actin spanning protein. The latter isofrom is shorter than any previously identified naturally occurring tropomyosin. The only tropomyosin of similar size previously characterised was an artificial construct that was shown to possess novel acto-myosin regulatory properties. The two isoforms are produced by alternative splicing from a single gene. We have shown that the shorter isoform is expressed at much lower levels than the larger one. We have cloned the two tropomyosins and characterised their actin binding and biophysical properties. As has been found with other recombinantly expressed tropomyosins, both isoforms need the addition of an N-terminal Ala-Ser dipeptide to bind to actin. This replaces the function of the N-acetyl group present in native tropomyosins and it is hence presumed the native forms are acetylated. As flexibility has been intimately related to tropomyosin function, we determined the thermal stability of these novel tropomyosins using circular dichroism. Surprisingly this was found to be significantly higher than that of the 161 residue *S.cerevisiae* and *S.pombe* tropomyosins, and the artificial 123 residue *S.cerevisiae* construct. We are currently assessing whether this indicative measurement is reflected in the acto-myosin regulation of reconstituted thin-filaments.

654-Pos Board B533

Multiple Isoforms of Fesselin (Avian Synaptopodin 2) are expressed in Smooth, Skeletal and Heart Muscle

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The synaptopodin 2 gene can be differently spliced, resulting in three mRNAs with varying 3′ ends coding for different C-termini (De Ganck et al 2008). The calculated molecular weights of these proteins are 117, 119, and 136 kDa. These isoforms require the expression of exons 1–3. However, only a single protein product has been detected in mammalian muscle lacking the product of these first exons.

We extracted four fesselin isoforms from avian smooth muscle tissue. These include the first isolated 79 and 103 kDa isoforms (Leinweber et al. 1999). The newly detected isoforms migrated on SDS gels with apparent molecular masses of 140 and 160 kDa. In contrast to our initial assumption that the 79 kDa was a proteolytic product of the 103 kDa protein we now show that they are different spliceforms. The 79 kDa isoform forms the core of synaptopodin 2. The other isoforms have different extensions at either the N- or C-terminal regions.

The different isoforms were differentially extracted by different buffers. Extractions were most complete under conditions that depolymerized both actin and intermediate filaments. Surprisingly although fesselin binds to actin and myosin none of the four different isoforms was extracted with the acto-myosin-complex. The extraction data suggest that fesselin functions in actin filament organization rather than in regulation of actin-myosin interactions. In contrast to smooth muscle tissue we detected one isoform of fesselin in skeletal and heart muscle tissue in agreement with the findings in mammalian tissue. In avian skeletal muscle we observed a 79 kDa isoform and in heart muscle a 170 kDa isoform. The reason for the differential expression of fesselin in different muscle types is unknown.

655-Pos Board B534

A Mechano-kinetic Model For The Myosin-V Walking Mechanism

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The double-headed, unconventional myosin, myosin-V, transports vesicles through cells by walking toward the plus end of actin filaments in a hand-over-hand fashion. Recent single molecule experiments with high spatial and temporal resolution have elucidated a number of performance features of myosin-V that can be used to test existing models for the underlying stepping mechanism.

We present a computational model that allows us to perform detailed tests of the compatibility of existing models with known details about the mechanical and kinetic properties of myosin V. Specifically, we use a coarse-grained physical model in which the neck domains are treated as semi-flexible filaments and the lever arm rotation of the leading head is realized through state-dependent changes in the equilibrium angle between the neck and head domains. The model is well constrained by experimental data on the mechanical properties of myosin V and on the kinetic cycle, and it reproduces key performance features of myosin-V, such as the run length, the distance of the working stroke, and the stall force. It also confirms the mechano-kinetic feasibility of a proposed gating mechanism based on intramolecular strain.

Because we explicitly model the thermal motion of all motor parts, we are able to present animations of motor stepping that realistically visualize the strong influence of thermal noise on motor stepping. In addition, our model allows us to make some predictions of parameters that are yet to be measured, including details of the molecule's flexibility, and establishes experimentally accessibly performance characteristics that can be used to test these predictions.

