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.

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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.

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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.

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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.

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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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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