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Review

Membrane fluidity and the perception of environmental signals in cyanobacteria and plants

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Abstract

Photosynthetic organisms, namely, plants and cyanobacteria, are directly exposed to changes in their environment and their survival depends on their ability to acclimate to such changes. Several lines of evidence suggest that temperature stress, such as unusually low or high temperatures, and osmotic stress might be perceived by plants and cyanobacteria via changes in the fluidity of their cell membranes. The availability of techniques for gene-targeted mutagenesis and gene transfer, as well as for the analysis of genomes and transcripts, has allowed us to examine and evaluate this hypothesis and its implications. In this review, we summarize recent studies of the regulation of gene expression by changes in the extent of unsaturation of fatty acids and membrane fluidity, and we present a discussion of the induction of gene expression by environmental stress and of sensors of environmental conditions and relationships between their activity and the fluidity of membranes in cyanobacteria and plants.

Contents

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Nomenclature

PG	phosphatidylglycerol
DPH	1, 6-diphenyl-1, 3, 5-hexatriene
Hik	histidine kinase
FTIR	Fourier transform infrared

1. Introduction

The plasma membranes and thylakoid membranes of cyanobacteria resemble the inner envelope and thylakoid membranes of the chloroplasts of higher plants in terms of both ultrastructure and lipid composition [1]. These membranes in cyanobacterial cells and chloroplasts are characterized by their unique lipid composition: the major components are glycolipids, such as monogalactosyl diacylglycerol, digalactosyl diacylglycerol and sulfoquinovosyl diacylglycerol [2]. Phosphatidylglycerol (PG) is the only phospholipid in these membranes [2] and accounts for approximately 10–20% of the total membrane lipids [3–8].

In plants, there is a very close relationship between the level of unsaturation of the fatty acids in PG and sensitivity to cold [9–11]. Genetic manipulation of levels of unsaturated fatty acids led to the eventual modification of the cold sensitivity of tobacco plants [12]. In cyanobacteria, sensitivity to cold is also closely correlated with the level of unsaturation of membrane lipids [13,14].

Thus, sensitivity to cold stress in photosynthetic organisms seems to be determined by the level of unsaturation of fatty acids in their unique membrane lipids.

Poikilothermic organisms, such as cyanobacteria and plants, modulate the composition of their membrane lipids in response to changes in environmental conditions [15], and modulation of levels of unsaturation of membrane lipids has been widely observed in a variety of species, such as cyanobacteria, *Tetrahymena thermophila*, *Acanthamoeba castellanii* and carp [16–20]. Modulation of the level of unsaturation of membrane lipids is regulated mainly by the low temperature-dependent desaturation of fatty acids. We have focussed our attention on a strain of cyanobacteria, *Synechocystis* sp. PCC 6803 (hereafter, *Synechocystis*), that introduces double bonds at the Δ6, Δ9, Δ12 and Δ15 (ω3) positions in C-18 fatty acids at the *sn-1* position in reactions catalysed by fatty acid desaturases that are encoded by the *desD*, *desC*, *desA* and *desB* genes, respectively [21,22].

It was postulated from the results of early experiments that the rigidification of membrane lipids might be the primary signal of cold stress [16–20] and that the activity of desaturases might be regulated by the fluidity of membrane lipids [23–25]. Indeed, a downward or an upward shift in temperature, as well as osmotic stress, induces variations in membrane fluidity that are due to the physical properties of the membrane lipids [26–29] and are related to the physical phase transition of membrane lipids from a liquid-crystalline phase to a gel phase [30,31].

To prove the above-mentioned hypothesis, an attempt was made to determine whether membrane fluidity could act directly to regulate the specific activity of desaturases [25,32]. Horváth et al. [25] suggested that desaturases might have a negative temperature coefficient, with higher activities at lower temperatures. However, a biochemical analysis of the dependence on temperature of the activity of cyanobacterial DesA, the Δ12 desaturase, which had been overexpressed in *Escherichia coli,* demonstrated that DesA has a positive temperature coefficient, as do most other enzymes [32]. Another mechanism proposed was that cold stress might enhance the expression of genes for desaturases. Los et al. [22] demonstrated that cold stress accelerated the transcription of the *desA*, *desB* and *desD* genes but not that of the *desC* gene. Such enhanced transcription of genes for desaturases has been observed in many poikilothermic organisms (see [18]). Transcription of *desA*, *desB* and *desD* genes in *Synechocystis* seems to be regulated via the rigidification of membrane lipids because catalytic hydrogenation, which decreases levels of unsaturated fatty acids in plasma membranes, induced a 10-fold increase in the expression of the *desA* gene with kinetics similar to those of the induction of this gene by cold [33]. These results imply that maintenance of the fluidity of membranes at physiological levels can be achieved under stress conditions in order to sustain the functional activity of membrane proteins and the membranes themselves [20].

The details of the involvement of membrane fluidity in the regulation of gene expression remain to be characterized. In *Saccharomyces cerevisiae*, addition of linoleic acid to the culture medium increases the temperature that is critical for the induction of heat-inducible genes [34]. Thus, the heat inducibility of such gene expression seems to depend on the level of unsaturated fatty acids and, thus, on the fluidity of membrane lipids. However, in *Synechocystis*, membrane fluidity might not be involved in the induction of gene expression by high temperature (see Section 3).

