Phosphorylation Regulates Myosin Driven Organelle Movements

Peter Duden and Andreas Nebenführ
Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, 37919

Abstract
Cytoplasmic streaming in plant cells is the continuous flow of cytoplasm and organelles throughout the cell, with the first observation of cytoplasmic streaming being published in 1774. However, the mechanism of cytoplasmic streaming remained unclear until components of the cytoskeleton were researched. Research now supports that the motive force generating cytoplasmic streaming is the interaction of myosin XI motor proteins with organelles while sliding along actin filaments. From this, a key topic of interest is how myosin-driven organelle movement is regulated. Our research focuses on whether phosphorylation affects the activity of myosin XI motor proteins. Specifically, we observed that the coiled-coil regions of coiled-coil region is required for stable targeting of MYA1 in Arabidopsis thaliana. We also observed that the coiled-coil regions of myosin XI isoforms are dimerized, and fluorescence-labeled organelles after addition of inhibitors. Our results support the conclusion that phosphorylation is a regulator of myosin-driven organelle movement. Recent research suggests the degree of phosphorylation is dependent upon the organism observed.

Introduction
Myosins are ATP-driven motor proteins that are involved in several important life processes, including muscle contraction and cell division. In plants, myosins XI's function is still not clear, although research has clearly linked myosin XI with organelle trafficking, the cytoskeleton, and cell polarization. For these reasons, myosin XI research offers insight into further understanding of important cell processes.

Golgi bodies have been shown to be moved by the acto-myosin system, and previous research in our lab supports that Golgi bodies are affected in movement by phosphorylation inhibitors, while actin filaments are not destroyed or disrupted by inhibitor treatments. With actin dynamics not being affected, the activity of myosin XI is considered. Additional support that myosin XI activity is regulated by phosphorylation is from myosin Va in mammals, which is supported to be regulated by phosphorylation. In order to study the impact of phosphorylation on myosin XI activity, this study uses the kinase inhibitor staurosporin and the phosphatase inhibitor okadaic acid. In general, the kinase inhibitor blocks the addition of a phosphate onto myosin, and the phosphatase inhibitor blocks the removal of a phosphate from myosin.

Methods
Five A. thaliana seed lines were used for the experiment. Seedlings were then placed on vertical plates (14 Murashige - Skoog) and incubated for five days. Fluorescence microscopy was then utilized in order to observe the signals from each of the green seedlings. A cover slip was placed with vacuum grease on two sides and gently placed over the seedling to create an observation chamber, which was filled with medium. A one minute time lapse with one picture per second was taken, in addition to pictures of five different cross sections of the root hair. After this, aliquots of 150 picomoles of dimethyl sulfoxide (DMSO), 1% in MSNO), staurosporin (200 nm in MSNO), okadaic acid (200 nm in MSNO, 1% DMSO), and okadaic acid (200 nm in MSNO, 1% DMSO) replaced the original medium. After ten minutes, data was again collected on the identical root hair. Analysis of velocities was conducted using an automated particle tracker in ImageJ.

Results and Discussion
Myosin MYA1 localization was observed across the root hair and most concentrated at the root hair tip. MYA1 signal was similar after inhibitor treatments (small increase in signal in shank of root hair). MYA1 velocities decreased across all data sets after okadaic acid treatments, and increased after staurosporins treatments (One way ANOVA, p-value > 0.01). RhAB4 velocity localization was also observed across the root hair and did not significantly change after inhibitor treatments (small increase of signal in shank of root hair). RhAB4 velocities were not affected in all data sets after administration of inhibitors (p-value > 0.01).

Conclusions
1. Phosphorylation decreases the movement of MYA1 and dephosphorylation increases the movement of MYA1.
2. RhAB4 movement is not regulated by phosphorylation as it is not affected by treatment with inhibitors.
3. Peroxisomes has increased movement when phosphorylated.
4. Golgi bodies has decreased movement when unphosphorylated.

From these conclusions, phosphorylation is supported to regulate myosin XI and its cargo in trafficking.

Further Research
- Optimization of particle tracking analysis to eliminate errors in data through matching accurate manual tracks with automated tracks
- Additional replications of organelle experiments
- Collecting data with different timelapses to ensure high velocities are not being cut off from data sets

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References