033(F033)

FUNCTIONAL ANALYSIS OF *API1* GENE ENCODING A KINESIN-LIKE PROTEIN, WHICH IS STRONGLY EXPRESSED IN AN APICAL CELL IN THE MOSS, *PHYSCOMITRELLA PATENS*.

Yuji HIWATASHI[1,2], Mitsuyasu HASEBE[1,2,3], [1]Dept. Mol. Biomechanics, Grad. Univ. Advanced Studies, Okazaki 444-8585, [2]Spec. Mechanisms 2, Natl. Inst. Basic Biol., Okazaki 444-8585, [3]PRESTO

In the moss, *Physcomitrella patens* subsp. *patens*, the differentiation of an apical cell in protonema and gametophore is regulated by auxin and cytokinin. To analyze the molecular mechanism of apical cell differentiation, we established gene-trap and enhancer-trap systems in *P. patens* and searched a marker gene. Using the systems, we identified a kinesin-like protein gene, named *API1* gene, which was strongly expressed in an apical cell of caulonema, rhizoid, and gametophore. This result suggests that a kinesin-like gene may be involved in the differentiation of an apical cell.

In this study, in order to investigate the function of *API1* gene, the intracellular localization of *API1* gene was examined. Full-length API1 protein was transiently expressed in protonema as a fusion protein with GFP at the C-terminus of API1 under the control of a constitutive promoter. The full-length API1-GFP fusion protein was specifically detected in nucleus, suggesting that API1 protein function in nucleus. Deletion of C-terminal region adjacent to motor domain of API1 protein affected the localization of API1-GFP fusion protein, suggesting C terminal region adjacent to motor domain is required for nucleus-specific localization of API1 protein. Genomic southern analysis with API1 as a probe revealed that *API1* gene was single copy gene. We are carrying out disruption of *API1* gene by the gene-targeting technique.

034(F034)

Isolation of monoclonal antibodies recognizing xylem cell wall components by using a phage display subtraction method

Naoki SHINOHARA, Taku DEMURA[1], and Hiroo FUKUDA Dept. Biol. Sci., Grad. Sch. Sci., Univ. Tokyo, Tokyo 113-0033, [1]Plant Sci. Centre, RIKEN, Wako 351-0198

Monoclonal antibodies (mAbs) recognizing differentiated cell-specific wall components are useful in distinguishing types of cells and dissecting developmental processes. We tried to generate such mAbs using a strategy consisting of (i) isolation of cell walls from synchronously differentiating cells of an *in vitro Zinnia* culture system (ii) construction of phage display library of recombinant antibody against the cell walls (iii) screening via biopannings with subtractive procedures. As a result, we succeeded in isolation of two mAbs, designated CN 8 and XD 3, which recognized epitopes on walls of xylem cells. In plants, CN 8 epitope localized in walls of immature tracheary elements (TEs) and xylem parenchyma cells, while XD 3 epitope localized in walls of TE precursors and immature fibre cells. These two mAbs recognized different types of cells cultured *in vitro*. These results indicate that they are useful molecular markers and tools to purify the wall components and to isolate specific xylem cells.

035(F035)

MORPHOLOGICAL CHANGES IN SUB-CULTURED SUNFLOWER ROOT TIPS UNDER IRON DEFICIENCY

Yuko TOMOTSUNE, Koji NOMURA, MS. Prog. Biosystem, U. Tsukuba, Tsukuba 305-8572

Iron deficiency induces transfer cells in root tips in many plants. Transfer cells change Fe(III) to Fe(II) to uptake iron. Along with differentiation of transfer cells, swelling and formation of root hairs are observed in iron deficient root tips. It is believed that aerial parts of a plant are necessary to induce transfer cells in root tips. However, there is no direct evidence to support this hypothesis.

In hydroponic sunflower, our previous results suggested that cytodifferentiaion of transfer cells under iron deficiency was a phenomenon mainly regulated in each root tip. To confirm this possibility, we try the organ culture of sunflower roots. We found that swelling and formation of root hairs in the subcultued root tips when they were transferred to iron free media. These morphological changes indicate regulatory mechanisms exist in the root itself.

036(F036)

PHOTOPERIOD-PHASE-SPECIFIC GENE EXPRESSION IN RICE LEAVES

Yuki SHIINA, Shinichiro KANDA[1], Hisashi TANAKA, Yasurou KURUSU and Masaru NIWA (Sch. Agr., Ibaraki U., Ami, Ibaraki 300-0393, [1]Aomori Pref. Agr. Exp. Sta., Towada 034-0041)

We obtained 5 kinds of RT-PCR product (cDNA fragments), which appeared photoperiod-phase-specifically, in rice leaves by means of the simple DD method (Kanda *et al.*, Breed. Sci. 48 (Suppl. 2): 625. 1998).

Two isogenic lines for the *Se-1* locus of rice were treated with the 12L12D light regime. Total DNA was extracted from leaf blades at the interval of 2h. Northern analysis was conducted using the above mentioned 5 cDNA fragments as probes. D70, one of the probes, performed strong signals at the end of the dark period in both of the lines. This photoperiod-phase-specific gene expression was also observed in the simple DD analysis.

Cloning, sequencing and southern analysis revealed that D70 was 447bp long and the product of the single copy in the rice genome.

Search of DDBJ revealed that the base sequence of D70 coincided partially with that of a cDNA which was obtained from the floral induced meristem of sorghum (ac. no. BE919317) at 95% homology.