The next question that researchers tried to answer was whether the genes for desaturases in *Synechocystis* or the regulators of their transcription perceive temperature directly or via membrane fluidity or, alternatively, whether there are sensors of temperature that perceive changes in

temperature and generate signals that regulate the expression of genes for desaturases. In the latter case, the identification of such temperature sensors, which might be expected to perceive changes in membrane fluidity, would be essential.

Until recently, we were only able to analyze the stress-induced expression of just a few genes, selected basically at random, by Northern blotting. However, it has become clear that each type of environmental stress activates the expression of a large number of genes [35–44]. Thus, to identify and characterize stress sensors, we need to determine how many genes and what kinds of gene are induced by individual kinds of stress and what fraction of the entire population of stress-inducible genes is regulated by each individual stress sensor. Northern blotting analysis provides only limited answers to these questions. The recent development of the DNA microarray technique has provided a new approach to the analysis of genome-wide patterns of transcription in organisms whose genomes have been sequenced. This technique is rapidly increasing our understanding of the way in which stress sensors recognize changes in membrane fluidity in cyanobacteria, in particular in *Synechocystis*, and in particular when this method is combined with systematic gene-targeted mutagenesis.

In this review, we shall focus on the relationship between the level of unsaturation of fatty acids in membrane lipids and the fluidity of membranes under cold, heat and hypersmotic stress, as well as on recent progress in the study of sensors of abiotic stress in cyanobacteria and in plants.

2. Modulation of membrane fluidity

2.1. Effects of temperature and hyperosmotic stress

The term of "membrane fluidity" refers to the dynamic properties of lipids in membrane bilayers, as defined by the extent of their disorder and molecular motion [17,20]. There are several methods that allow us to monitor membrane fluidity under physiological conditions. The most common method involves measurements of anisotropy by fluorescence polarization in the presence of 1,6-diphenyl-1,3,5-hexatriene (DPH) [29]. This compound is incorporated into membranes in parallel to the acyl chains of membrane lipids and fluorescence from it is strong when it interacts stably with rigidified membranes [45]. Thus, the measured anisotropy decreases when membrane fluidity increases. However, since DPH is not completely free to rotate or move in membranes, the extent of the interaction between DPH and membrane lipids is restricted [45]. Thus, it is impossible to measure membrane fluidity quantitatively by this method. By contrast, Fourier transform infrared (FTIR) spectroscopy [28] can be used for quantitative analysis of the physical state of membranes over a physiological range of temperatures. FTIR allows us to monitor the disorder of acyl chains of glycerolipids and the interactions between lipids and membrane proteins, using as the measured parameter, the frequency of the symmetric CH_2 stretching mode near 2851 cm^{-1}. This frequency decreases by approximately 2–5 cm^{-1} upon the transition from an ordered to a disordered state. Thus, a low and high frequencies of the CH_2 stretching mode correspond to the rigidified and fluid states of membranes, respectively.

The effects of changes in temperature on membrane fluidity were demonstrated recently by FTIR spectroscopy [28]. As shown in Fig. 1A and B, the frequencies of the CH_2 stretching mode in both isolated plasma membranes and isolated thylakoid membranes from wild-type *Synechocystis*

cells that had been grown at 35 and 25 °C decreased with a decrease in temperature [28]. Thus, it was clear that changes in temperature modulated membrane fluidity. The differences in the degree of disorder between plasma membranes and thylakoid membranes in Fig. 1A and B were due to differences in the levels of unsaturation of membrane lipids and in the ratios of protein to lipid in these membranes [28].

The effects of hyperosmotic stress on membrane fluidity have also been examined using DPH [27,29]. When phospholipid vesicles were exposed to hyperosmotic stress due to the addition of polyethylene glycol to the medium, an increase in anisotropy was observed [27]. Moreover, when the osmotic pressure of the medium surrounding intact yeast cells was increased by the addition of glucose, an increase in anisotropy was also observed [29]. These findings suggest that hyperosmotic

