

## **T 18: The Arp2/3 complex in *Physcomitrella patens*: possible role in the gravitropic response**

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The Arp 2/3 complex is ubiquitous in non plant eukaryotes (see Mullins and Pollard, 1999) and plays a crucial role in two major properties of the actin network: (1) it favors the polarization of F-actin by capping the barbed end of the actin filament, and (2) it permits branching of actin filaments by binding the pointed end of a filament to the lateral side of another filament. The complex is composed of seven subunits (Arp2, Arp3 and ARPC1 through 5 - see May, 2001), all of which are required for the two functions but the role of each subunit is not fully understood. In plants, no functional evidence for the presence of this complex has been described. However, analysis of different cDNA and gDNA sequences available in databases confirms the presence of all subunits in *Arabidopsis* and other plants.

*Physcomitrella patens* caulonema cells show a negative gravitropic response in the dark. Moreover, as in other mosses, the response is confined to the tip growing cells. Organisms respond to the gravity vector by a mechanism involving two main components: a "sensor" or statolith which could be a subcellular organelle, and, a network of actin and/or microtubules which play a role of integrator of the signal and triggers subsequent polar tip growth (Braun, 1997). Although a precise role of the actin cytoskeleton in this polar response remains unclear, the regulatory function of the Arp2/3 complex could be a key factor in the understanding of polar tip growth in response to gravity.

In this presentation, we will focus our interest on the subunit Pp-p20 (ARPC4) of the Arp2/3 complex in *P. patens*. Using RT PCR approach, a cDNA coding for a p20 has been isolated. It codes for a 169 amino acid protein with a molecular weight of about 20kDa. The amino acid sequence of Pp-p20 is 66% identical and 85% similar to the human p21 homologue and appears to be represented in the *P. patens* genome as a single copy gene, as determined by Southern analysis.

Protoplasts transformation has been performed with a construct containing Pp-p20 cDNA containing a triple HA epitope tag at the 5' end. Stable lines of *P. patens* containing this construct were analyzed for the accumulation of the protein. Lines positive for the presence of the PP-p20 protein were then tested for their response to gravity. We will report on the altered gravitropic response in some of these lines and discuss the possible role of the Arp2/3 complex in the gravitropic response in *P. patens*.

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