

Putting the brakes on a myosin motor

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Casey Eddington and Margaret A. Titus¹

From the Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, Minnesota 55455

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Insulin-stimulated trafficking of GLUT4 requires the myosin motor Myo1C and signaling adaptor 14-3-3 β . Originally, it was thought that 14-3-3 β promotes GLUT4 transport by binding the Myo1C lever arm and activating the Myo1C motor. New work by Ji and Ostap using *in vitro* assays reveals that 14-3-3 β binding actually inhibits Myo1C motility, prompting reconsideration of the functional relationship between 14-3-3 β and Myo1C and the regulatory potential of atypical light chains.

Glucose homeostasis, crucial for cell viability, is regulated by insulin. Specifically, insulin signaling promotes a rise in intracellular calcium that stimulates the mobilization of glucose transporter type 4 (GLUT4) storage vesicles (GVs)² and subsequent translocation of these vesicles to the membrane, where GLUT4 takes up circulating glucose into adipocytes and muscle cells (1). To reach the plasma membrane, GV's must navigate the dense cortical actin filament network, requiring the action of vesicle-associated myosin motors. The myosin motor Myo1C, in particular, plays several vital roles during key stages of GV trafficking (2, 3). Specifically, Myo1C is implicated in transporting or tethering GV's to the cytoskeleton by binding actin via its motor domain and GV's via its phosphoinositide binding tail (4). The signaling events that lead to the myosin-dependent transport of GV's have been described, but the steps that activate Myo1C and the exact role this myosin plays in this process are not fully known. New *in vitro* work from Ji and Ostap explores a proposed regulator of this process, the signaling adaptor 14-3-3 β , revealing an unexpected function with interesting implications for the control of Myo1C motor activity (5).

Myo1C is a widely expressed myosin motor containing a typical myosin motor domain followed by a lever arm that binds light chains (LCs) and finally a tail that contains a polybasic region capable of phosphoinositide phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P₃) binding (Fig. 1). The lever arm contains three IQ motifs that typically serve as binding sites for a total of three molecules of calmodulin (CaM),

but other proteins, including members of the CaM superfamily, can bind to the IQ motifs as well (6, 7). The lever arm plays a critical role in force transmission, and LC binding to the IQ motifs is thought to stabilize this structure (8, 9). The interactions of Myo1C's first IQ motif immediately adjacent to the core motor domain, IQ1, with CaM depend on intracellular calcium levels (6, 7, 10). CaM associates with the Myo1C lever arm when calcium is low; however, an increase in calcium causes dissociation of CaM from IQ1 (7). This provides an opportunity for the other binding proteins to compete with CaM for this site. One such protein is RalA, which has been implicated in linking Myo1C to the exocyst complex that tethers vesicles to the plasma membrane prior to vesicle fusion (6). The signaling adaptor 14-3-3 β was also identified as a calcium-sensitive Myo1C-interacting partner (10). 14-3-3 β is a member of a protein family that mediates signal transduction by promiscuous binding near phosphorylated residues and was shown to be essential for GLUT4 transport and tethering (10). Specifically, calcium-stimulated phosphorylation of Myo1C at Ser-701, a residue that is located close to the motor domain-IQ1 interface, by CaMKII was previously shown to correlate with Myo1C:14-3-3 β binding, increased Myo1C ATPase activity, and increased GLUT4 translocation (10). Therefore, it was proposed that 14-3-3 β binding activates Myo1C motor activity, resulting in transport of GV's to the membrane. However, a direct biochemical test of this proposal was lacking.

Ji and Ostap now provide this test, examining the impact of exchanging bound CaM for 14-3-3 β on Myo1C motor function *in vitro* (5). They confirm the original finding that calcium regulates the Myo1C:14-3-3 β interaction. The addition of excess dimeric 14-3-3 β to purified Myo1C results in low levels of binding; however, the addition of 40 μ M Ca²⁺ causes increased binding, up to 1 dimer per Myo1C heavy chain. The work also confirms that 14-3-3 β binds to IQ1 (5, 11). Unexpectedly, the authors discovered that 14-3-3 β binding to Myo1C IQ1 is independent of Ser-701 phosphorylation. It instead relies solely on CaM dissociation from IQ1 that is stimulated at high calcium concentrations (Fig. 1A and B). As the loss of CaM would destabilize the lever arm, leading to inhibition of Myo1C motor function, the authors tested the ability of 14-3-3 β to rescue potentially impaired Myo1C motor function at high calcium levels using *in vitro* motility assays. Surprisingly, whereas 14-3-3 β did not alter the actin-activated ATPase of Myo1C, it strongly inhibited motility in the presence of calcium, the opposite of what was predicted from previous *in vivo* data. If Myo1C transport function is inactivated when bound to 14-3-3 β , why is the Myo1C:14-3-3 β interaction essential for GV transport during elevated calcium? One interesting possibility is that 14-3-3 β

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¹ To whom correspondence should be addressed: Dept. of Genetics, Cell Biology, and Development, 6-160 Jackson Hall, 321 Church St. S.E., University of Minnesota, Minneapolis, MN 55455. Tel.: 612-625-8498; E-mail: titus004@umn.edu.

