E232 Isolation and Expression Patterns of a cDNA encoding Phytoene Synthase in Citrus

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A cDNA clone (Psy1) encoding phytoene synthase was isolated from the fruit cDNA library of Citrus (*Citrus unshiu* Marc.). Sequence analyses and phylogenetic dendrogram revealed that the cDNA contains an open reading frame encoding 437 amino acids (47 kDa), which showed significant similarity to those of phytoene synthases of fruit-producing plants. RNA blot analysis showed that the mRNA is expressed in the edible parts and peels of fruits, leaves, and flowers, as a single transcript. Also, during the ripening of fruits, the Psy1 transcripts were detected in all stages and its expression markedly increased to the maximum level in the latest stage. A similar pattern was also detected in peels. Also, the level of Psy1 transcripts is changed in the process of leaf development. Our results suggested that Psy1 is an important regulatory enzyme in carotenoid accumulation during fruit ripening. This is the first report to characterize the relationship between the expression of Psy and fruit development in a woody plant.

E233 Molecular Characterization of cDNA Clones for ADP-Glucose Pyrophosphorylase from Citrus

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Two cDNA clones encoding ADP-glucose pyrophosphorylases have been isolated from fruit and leaf cDNA libraries of Citrus (*Citrus unshiu* Marc.) in which one was designated as agpS for the small subunit and the other as agpL for the large subunit. The deduced amino acid sequence of agpS has a unique feature. That is, it lacks a cystein residue (Cys-12) which is usually conserved in all other dicot enzymes. This is the first report of agpS lacking Cys-12 among dicot small subunits. During leaf development, the transcripts of agpS and agpL showed a higher expression level at younger stages. During fruit development, the expression level of both subunits was observed as highest in the mini-green stage, but it decreased in the small green stage and it increased again towards the maturing stage. These results suggest that both subunits may play important role in the regulation of Citrus fruit and leaf development.

E234 Changes in Phosphorylation of 50 and 53 kD Soluble Proteins in Graviresponding Oat (Avena sativa) Shoots

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The present work shows that phosphorylation of 50 and 53 kD soluble proteins is involved in the gravitropic response graviresponsive pulvini of oat (*Avena sativa*) shoots. Differential phosphorylation of these proteins between lower and upper halves of pulvini was detected only when stem segments were gravistimulated. The differential phosphorylation of the two proteins occurred as early as 5 min after the initiation of gravistimulation treatment. This is five min earlier than the presentation

time of 10 min. This differential phosphorylation was changed by cycloheximide and okadaic acid, imply that (newly synthesized) phosphoprotein phosphatase(s) may play important roles in the gravitropic response. These findings indicate that the differential phosphorylation of the 50 and 53 kD soluble proteins in graviresponding oat shoots may be an importnat component of the gravity signal transduction pathway.

E401 Effect of an acid pH shock on physiological changes of Chlamydomonas acidophila, UTCC 122

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The effect of low pH on physiological changes were studied with the acidophilic green alga, Clamydomonas acidophila, UTCC 122. pH-dependent physiological parameters such as growth rate, pigmenets, cell volumes, cell surface areas, quantitative protein profiles, carbonic anhydrase (CA) activity, and CA contents were measured. The growth rates were identical, 0.5~0.7 cells day^{-1}, at pH 3.7 to pH 6.7, showing cell volume reduced gradually as they were growing, whereas that at pH 2.7 was stoped and cell volume was increased dramatically. The chlorophyll *a* concentration of all the cultures incubated for 1 day was 191~255 pg $cell^{-1}$, after then that was declined from 60~103 pg $cell^{-1}$ at pH 3.7~6.7 except 210 pg $cell^{-1}$ at pH 2.7, which was directly related with cell volume. The external carbonic anhydrase activity was varied from 0.11 to 0.34 E.U. 5.4 X 10^6cells, showing the gradual increase during culture, although the total CA activity was not shown any patterns. Proteins of apparent molecular masses of 17 kDa was increased at pH 2.7, whereas 41 and 63 kDa was disappeared. The CA molecular mass of Clamydomonas acidophila was 29 kDa and the concentration of that was same in all acid cultures.

E801 A Novel Protein in Mitochondria Interacts with Death Domain of Fadd and Induces Mitochondria-Mediated Cell Death

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Upon receptor trimerization with Fas ligand or an anti-APO-1 antibody, Fas is activated and recruits the adaptor molecule FADD(MORT-1) to its cytoplasmic region via their corresponding conserved death domains(DD). Death adaptors gave a homologous sequence termed the 'death domain(DD)'. To identify regulatory proteins interacting with FADD, we have screened $3.0{10}^6$ cDNA transformants using yeast two-hybrid assay. D22 clone is one of the putative positive clones that interact with FADD. In vitro binding assay using a GST-FADD fusion protein purified from bacteria shows that D22 interacts with the full-length and the DD of FADD, indicating that this protein interacts with FADD through the DD. Overexpression of D22 in NIH3T3 cells and HeLa cells efficiently induces cell death, which is partially attenuated by zVAD(a broad Inhibitor of caspases), LEHD(caspase-9 inhibitor), and Bcl-xL, indicating that D22-induced cell death may be mediated through the mitochondria event. Overexpression of D22 shows localization in mitochondria and release into cytosol when apoptotic signals are turned on. These results suggest that D22 may be a death-adaptor molecule interacting with FADD to transmit a mitochondria-mediated apoptotic signal.

E802 Nodulin-24, peribacteroid membrane protein, is targeted to the vacuole in yeast and Arabidopsis

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Nodulin-24 is a membrane protein of