Phosphorylation Regulates Myosin Driven Organelle Movements

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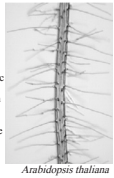
Abstract

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[1]. However, the mechanism of cytoplasmic streaming remained unclear until components of the cytoskeleton were researched[2]. 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[3, 4, 5]. 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, the goal of our research is to determine the presence of regulation of myosin XI motors by phosphorylation, and whether phosphorylation has a significant effect on cytoplasmic streaming.



Arabidopsis thaliana

(Diet et al. 2006)

The effect of phosphorylation on myosin XI trafficking was determined with kinase and phosphatase inhibitors on myosin and organelle localization and movement in *Arabidopsis thaliana*. We observed significant changes in the velocities of myosin isoform MYA1 and fluorescently tagged 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 regulation by phosphorylation is dependent upon the organelle observed.

Introduction

Myosin XI

(Li and Nebenführ, 2008)

Myosins are ATP-driven motor proteins that are involved in several important life processes, including muscle contraction and cell division. In plants, myosin XI's function is still not clear, although research has clearly linked myosin XI with organelle trafficking, the cytoskeleton, and cell polarization[6]. 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 phosphatase inhibitors, while actin filaments are not destroyed or disrupted by inhibitor treatments[7]. 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 phisophorylation[8]. 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

Apparatus to administer inhibitor

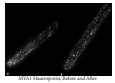
Five *A. thaliana* seed lines were used for the experiment. Seedlings were then placed on vertical plates (1/4 Murashige - Skoog) and incubated for five days. Fluorescence microscopy was then utilized in order to observe the signals from each of the grown seedlings. A cover slip was pasted with vacuum grease on two sides and gently placed over the seedling to create an observation chamber, which was filled with medium. A one minute timelapse with one picture per second was taken, in addition to pictures of five different cross sections of the root hair to observe localization. After this, three aliquots of 100 microliters of dimethyl sulfoxide (DMSO; 1% in MSNS), staurosporin (200 nM in MSNS and 1% DMSO), or okadaic acid (200 nM in MSNS and 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.

Central Questions

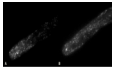
How does phosphorylation affect myosin localization and velocity?
Does the effect of phosphorylation differ among organelles?

Results and Discussion

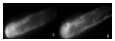
MYA1 Okadaic Acid, Before and After



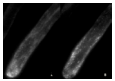
MYA1 Staurosporin, Before and After



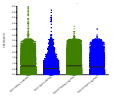
RabA4b Okadaic Acid, Before and After



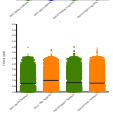
RabA4b Staurosporin, Before and After



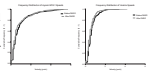
Myosin MYA1 localization was observed across the root hair and was most concentrated at the root hair tip. MYA1 signal was similar after inhibitor treatments (small increase in signal in shank of root hair). MYA1 velocities decreased across all data sets after okadaic acid treatments, and increased after staurosporin treatments (One way ANOVA, p-value <0.01).

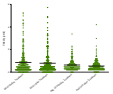


RabA4b vesicle localization was also observed across the root hair and did not significantly change after inhibitor treatments (small increase of signal in shank of root hair). RabA4b velocities were not affected in all data sets after administration of inhibitors (p-value > 0.01).

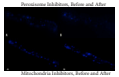


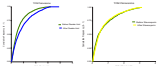
Myosin MYA1 and RabA4b velocities and localization did not significantly change after administration of 1% DMSO.



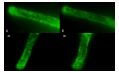


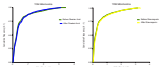
Peroxisome Inhibition, Before and After



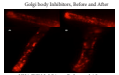


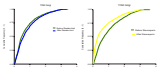
Mitochondria Inhibition, Before and After



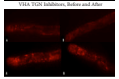


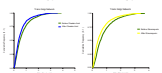
Golgi body Inhibition, Before and After



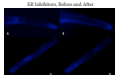


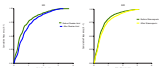
VHA TGN Inhibition, Before and After



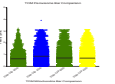


ER Inhibition, Before and After

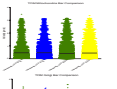




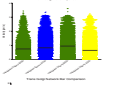
Peroxisome signal increased in velocity after administration of okadaic acid (p-value < 0.01). Velocities were not affected by administration of staurosporin. Localization was throughout the length of the root hair. Inhibitors had small effects on localization (small increase in spot size).



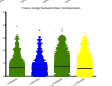
Mitochondria localization was packed and numerous, with the signal being globular and slightly diffuse. Mitochondria localization did not change after inhibitor treatments. Velocities were also not affected by inhibitor treatments.



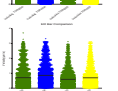
Golgi bodies were similar to mitochondria in signal localization, with globular signal as well as diffuse signal througout the root hair. Golgi body localization was not affected by inhibitors. Slightly faster movements were observed after treatment with okadaic acid, and had slower velocities after staurosporin treatment (p-value < 0.01).



VHA trans Golgi network localization was similar to Golgi bodies througout the root hair. No localization changes were seen after inhibitor treatments. Okadaic acid and staurosporin were observed to cause slightly lower velocities (p - value < 0.01).



Motile ER signal was observed as very diffuse, and the signal intensity had to be diminished in order to track signal spots. The wave like patterns expressed in the graphs are likely the result of the tracking program inaccurately measuring velocities. Both inhibitors caused an increase in motile ER movement (p - value < 0.01).



Conclusions

1. Phosphorylation decreases the movement of MYA1 and dephosphorylation increases the movement of MYA1.

2. RabA4b 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

Acknowledgements

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