Developmental and electrophysiological studies have shown that the cochlear function is altered in these mice. We carried out a detailed kinetic analysis of the baculoviral expressed truncated shaker-1 construct to assess whether the ATP hydrolysis mechanism is altered in these mice. The basal steady-state rate (0.03 s^{-1}) is activated by actin only 1.5 -fold. ATP binding is relatively fast (1.22 $uM^{-1}s^{-1}$) and the dissociation of the myosin from the actomyosin complex is the same as for the wild type myosin VIIa (400 s^{-1}). ADP binding to (1.8 $uM^{-1}s^{-1}$) and the dissociation (1.5 s^{-1}) from the actomyosin complex are also similar to the wild type. Phosphate release from the M-ADP-P_i complex is slow (0.07 s^{-1}) and is not activated by actin. Quenched flow experiments show that there is no Pi burst and the rate of the hydrolysis is 0.12 s^{-1}. The R502 residue is in a small loop that is in the middle of an α-helical region which aligns with the switch-2 helix and is the continuation of the relay-loop. The sequence in the loop is changed from NRPM to NPPM which is likely to alter the conformation of the helix-loop-helix structure that is relatively straight in all myosins.
Consequently the rate constants of the steps leading into the power stroke (hydrolysis and phosphate dissociation) are reduced greater than 10-fold whereas the post-power stoke steps are essentially unchanged. As a result the shaker-1 mutant protein has a severely compromised ATP hydrolysis mechanism in which $<$ 10% of the normal fraction of the force-producing crossbridges are formed.
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2896-Pos Board B666
Single Molecule Movement of Full-Length Myosin X
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Myosin X is an unconventional myosin that is critical for cargo transportation to filopodia, and is associated with the mechanism underlying filopodia formation and extension.
We previously reported that myosin X-HMM is a processive motor with large ~34 nm step size. Our myosin X-HMM dominantly shows the large step size on both single actin filament and fascin-actin bundle although a minor ~18 nm step size can be seen on fascin-actin bundle [Sun *et al.* (2010) Nat. Struct. Mol. Biol., 17, 485-491]. On the other hands, Rock and his colleagues recently reported that their myosin X-HMM only showed ~18 nm step size [Nagy *et al.* (2010) J. Biol. Chem., 285, 26608-26617; Kapitein *et al.* (2010) Biophys. J., 99, 2143-2152]. Thus, the actual step size of myosin X remains to be elucidated.
To address the question of innate step size of myosin X molecules, we produced the full-length myosin X construct with a leucine zipper motif at the C-terminal end ($M10^{Full}LZ$) to ensure the dimer formation. We first compared the localization of $M10^{Full}LZ$ with the wild type myosin X full-length ($M10^{Full}$) in COS7 cells, and confirmed that $M10^{Full}LZ$ localized at the tip of filopodia, which is similar to $M10^{Full}$.
We next expressed $M10^{Full}LZ$ in Sf9 cells and purified for motility assay. The $M10^{Full}LZ$ could move actin filaments in PIP_3 dependent manner by *in vitro* multi-molecule motility assay, consistent with the result of $M10^{Full}$. We then analyzed the single molecular step size of $M10^{Full}LZ$ using a total internal reflection fluorescence microscope. The mean step size of $M10^{Full}LZ$ was the large ~34 nm on single actin filaments. This result suggests that the innate step size of myosin X is ~34 nm. The step size of $M10^{Full}LZ$ on actin bundles in filopodia is now underway.

