

## **Arp3 is involved in plant cell elongation mediated by rearrangement of actin microfilaments.**

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Cellular polarity is a cell feature in all eucaryote cells that results in the asymmetrical distribution of cell compounds and organelles influencing growth and cell division. Extracellular or intracellular signals can be propagated by pathways mediated by small Rho-GTPases leading to final rearrangement of cytoskeleton structures.

Three different types of linear proteinaceous polymers make the cytoskeleton: actin filaments, microtubules and intermediate filaments. In plants, research has focused on the actin and the microtubule network, but there is still little known about regulation of architecture and dynamics of actin cytoskeleton. Comparisons of yeasts, moulds and animal cells have revealed existence of several actin-related proteins (ARPs) that share 20-60% sequence identities with conventional actins. Arp2 and Arp3 are part of a seven-subunit protein complex that choreographs the formation of branched actin network, binds profilin, and nucleates and polymerises F-actin.

We have isolated *arp3* gene from a lambda-FIX-II genomic library (Leeds) of *Physcomitrella patens* by heterologous screening using the Arabidopsis *arp3* genomic sequence as a probe. The length of the *Pparp3* genomic sequence is 2811 bp and contains nine exons that encode a predicted 417 aminoacid peptide that shows a high degree of conservation compared to Arp3 from other eucaryotes. Disruption of the *Pparp3* gene is performed by deletion of 900 bp including exons 4, 5, 6 and partially exon 7 and replacing it by 35S hygromycin phosphotransferase flanked by P-lox sites. This *arp3-KO* vector was employed to transform the protoplasts. 72 independent hygromycin resistant colonies displayed a marked morphological specificity. Chloronemal cells were short with a length/width ratio of almost 1:1 compared to a wild type ratio of 7:1. The caulonema phase is completely absent and auxin treatment did not induce the reappearance of this cell type. Buds formed directly on chloronemal cells and developed into gametophores. Gametophores are shorter than wild type ones but leaf morphology remains unchanged. During gravitropic experiments in darkness young gametophores display the typical etiolation response.

In order to investigate subcellular structure in the knockout lines we retransformed a GFP-Tn-expressing strain with the *arp3-KO* vector bearing neomycin-phosphotransferase. GFP-Tn binds to F-actin and brightly labels a cortical branched network of actin cables that are aligned parallel to the axis of growth as are the cortical star-like structures connected with them. In the *arp3-KO* strains the actin network is disorganised and completely lacks actin bundles and star-like structures. Further detailed analysis will be provided using confocal microscopy.

This is the first report of an Arp3 knockout in any multicellular organism with a viable phenotype. Arp2/3 complex as part of cytoskeleton machinery appears to be important for plant cell elongation processes. The role of Arp3 also seems to be crucial in the execution of signalling induced by auxin. Point mutation approach in combination with two-hybrid yeast system could provide further information about plant actin cytoskeleton structure and dynamics.