656-Pos Board B535

Regional Variations in Flexibilities Limit Continuum Rod Description of Long Coiled Coils

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Continuum rod description of coiled coils yielded a persistence length that range between 150–300 nm (Hvidt, S. et al., 1982. Biochem. 21, 4064, Adamovic, I. Et al. 2008. Biophys. J. 94, 3779). However, recent studies show that long coiled coils such as the stalk of a Rad50 complex exhibit regional variations in flexibilities (van Noort, J. et al., 2003. PNAS, 100, 7581). Apart from a length dependence due to non-bonded interactions (Lakkaraju, S. et al., 2008. BioPhys J. 94, 2404-Pos) our investigations reveal that stiffness of coiled coils is also strongly sequence dependent, hence limiting the region within which a coiled coil maybe described as a continuum rod. Bending stiffness (K_b) of a 161 Å long coiled coil with a leucine zipper periodicity calculated using normal mode analysis, forced bending dynamics and thermal fluctuation analysis (TFA) with locally built triad systems is about $1.86X10^{-27}$ Nm^2. Replacing the apolar residues at the a and d positions of the heptad repeat that are involved in the knob-into hole packing, with a hydrophilic (Glu and Lys) or polar charged residues (Ser), decreased K_b to about $0.864X10^{-27}$ Nm^2. Importantly, TFA of tropomyosin (PDB: 1C1G) mapped regions of varied flexibilities whose K_b varied between $0.33X10^{-27}$ Nm^2 (Ala 211 to Lys 221) to about $1.83X10^{-27}$ Nm^2 (Ser 36 to Leu 46), which confirms that stiffness of the structure decreases towards the C-terminus. Four of the seven actin binding sites (Brown, J.H. et al., 1986. Adv. Prot. Chem. 71, 121) lie within the regions of increased flexibility ($K_b \sim (0.75–1.36)X10^{-27}$ Nm^2), which possibly has a mechanical role during the on state of muscle contraction.

657-Pos Board B536

The Striated Organelle: A Molecular Motor In Vestibular Type I Hair Cells

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The striated organelle (STO) is a structure located in the subcuticular region of hair cells, consisting of alternating thick and thin bands (Friedman, 1965; Ross and Bourne, 1983). Although present in all cochlear and vestibular hair cells, the STO is particularly well-developed in type I hair cells, where it is shaped like an inverted open cone that contacts the cell membrane along its entire circumference. It is separated from the cuticular plate by a layer of mitochondria. In other hair cells, it is a much smaller structure and appears to be free-floating. We studied its structure using electron microscopic (EM) tomography in type I hair cells. In three-dimensional reconstructions, we found that it is connected to at least some actin rootlets. It may also be associated with microtubules, mitochondria and smooth endoplasmic reticulum. Confocal immunohistochemistry places yotiao (an AKAP protein) in the same area as the STO, and the actin-binding protein, alpha-fodrin (non-erythroid spectrin), where the STO contacts the cell membrane. The contact with the rootlets suggests that the STO might regulate hair-bundle stiffness. Its association with the cell membrane suggests that the STO may help in the formation of the constricted neck characteristic of type I hair cells.

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658-Pos Board B537

Dystrophin and Utrophin have Distinct Effects on the Microsecond Dynamics of Actin

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This study addresses the molecular mechanisms of actin's interaction with dystrophin and utrophin, in relationship to the pathology of muscular dystrophy. Dystrophin and utrophin bind actin *in vitro* with similar affinities, but with different molecular contacts. It has been proposed that these differences alter the elasticity of actin-dystrophin and actin-utrophin linkages to the sarcolemma, affecting the cell's response to muscle stretches. To test this hypothesis, we have determined the effects of dystrophin and utrophin on the microsecond dynamics of erythrosin iodoacetamide-labeled actin using transient phosphorescence anisotropy (TPA). Binding of dystrophin or utrophin to actin resulted in significant changes in the TPA decay, revealing similarities as well as differences in the structural effects of each protein on actin. At a low level of actin saturation ($\leq$20%) both proteins induced similar changes in actin dynamics, but at higher levels of saturation, utrophin was more effective than dystrophin and induced more pronounced changes in the final anisotropy, correlation time, and initial anisotropy of actin. The simplest interpretation of these changes is that utrophin restricted the amplitude and increased the rates of motion of the probe to a substantially larger extent than dystrophin. Further analysis indicated that the actin-utrophin complex is much more torsionally flexible than the actin-dystrophin complex. We propose that these differences between dystrophin and utrophin in their effects

on actin dynamics affect elastic properties of actin-mediated linkages with the sarcolemma. Future experiments using expressed fragments of dystrophin and utrophin and their functionally relevant mutants will determine which structural elements of these proteins are critical in determination of the flexibility of actin filaments and what level of actin flexibility is physiologically optimal.

