Regulation of fission yeast myosin-I force production and cellular levels at endocytic actin patches by a novel light chain

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Actin-dependent internalization of endocytic vesicles, a necessary life process, is driven by a force producing motor protein called myosin-I (called Myo1p in fission yeast). Myo1p consists of three domains: the catalytic actin-binding motor ('head'), light chain-binding domain ('neck'), and the 'tail' which anchors the protein at the plasma membrane. The motor converts the free energy from ATP hydrolysis into mechanical work through a conformational change which displaces an attached actin filament resulting in force production. This study assessed the role of a novel Myo1p light chain (Cam2p) in Myo1p function. We employed time-lapse epi-fluorescence microscopy to track Myo1p at endocytic patches and i*n vitro* assays to assess Myo1p motor activity in the fission yeast *Schizosaccharomyces pombe*. Cam2p localizes with Myo1p at actin patches in a wild-type background. Both *myo1Δ* and *cam2Δ* strains are temperature-sensitive and exhibit similar defects in cell shape at elevated temperatures. These defects were not exacerbated in a *myo1Δ cam2Δ* double mutant, suggesting that the primary role of Cam2p lies with Myo1p. The levels and lifetime of Myo1p at endocytic patches decreased in a *cam2Δ* background. *In vitro*, the actin-activated ATPase activity of Myo1p was not affected by the loss of Cam2p, however, loss of Cam2p decreased the rate of actin filament gliding by approximately 50% in the *in vitro* motility assays. This suggests that Cam2p aids in the stabilization of the Myo1p neck in order to maximize actin filament displacement. Overall, our work suggests that Cam2p is necessary for maintaining Myo1p levels and motor-dependent force production at endocytic actin patches.