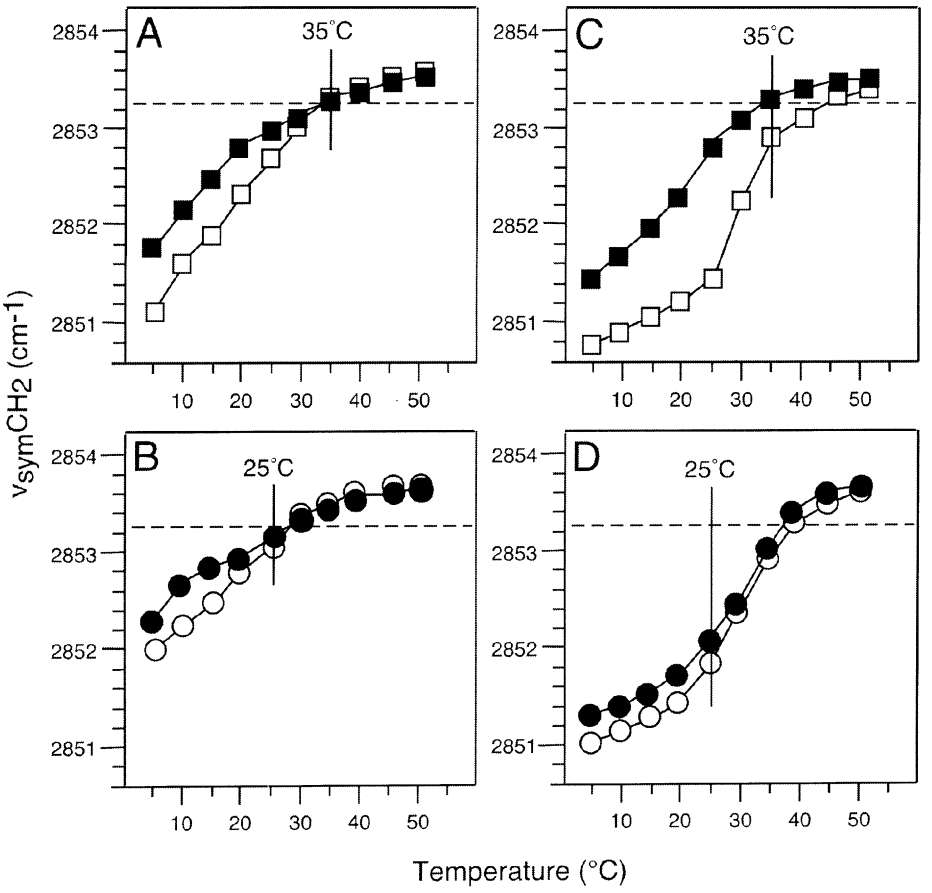

Fig. 1. FTIR analysis of the lipid fluidity of membranes from wild-type and *desA*$^{-}$/*desD*$^{-}$ mutant *Synechocystis* cells. Results are shown for plasma membranes (open symbols) and thylakoid membranes (closed symbols) from wild-type and mutant cells: (A) wild-type cells grown at 35 °C; (B) wild-type cells grown at 25 °C; (C) mutant cells grown at 35 °C; (D) mutant cells grown at 25 °C. The vertical solid lines indicate the growth temperature (35 °C or 25 °C). The horizontal dashed lines indicate the extent of lipid disorder in wild-type cells, which was identical at 25 °C and 35 °C. Taken from [28] with modifications.

stress might reduce membrane fluidity. However, FTIR analysis is necessary to confirm this possibility.

2.2. Effects of the unsaturation of fatty acids in membrane lipids

Cyanobacterial membranes are suitable systems for studies of the way in which the unsaturation of fatty acids affects the fluidity of membrane lipids because the number of unsaturated bonds in the fatty acids in membrane lipids can be altered by genetic manipulation of fatty acid desaturases [13,14]. When the *desA* gene from *Synechocystis* was introduced into the genome of *Synechococcus* sp. PCC 7942, which contains only monounsaturated fatty acids, the resultant *desA*$^+$ cells produced considerable amounts of diunsaturated fatty acids [13]. Moreover, when both *desA* and *desD* genes were genetically inactivated in *Synechocystis*, the resultant *desA*$^-$/*desD*$^-$ cells synthesized only monounsaturated lipids and no polyunsaturated lipids [14].

FTIR spectroscopy provided evidence for changes in membrane fluidity as a result of the unsaturation of fatty acids [28]. The frequency of symmetric CH_2 stretching modes at 25 °C in both plasma membranes and thylakoid membranes isolated from *desA*$^-$/*desD*$^-$ cells grown at 25 °C was approximately 5 cm^{-1} lower than that in both types of membrane from wild-type cells grown at the same temperature (compare Fig. 1B and D). By contrast, similar levels of the frequency of the CH_2 stretching mode were observed in plasma membranes and thylakoid membranes from wild-type and mutant cells, after both had been grown at 35 °C (Fig. 1A and C). Since a decrease in the frequency of symmetric CH_2 stretching modes corresponds to rigidification of membranes [28], these findings indicate that plasma membranes and thylakoid membranes from *desA*$^-$/*desD*$^-$ cells grown at 25 °C were more rigidified than those from wild-type cells grown at 25 °C, whereas both types of membrane were similarly fluid in wild-type and mutant cells that had been grown at 35 °C.

A decrease in membrane fluidity was also observed in transgenic tobacco plants that carried a gene for glycerol-3-phosphate acyltransferase that is expressed in the chloroplasts of squash plants. These transgenic tobacco plants were sensitive to chilling as a result of increased levels of saturated fatty acids in PG [12]. FTIR studies indicated that isolated thylakoid membranes from these transgenic plants were dramatically rigidified below 25 °C, whereas those from control plants, which had been transformed with the empty vector, remained much more fluid [46].

These findings indicate that membrane fluidity depends on the level of unsaturation of membrane lipids in cyanobacteria and chloroplasts.

2.3. Homeostatic regulation of membrane fluidity by fatty acid desaturation

The cold-dependent regulation of the unsaturation of fatty acids was investigated in detail in *Anabaena variabilis* [47]. A decrease in growth temperature accelerated the desaturation of membrane lipids, with suppression of the synthesis of lipids and a resultant increase in the level of unsaturation of fatty acids in membrane lipids. An increase in the unsaturation of fatty acids at low temperature depends on the synthesis de novo of fatty acid desaturases via the cold-inducible expression of genes for these enzymes [22]. By contrast, an increase in growth temperature stimulated the synthesis of membrane lipids but reduced the desaturation of fatty acids, with a resultant decrease in the levels of unsaturation of fatty acids in membrane lipids. These findings

indicate that, under stress conditions, homeostatic regulation of the physiological fluidity of membrane lipids is regulated by a balance between the desaturation of fatty acids and the synthesis of membrane lipids.

