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

soybean root nodules. It was shown to have a cleavable signal sequence and localized to the membrane enclosing the symbiotic bacteria. For understanding the targeting process of nodulin-24, nodulin-24 cDNA was fused to -glucuronidase (GUS) gene and the fused construct was introduced into yeasts. Subcellular fractionation and marker enzyme assays were performed for localization of the fusion protein; The GUS activity was concentratedly found in the P13 fraction which supposedly contains ER and vacuoles. Isolated vacuoles by discontinuous ficoll gradient centrifugation also have high GUS activity. It was concluded that the fusion was targeted to vacuole in yeast. For nodulin-24 targeting in Arabidopsis, the fusions were introduced into Arabidopsis. The transient expression of nodulin-24/green fluorescent protein (GFP) fusion showed that nodulin-24 is targeted to the central vacuole. These results may suggest that the symbiosome is an organelle equivalent to vacuoles.

E803 Development of gene therapy for Inuslin-Dependent Diabetes Mellitus (type I, IDDM) by transfer of human pro-insulin gene to skeletal muscle.

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We have established the gene theraputical method on type I insulin dependent diabetes mellitus (IDDM) using Drosophila transposable P-element (Suh et al., 2001 in preparation). In this approach, human keratin-14 promoter was used and human pro-insulin was desinged to be expressed from the human keratinocyte in the skin tissue. The system has shown its effectiveness and convenience in transferring human pro-insulin gene to the mouse keratinocytes. However, the regulation of the gene expression was not completely resolved. This problem could be overcome is by introducing the promoter of the gene encodes enzyme which related to the glycolysis. In this study, the skeletal muscle tissue was selected as a target tissue and the promoter region of skeletal muscle specific hexokinase II, phosphofructo- kinase and pyruvatekinase gene will be used accordingly. The possibility of insulin expression in muscle cells and regulation of insulin expression by blood glucose level will be discussed.

E804 Characterization of the 5'flanking region of the rat SPARC gene

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ECM (Extracellular matrix protein) family 중 하나인 SPARC은 (Secreted Protein, Acidic and Rich in Cysteine) 분비성 당단백질로 종양세포로의 발생(tumorigenicity) 및 신생혈관형성(angiogenesis)시 ECM complex에 대한 다양한 조절기능을 담당한다고 보고되어있다. 위암에서 SPARC 유전자가 특이하게 발현된다는 예비결과에 근거하여 본 실험실에서는 SPAPRC의 위암조직 특이적 발현에 관여하는 cis-acting element를 확인하고, 이를 토대로 유전자치료제에서의 문제인 유전자 전달에 보다 효율적인 방법을 구축하고자 하였다. 기존에 발표된 SPARC 유전자의 cDNA 서열에 근거하여 genome walker library를 (Clontech Co., USA) PCR로 검색하였으며 이를 통해 SPARC 유전자의 -460 bp에서 +40 bp에 해당되는 5' flanking region을 얻었고, 제한효소를 이용하여 3개의 deletion construct를 제작하였다. Western blotting을 통하여 위암기원 세포주(AG5, SNU638, SNU719)에서 SPARC의 발현을 확인한 후, 상기 construct들을 이들 세포주에 transfection시켜 luciferase의 활성을 측정하였다. 조사한 모든 세포주에서 -300 bp의 flanking region을 갖는 construct에서 luciferase의 활성이 가장 높음을 확인하였다.