

Myosin-VIII Participates in Proper Branch Formation in *Physcomitrella patens*

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Myosins are molecular motors that use the actin cytoskeleton as tracks to power their movement. Plant cells have two distinct classes of myosins, class VIII and XI. Class XI myosins are thought to be responsible for cytoplasmic streaming and much of the organelle transport within the cell. Class VIII myosins remain more enigmatic in function. To date there have been no reports on loss-of-function mutations in myosin-VIII in any plant system. Here we present evidence that class VIII myosins in *Physcomitrella* play a role in proper branch formation in protonemal tissue.

Physcomitrella has five class VIII myosin genes. Using transient RNAi analysis, we reduced the expression of class VIII myosins in *Physcomitrella* and discovered that plants lacking myosin VIII regenerate after transformation with the RNAi construct to varying degrees, but are unable to form viable plants. The ability to regenerate may vary depending on the amount of myosin VIII present in the original transformed protoplast. The inability to form a viable plant suggests that class VIII myosins are essential. However the terminal RNAi phenotype is pleiotropic, and thus not indicative of a specific growth defect.

To address the function of specific myosin genes, we have used homologous recombination to generate *Physcomitrella* lines singly disrupted for myosins 8B, 8C and 8D. We have also generated double nulls and the triple null, $\Delta 8BCD$. Under normal growth conditions all myosin null lines grow normally and appear wildtype in morphology, indicative of highly redundant functions amongst these three myosin genes. However when moss tissue is exposed to uni-directional light, we uncover defects associated with loss of myosin function particularly in the triple null line.

In uni-directional red light, wildtype protonema form a branched network of filaments that grow towards the direction of light. $\Delta 8BCD$ primary protonemal filaments continue to grow as in wildtype, but the secondary branches appear unable to detect the direction of light, often curving around the mother filament. In uni-directional white light $\Delta 8BCD$ protonemal cells are shorter than wildtype, due to a decrease in overall cell size. Interestingly in uni-directional blue light, $\Delta 8BCD$ protonema grow away from the light source, as opposed to wildtype that does not respond to this light cue. These results suggest that myosin-VIII is downstream of light signaling and functions to regulate proper cell morphogenesis.

To address how myosin-VIII may be affecting cell morphogenesis, we determined the localization of arabino-galactan (AGP) proteins in the myosin-VIII knockout lines. Under normal conditions we discovered that the staining pattern of AGP proteins as detected by LM-6 immunofluorescence, differs with respect to wildtype. We observed that, as opposed to wildtype cells that only stain in at the very tip of their apical cells, all myosin VIII null lines show uniform staining in the apical cell as well as several sub-apical cells. This suggests that some wall components of the apical cells do not polarize in myosin VIII mutants to the same extent as in wildtype. Proper localization of cell wall components may be very important for detecting and responding to light signals.