659-Pos Board B538

Myosin-induced Movement Of Tropomyosin Isoforms On Actin Filament

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Tropomyosins (TMs) are a family of proteins which regulate actin interactions with myosins and other actin-binding proteins. TMs flexibility is thought to underlie the mechanism of TM-dependent regulation. In this work involvement of C-terminal sequence of TM in myosin-induced shift of TM during filament activation was analyzed. Recombinant αTM variants with C-terminus encoded by exon 9a (TM1b9a) or 9d (TM5a) were used. Both isoforms belong to short class of TMs with N-terminus encoded by exon 1b. To assess the degree of TM flexibility we measured FRET between AEDANS, which was attached to each TM isoform either in Cys^{153} or in Cys^{28}, and DABMI bound to actin in Cys^{374}. TM's Cys^{153} is a native residue located in central region of TM, Cys^{28} was introduced in N-terminal region using recombinant DNA methods. When bound to actin alone both TM isoforms show moderate flexibility with TM-actin distances ranging between 38.5 and 44.3 Å. Myosin S1 induced >10 Å movement of N-terminal region of TM5a, whereas it's central region was moved by about 4 Å. More uniform movement of the central and N-terminal regions was observed for TM1b9a. We conclude that TM's C-terminal sequence determines flexibility of the molecule. Supported by The Wellcome Trust.

660-Pos Board B539

Interactions of the Pleckstrin Homology Domains of M-RIP (p116Rip) with F-actin

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M-RIP has been shown to interact with actin, myosin, RhoA and the targeting subunit of myosin phosphatase, and has been proposed to be a scaffolding protein that anchors RhoA and Rho kinase onto myosin phosphatase and the actomyosin cytoskeleton. The N-terminal portion of M-RIP has 2 pleckstrin homology domains at residues 44–152 and 387–484 (PH1 and PH2, respectively). Mulder *et al.* (*J. Biol. Chem.* **278**, 27216–23, 2003) showed that the actin-binding activity of M-RIP resides in its N-terminal portion, and characterized the interaction between PH1 and F-actin, but did not investigate the role of PH2 in M-RIP's actin binding activity. In this work we examined the contributions of PH1 and PH2 to the M-RIP-F-actin interaction by, first, constructing 3 deletion mutants of M-RIP: M-RIP(1-386) (PH1), M-RIP(146-492) (PH2) and M-RIP(1-492) (PH1 and PH2). Co-sedimentation experiments with F-actin were then carried out at low or high speeds to sediment M-RIP-bound to F-actin bundles or filaments, respectively. The following results were obtained: 1) The extent of bundle formation increases in the order M-RIP(1-386) < M-RIP(146-492) < M-RIP(1-492). 2) The extent of total actin binding increases in the order M-RIP(1-386) < M-RIP(146-492) < M-RIP(1-492). Sequence homology analysis revealed that certain basic residues known to be important for actin binding in other PH domain-containing proteins (Yao *et al. J. Biol. Chem.* **274**, 19752–61, 1999) are present in PH2. Mutagenesis of three such residues, Lys404, 405 and 396, into Ala eliminated the binding between M-RIP(1-492) and F-actin. Taken together our results show that the binding of M-RIP to F-actin is mediated primarily via its PH2 domain, in particular via ionic interactions between certain basic residues in PH2 and acidic residues in actin. (Supported by NIH AR41637 and AR49066).

661-Pos Board B540

βCaMKII Regulates Actin Assembly and Structure

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Recently Ca^{2+}-Calmodulin-dependent protein kinase II (CaMKII), an abundant synaptic protein, was shown to be important in the organization of actin filaments at synapses. We report the binding properties and mechanistic role of βCaMKII as an actin binding protein *in vitro*. A particular case is the ability of βCaMKII to bundle actin filaments, which we corroborated by cryo-electron tomography. In addition, a pyrene-actin fluorescent polymerization assay was used to determine that the enzyme reduced the rate of polymerization by ~ 80%, suggesting that βCaMKII either serves as a capping protein or binds monomeric actin reducing the amount of freely available monomers to nucleate polymer assembly. Finally, by means of fluorescent cross correlation spectroscopy we determined that the β isoform of CaMKII does bind to monomeric actin, reaching saturation at a stoichiometry of 6:1 actin mononmers per βCaMKII holoenzyme with a binding affinity of ~2 μM. In conclusion, βCaMKII has a dual functional role; it can sequester monomeric actin to reduce actin polymerization and can also bundle actin filaments. Together, these effects would impact both the dynamics of assembly of actin filaments and enhance the structural rigidity of the filaments once formed, significantly impacting the structure of synapses.