2897-Pos Board B667
Exploring Bundle Selectivity of Myosin X Forced Dimer Constructs in vivo
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Cells organize their contents and regulate cell shape and mechanics through molecular motors functioning on cytoskeletal filaments. Myosin X, an actin-based motor that concentrates at the distal tips of filopodia of mammalian cells, selects the fascin-actin bundle at the filopodial core for motility. While poorly processive on single actin filaments, it takes processive runs on actin bundled by fascin. Recently we showed that the post-IQ region is the main contributor to myosin X's selectivity. This region contains a charged single alpha-helix (SAH), which may impart unique mechanical or affinity properties to the motor. The structural character of this region was perturbed by insertion of a free swivel (GSGGSG flexible linker) after the SAH domain. The post-SAH swivel mutant showed no preference for bundled actin for motility in vitro, thus providing support to a selectivity model where the search space of the forward head for the next binding site is constrained to neighboring filaments in a bundle. Here we investigate if interrupting the "selectivity module" also has the described effect in live cells.
We overexpressed in the U2OS cell line three fluorescently tagged forms of myosin X: the GCN4 forced dimer used in in vivo experiments, the post-SAH swivel mutant of GCN4 dimer and the wild type with a native dimerization domain. GCN4 forced dimer performs in the cell in the same manner as full- length myosin X. Introducing a free swivel in the SAH domain region affects the selective nature of myosin X in the natural cytoskeleton environment of the cell interior as co-localization of GFP labeled protein with filopodial tips does not occur for the Swivel mutant.

2898-Pos Board B668
Functional Characterization of Human Myosin-18A and its Interaction Partners
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Molecular motors of the myosin superfamily share a generic motor domain region. They bind actin in an ATP-sensitive manner, exhibit actin-activated ATPase activity, and generate force and movement in this interaction. Many myosins, such as the members of class-18 have not been characterized in-depth. Class-18 myosins form heavy chain dimers and contain protein interaction domains outside the motor domain. Changes of otherwise highly conserved active site residues have raised the question whether myosin-18 can productively interact with ATP in the same way as other myosins. A recently described interaction of mammalian myosin-18A with the Golgi-associated phosphoprotein GOLPH3 was proposed to link the Golgi apparatus to the actin-based cytoskeleton. Here, we characterized human myosin-18A function and its interaction with GOLPH3 in vitro. We found that the interaction of the myosin-18A motor domain with actin filaments appears to be unusual, as the ADP state displays the highest actin-affinity. We performed negative-stain electron microscopy with myosin-18A motor domain decorated F-actin filaments in different nucleotide states and observed the most homogenous and continuous decoration in the ADP-state. The isolated N-terminal subdomain contains a region rich in lysine and glutamate (KE) and a PDZ module. We show that the KE-rich region displays ATP-independent binding to F-actin, whereas the PDZ module mediates the binding of myosin-18A to GOLPH3 and thereby to the Golgi apparatus. We propose that human myosin-18A connects the actin cytoskeleton with the surfaces of organelles such as the Golgi apparatus and that it can target interacting proteins or complexes to the actin cytoskeleton via its PDZ module.

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Parasite Motility: Mechanisms of a Novel Molecular Motor, Myosin XXI
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Myosin XXI is a motor found in the disease-causing organism Leishmania. Genome analysis identified only two myosin genes, a class IB and a class XXI. While no expression of myosin IB has been found in the organism to date, myosin XXI has been detected in both the promastigote and the amastigote stages of the Leishmania life cycle, where it is preferentially localised to the proximal region of the flagellum. The presence of only a single myosin isoform suggests that this myosin carries out a variety of functions within the protozoa, including possible roles in membrane anchorage as well as longer range directed movements with cargo. We have co-expressed myosin-XXI and calmodulin in a baculovirus system, and found that it binds a single calmodulin at its neck domain which is required for motility, although not for ATPase activity. Myosin XXI transports actin filaments in motility assays, and is insensitive to both high salt and Ca2+ concentrations up to pCa 4. Sequence analysis of myosin XXI identifies a leucine zipper as well as two short coiled-coil regions, suggesting that myosin XXI is able to dimerise. To confirm this hypothesis we expressed a variety of tail constructs and studied formation of dimerisation using gel filtration. We found that the tail fragment binds calmodulin and dimerises. Intriguingly the full-length myosin XXI initially appeared to be a monomer. Further studies showed the tails dimerise in a temperature dependent manner. While the tails are stable monomers below 20 oC, they form dimers above this. When this was repeated with the full-length protein we also observed the creation of dimers or higher state oligomers. We are further characterising this observation using electron microscopy and TIRF microscopy photobleaching experiments.
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