Once the temperature-acclimated level of unsaturation of the fatty acids in membrane lipids has been achieved by homeostatic regulation, no further changes in membrane fluidity occur and the activity of signal transduction pathways and the cold-inducible expression of genes ceases. The temperature-acclimated level of unsaturation of fatty acids now maintains the physiologically appropriate fluidity of the membranes. Thus, for the homeostatic regulation of membrane fluidity, the temperature-dependent expression of genes and, in particular, that of genes for desaturases, is transient, as demonstrated by Los et al. [22] in the case of the inducibility of genes for desaturases by low temperature in *Synechocystis*. Since cold stress rigidifies membranes [28], it seems reasonable to postulate that the expression of genes for desaturases might be induced in a ridification-dependent manner. It is possible that a feedback link, which depends on changes in membrane fluidity, might control the activation and repression of sensors of cold and heat stresses.

As mentioned above, hyperosmotic stress might decrease membrane fluidity [27,29], a possibility that suggests the existence of a system for the homeostatic regulation of membrane fluidity under hyperosmotic stress. However, genome-wide analysis of transcription using DNA microarrays indicates that hyperosmotic stress does not induce the expression of genes for desaturases in *Synechocystis* [41,42]. Little is known about the regulation in response to hyperosmotic stress of the desaturation of fatty acids and about the induction of the expression of genes for desaturases under hyperosmotic stress.

3. Perception of low temperature

3.1. Cold-induced gene expression in cyanobacteria

Using DNA microarrays to analyze genome-wide transcription, we identified 45 cold-inducible genes in *Synechocystis* [37,42]. They include genes that are involved in various cellular processes, as follows (each gene is followed by its product): genes involved in the regulation of gene expression, such as *rpoA*, the α-subunit of RNA polymerase; *sigD*, sigma factor D; *nusG*, transcription termination factor; *rbpA*, RNA-binding protein A; *crh*, DEAD-box RNA helicase; *fus*, elongation factor EF-G; genes for many ribosomal proteins; genes involved in the regulation of photosynthesis, such as *hliA, hliB* and *hliC*, which encode high light-inducible proteins; *ndhD2*, subunit 4 of NADH dehydrogenase; *cytM*, cytochrome *C*M; and genes that are expressed in response to oxidative stress, such as *xthA*, exodeoxyribonuclease III and *gshB*, glutathione synthase [42]. Thus, it is likely that cold stress induces the expression of genes involved not only to the desaturation of membrane lipids but also in the regulation of photosynthesis and gene expression under low-temperature conditions (Fig. 2A). It also seems likely that, in addition to increases in the unsaturation of fatty acids, maintenance and/or the reconstruction of the machinery for photosynthesis and the expression of appropriate genes are required for acclimation to low temperature. However, 60% of the cold-inducible genes that encode proteins of unknown function [37,42], and these genes might include genes that are functionally important under cold stress. To

