STUDIES ON *PHYSCOMITRELLA PATENS* CYTOSKELETON

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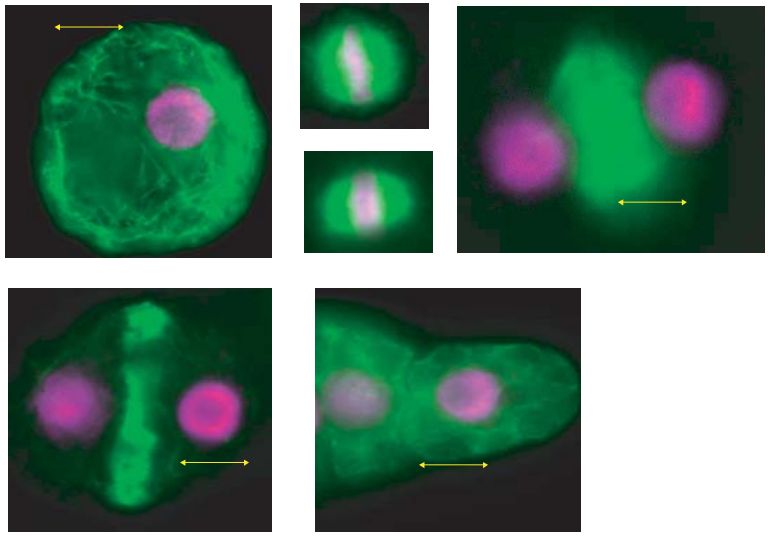
The haplobiontic and monoecious moss (Plantae - Bryophytae) *Physcomitrella patens* is known to have a high frequency (90%) of homologous recombination ((Schaefer and Zryd, 1997), allowing the use, for the first time in studies of land plants biology, of all reverse genetics approaches that have proved so efficient in yeast genetics. cDNA based replacement vectors with homologous sequences as short as 50 to 200 bp can be used for efficient targeted transformation. When the target sequence is known, the use of *P. patens*, for knockout or gene replacement strategies is faster and very efficient.

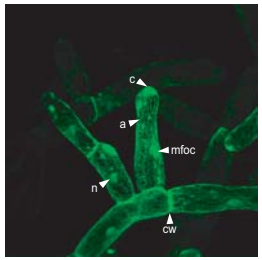
Mosses have been the object of many studies in the area of cell polarity. Different tools, including light of specific wavelength or specific polarization, can be used to alter cell polarity. We are interested in understanding the factors that control the orientation of cell division and the position of the new cell wall.

Tubulin-microtubule structure has been studied by immuno-labeling during the first moss protoplast division induced by linearly polarized light. Microtubule do not display an oriented pattern before the onset of mitosis, then a polarized arrangement appears which precedes any change in cell shape

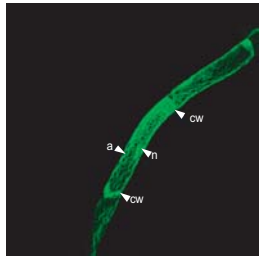


The double arrow indicate both the direction of the EV of the polarized light and the scale (10 micrometer. The whole process of division takes approximately 48 hours

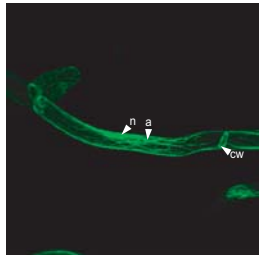
We have transformed *Physcomitrella patens* wild type with a 35S GFP-talin cassette and report here a preliminary description of the moss actin cytoskeleton observed in vivo by confocal microscopy.



chloronema apical cell

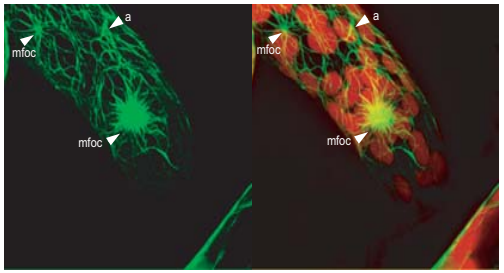


chloronema sub apical cell



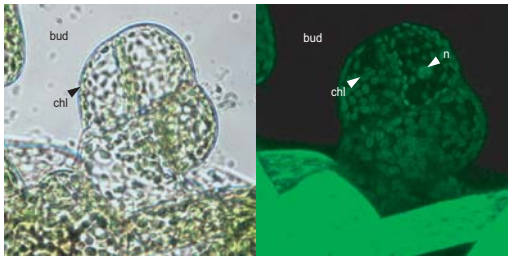
caulonema sub apical cell

Five different actin structures can be recognised in protonema cells: a branched network of cortical cables (a), a diffused fluorescence surrounding the nucleus (n), a cap-like structure associated with the growing tip of apical cells (c), a strong accumulation of actin near the cell wall separating two cells (cw) and intensively labelled star-like cortical structures which may correspond to microfilament organising center (mfoc). Actin cables are predominantly oriented parallel to the cell axis and seems to display a polar distribution in chloronema subapical cells, being more dense at the apical end. With the exception of mfoc, these structures correspond to actin structures previously described in plant cells (pictures are projections of 1.5 µm slices, magnification is 200 x).

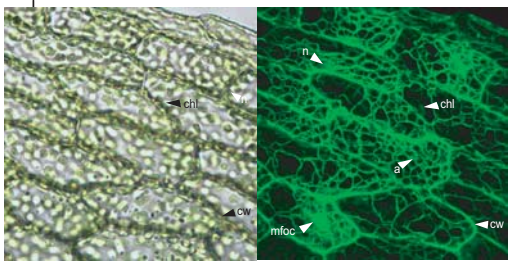


putative microfilament organising centres

Larger magnification of a putative microfilament organising center observed in a chloronema cell. These star like structures seems to contain high concentration of F-actin and are directly connected with the cortical actin cables. We hypothesise that they may be centres where actin polymerisation and/or bundle formation occur (projection of 0.3 µm slices, magnification 500 x)



3-cells stage bud



leaf tissue

A weak fluorescence surrounding chloroplasts (chl) and nucleus (n) can be detected in buds at the 3-cell stage, but brightly labelled F-actin structures are not observed. Yet GFP labelling of cortical cables (a), cell walls (cw), microfilaments organising centers (mfoc) and nuclei is clearly observed in cells of differentiated leaves of the gametophore. The absence of GFP labelled cortical structures in buds is not clearly understood and deserves further studies.