662-Pos Board B541

Coactosin, A Cofilin Like Protein, Does Not Change The Twist of F-actin

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Actin cytoskeletal rearrangements are orchestrated by a vast number of actin binding proteins. Coactosin is a 17kDa actin binding protein originally discovered in *Dictyostelium discoideum*. The protein has an ADF-homology domain (ADF-H-domain) also found in cofilin, twinfilin, and drebrin. It was shown that coactosin interferes with capping proteins, but in the absence of capping proteins it does not influence on actin polymerization. We used electron microscopy and the IHRSR method to reconstruct actin filaments stoichiometrically decorated with coactosin. In contrast toADF/cofilin, coactosin does not change the twist of F-actin. Nevertheless, coactosin utilizes a similar interface on F-actin to that used by ADF/cofilin. Our data are consistent with the solution structural studies on coactosin showing that the regions of ADF/cofilin involved in the interaction with F-actin are structurally conserved in coactosin.

663-Pos Board B542

Conformational Changes Of Arp2/3 Complex During Activation

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Kinetic and structural studies of Actin-related protein (Arp) 2/3 complex point to a transition from inactive to active conformations at an actin branch point. The inactive conformation is greatly favored for purified Arp2/3 complex. Crystal structures of inactive Arp2/3 complex show that Arp2 and Arp3 are separated by about 3 nm compared to their positions as the first two subunits of the daughter filament in low resolution models based in reconstructions from electron tomograms of actin filament branch junctions. Here, we use atomistic-scale molecular dynamics simulations to study activation of Arp2/3 complex. Starting in the ATP-bound inactive crystal structure, we apply forces to Arp2 and Arp3 so that the energy of the system is at a minimum when Arp2 and Arp3 are positioned like the first and second subunits of the daughter filament. Arp2 does not detach from the other subunits during activation as proposed from biochemical experiments. The long C-terminal α-helix of p34 rotates along with Arp2 and p40 through interactions with p20. Residues His^{30}-Leu^{35} of p20 stay in contact with the residues of the αG helix of Arp2 throughout the course of the simulation. Also, residues Arg^{106}-Lys^{107} of p20 interact with the region of Arp2 surrounding Leu^{235} between the αF and αG loops. The main impediment to a smooth transition is the αK helix of subdomain 3 of Arp3, which collides with the DNase binding loop of Arp2. This study provides the first insights into the conformational changes of Arp2/3 complex forms an actin filament branch.

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Acidic Calponin controls ERK1/2 translocation and l-Caldesmon phosphorylation in fibroblasts

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Calponin is an actin binding, troponin-like molecule, first isolated from chicken gizzard smooth muscle cells where it is involved in regulation of contractility. So far there are three known isoforms, basic, acidic and neutral Calponin, each encoded by a separate gene. Expression of the acidic Calponin isoform is, unlike basic Calponin, not restricted to smooth muscle cells but is expressed in a variety of tissues and cell types, especially in the brain. For basic Calponin it is known that the molecule affects smooth muscle cell contractility by regulation of the PKC/ERK signaling pathway. Here we could show that acidic Calponin co-precipitates together with ERK1/2 and PKCalpha in the cultured rat fibroblast cell line REF52.2. Moreover it colocalises with ERK1/2 and PKCalpha in podosome-like structures and at the cell cortex after Phorbol-12,13-Dibutyrate (PDBu) treatment. Knockdown of acidic Calponin expression in these cells results in inhibition of PDBu-mediated ERK1/2 translocation to these structures, whereas PKCalpha targeting is not affected. Both endogenous ERK1/2 activity and phosphorylation of the ERK1/2 substrate l-Caldesmon are blocked by acidic Calponin knockdown, indicating an important role for the acidic Calponin isoform in regulation of ERK1/2 function in REF52.2 cells. Abstract sponsored by John Gergely, Boston Biomedical Research Institute, Watertown 02472, MA, USA
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