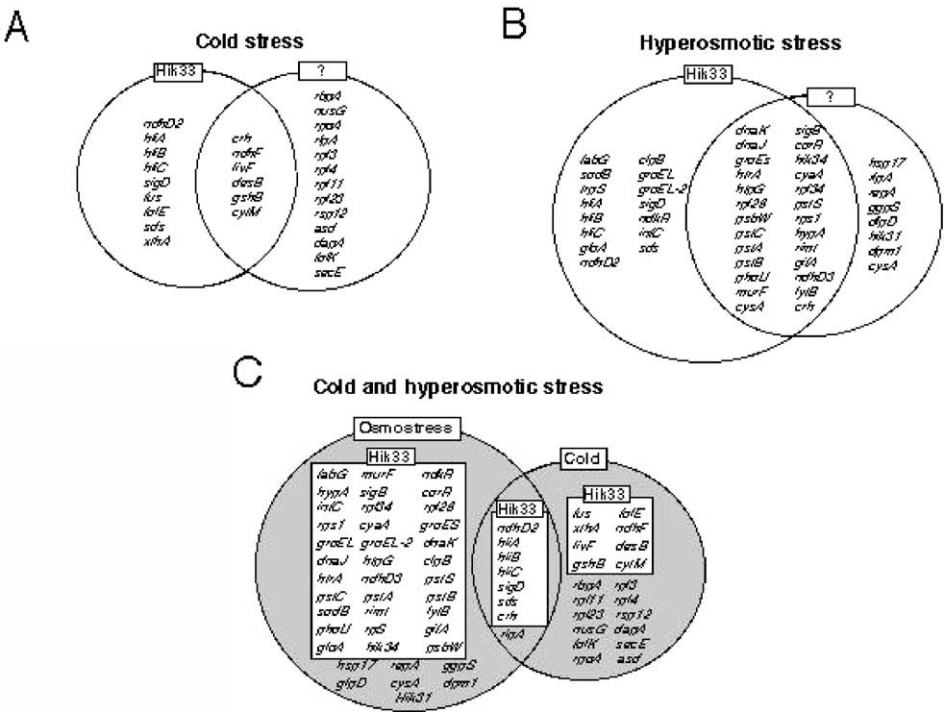

Fig. 2. Differential involvement of Hik33 in the regulation in *Synechocystis* of expression of cold-inducible and hyperosmotic stress-inducible genes. Genes whose expression is regulated by Hik33 or by an unidentified sensor(s) are grouped according to the results of DNA microarray experiments [37,42]. (A) Genes induced by cold stress. (B) Genes induced by hyperosmotic stress. Circles surround genes whose stress-inducible expression is regulated by Hik33 or by an unidentified sensor(s). The overlapping regions of circles encompass genes whose stress-inducible expression is regulated by both Hik33 and an unknown sensor(s). (C) Comparison of genes whose expression is induced by hyperosmotic stress and/or cold stress in wild-type *Synechocystis*. Circles surround genes whose expression was induced by osmotic stress and cold stress, as mentioned above. The rectangles in these circles enclose genes whose expression is regulated by Hik33 under stress conditions, whereas genes outside rectangles appear to be regulated by other as yet unknown sensors. The rectangle in the overlapping region of the two circles encloses genes whose Hik33-regulated expression was observed under both kinds of stress. The products of the indicated genes are identified in Cyanobase (http://www.kazusa.or.jp/cyano/) and the *Synechocystis* PCC6803 gene Annotation Database in GenomeNet Community Databases (http://orf.genome.ad.jp/.).

understand the entire mechanism of the response and acclimation to cold stress in *Synechocystis,* a complete analysis of the stress-inducible genes for proteins of unknown function is now necessary.

3.2. Sensors of low temperature

The cold sensor Hik33 (histidine kinase 33) was originally identified in *Synechocystis* as a regulator of the cold-inducible expression of the *desB* gene after targeted mutagenesis of almost all of this microorganism's genes for histidine kinases [48]. Subsequent analysis of the genome-wide

pattern of gene expression using DNA microarrays demonstrated that Hik33 regulates the expression of 28 of the 45 cold-inducible genes (Fig. 2A) [37,42].

Analysis of the deduced amino acid sequence of Hik33 revealed a HAMP region [49] (also known as a type-P linker [50]), a leucine zipper and a PAS (Per, Arnt, Sim, and phytochrome [51]) domain (Fig. 3A). The HAMP region consists of two helical regions in tandem that transduce stress signals via intramolecular structural changes that occur as a result of interactions between the two helical regions and lead to the intermolecular dimerization of membrane proteins [49,50]. The PAS domain appears to be involved in the sensing of oxidative stress by a variety of proteins [51] but its function in Hik33 remains unknown. The structural characteristics of Hik33 suggest that cold stress might promote a conformational change in the HAMP region, with the subsequent activation of Hik33 as a result of the dimerization of this protein (Fig. 3A) [42]. Thus, perception of cold stress would trigger the dimerization of Hik33.

There are two transmembrane domains in the amino-terminal region of Hik33 (Fig. 3A) [52]. The results of FTIR analysis [28] strongly suggest that Hik33 might recognize a change in membrane fluidity at low temperatures [17,19]. This possibility was examined by mutation of the *hik33* gene in *desA*$^{-}$/*desD*$^{-}$ cells. In the resultant *desA*$^{-}$/*desD*$^{-}$/*hik33*$^{-}$ cells, the expression of Hik33-regulated genes, such as the *hliA, hliB* and *sigD* genes, was no longer inducible by cold [44]. Thus, it appears that Hik33 perceives a decrease in membrane fluidity, which depends on an increase in the extent of unsaturation of fatty acids, as a primary signal of cold stress. However, the way in which Hik33 perceives cold-induced changes in membrane fluidity at the submolecular level remains to be clarified.

Another histidine kinase, DesK, was recently identified as a cold sensor that regulates the cold-inducible expression of the *des* gene for the Δ5 desaturase in *Bacillus subtilis* (Fig. 3B) [53]. The *desK* gene forms an operon with the *desR* gene, which encodes a response regulator that binds specifically to the promoter region of the *des* gene. It is likely that the DesK–DesR two-component system regulates the expression of the *des* gene only (D. de Mendoza, personal communication). Induction of the *des* gene in *B. subtilis* by the DesK–DesR system was inhibited in the presence of an exogenous supply of unsaturated fatty acids or of isoleucine [54]. However, the signal that is perceived by DesK upon exposure of cells to cold stress remains to be identified.

3.3. Cold-inducible gene expression and transduction of cold signals in plants

Large numbers of cold-inducible genes have been identified in plants [36,39], and analysis of the transcriptional control of cold-inducible genes, such as *rd29A* and *corl5a*, in *A. thaliana* identified a cold-responsive element (DRE/CRT) in their promoters, as well as transcription factors that bind specifically to this element (DREB1 and CBF) [55,56]. Factors that regulate the activity and/or expression of DREB1 and CBF were also identified by the molecular cloning of genes that are required for the regulation of the DREB1/CBF-regulated expression of cold-inducible genes [57]. For examples, a RING finger protein, Hos1, which is a homolog of E3 ubiquitin ligases, represses cold signaling, probably via degradation of positive regulators of signaling [58]. By contrast, LOSS/ABA3, which is a sulfurase that transfers sulfur from a sulfur donor to the desulfo/dioxyo form of molybdenum cofactor (MoCo) to generate the sulfide form of MoCo, is required for the positive regulation of the expression of cold-inducible genes [59]. In addition, involvement of a mitogen-activated protein (MAP) kinase cascade in the transduction of cold signals has been demonstrated in alfalfa (*Medicago sativa*) and in *A. thaliana* [60–62].

