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cDNA CLONING OF A MITOCHONDRIAL PHOSPHATE TRANSPORTER GENE FROM *Lotus japonicus*

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Mitochondrial phosphate transporter (MPT) is located in the inner membrane. It transports inorganic phosphate into the mitochondrial matrix and plays an essential role in the oxidative phosphorylation of ADP to ATP.

We isolated an MPT cDNA, *LjMPT2*, from a *Lotus japonicus* nodule library with a soybean MPT cDNA as a probe. *LjMPT2* contained an entire coding region for a typical MPT with 356 amino acids. Northern blot analysis and *in situ* hybridization is under way. LjMPT2 protein has a characteristic Cys residue which is highly conserved among other mammalian and plant MPTs. We will check if the Cys residue is essential for the transport activity by site-directed mutagenesis. *LjMPT2* may also serve as a molecular probe for examination of oxidative phosphorylation activity of mitochondria under anaerobic conditions in root nodule cells.

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PURIDICATION AND CHARACTERIZATION OF NITRATE REDUCTASE FROM COCCOLITHOPHORIDS, *EMILIANIA HUXLEYI*

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Marine coccolithophorids produce huge biomass by blooming in the ocean and affect marine environment.The growth of *Emiliania huxkeyi* is strongly regulated by nitrate concentration. To characterize the mechanism of nitrate utilization is very important to know how the *Emiliania* bloom is controlled.

In this study, we focus on nitrate reductase (NR: EC 1.6.6.1) as a key factor of the utilization system of nitrate. The enzyme was highly purified by a combination of chromatography on blue Sepharose CL-6B, Mono-Q and Superdex 200 gel filtration. Molecular masses of the native form and the subunit of the enzyme was 500,00 (gel-filtration) and 85,000 (SDS-PAGE), respectively. The results indicated that the native NR was composed of six subunits. The enzyme utilized NADH, but not NADPH, as an electron donor. The K_m values for NADH and KNO_3 were 54 μM and 355 μM, respectively.

The enzyme showed reducing activities of NADH:Cyt *c*, MV:KNO_3 and NADH:KNO_3. The pH optimum of the NADH:KNO_3 activity was 8.0. Inhibitor-experiments suggested that sulfhyl groups and heme- and non heme-irons are concerning to the activity. These enzymatic characters indicate that NR of *E. huxleyi* is very unique among plants.

667(S713)

Rma1, a novel type of RING finger protein conserved from Arabidopsis to human, is a membrane-bound ubiquitin ligase

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Rma1 is a protein with a RING finger motif and a C-terminal membrane-anchoring domain and is well conserved among higher eukaryotes. We have shown that a fusion protein between maltose binding protein (MBP) and Arabidopsis Rma1 (AtRma1) is polyubiquitinated when incubated with the wheat germ lysate. The polyubiquitination of MBP-AtRma1 has been reconstituted by incubation with purified ubiquitin, the ubiquitin-activating enzyme E1, and one of the two ubiquitin-conjugating enzyme E2's (Ubc4 or UbcH5a). This indicates that AtRma1 possesses a ubiquitin ligase activity, which depends on Ubc4 or UbcH5a. Similar results were obtained with Human Rma1. Taken together, Rma1 represents a novel, membrane-bound type of ubiquitin ligase E3, which functions with the Ubc4/5 subfamily of E2.

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RELATION BETWEEN THE SUBUNIT DIVERSITY AND ITS FUNCTIONAL ROLES IN SOYBEAN FERRITIN

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Ferritin is a multimeric iron storage protein composed of 24 subunits. Ferritin purified from dried soybean seed resolves into 26.5 and 28 kDa peptides. Amino acid sequence analysis determined that the 28 kDa subunit is novel, whereas the 26.5 kDa subunit is identical to one that was identified previously. The 26.5 kDa subunit lacks the C-terminal 16 residues. The cleavage of the C-terminal domain from the 28 kDa subunit contributes to the stability of the ferritin protein shell and functions in iron release. The corresponding region in the 28 kDa soybean ferritin subunit identified in this research is not susceptible to cleavage. The novel ferritin subunit stabilizes the ferritin shell by co-existing with the cleaved 26.5 kDa subunit. These data demonstrate that soybean ferritin is composed of at least two different subunits and these subunits have co-operative functional roles in soybean seeds.