Unconventional Myosins I (849 - 863)

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Mice null for brush border myosin I assemble brush borders and lack an overt phenotype

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Brush border myosin I (BBMI) is a single-headed, actin-based motor protein found in the apical brush border domain of intestinal epithelial cells. Although BBMI has been characterized extensively in vitro, a clear understanding of its in vivo function is lacking. To gain insight concerning the role of this motor within the brush border, we have generated a mouse line null for the BBMI gene. Mice homozygous for the deletion (BBMI -/-) are fertile, eat normally, are of comparable weight, and behave in a manner similar to wildtype animals. Gross level analysis of small intestine morphology and light microscopic analysis of brush border morphology indicate that at these levels, BBMI -/- mice are similar to controls. However, ultrastructural analysis of BBMI -/- microvilli reveals that the "bridges" normally found linking actin bundles and plasma membrane (classically thought to represent BBMI) are missing in the knockout animals. Western blot analyses of isolated brush borders from BBMI -/- mice indicate that myosins Ic, II, VI, VIIa and villin are retained in these structures at levels comparable to control. Interestingly, calmodulin levels in BBMI -/- brush borders are markedly reduced, a finding consistent with BBMI acting as the major calmodulin-binding protein in the brush border. Given the presence of an intact brush border and the lack of any pronounced phenotype in BBMI -/- mice, we postulate that BBMI may be involved in the plasticity of the brush border structure in response to dietary stress or cell injury. Experiments involving mucosal injury and intestinal pathogen exposure are now underway.

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***Drosphila* Intestinal Epithelial Cells Lacking the Brush Border Specific Myosin-Ib, Assemble a Brush Border but Starve to Death as Larvae.**

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Drosophila myosin IB (MIB), one of two myosins I in *Drosophila*, is the apparent ortholog of mammalian brush border (BB) myosin I. MIb localizes to the BB of the intestinal epithelial cells at all stages of devleopment. To study the function of BBMIs we have pursued a reverse genetic strategy. Excision of a transposon 394 nucleotides 3' of MIB resulted in a deletion mutation that removed ~3/4ths of the MIB gene, including the region encoding the actin binding site. Immunoblot analysis indicates that no MIB protein is produced in flies homozygous for this deletion. Embryos hatch but arrest as 1st instar larvae, fail to grow, and die after ~7 days, a phenocopy of starvation. At the ultrastructural level, these animals have normal appearing BBs, but lack microvillar cross bridges between the actin core and the membrane. To further characterize the effect of MIB loss, additional mutant alleles were generated by EMS mutagenesis. Flies from 7 of 8 mutant lines produced no full length MIB; 1 line does express full length MIb protein. All mutant alleles are homozygous lethal and show developmental and growth defects similar to the deletion mutation. To determine if lethality is due to failure to digest yeast into absorbable nutrients, mutant larvae were fed a complete predigested diet. This diet did not result in greater growth or continued development. Rescue with MIB cDNA transgene constructs is underway. These results indicate that 1) Mb is not required for assembly of the BB cytoskeleton, and 2) may be required for nutrient uptake by the gut.

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Regulation of XenM-1β suggests its involvement in meiotic maturation and/or fertilization

