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The research efforts of this division are aimed at developing a full understanding of the molecular mechanisms by which plants are able to acclimate to and tolerate stresses that arise from changes in environmental conditions, with particular emphasis on temperature stress and salt stress. In 1999, using higher plants and cyanobacteria as our experimental materials, we made significant progress in the following areas.

I. The perception and transduction of low-temperature signals in *Synechocystis*

Low temperature is an important environmental factor that affects the growth of all living organisms. Many organisms are known to acclimate to low temperatures by expressing various low temperature-inducible genes. However, mechanisms for the perception and transduction of low-temperature signals remain to be characterized. In the cyanobacterium *Synechocystis* sp. PCC 6803, expression of the genes for fatty acid desaturases is enhanced by low temperature. Moreover, the decrease in membrane fluidity induced by low 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 putative genes for histidine kinases in *Synechocystis* sp. PCC 6803 and monitored the subsequent response of the promoter of the gene for the ω3 fatty acid desaturase, *desB*, to low temperature. Among 41 mutant lines with disrupted genes for histidine kinases, we identified two mutants, in which, respectively, the *hik19* gene and the *hik33* gene had been inactivated and in which no induction of the reporter gene for luciferase occurred upon exposure of cells to low temperature. This result indicated that mutation of the two genes for histidine kinases abolished the inducibility by low temperature of the activity of the *desB* promoter. Mutation of these two genes also reduced the extent of induction at low temperature of other low temperature-inducible genes, such as the *desD* gene for Δ6 desaturase and the *crh* gene for RNA helicase.

We also introduced an antibiotic-resistance gene cassette randomly into the chromosomes of *Synechocystis* and screened mutants for altered expression of the *desB* gene. In one of the mutants, 2C, in which the *desB* promoter was insensitive to low temperature, we found that the gene for a response regulator, Rer1, had been inactivated. The extent of the low temperature-dependent increase in the level of the *desB* transcript in *Δrer1* cells was reduced to half of that in wild-type cells. By contrast, the inducibility by low temperature of the *desD* and *crh* genes was unaffected by the mutation. Thus, it is possible that Rer1 might regulate the expression of the *desB* gene specifically and might not affect the expression of the other genes examined.

Figure 1 shows a hypothetical scheme for the transduction of low-temperature signals. In this scheme, Hik33 spans the plasma membrane twice and forms a dimer, whose structure is influenced by the physical characteristics of the lipids in the plasma membrane. Such characteristics include fluidity, which is controlled by temperature. When the temperature is decreased, the histidine residue in the histidine kinase domain is phosphorylated. The phosphate group is then transferred to Hik19 and eventually to Rer1 which regulates the expression of the *desB* gene.

II. Genetic modification of plants to enhance stress tolerance: introduction of the capacity for the synthesis of glycinebetaine into *Arabidopsis*

Metabolic acclimation *via* the accumulation of compatible solutes is considered to be a fundamental strategy for the protection and survival of plants when they

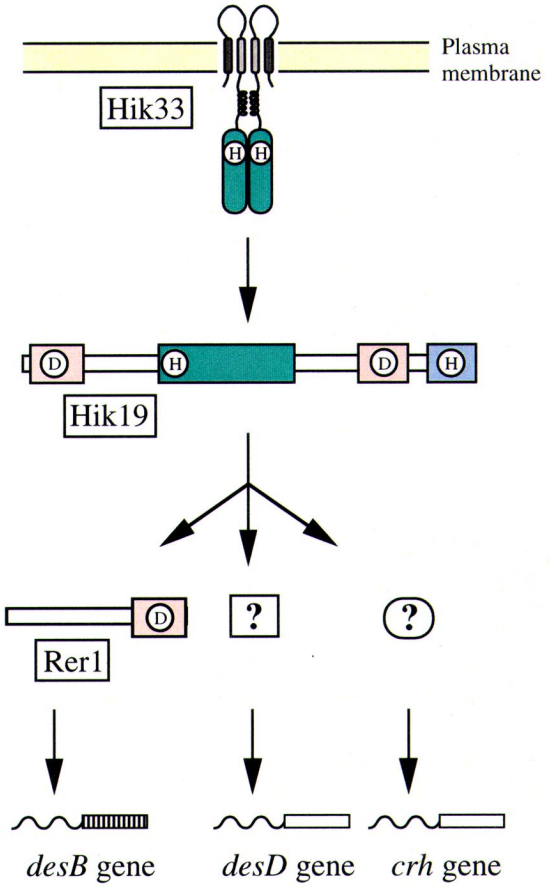

Figure 1. A hypothetical scheme showing the pathway for transduction of low-temperature signals in *Synechocystis*. The histidine kinase domains of Hik33, Hik19, the receiver domains of Hik19 and Rer1, and the histidine phosphate-transfer domain of Hik19 are shown in turquoise, pink and blue, respectively. The plasma membarne is shown in yellow. The histidine and aspartate residues that might be involved in the phospho-relay reaction are indicated by H and D in circles, respectively. Adapted from I. Suzuki, D.A. Los, Y. Kanesaki, K. Mikami and N. Murata, *EMBO J.*, 19, 1327-1334 (2000).

are exposed to extreme environments. In response to stress, such as high levels of salt, cold and drought, certain plants accumulate glycinebetaine (hereafter called betaine), a quaternary amine that protects cellular components, such as complex proteins and membranes, from the harmful effects of high levels of salt or extreme temperatures *in vitro*.

