The Arp2/3 complex is required for cell differentiation and cell elongation in *Physcomitrella patens*

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Cell polarity is considered as a primary event determining eukaryotic cell function and developmental fate. The actin cytoskeleton plays an essential role in the establishment of cell polarity, being involved both in the local reinforcement of the spatial information and in the final reorganization of the cell. The ARP2/3 complex is involved in the regulation of actin polymerisation and nucleation and therefore directly controls the formation of the cytoskeletal microfilaments. In plants, genes and ESTs for the seven subunits of the ARP2/3 complex have been identified but the role of the complex in plant morphogenesis and cell differentiation is still poorly understood.

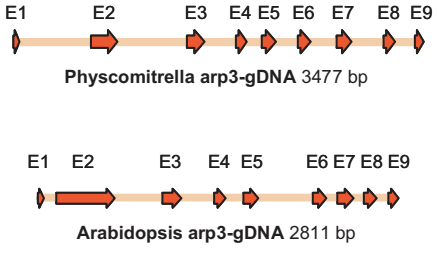

Fig1. Exon-intron structure of genomic sequence of Arp3 of *Physcomitrella patens* compared with *Arabidopsis* one.

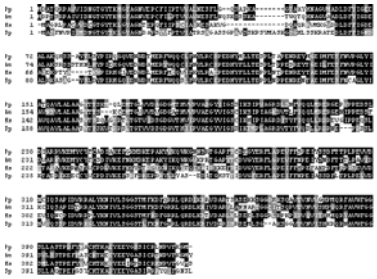

Fig2. Amino acid sequence alignment of *Physcomitrella patens* ARP3 with several known homologues

We have isolated and characterized the genomic sequence of the Arp3 gene from the moss *Physcomitrella patens* which contains nine exons as its ortholog in *Arabidopsis* . It encodes a predicted 425 amino acid peptide that shares 76% amino acid identities with the *A. thaliana* but 56% and 58% with *S. pombe* and *H. sapiens* homologues, respectively.

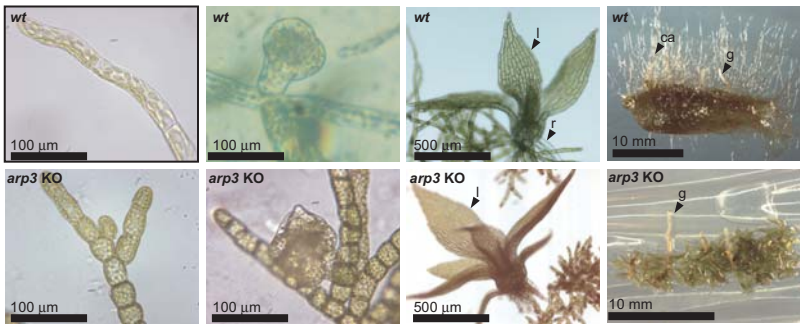

Fig3. Comparison of different developmental stages in wild type (**wt**) and Arp3-knock-out strain (**Arp3 KO**)

Disruption of Arp3 by gene targeting generated moss strains with a complex developmental phenotype. In Arp3 knock-out mutants, the filamentous protonema is composed exclusively of chloronemal cells without any caulonemal cells (**ca**). Additionally, chloronemal cells do not fully elongate and display a length to width ratio of 1:1 whereas this ratio is 7:1 in WT cells. Buds differentiate directly from chloronemata to form stunted gametophores with shorter internodes, but carrying normally differentiated leaves (**l**). Rhizoids (**r**), which differentiate from the basis and the stem of the gametophore in WT, are absent in Arp3 knock-out strains. Finally, tropic responses are apparently not impaired in Arp3 knock-out since protonemal cells display normal photo- and polarotropic responses whereas negative gravitropism can be observed in gametophores (**g**) grown in darkness.

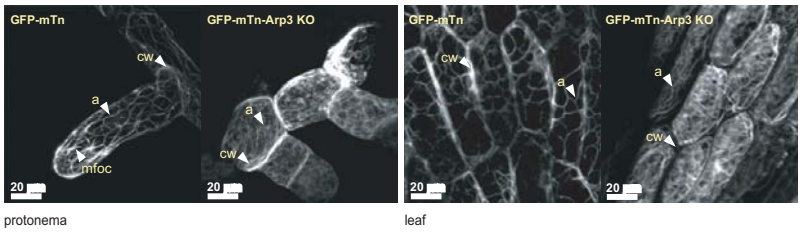

Fig4. Comparison of actin network in the protonema and leaf at GFP-mTn transformant and GFP-mTn-Arp3 KO strain.

The actin cytoskeleton was visualised in vivo in strains expressing a GFP-talin construct. In GFP-talin / wild-type strains, F-actin is brightly labelled and forms a highly organised cortical branched network of cables (**a**) essentially aligned parallel to the axis of growth as well as cortical star-like structures (**mfoc**) connected with them. In GFP-talin / Arp3-KO strains the actin network is completely disorganised. The phenotype described here suggests that the actin cytoskeleton is important for chloronema elongation, for caulonema and rhizoid differentiation but not for leaf morphogenesis in.

This is the first report of an Arp3 knockout in plant. Our data support the essential role of the actin network in plant cell differentiation and growth and suggest that this function could be cell specific. Further studies will certainly clarify the relation between actin and plant morphogenesis in Physcomitrella.

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