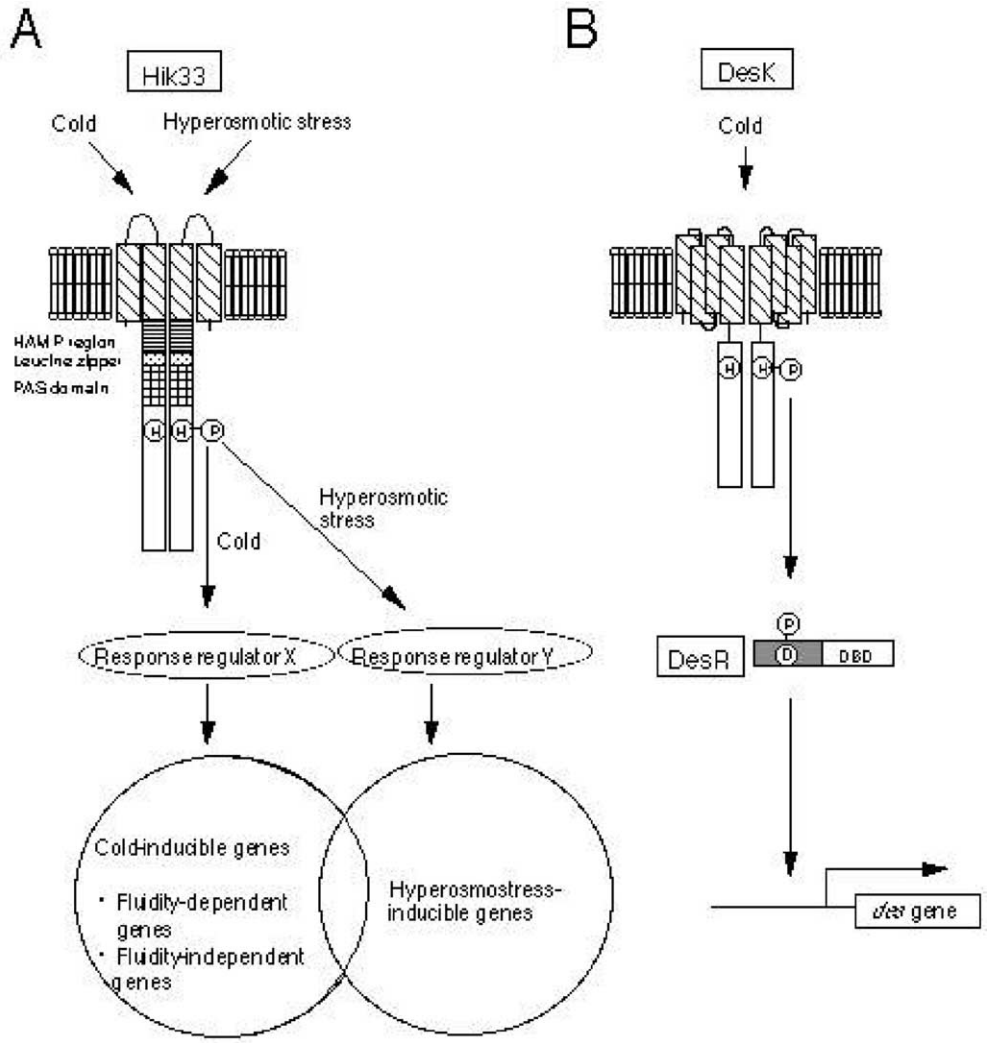

Fig. 3. Schematic representation of hypothetical structures and proposed signal-transduction pathways of Hik33 and DesK. (A) Hypothetical structure of Hik33 and proposed signaling pathways under cold stress and hyperosmotic stress in *Synechocystis*. Hik33 perceives cold stress and hyperosmotic stress and induces the expression of two distinct but partially overlapping sets of stress-specific genes. The response regulators, X and Y, that function downstream of Hik33 have not yet been identified. (B) Hypothetical structure of DesK and the cold-stress signaling pathway in *B. subtilis*. DesK is shown with the typical schematic structure proposed for classical histidine kinases with four transmembrane regions [53]. The response regulator DesR consists of a receiver domain (shaded ellipse) and a DNA-binding domain (DBD), which binds to the promoter region of the *des* gene. The DesK–DesR signal-transduction pathway is inhibited when unsaturated fatty acids or isoleucine are included in the culture medium [54]. H and D indicate histidine and aspartate residues, respectively, whose phosphorylation results in the association with a phosphoryl group (P).

The DNA microarray technique has been used to identify cold-inducible genes in *A. thaliana* [39], demonstrating that low temperature induces the expression of 218 genes, whose products can be divided into several groups, such as transcription factors, signal transducers, transporters, enzymes involved in the synthesis of the cell wall and enzymes involved in responses to oxidative

stress. Moreover, at normal growth temperature, overexpression of CBF1, CBF2 and CBF3 in transgenic plants of *A. thaliana* enhanced the expression of 41 genes, 30 of which had been identified as cold-inducible genes in wild-type plants [39]. Thus, CBF transcription factors might regulate the expression not only of cold-inducible genes but also of genes induced by other signals.

Despite the accumulation of important information about the tranduction of cold signals, little is known about cold sensors and the mechanisms responsible for the perception of low temperature in plants. Experiments in *M. sativa* using fluidisers and rigidifiers of membrane lipids have suggested the possible involvement of membrane fluidity in the sensing of cold signals [60,63,64]. However, it is still unclear whether these chemicals modulate gene expression via changes in membrane fluidity or whether they affect gene expression directly by some as yet unknown mechanism.

