DIVISION OF CELLULAR REGULATION

Professor:	*Norio Murata*
Associate Professor:	*Koji Mikami*
Research Associates:	*Atsushi Sakamoto*
	Yoshitaka Nishiyama
	Iwane Suzuki
Technical Staff:	*Hideko Iinuma*
	Katsushi Yamaguchi
Monbusho Foreign Scientist:	*Dmitry A. Los*[1)]
JSPS Fellows:	*Suleyman I. Allakhverdiev*[2)]
	S. Shivaji [3)]
	Prasanna Mohanty [4)]
HFSP Fellow:	*Masami Inaba*
Visiting Scientists:	*Tony H. H. Chen* [5)]
	Patcharaporn Deshnium [6)]
	Richard A. Dilley [7)]
	Martin Fulda [8)]
	Gabor Galiba [9)]
	Martin Hagemann [10)]
	Lisa Heins [11)]
	Cai-Xia Hou [12)]
	Larissa Kisseleva [1)]
	Alexander A. Lyukevich [1)]
	Jogadhere S. S. Prakash [3)]
	Kostas Stamatakis [13)]
	Balazs Szalontai [14)]
Graduate Students:	*Yuji Tanaka*
	Ryoma Suzuki
	Yu Kanesaki
	Ali Ferjani

[1)] *from the Institute of Plant Physiology, Moscow, Russia*
[2)] *from the Institute of Basic Biological Problems, Pushchino, Russia*
[3)] *from the Centre for Cellular and Molecular Biology, Hyderabad, India*
[4)] *from the Regional Plant Resource Centre, Bhubaneswar, India*
[5)] *from Oregon State University, Corvallis, OR, USA*
[6)] *from the National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand*
[7)] *from Purdue University, Lafayette, IN, USA*
[8)] *from the University of Hamburg, Hamburg, Germany*
[9)] *from the Agricultural Research Institute, Martonvásár, Hungary*
[10)] *from the University of Rostock, Rostock, Germany*
[11)] *from the University of Kiel, Kiel, Germany*
[12)] *from Xinjiang University, Urumqi, China*
[13)] *from the National Center for Scientific Research "Demokritos", Athens, Greece*
[14)] *from the Biological Research Center, Szeged, Hungary*

The research efforts of this division are devoted to developing a full understanding of the molecular mechanisms that allow plants to acclimate to and tolerate various kinds of stress that arise from changes in environmental conditions, with particular emphasis on extreme temperatures and high salinity. In 2000, significant progress was made in the following areas as a result of studies of higher plants and cyanobacteria.

I. A sensor for low-temperature signals

Low temperature is an important environmental factor that affects the growth and behavior of all living organisms. Many organisms are able to acclimate to low temperatures by regulating the expression of various genes. However, mechanisms for the perception and transduction of low-temperature signals remain to be characterized. In the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*), expression of the genes for fatty acid desaturases is enhanced by low temperature. Moreover, the decrease in membrane fluidity that occurs upon a downward shift in temperature appears to be a primary signal for induction of the expression of the genes for these desaturases.

In various bacteria, yeast and plants, physical and chemical stimuli are perceived by a group of proteins that includes histidine kinases. We attempted to disrupt individually all the putative genes for histidine kinase in *Synechocystis* and then we examined the subsequent response to low temperature of the promoter of the *desB* gene, which encodes the ω3 fatty acid desaturase, by monitoring the activity of a reporter gene for luciferase. Among 41 mutant lines with disrupted genes for histidine kinase, we identified two mutants, in which, respectively, the *hik19* gene and the *hik33* gene had been inactivated. Mutation of these two genes also reduced the extent of induction at low temperature of other genes whose expression is induced by low temperature, such as the *desD* gene for the Δ6 desaturase and the *crh* gene for RNA helicase. Hik33 has two membrane-spanning domains at its amino terminus and it is likely that this enzyme is localized on the cell membrane. By contrast, it is likely that Hik19 is a soluble protein. Therefore, Hik33 might be a primary sensor and Hik19 might be a signal transducer. This is, to our knowledge, the first discovery of a cold-sensing protein in any type of cell.

II. Analysis of the regulation of gene expression using a DNA microarray

The *Synechocystis* DNA microarray, CyanoCHIP™, covers 97% of all the open reading frames (ORFs) found in the genome of this cyanobacterium. Analysis of temperature-dependent gene expression with this DNA microarray revealed that genes whose expression is enhanced by low temperature encode proteins that are, for the most part, subunits of the transcriptional and translational machinery, such as the α subunit of the RNA polymerase, a σ factor, and some protein subunits of the ribosome. These proteins might be essential for reversal of suppression of protein-synthesizing activity by low temperature. By contrast, most cold-repressible genes were found to encode components of the photosynthetic machinery, such as subunits of photosystem I and of phycobilisomes. These changes in gene expression might represent the acclimative responses of photosynthetic organisms that allow maintenance of photosynthetic activity at a certain level regardless of temperature. To our surprise, we found that the expression of a number of genes for proteins of unknown function was also enhanced or repressed by low temperature.

