DIVISION OF DEVELOPMENTAL BIOLOGY (ADJUNCT)

Professor: *Kenzo Nakamura (Nagoya University)*
Associate Professor: *Tsukaho Hattori (Mie University)*
Assistant Professor: *Masa-aki Ohto*
Technician Staff. *Etsuko Aoki*, *Yasuko Furukawa*
Postdoctoral Fellow: *Kiyoshi Onai*
Graduate Students: *Yasuhiro Koide (Nagoya University)*, *Yukiko Iwata (Nagoya University)*, *Shingo Hayashi (Nagoya University)*

Availability of sugars and the inter-organ transport and distribution of sugars are essential in the growth and development of the plant body. Expression of a variety of plant genes is regulated, either positively or negatively depending on the gene, by the level of sugars at the sugar-importing sink sites as well as at the sugar-exporting source sites of the plant body. Thus sugars are not only important as sources for cellular energy and the synthesis of macromolecules but also as a signal controlling the growth and development of plants by changing the pattern of gene expression. Our research attention is focused to elucidate the mechanisms involved in the regulation of gene expression in response to sugars, especially the activation of gene expression by increased-levels of sugars, and the role of such regulation in the organ development in plants. In addition, mechanisms involved in the accumulation of storage proteins in plant vacuoles are also studied.

I. Molecular and genetic approaches for the analysis of the sugar-signalling during growth and development of *Arabidopsis thaliana*.

During the growth of plants, new organs develop as carbohydrate sink, and many vegetative organs shows sink to source transition after their maturation. Many aspects of the organ development in higher plants are thought to be affected, to some degree, by the levels of sugars. Sugars seem to have influence on the meristematic transition in long day plants from vegetative to reproductive growth. To obtain insights into the role of sugar-regulated gene expression in the growth and organ development in plants, we are screening for mutants of Arabidopsis with defects or anomalies both in the sugar-regulated gene expression and in the developmental processes such as leaf development and the determination of the flowering time. To aid this purpose, we have established more than 7,000 independent lines of Arabidopsis plants transformed with T-DNA containing multiple copies of the enhancer sequence. We have identified more than 20 of mutant lines with defects in the development of leaves or anormalies in the flowering time which also show the altered patterns of the expression of sugar-inducible genes, such as β-amylase gene (*Atβ-Amy*) (Fig. 1). The mutants were named as *uns* (*unusual sugar response*) after their abnormal responses to sugars concerning the gene expression, *in vitro* flowering and chlorophyll content in leaves of *in vitro*-cultured plant.

II. Regulatory factors involved in the sugar-inducible expression of plant genes

Expression of genes coding for sporamin and β-amylase, two major proteins of the storage roots of sweet potato, is inducible by high levels of sugars in various vegetative tissues. The GUS reporter genes under the control of the promoters of these genes are also inducible by sugars in leaves of transgenic tobacco plants, and these fusion genes are expressed in tubers of transgenic potato plants. Although the induction of expression of these fusion genes requires the activity of hexokinase, phosphorylation of hexose by hexokinase is not sufficient to cause the induction. The induction requires Ca^{2+}-signalling and the activity of protein kinase.

Eight different cDNAs for the isoforms of calcium-dependent protein kinase (CDPK) were isolated from leaves of tobacco, and transcripts of two of them were found to be increased upon treatment of leaves with various metabolizable sugars. Antibodies against a fragment of one of these isoforms cross-reacted strongly with the 57 kDa-protein in the soluble fraction from the young leaves. The level of this 57 kDa-protein decreased significantly as leaf matures, while the level of this 57-kDa protein in mature leaves increased significantly after the treatment of leaves with sugars. The sugar-induction of the 57-kDa protein occured preceding the induction of expression of the β-amylase:GUS reporter gene. In addition, a 54 kDa-protein with autophosphorylation activity in the plasma membrane of mature leaves also increased significantly upon treatment of leaves with sugars. This protein was purified to about 1,000-fold compared to the crude

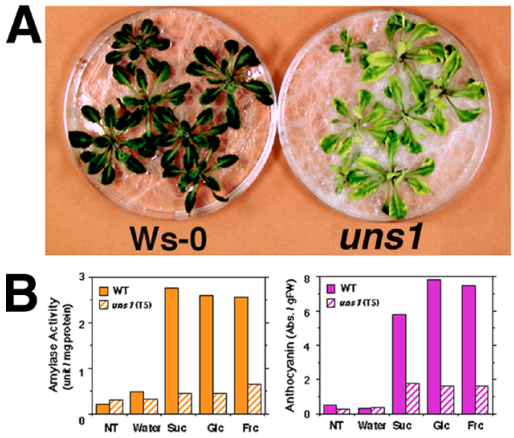

Fig.1 Phenotypes of wild type and homozygous mutant line (*uns1*) grown for 3 weeks. The chlorophyll content of the mutant plants (Fig.1A, right) is much lower than that of wild type plants (Fig.1A, left). Mutant plants also showed the reduced levels of the sugar-inducible increase of β–amylase (Fig.1B, left) and anthocyanin (Fig.1B, right) when leaf explants were treated with high levels (5%) of sugars.

extract. It phosphorylated histone IIIS in a Ca^{2+}-dependent manner and cross-reacted with an antibody against CDPK of *Arabidopsis thaliana*. These results suggest the possible involvement of CDPKs in the sugar-inducible gene expression and the development of leaves.

Selected Publication:

Iwata, Y., Kuriyama, M., Nakakita, M., Kojima, H., Ohto, M. and Nakamura, K., (1998) Characterization of a calcium depedent protein kinase of tobacco leaves that is asociated with the plasma membrane and is inducible by sucrose. *Plant Cell Physiol.* **39**(11): 1176-1183.

Koide, Y., Matsuoka, K., Ohto, M. and Nakamura, K., (1999) The N-terminal propeptide and the C terminus of the precursor to 20-kilo-dalton potato tuber protein can function as different types of vaculor sorting signals. *Plant Cell Physiol*. **40**(11): 1152-1159.