² The abbreviations used are: GV, GLUT4 storage vesicle; LC, light chain; CaM, calmodulin; CaMKII, Ca²⁺/calmodulin-dependent kinase II.

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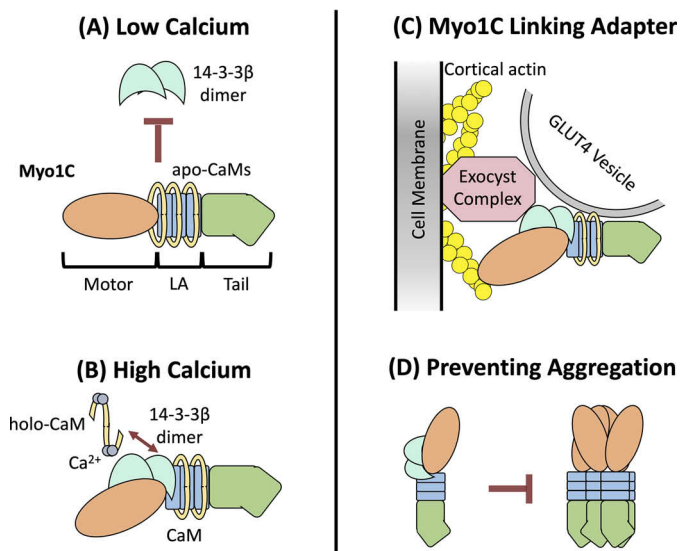


Figure 1. 14-3-3 β competes with CaM for Myo1C binding to the lever arm. A, three apo-CaM molecules bind the Myo1C lever arm (LA) when intracellular calcium is low, interfering with 14-3-3 β association with Myo1C. B, insulin stimulation causes an increase in intracellular calcium, dissociating CaM from the first IQ domain (IQ1), allowing the 14-3-3 β dimer to bind. C, 14-3-3 β could link Myo1C to GLUT4-containing vesicles and membrane fusion machinery to the membrane, allowing the motor to tether the vesicle to the cortical actin network. D, high calcium causes CaM dissociation from the Myo1C lever arm, exposing hydrophobic patches, which can cause Myo1C aggregation. 14-3-3 β binding the lever arm may prevent Myo1C aggregation when calcium is high.

binding stabilizes Myo1C as well as linking Myo1C to key players involved in GV transport and fusion, as has been suggested for RalA (8). For example, 14-3-3 β could allow Myo1C to tether GLUT4-containing vesicles to the cortical actin network until the exocytic machinery can be engaged and promote fusion with the plasma membrane (Fig. 1C). The binding of 14-3-3 β to IQ1 would also prevent Myo1C aggregation when high Ca²⁺ levels cause loss of CaM, resulting in exposure of a hydrophobic stretch prone to aggregation (Fig. 1D). Further work to examine the *in vivo* impact of these associations in promoting transport and membrane fusion of the GVs is eagerly awaited.

The results of Ji and Ostap do not fully agree with earlier observations showing that phosphorylation at Ser-701 by CaMKII increases both 14-3-3 β binding and motor activity. Previous experiments were performed using Myo1C immunoprecipitated from a total cell lysate or using a purified motor-IQ fragment (10, 11). The two different studies each demonstrate phospho-Ser-701-dependent binding of 14-3-3 β to Myo1C. However, in the case of the experiments with cell lysates (10), it is possible that another protein impacts Myo1C in general or the 14-3-3 β interaction with Myo1C in a CaMKII-dependent manner. As for the motor-IQ fragment (11), it is possible that the short lever arm adopts a conformation that makes 14-3-3 β binding more sensitive to Ser-701 phosphorylation. Identifying the full complement of Myo1C binding partners under basal and insulin-stimulated conditions and characterization of the impact of Ser-701 phosphorylation of full-length Myo1C on

14-3-3 β binding and ATPase activity should help to resolve the discrepancies and provide new insights into how insulin signaling mobilizes Myo1C for increasing GLUT4 levels at the plasma membrane.

The work presented by Ji and Ostap also highlights the importance of the role of the Myo1C lever arm in determining its function. Myosin LCs typically regulate the activity of the motor and stabilize the lever arm (9), enabling an effective power stroke. However, it is now apparent that the Myo1C lever arm acts as a dynamic signaling hub for calcium-dependent interactions with LCs, such as RalA, CaBP1, CIB1, and 14-3-3 β , that can alter force generation or potentially even step size. It is interesting to consider that perhaps the lever arms of other unconventional myosins, particularly those that exhibit Ca²⁺-sensitive CaM binding, could undergo LC switching as is seen for Myo1C. The ability of the lever arm to bind to different LCs or binding partners as a result of cell signaling or changing cellular conditions might alter the localization and/or activity of a number of different myosins, thereby expanding the diverse range of roles for the myosin superfamily.

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