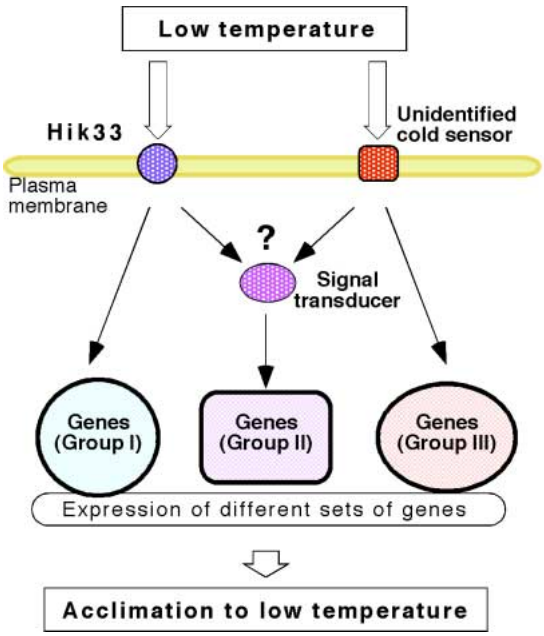

Figure 1. A hypothetical scheme for transduction of low-temperature signal.

Our examination of the cold-dependent regulation of gene expression with the DNA microarray also revealed that the cold-regulated genes could be divided into three groups by reference to the effect of inactivation by mutation of Hik33. In the first group, regulation of gene expression by low temperature was totally abolished upon inactivation of Hik33; in the second group, the extent of such regulation was reduced by half; and, in the third group, such regulation was totally unaffected. These observations indicate that Hik33 might regulate the expression of certain cold-regulated genes, while expression of those genes that were unaffected by inactivation of Hik33 might be regulated by another system for transduction of the low-temperature signal (Fig. 1; Suzuki, Kanesaki, Mikami, Kanehisa, and Murata *Mol. Microbiol*. in press).

III. The importance of membrane lipids in protection of the photosynthetic machinery against salt stress

High salinity is one of the main environmental factors that severely limit the growth and the productivity of plants and microorganisms. We showed previously that membrane lipids are intimately involved in the protection of both plants and microorganisms against low-temperature stress. To provide further insight into the importance of membrane lipids in stress tolerance, we examined the tolerance to salt stress of the photosynthetic machinery of *Synechococcus* sp. PCC 7942 in which we had genetically enhanced the unsaturation of fatty acids in the membrane lipids. Our results revealed that the unsaturation of fatty acids enhanced the salt tolerance of cells at least in terms of the activities of photosystem I, photosystem II and the Na^+/H^+ antiport systems. The enhanced tolerance was mediated, at least in part, by the increased tolerance of the photosynthetic machinery to salt-induced damage and by an increase in the cell's ability to repair the photosynthetic and Na^+/H^+ antiport systems. These results serve to emphasize the physiological importance of the repair of the photosynthetic machinery and the Na^+/H^+ antiport system in the protection of *Synechococcus* cells from salt-induced damage.

IV. Characterization of the Na^+/H^+ antiporters of *Synechocystis*

Control of membrane permeability to Na^+ ions and to counteracting K^+ ions is one of the most important aspects of the acclimation of cells to high-salt environments. Na^+/H^+ antiporters are membrane-bound proteins that play an important role in maintenance of the balance of intracellular concentrations of Na^+ and K^+ ions in plant, fungal and bacterial cells. We used a mutant of *Escherichia coli* that is deficient in Na^+/H^+ antiport activity as a host to examine functional complementation with genes for putative Na^+/H^+ antiporters from the genome of *Synechocystis*. We tested five genes and found that at least two genes encoded Na^+/H^+ antiporters with different respective affinities for Na^+ and Li^+ ions. To our knowledge, this is the first functional characterization of Na^+/H^+ antiporters from a cyanobacterium. The coexistence of high-affinity and low-affinity Na^+/H^+ antiporters in *Synechocystis* is consistent with the ability of this organism to acclimate to a wide range of extracellular concentrations of Na^+ ions (Inaba, Sakamoto, and Murata *J. Bacteriol*. in press).

V. The role of glycinebetaine in tolerance to freezing temperatures

Glycinebetaine (GB) is a zwitterionic quaternary amine that is found in a large variety of microorganisms, plants and animals. It belongs to a group of compounds known as compatible solutes, which act very efficiently to stabilize the structure and function of cellular macromolecules such as protein complexes and membranes. In a previous study, we cloned the *codA* gene for choline oxidase from *Arthrobacter globiformis.* Choline oxidase catalyzes the conversion of choline to GB. We introduced the gene for this enzyme into *Arabidopsis thaliana,* which does not synthesize GB. The resultant transformed plants not only accumulated high levels of GB but also exhibited significantly elevated tolerance to a broad spectrum of environmental stresses, such as high salt, low and high temperatures, and strong light.