The information about cold sensors in plants is very limited. In the mammalian nervous system, Ca^{2+}-permeable channels which belong to the transient receptor potential family were recently identified as cold sensors [65,66]. When plant cells were incubated at low temperature, a rapid influx of Ca^{2+} ions was observed [67,68]. Since some chemicals that enhance the influx of Ca^{2+} ions also induce the expression of cold-inducible genes in *M. sativa* [60,64], it is possible that Ca^{2+} channels or non-specific ion channels in plasma membranes might function as cold sensors in plants. Cloning and characterization of the Ca^{2+} channels that respond to cold stress might shed some light on this hypothesis.

4. Perception of high temperature

4.1. High temperature-induced gene expression

The heat shock response is the name given to the strong and transient induction of genes for so-called heat shock proteins at high temperature [69,70]. DNA microarray analysis indicated that, in *Synechocystis*, heat shock induces the expression of genes for additional proteins, such as *sigB* (for a sigma factor B), *ama* (for *N*-acyl-L-amino acid amidohydrolase), *hypAl* (for a component of a hydrogenase), *sodB* (for superoxide dismutase) and *ctpA* (for a carboxy-terminal processing protease) [44]. Thus, heat stress induces the expression of genes involved not only in the folding and turnover of proteins but also in the regulation of gene expression and responses to oxidative stress. Moreover, as observed in the case of cold-inducible genes, a number of genes for proteins of unknown function were also induced by heat stress [45]. Identification of the functional roles of these genes is required if we are to understand all the details of the mechanisms responsible for the response and acclimation to heat stress.

4.2. Sensors of high temperature

Heat stress increases membrane fluidity [71,72]. However, we observed that the replacement of polyunsaturated lipids by monounsaturated lipids in membranes of *Synechocystis* by mutation of the *desA* and *desD* genes did not affect the heat-induced expression of genes [44]. Therefore, heat stress might not be perceived via changes in membrane fluidity under heat stress in *Synechocystis*.

In their studies of *Synechocystis,* Horváth et al. [73] observed that benzylalcohol, a fluidizer of membranes, induced the expression of heat shock genes, such as *dnaK*, *groES* and *hspA*, and they proposed that heat might be perceived via changes in membrane fluidity. However, our analysis of genome-wide patterns of transcription in *Synechocystis* indicated that heat stress and benzylalcohol induce the expression of distinctly different sets of genes, apart from a small number of genes whose expression is induced both by heat and by benzylalcohol [44]. Moreover, photosynthetic activity was depressed by treatment of cells with benzylalcohol [73], suggesting that benzylalcohol might have some deleterious effects on cell physiology. By contrast, a decrease in the unsaturation of membrane lipids due to genetic modification had no effect on photosynthetic activity at high temperatures [74,75]. Therefore, it is likely that *Synechocystis* recognizes heat and benzylalcohol as different signals and that treatment with benzylalcohol is not equivalent to exposure to an elevated temperature.

In *E. coli*, a high-temperature signal is transduced, in part, via the CpxA–CpxR phosphorelay system [76]. CpxA is a histidine kinase that contains two transmembrane regions and CpxR is a response regulator that functions as a transcription factor to regulate the expression of heat-inducible genes [77–79]. The activity of CpxA is influenced by the composition of membrane lipids [80], suggesting that CpxA might sense changes in the physical state of membrane lipids.

The CpxA–CpxR system has also been found in *Salmonella typhi* and *Yersinia pestis* but not in any other bacteria examined [81]. Thus, other microorganisms probably have heat sensors that are different from CpxA in terms both of structure and mode of activation. A histidine kinase, HsfA, and a response regulator, HsfB, were recently identified as factors that are involved in transduction of the heat-stress signal in *Myxococcus xanthus* [82]. However, since HsfA is a cytoplasmic protein with a phosphate-receiver domain, it is likely that HsfA is a signal transducer and not a heat sensor [82]. In contrast, a heat-sensitive transient receptor potential channel has been identified as a heat sensor in mammalian keratinocytes [83]. Heat sensors have not yet been identified in any photosynthetic organism.

5. Perception of hyperosmotic stress

5.1. Hyperosmotic stress-induced gene expression

An increase of extracellular osmolarity causes the movement of water and solutes out of the cells and decreases cell turgor. Eventually, cell structures are modified to such an extent that plasmolysis is induced. Studies on the response and acclimation of cells to hyperosmotic stress have focused mainly on increases in intracellular osmolarity via accumulation of so-called compatible solutes, such as proline, glycinebetaine, trehalose and sucrose, all of which can be accumulated to molar levels without having negative effects on cells [84–86].

Our recent analysis of genome-wide patterns of transcription in *Synechocystis* exposed to hyperosmotic shock indicated that hyperosmotic stress induced the expression of 257 genes, whose products are involved in the synthesis and maintenance of cell walls and membranes, the formation of the phosphate-transport system and the regulation of photosynthesis (Fig. 2) [41,42]. Osmostress-inducible genes also include genes for factors that are involved in the regulation of signal transduction, gene expression and the turnover of proteins (Fig. 2) [42].

