

Ethylene functions in the moss, *Physcomitrella patens*.

Kenjiro Fujiwara and Akio Toh-e

Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Bunkyo-ku, 113-0033, JAPAN

Contact e-mail: fujiwara@biol.s.u-tokyo.ac.jp

Ethylene is one of the typical plant hormones that work in many processes, such as seed germination, root hair development, fruit ripening and stress responses in higher plants. The putative ethylene receptor gene, ETR1 was first isolated in *Arabidopsis* and its homolog is found in many higher plants now. The sequence of ETR1 resembled the prokaryotic two-component sensory His kinase and the N-terminus of Etr1 protein was found to bind directly to ethylene molecule. To date, there is no clear evidence that lower plants utilize ethylene in some way, and ethylene is thought to be the plant hormone only working in higher plants.

We have isolated the putative ETR1 gene from the moss, *Physcomitrella patens* genome and named the gene as PpETR1. To investigate its function, we first over-expressed PpETR1 and its truncated form in *P.patens* protoplasts. While neither the full size PpETR1 gene, expressed under the control of the CaMV35S promoter, nor the C-terminally truncated form inhibited protoplast growth, the N-terminally truncated form of PpETR1 had a severe inhibitory effect.

Because a dominant negative *etr1-1* mutant and its putative downstream *ctr1* mutant of *Arabidopsis* were sensitive to high osmolality, we considered the reason of inhibitory effect of the N-terminal segment of PpETR1 might be through osmotic status of the protoplast embedded in 8% mannitol. So we next investigated ethylene function in *P. patens* using the well-known precursor 1-aminocyclopropane-1-carboxylic acid (ACC), its competitive inhibitor aminooxyacetic acid (AOA), and silver nitrate which is thought to inhibit ethylene perception process of Etr1 protein through its copper binding domain.

When cultured on a plate, 50 μ M of ACC seemed to have no clear effect as was reported previously. When cultured in liquid medium in a closed bottle, ACC did not inhibit growth at all and many gametophytes were produced, but growth in liquid medium in air was inhibited by ACC. When tissues were transported from plate to liquid medium with 15 μ M AOA or 1 μ M silver nitrate, they all died soon.

These results indicated that *P. patens* utilized ethylene in order to adapt to the external osmotic status, and this function is especially important for growing from water into the air. Considering the reports that ACC synthetase changed its activity in relation to the external osmotic status in tomato and tobacco cultured cells, this ethylene function of regulating osmotic status may be common in higher and lower plants. And, since there was no report of ethylene function in algae, ethylene may have played an important role for the colonization of land by plants.