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FULL-LENGTH cDNA ACTIVATION SYSTEM OF *PHYSCOMITRELLA PATENS* TO STUDY AUXIN AND CYTOKININ FUNCTION

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Auxin and cytokinin are essential phytohormones for cell differentiation and growth. It, however, remains to be elusive about their molecular mechanism due to their complicated network, though it has been intensively studied in some model angiosperms. In moss, *Physcomitrella patens*, auxin and cytokinin are also important for cell differentiation. Auxin stimulates differentiation of rhizoid or a certain type of protonema, and cytokinin triggers bud formation of gametophore from protonema. It is easily possible to screen mutants of such kinds of cell differentiation. *P. patens* takes another advantage that it is the only plant in which gene targeting is easily conducted.

We have reported screenings of tagging mutants and gene- or enhancer-trap lines of *P. patens* to study a molecular mechanism of the hormones. We recently started a new project, full-length cDNA activation system. We made a full-length cDNA library of the moss and determine DNA sequences of each clone, which will serve as a good source of EST database. Then every clone is targeted by homologous recombination and overexpressed in a locus of the moss genome. This gain-of-function based screening could work complementarily to our previous loss-of function based screening. We will present a detail of this project and a progress of the EST.

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TRANSDIFFERENTIATION TO THE SECONDARY ABSCISSION INDUCED BY METHYL JASMONATE IN *BRYOPHYLLUM CALYCINUM*

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Methyl jasmonate (JA-Me, 0.5%, w/w) strongly induced the formation of the secondary abscission zone above and below the treatment after 4 to 6 days, when it was applied as lanolin paste in the middle part of internode segments of *Bryophyllum calycinum*. Ethephon (2-chloroethyl phosphonic acid, Ethrel, 1%, w/w) in lanolin also induced the formation of the secondary abscission zone in internode segments. Auxin (IAA, 0.1%, w/w) applied to the apical side of internode segments in lanolin extremely inhibited the formation of the secondary abscission zone induced by both JA-Me and ethephon. Application of JA-Me and ethephon little affected ethylene production and endogenous level of jasmonates (JAs) in internode segments, respectively. These results suggest that endogenous JAs and ethylene interacting with IAA have an important role in transdifferentiation to the secondary abscission zone, while mechanism by which JAs and ethylene induce the formation of the secondary abscission zone seems to be independent.

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The Role of Auxin Polar Transport in Morphogenesis in Monocot Plant.

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Auxin polar transport plays important roles in many morphological events in plant. In *Arabidopsis*, several *PIN* genes encoding auxin efflux carrier protein have been identified. Through the molecular genetic studies on the *PIN* genes, the role of auxin polar transport in morphogenesis in dicot is vigorously being elucidated. Although, there are some obvious differences between dicot and monocot plant in the pattern formation during embryogenesis and vascular patterning which are thought to be closely related with auxin polar transport, there are few studies on the role of auxin polar transport in monocot plant.

In order to investigate the role of auxin transport in monocot plant, we have isolated four full-length cDNA clones encoding PIN-family auxin efflux carrier proteins by screening cDNA libraries derived from rice embryo. We are now conducting precise expression analyses by RNA gel blot analysis and *in situ* hybridization experiment, intracellular localization analysis by immuno-histochemistry, and functional disruption by RNA interference. Based on these results, we will discuss the role of auxin polar transport in embryogenesis and postembryonic morphogenesis in a monocot plant, rice.

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GENE-EXPRESSION ANALYSIS OF *MSG2/IAA19* OF ARABIDOPSIS USING THE PROMOTER-*GUS* FUSION

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Dominant mutation of *MSG2/IAA19*, a member of the *Aux/IAA* auxin early gene family, abolishes the auxin-dependent differential growth responses of hypocotyls. We introduced the *IAA19* promoter-*GUS* fusion gene into Arabidopsis, and examined expression of the gene by histochemical analyses. In etiolated seedlings, *GUS* staining was detected in vascular tissue just under the elongating zone of hypocotyl, and weak staining was observed in vasucular tissue of root. The expression was seen in primordium of lateral root. The staininig of hypocotyl was increased by either gravistimulation or unilateral irradiation with blue light for 2 hr. It also increased in both hypocotyl and root by treatment with 50 μM 2,4-D for 24 hr. Inflorescence stem just under the elongating zone was also stained, while rosette and cauline leaf did not show any staining. The staining was observed in style and funiculus of pistil and vascular tissue of petal and sepal.