5.2. Sensors of hyperosmotic stress

In *E. coli*, two membrane-integrated histidine kinases, EnvZ and KdpD, have been proposed as osmosensors that perceive hyperosmotic stress and transmit signals that lead to the expression of hyperosmotic stress-inducible genes [87,88]. EnvZ regulates the expression of the *OmpC* and *OmpF* genes, which encode outer-membrane porins, whereas KdpD regulates the expression of the *kdp* operon for the K^+-uptake system [89,90]. *S. cerevisiae* has only one histidine kinase, Slnl, that acts as an osmosensor and activates the Hog1 pathway for the expression of genes that are involved in the synthesis of glycerol, which is a major compatible solute in this yeast [91,92]. In *Synechocystis*, Hik33, which was identified as a cold sensor as discussed above, also acts as a sensor of hyperosmotic stress [42].

Genome-wide analysis of transcription in *Synechosystis* using DNA microarrays indicated that Hik33 contributes fully and to some extent to the inducibility by hyperosmotic stress of 23% and 58%, respectively, of all osmostress-inducible genes (Fig. 2) [42]. The osmostress-inducible expression of the gene for nucleoside-diphosphate kinase (NdpK; Fig. 2) seems to be particularly important because NdpK catalyses the synthesis of nucleoside triphosphates [93] and acts as a regulator of signal transduction via histidine-specific phosphorylation of histidine kinases, such as EnvZ and CheA [94]. In addition, the osmostress-inducible expression of the phosphate-transport system might supply phosphate for the production of nucleoside triphosphates and for the phosphorylation of proteins.

The activity of KdpD is affected by changes in the physical state of membrane lipids, which can be achieved by addition of a membrane fluidizer, procaine, or by removal of phosphatidylethanolamine from membranes [90,95]. Procaine also influences the activation of EnvZ [94,96]. These findings suggest that osmosensors, such as EnvZ and KdpD, might recognize changes in membrane fluidity that are induced by hyperosmotic stress. However, it is still unclear whether Hik33 perceives hyperosmotic stress via changes in membrane fluidity.

The genome of *A. thaliana* includes 11 genes for histidine kinases [97]. Among them, only one, AtHK1, is a candidate for an osmosensor [98] and the functional characterization of this protein should provide useful information.

6. A multi-stress sensor in *Synechocystis*

DNA microarray experiments have demonstrated clearly that Hik33 perceives both cold stress and osmotic stress (Figs. 2 and 3) [42]. This observation is inconsistent with the concept that a single sensor specifically perceives a single stress [99]. Moreover, the results of DNA microarray experiments also indicate that Hik33 regulates different sets of genes under cold stress and hyperosmotic stress conditions (Figs. 2 and 3) [42], suggesting that Hik33 might recognize osmotic stress and cold stress as different signals. It is very likely, as mentioned above, that Hik33 recognizes changes in membrane fluidity as the primary signal of cold stress and hyperosmotic stress. It is unclear how Hik33 can perceive these different signals via changes in membrane fluidity.

Hik33 was first identified as a chemical sensor of drugs, such as inhibitors of photosynthesis [100], while Nb1S, a putative homolog of Hik33 in *Synechococcus elongatus* PCC 7942, was

identified as a sensor of strong light and nutrient stress [101]. These findings suggest that Hik33 might also perceive strong light and nutrient stress. Thus, Hik33 can be considered to be a ''multi-stress sensor'' in *Synechocystis*.

In *S. cerevisiae*, Slnl is involved in sensing osmotic stress and oxidative stress [102,103], and it can also perceive heat stress [104]. In *Schizosaccharomyces pombe,* three membrane-bound His kinases, Mak1, Mak2, and Mak3 (Mcs4p-activating kinases), each seem to be able to perceive oxidative stress, salt stress and cold stress [105–107]. Therefore, the ability to perceive several types of stress is not unique to Hik33. It is noteworthy that Sln1 phosphorylates Sskl and Skn7, which are both response regulators, under hyperosmotic and oxidative conditions, respectively [103,108].

7. Conclusion and future perspectives

It has been postulated that a change in the fluidity of membrane lipids is the primary signal in the perception of cold, heat and, possibly, osmotic stress in many organisms. However, information about the molecular mechanisms responsible for the perception and transduction of environmental signals is limited and this hypothesis has not been fully validated. The availability of the sequences of the genomes of many organisms and the development of genome-wide DNA microarrays have allowed us to identify families of genes that are specifically induced by individual stresses and to identify the sensors and tranducers of environmental stress signals.

In cyanobacteraia, it is very likely that the rigidification of membranes at low temperatures is the primary signal of cold stress that is perceived by Hik33 and an as yet unknown sensor. However, we cannot exclude the possibility that the cold signal is perceived and transduced without any involvement of membrane fluidity. It appears less likely that high temperature is perceived via changes in membrane fluidity and it is not known whether membrane fluidity is involved in the perception of osmotic stress and transduction of the osmostress signal. More precise and thorough investigations are necessary to determine how membrane fluidity contributes to the perception of environmental signals.

Some histidine kinases have been identified that very possibly perceive temperature stress and osmotic stress via changes in membrane fluidity. It remains to be determine how these kinases recognize a change in membrane fluidity, and the domains and amino acids involved in signal perception remain to be identified. Furthermore, it is also critical to identify the lipid molecules, among all the membrane lipids, that interact with the sensors to activate or inactivate them. Future research should provide answers to these important questions.

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