In order to evaluate the role of betaine in stress tolerance *in vivo*, we transformed *Arabidopsis thaliana*, which does not normally accumulate betaine, with the *codA* gene for choline oxidase (which catalyzes the conversion of choline to betaine) from the soil bacterium *Arthrobacter globiformis*. In transformed *Arabidopsis*, the accumulation of betaine, which was the result of the expression of functional choline oxidase in chloroplasts, significantly enhanced the tolerance of the plants to a wide variety of environmental stresses, such as high levels of salt, and low and high temperatures. Furthermore, enhancement of such tolerance was not confined to a specific stage of development but was apparent throughout developmental stages that included the imbibition and germination of seeds and the early and later stages of vegetative growth.

In transformed *Arabidopsis*, enhanced tolerance to stress was also evident at the cellular level. Photosystem II in the chloroplasts of transformed plants was less susceptible than that of wild-type plants to photoinhibition by high-intensity light. We demonstrated that the enhanced tolerance of the photosynthetic machinery to high-intensity light resulted from the accelerated recovery of the photosystem II complex from photoinduced inactivation. The extent of such accelerated recovery was reduced when the synthesis of proteins was blocked by lincomycin, suggesting that betaine, accumulated *in vivo*, might promote protein synthesis *de novo*, which is considered to be crucial for the recovery of cells from stress-induced damage.

III. Mechanisms of inactivation of the photosynthetic machinery by salt stress in cyanobacteria

High-salt stress is an environmental factor of major importance that limits the growth and productivity of plants. We studied the mechanism of the salt-induced inactivation of the photosynthetic machinery in the cyanobacterium *Synechococcus* sp. PCC 7942. Incubation of cells in medium prepared with 0.5 M NaCl resulted first in the rapid and reversible inactivation of photosystems I and II, which was followed by the slow and irreversible inactivation of both photosystems. The rapid inactivation resembled the effects of 1.0 M sorbitol. The slow inactivation was prevented by a blocker of Na^+ channels. The presence of blockers of Na^+ channels and water channels together protected both photosystems I and II against the short-term and the long-term effects of 0.5 M NaCl. Thus, it seems likely that NaCl has both osmotic and ionic effects. Our results suggest the following mechanism for the salt-induced inactivation of photosynthesis. The osmotic effect of NaCl decreases the amount of water in the cytosol, rapidly increasing the intracellular concentrations of salts. The ionic effect of NaCl is caused by an influx of Na^+ ions through K^+/Na^+ channels that also increases concentrations of salts in the cytosol, which results in the irreversible inactivation of photosystems I and II.

We also investigated the role of polyunsaturated lipids in cell membranes in the tolerance of the photosynthetic machinery to high-salt stress by comparing the *desA*$^-$*/desD*$^-$ mutant of *Synechocystis* sp. PCC 6803, which contained monounsaturated fatty acids, with the wild-type strain, which contained the full complement of polyunsaturated fatty acids. The oxygen-evolving activity of *desA*$^-$*/desD*$^-$ cells was more sensitive to high-salt stress than was that of wild-type cells. Moreover, the activity of the Na^+/H^+ antiport in *desA*$^-$*/desD*$^-$ cells was suppressed to a greater extent than that of the antiport in wild-type cells under high-salt stress. These observations suggest that polyunsaturated fatty acids in

membrane lipids might stimulate the activity and/or the synthesis of the Na^+/H^+ antiport system to protect the photosynthetic machinery against salt-induced inactivation.

IV. Acclimation of the photosynthetic machinery to high temperature

High-temperature stress causes the irreversible inactivation of the photosynthetic machinery. However, when photosynthetic organisms have acclimated to moderately high temperatures, their photosynthetic machinery exhibits enhanced thermal stability. We have been studying the molecular mechanisms that underlie the acclimation of the photosynthetic machinery to high temperature in a cyanobacterium and in *Chlamydomonas*.

In the cyanobacterium *Synechococcus* sp. PCC 7002, cytochrome c_{550} and PsbU, the extrinsic proteins of the photosystem II complex, stabilize the oxygen-evolving machinery at high temperatures. To clarify the role of PsbU *in vivo*, we inactivated the *psbU* gene in *Synechococcus* sp. PCC 7002 by targeted mutagenesis. Not only the thermal stability of the oxygen-evolving machinery did not increase in the mutated cells at a moderately high temperature, but these cells were also unable to develop cellular thermotolerance upon acclimation to such temperatures. These results suggest that PsbU might play an important role in enhancing the thermal stability of the oxygen-evolving machinery at high temperatures and, moreover, that the stabilization of the machinery might be crucial for the acquisition of cellular thermotolerance.

In *Chlamydomonas reinhardtii*, enhancement of the thermal stability of the oxygen-evolving machinery was prevented by cycloheximide and lincomycin. The specificity and effects of these drugs suggest that the synthesis of proteins encoded by both the nuclear genome and the chloroplast genome is required for this enhancement. No synthesis of homologs of three heat-shock proteins, namely, Hsp60, Hsp70 and Hsp22, was induced at the moderately high temperatures that induce the enhanced thermal stability of the oxygen-evolving machinery. Thus, it appears likely that heat-shock proteins are not involved in the acclimation of the photosynthetic machinery to high temperature.

Publication List:

(1) Original articles

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Yamamoto, H., Miyake, C., Dietz, K.-J., Tomizawa, K.-I., Murata, N. and Yokota, A. (1999) Thioredoxin peroxidase in the cyanobacterium *Synechocystis* sp. PCC 6803. *FEBS Lett*., **447**, 269-273.

(2) Review article

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