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. The root hairs of Arabidopsis thaliana provide an excellent model to study the phenomenon due to its rapid polarized growth at the root hair tip (Figure 1). Cytoplasmic streaming in plants may be through 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 regulation of myosin XI motor proteins. Specifically, the goal of our research is to determine the presence of phosphorylation of myosin XI motors by phosphorylation, and whether phosphorylation has a significant effect on cytoplasmic streaming.

The presence of phosphorylation regulation on myosin XI trafficking was determined with time-lapse and pharmacological inhibition on myosin and organelle localization and movement in Arabidopsis thaliana. We observed significant changes in the velocities of myosin XI myosins and fluorescently tagged organelles after addition of inhibitors. The number of unique tracks also changed after inhibition, indicating increased cytoplasmic streaming removal. The localization of organelles and the myosin pattern remained the before and after addition of inhibitors. Our results support the conclusion that phosphorylation is a regulator of myosin driven organelle movement. The change in velocities caused by phosphorylation appears to vary among different types of organelles.

Introduction

Cytoplasmic streaming is a forceful movement through the cell that distributes organelles, metabolites, and cytokines. Although cytosolic movement is present in both plant and animal cells, the cytoplasmic streaming in the former is more prominent as a result of an inability to change shape or position from their cell wall. Essential to cell viability, a significant amount of research is aimed at discovering its mechanism. The root hairs of Arabidopsis provide an excellent model to study cytoplasmic streaming, as they are unicellular, require significant cytoplasmic streaming as a result of post fertilization growth (greater than 1 µm), and are positioned away from the main root for clear observation.

When cytoplasm from plant cells was observed to be insufficient in generating cytoplasmic streaming alone, a molecular motor was hypothesized as the possible cause.1 Myosin, known as ATP-dependent motor proteins, are now most supported to be the motor. Myosin motors are in close interaction with actin filaments, which function as tracks longitudinally across the cell. Of the 35 known classes, VIII and XI myosins are found in plants and play active roles in cytoplasmic streaming. Support of these acto-myosin systems in cytoplasmic streaming includes research that inhibition of plant myosin in pollen tubes results in halted cytoplasmic streaming, and that isolated myosin motor proteins move at the same velocity as cytoplasmic streaming in identical cell types.2

MYXII-X is the fastest known myosin motor, processively moving along actin at 7 µm/s due to its high rate of ATP hydrolysis and large displacement in each step. As such, myosin II is optimal for carrying organelles across a growing cell, with many organelle interactions being discovered in recent research.3 Current research in our lab focuses on the regulation of myosin XII and its organelle cargo in relation to cytoplasmic streaming. Regulation of class V myosins in mammals, which has many conserved substructures in comparison to class XI myosins, is facilitated by phosphorylation (Figure 2). Previous research has shown that Golgi bodies have been affected by phosphorylation in vitro, while actin filaments do not become disrupted or desorbed (Nebenführ, 1989). In the experiment, two inhibitions of phosphorylation were utilized in order to observe the regulatory role phosphorylation has on organelle velocities carried by myosin XI. The kinase inhibitor staurosporin and the phosphatase inhibitor okadaic acid were used to determine the effects of phosphorylation.

Methods

Plant Lines and Seed Preparation

Three Arabidopsis seed lines were used for this experiment. All single GFP and DCP markers for MYA1 and RabA4b seed lines were created in our lab previously. A triple organelle marker (TOM) was also utilized, containing peroxisome-CFP, Mitochondrion-YFP, and Goldi-mCherry in one binary vector. TOM was created through combining Golgi-CFP with Mitochondrion-YFP and Peroxisome-CFP by individual 35S promoters in binary vector pBGR6. For growth conditions, RabA4b, MYA1, and TOM seedlings were placed on vertical plates (1/4 Murashige - Skoog) and incubated for 5-days.

Fluorescence Microscopy

Fluorescence microscopy was then utilized in order to observe the movement of each of the given 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 time-lapse 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 μl of dimethyl sulfoxide (DMSO) - 1% in MS10, staurosporin (200 µM in DMSO and 1% DMSO), okadaic acid (300 µM in DMSO) and 1% DMSO replaced the original medium. The medium was removed by allowing a piece of paper to contact the observation chamber on the opposite side of where the inhibitor or control is being added, allowing the medium to enter the paper as the inhibitor pushes the medium (Figure 3). After 10 minutes, data collection was repeated.

Statistical analysis of MYA1, RabA4b, and TOM

Organelle signals in timelapses were tracked through an automated particle tracker in ImageJ. Parameters were adjusted until the tracker identified most signals correctly with the least amount of tracks being reported (Figure 4). A final macro was run to visualize remaining errors and to provide data so that the total percentage of incorrect tracks could be calculated. The optimized data contains the velocities and number of utilized tracks present in each root hair. A minimum of 4 root hairs for each signal were used for analysis.

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 have increased movement when phosphorylated.
4. Golgi bodies have decreased movement when unphosphorylated and increased movement from phosphorylation.

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

Further Research

• Continue optimizing particle tracking analysis to eliminate remaining errors in data
• Determine novel approaches in tracking signals that are too diffuse or numerous with current macro (Endoplasmic Reticulum, Trans Golgi Network, Mitochondrion)
• Determine the proportion of signals actively streaming against stationary signals, and develop coding to filter stationary signals accurately
• Utilization of other kinase/phosphatase inhibitors to observe if effect is replicated
• Control studies using actin polymerization inhibitor (latrunculin) to account for actin dynamics
• Observe growth rate of root hairs under continued phosphorylated/dephosphorylated myosin XI

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References

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 regulation 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.

The presence of phosphorylation regulation 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 change in velocities caused by phosphorylation varies among different types of organelles.