In 2000, we found that transformation of *A. thaliana* with the *codA* gene also enhanced the freezing tolerance of transformed plants. The accumulation of GB in chloroplasts dramatically improved the survival of mature plants, stabilized membranes, and efficiently protected the photosynthetic machinery at freezing temperatures (Fig. 2). The observation that the

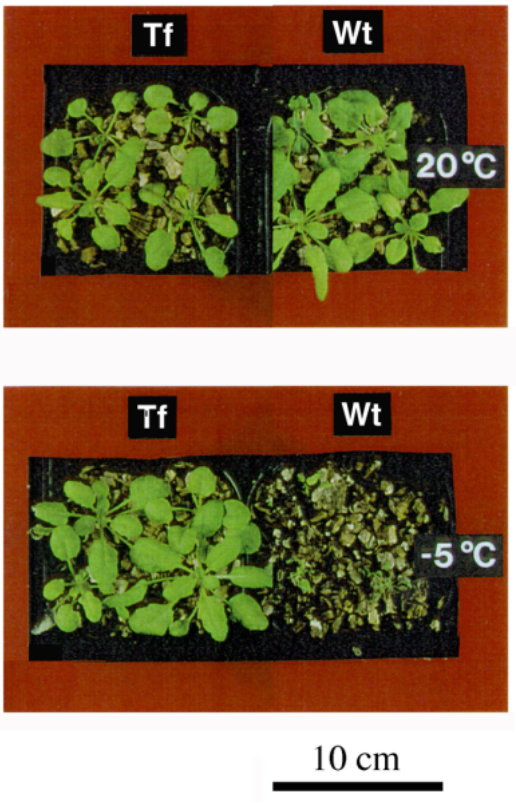

Figure 2. Enhanced freezing tolerance of *Arabidopsis* plants that have been transformed with the *codA* gene for choline oxidase.
Wild-type (Wt) and transformed (Tf) plants were grown at 22˚C for 33 days. They were then incubated at 20˚C or -5˚C for 2 h and subsequently incubated at 22˚C for seven days to examine survival. The transformed plants accumulated glycinebetain (GB) at 0.90 μmol g^{-1} fresh weight, whereas wild-type plants contained no GB. Adapted from Sakamoto *et al.*, *Plant J*., **22**, 449-453 (2000).

transformed plants exhibited tolerance to various kinds of abiotic stress suggests that, in addition to its stabilizing effect on proteins and membranes, GB might, under stress conditions, contribute to the maintenance of cellular functions of fundamental importance, such as transcription and translation. We are now focusing our attention on the molecular mechanisms by which GB enhances the tolerance to various kinds of stress, with particular emphasis on possible protection of the transcriptional and translational apparatus from stress-induced damage.

List of publication:

(1) Original articles

Allakhverdiev, S.I., Sakamoto, A., Nishiyama, Y., Inaba, M., and Murata, N. (2000) Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant Physiol*., **123**, 1047-1056.

Allakhverdiev, S.I., Sakamoto, A., Nishiyama, Y., and Murata, N. (2000) Inactivation of photosystems I and II in response to osmotic stress in *Synechococcus*. Contribution of water channels. *Plant Physiol*., **122**, 1201-1208.

Gao, M., Sakamoto, A., Miura, K., Murata, N., Sugiura, A., and Tao, R. (2000) Transformation of Japanese persimmon (*Diospyros kaki* Thunb.) with a bacterial gene for choline oxidase. *Mol. Breed.*, **6**, 501-510.

Pardha Saradhi, P., Suzuki, I., Katoh, A., Sakamoto, A., Sharmila, P., Shi, D.-J., and Murata, N. (2000) Protection against the photo-induced inactivation of the photosystem II complex by abscisic acid. *Plant Cell Environ.*, **23**, 711-718.

Sakamoto, A., Valverde, R., Alia, Chen, T.H.H., and Murata, N. (2000) Transformation of *Arabidopsis* with the *codA* gene for choline oxidase enhances freezing tolerance of plants. *Plant J.*, **22,** 449-453.

Suzuki, I., Los, D.A., Kanesaki, Y., Mikami, K., and Murata, N. (2000) The pathway for perception and transduction of low-temperature signals in *Synechocystis*. *EMBO J.*, **19**, 1327-1334.

Szalontai, B., Nishiyama, Y., Gombos, Z., and Murata, N. (2000) Membrane dynamics as seen by Fourier transform infrared spectroscopy in a cyanobacterium, *Synechocystis*. PCC 6803. The effects of lipid unsaturation and the protein to lipid ratio. *Biochim. Biophys. Acta*, **1509**, 409-419.

Tanaka, Y., Nishiyama, Y., and Murata, N. (2000) Acclimation of the photosynthetic machinery to high temperature in *Chlamydomonas reinhardtii* requires synthesis *de novo* of proteins encoded by the nuclear and chloroplast genomes. *Plant Physiol*., **124**, 441-449.

(2) Review articles

Los, D.A., and Murata, N. (2000) Regulation of enzymatic activity and gene expression by membrane fluidity. *Science's STKE*, http://www.stke.org/cgi/content/full/OC_sigtrans;2000/62/pe1.

Sakamoto, A., and Murata, N. (2000) Genetic engineering of glycinebetaine synthesis in plants: current status and implications for enhancement of stress tolerance. *J. Exp. Bot.*, **51**, 81-88.