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Diverse cellular roles have been proposed for unconventional myosin-1β. While vertebrate myosin-1β of the inner ear hair cell has been implicated as the adaptation motor that gates mechanotransductive ion channels, the localization of myosin-1β in filopodia, lamellipodia, ruffles and growth cones of motile cultured cells also supports a possible role in actin/plasma membrane dynamics. We previously reported that myosin-1β (Xen-1β) is expressed in the full-grown *Xenopus* oocyte. Developmental Northern analysis of polyA$^+$ RNA fractions detected a sharp up-regulation of transcript during progesterone-induced meiotic maturation, the process by which the meiosis I-arrested oocyte is converted into the fertilization-competent, meiosis II-arrested egg. This result is in contrast to actin, myosin-2A and other 'housekeeping' transcripts that are down-regulated by deadenylation during *Xenopus* meiotic maturation. Comparison of total RNA fractions to polyA$^+$ RNA fractions between control oocytes and eggs showed that while the level of total XenM-1β RNA remained constant, the level of XenM-1β polyA$^+$ RNA increased dramatically, demonstrating that XenM-1β transcript was selectively polyadenylated during maturation. A corresponding increase in XenM-1β protein was detected by immunoblotting. Other transcripts known to be translationally activated via selective polyadenylation during *Xenopus* meiotic maturation (mos, cyclins A_1, B_1, B_2) are required for progression through meiotic maturation and fertilization suggesting XenM-1β involvement in the rapid ion fluxes and/or actin/plasma membrane dynamics that accompany these events. Consistent with this possibility, preliminary confocal immunofluorescence analysis showed a striking, transient relocalization of XenM-1β from the cytoplasm to the cell periphery upon egg activation with the artificial fertilization stimulus, Ca^{+2} ionophore. Using antisense oligonucleotide techniques for protein ablation in *Xenopus* oocytes, eggs and early embryos, we are now examining the cellular role of XenM-1β during meiotic maturation and fertilization.

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Design of myosin mutants to provide specific inhibition

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We have developed a mutant/inhibitor strategy that allows us to test the role of a myosin isozyme in a given cellular function. We mutate myosin in a manner that maintains normal ATP hydrolytic and chemomechanical activity, but that allows specific binding of an inhibitor to the mutant myosin, but not the wild-type myosin from which it was derived. We have designed such a mutation in the ATP-binding pocket of myosin I-beta that sensitizes the mutant protein to N6-modified ADP analogs when expressed in a baculovirus system, while it retains nearly normal activity in the presence of ATP. Transgenic mice expressing the mutant protein show specific inhibition of slow adaptation in utricular hair cells, unequivocally identifying myosin I-beta as the adaptation motor and an important part of the sensory transduction apparatus.

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Characterization of a Nuclear Isoform of Myosin I

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Myosin I is a single headed non-filamentous member of the myosin superfamily of actin based molecular motors. Immunological, biochemical and molecular biology approaches have shown the presence of myosin I in the nucleus. Microsequencing has revealed that the 120 kDa protein immunoprecipitated from the nucleus is a form of myosin Iβ that contains an unique 16 amino acid NH_2-terminal extension. Analyses of the cloned nuclear myosin I cDNA and the mouse myosin I
β gene on chromosome 11 have uncovered an upstream exon that contains a start site that is in frame with the consensus myosin I β start site in Exon 1. The unique 16 amino acid peptide is coded by the nucleotides between these 2 start sites. Expression of the cDNA for the nuclear isoform of myosin I β resulted in nuclear localization of an epitope tag. In contrast, expression of the same cDNA without the nucleotide sequence for the peptide resulted in cytoplasmic localization, exclusively, of the epitope tag. Affinity purified antibodies to the 16 amino acid peptide also demonstrated nuclear staining. Confocal and electron microscopy revealed that nuclear myosin I co-localizes with RNA polymerase II in an α-amanitin and actinomycin D dependent manner. Protein immunoblots confirmed co-immunoprecipitation of NMI and RNA Polymerase II. Moreover, the affinity-purified antibodies to the 16 amino acid peptide block *in vitro* RNA synthesis. These data establish the presence of an actin based motor in the nucleus and suggest an important functional role for nuclear myosin I.

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Analysis of Myosin I b GFP constructs expressed in multiple cell types

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Myosin I b has been found in numerous cell and tissue types though very little information concerning its cellular function has been obtained. We have fused green fluorescent protein to both the amino and carboxy termini of this protein and observed its distribution in several cell types. Transient transfection of LLCPK1 cells found the GFP construct in microvilli, lamellipodia, cell-cell borders and also in a punctate pattern in the cytoplasm. These findings are consistent with immunofluorescent data. We