolymerization process in real-time at the single-molecule level. Previous experiments with Atomic Force Microscopy (AFM), which provide both high structural resolution and dynamics information under buffer solution, have reported complex dynamic behaviour for FtsZ filament network deposited onto a mica surface[1]. A recent model considering a labile longitudinal monomer-monomer bond with a preferential curvature and flexibility in addition to lateral attraction between monomers is enough to account for this rich dynamic behaviour[2].

In order to correlate GTPase activity with the labile nature of longitudinal bond between FtsZ monomers we used slow hydrolizable GTP analogues. We found a strong decrease on depolymerization velocity when GTPase activity is diminished allowing us to reveal cyclization, fragmentation, reannealing and release of monomers from either end or from both simultaneously as the essential features of a single FtsZ filament dynamics.

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2866-Pos

The Mechanism of E-Ring Formation During Min Oscillations

Martin Loose[1], Elisabeth Fischer-Friedrich[2,3], Karsten Kruse[4], Petra Schwille[1].
[1]Biophysics, Biotec, TU Dresden, Dresden, Germany, [2]Department of Physical Chemistry, Weizmann Institute of Science, Rehovot, Israel, [3]Max-Planck-Institute for Physics of Complex Systems, Dresden, Germany, [4]Theoretische Physik, Universitaet des Saarlandes, Saarbruecken, Germany.

In the bacterium Escherichia coli, the Min proteins oscillate between the cell poles to select the cell center as division site. This dynamic pattern has been proposed to arise by self-organization of these proteins, and several models have suggested a reaction-diffusion type mechanism. Recently, we have found that the proteins MinE and MinE are able to spontaneously form planar surface waves on a flat membrane in vitro. One particular feature of the patterns in vivo and in vitro is the so-called MinE ring, which defines the directionality of the traveling wave. How this accumulation of MinE at the trailing edge of the wave arises and its functional role during Min oscillation is not yet fully understood. We have applied FRET, TIRF and single molecule imaging techniques to study the behavior and interdependence of the three Min proteins in more detail. This helped us to propose a novel mechanism of Min protein wave propagation and E-ring formation.

2867-Pos

Kinetic Analysis of Actin Dendritic Nucleation from a Physicist's Standpoint

Pavel Kraikivski, Boris M. Slepchenko.
Richard D. Berlin Center for Cell Analysis and Modeling, Department of Cell Biology, University of Connecticut Health Center, Farmington, CT, USA.

Arp2/3-mediated polymerization of actin is an important mechanism by which cells dynamically change their shapes. Recent advances in deciphering a pathway of dendritic nucleation lay the groundwork for kinetic analyses that would estimate rate constants of the reaction steps involved in the process. Using kinetic models with various assumptions about a pathway's rate-limiting step, we have analyzed published quantitative data obtained from pyrene-labeled actin assays. All the available data are fitted well by two different models: one assumes that the nucleation is limited by the binding of the Arp2/3 complex to a mother filament whereas in the other, the rate-limiting step is the subsequent activation of the bound complex. To distinguish between these possibilities conclusively, we propose experiments with varying initial concentrations of actin monomers. The experiments need to be performed in nonequilibrium conditions, in which spontaneous formation of aster-like actin structures have been recently observed. For these conditions, the two models exhibit qualitatively different dependences of fluorescence intensity on the initial concentrations of G-actin. The work is supported by National Institutes of Health through grants 1U54 -RR022232, P41-RR13186, and 1U54-GM64346-01.

2868-Pos

Simulation Reveals Fundamental Behavior of the Actin Filament and Arp2/3 Branch Junction

Jim Pfaendtner, Gregory A. Voth.
University of Utah, Salt Lake City, UT, USA.

Molecular modeling and simulation now offer unprecedented opportunity to study biological events at the atomic level. Unfortunately, broad application of these approaches is elusive due to the difficulty of simulating biologically relevant length and time scales. I will use our recent investigation into key processes within the actin cytoskeleton to highlight progress we have made on these challenges.

The self-assembly of actin into a branched network of filaments is a vital process within the cytoskeleton of eukaryotic cells. This is chiefly regulated by the controlled hydrolysis of actin's bound ATP nucleotide. Although it is well established that ATP hydrolysis induces a change in actin's structure and dynamics, the exact mechanism by which this occurs is still unknown. We have used a novel computational approach to simulate various hydrolysis-induced structural transformations that have been suggested in the literature. Based on these results we are able to suggest a mechanism by which ATP hydrolysis regulates actin's structure and dynamics.

Equally important to the dynamics of the cytoskeleton is the process by which actin filaments are depolymerized in a controlled fashion. Recent experimental studies have yielded a number of hypotheses regarding the process by which actin depolymerization factor cofilin severs and depolymerizes filaments. We will present molecular and coarse-grained analyses of cofilin-bound actin filaments, and provide a molecular view of this process.

Finally, we have performed simulations of the actin branch junction - a key structural building block in the cytoskeleton. Our simulations reveal how small features of the actin protein give rise to the underlying dynamics of the entire branch junction. Strategies for coarse-graining supramolecular structures will be discussed in context of the branch junction.

2869-Pos

Toward De Novo Recapitulation of Cytoskeleton Dynamics with DNA Nanotubes

Rizal F. Hariadi[1], Bernard Yurke[2], Erik Winfree[1].
[1]California Institute of Technology, Pasadena, CA, USA.
[2]Boise State University, Boise, ID, USA.

The polymerization and depolymerization of cytoskeleton can transduce chemical energy into mechanical energy that drives cellular motility, such as chromosome segregation to cell protrusion. De novo recapitulation of the cytoskeletal phenomena with synthetic material would test our understanding of the design principles of polymerization motors. In the DNA nanotechnology tool box, DNA nanotubes are arguably the closest counterparts of cytoskeleton