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Review

Membrane fluidity and its roles in the perception of environmental signals

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Abstract

Poikilothermic organisms are exposed to frequent changes in environmental conditions and their survival depends on their ability to acclimate to such changes. Changes in ambient temperature and osmolarity cause fluctuations in the fluidity of cell membranes. Such fluctuations are considered to be critical to the initiation of the regulatory reactions that ultimately lead to acclimation. The mechanisms responsible for the perception of changes in membrane fluidity have not been fully characterized. However, the analysis of genome-wide gene expression using DNA microarrays has provided a powerful new approach to studies of the contribution of membrane fluidity to gene expression and to the identification of environmental sensors. In this review, we focus on the mechanisms that regulate membrane fluidity, on putative sensors that perceive changes in membrane fluidity, and on the subsequent expression of genes that ensures acclimation to a new set of environmental conditions.

Keywords: Cold sensor; DNA microarray; Environment; Membrane fluidity; Membrane lipid; Osmosensor; Temperature stress

Contents

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1. Introduction

Various kinds of environmental stress, such as temperature stress and osmotic stress, cause alterations in the physical properties of the membrane lipids in living cells. It seems likely that cells perceive these alterations via sensory proteins embedded in their membranes. These proteins transfer the signals from the environment to networks of signal-transduction pathways, with the resultant regulation of gene expression [1,2]. Chemical and genetic modification of the physical properties of membrane lipids can have similar effects on the expression of genes that are involved in the acclimation of cells to various conditions [3–5]. The physical state of membrane lipids also acts directly to regulate the activity of membrane-bound proteins, such as the translocators of small molecules, ion channels [6], receptor-associated protein kinases [7,8], and sensor proteins [9,10].

Until recently, studies of the influence of membrane fluidity on gene expression have been limited to studies of a small number of genes whose expression has been examined by Northern blotting, focusing on the feedback regulation of membrane fluidity and on genes that are responsible for the maintenance of the physical properties of membrane lipids [1–3,8]. However, since DNA microarrays that cover the entire genomes of various organisms have become available, it has been possible to analyze the genome-wide expression of genes, which are associated with acclimation. The use of DNA microarrays, in combination with the site-directed mutagenesis of genes that are responsible for the maintenance of membrane fluidity, has provided a very powerful method for studies of the contribution of membrane fluidity to gene expression and for identification of various sensors of environmental conditions.

In this review, we shall focus on the mechanisms that regulate the fluidity of membrane lipids on putative sensors that perceive changes in membrane fluidity, and on the subsequent expression of genes that ensure acclimation to a new set of environmental conditions. In particular, we shall discuss findings obtained by the analysis of genome-wide gene expression with DNA microarrays.

2. Modulation of membrane fluidity

2.1. Measurements of membrane fluidity

The extent of molecular disorder and molecular motion within a lipid bilayer is referred to as the fluidity of the membrane. Several methods can be used to monitor membrane fluidity. The most common method involves measurements of the anisotropy of the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) [11–13]. DPH is incorporated into membranes in parallel to the acyl chains of membrane lipids and the fluorescence of DPH is only weakly depolarized when it interacts stably with rigidified membranes [12]. However, since DPH is not completely free to rotate within membranes, the extent of the interactions between DPH and membrane lipids is restricted. Thus, this method is unsuitable for quantitative measurements of membrane fluidity. Another method, Fourier transform infrared (FTIR) spectroscopy, can be used for quantitative analysis of the physical state of membrane lipids [14]. FTIR spectroscopy allows us to monitor the disorder of the acyl chains of lipids and the interactions between lipids and membrane proteins in terms of the frequency of the symmetric CH_2 stretching mode near 2.81 cm^{-1}. This frequency decreases by approximately 2–5 cm^{-1} upon the transition of the membrane lipids from an ordered to a disordered state. Thus, low and high frequencies of the CH_2 stretching mode correspond to the rigidified and fluid states of membrane lipids, respectively.

2.2. Effects of changes in temperature

The effects of changes in temperature on membrane fluidity have been demonstrated by DPH fluorescence polarization in fish [15,16], bacteria [17], and cyanobacteria [5]. The cited studies focused on the effects of low temperature and demonstrated clearly that membrane fluidity decreases with a decrease in temperature (Fig. 1). Recently, these changes have also been examined by FTIR spectroscopy [14,18]. In the cyanobacterium *Synechocystis*, the frequency of the CH_2 stretching mode in isolated cytoplasmic membranes and in isolated thylakoid membranes decreases with a decrease in temperature [14,18].

The effects of high temperatures on the physical state of membranes have also been studied, albeit less extensively [4,19]. High temperatures cause the fluidization of membranes (Fig. 1), which can lead to disintegration of the lipid bilayer. It is clear that both increases and decreases in temperature modulate membrane fluidity.

2.3. Effects of osmotic stress

The effects of hyperosmotic stress on membrane fluidity have been examined using DPH [13,20]. When phospholipid vesicles were exposed to hyperosmotic stress that was

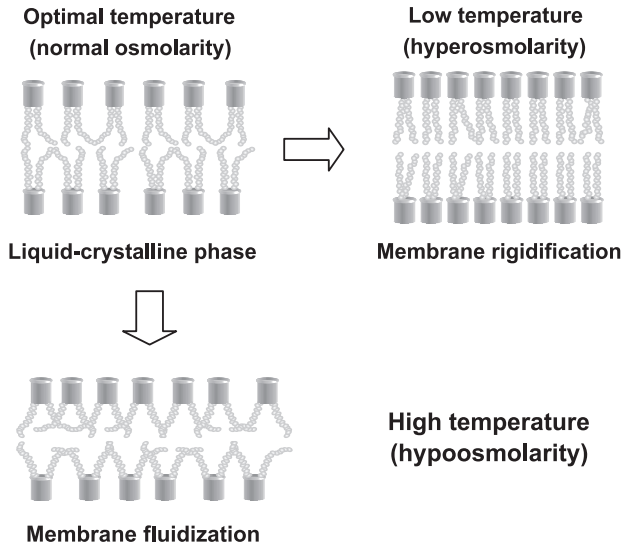

Fig. 1. Schematic representation of changes in membrane structure and the behavior of lipid bilayers under low- and high-temperature stress. Low temperatures cause "rigidification" of membranes, whereas high temperatures cause "fluidization" of membranes.

due to the addition of polyethylene glycol to the medium, an increase in anisotropy was observed [20]. A similar effect was observed in yeast when glucose was added to the medium to raise the osmolarity [13]. These findings suggest that hyperosmotic stress might reduce membrane fluidity similarly to low-temperature stress. However, more studies are necessary to confirm this possibility in the biological membranes of living organisms.

The effects of hypoosmotic stress on membrane fluidity have not been studied in detail but it has been suggested that hypotonic stress might fluidize membranes similarly to heat stress.

Aliphatic alcohols cause the fluidization of membranes and they are often used to simulate heat and hypotonic stress [5,21,22]. However, the relevance of such an approach is questionable, since heat stress and benzyl alcohol (both of which provoke the fluidization of membranes) induce different sets of genes in *Synechocystis* [18]. Thus, the physiological impact of heat stress and that of membrane fluidizers on cell membranes might be rather different.

2.4. Effects of the unsaturation of fatty acids

The dependence of membrane fluidity on the extent of unsaturation of fatty acids in membrane lipids is a well-characterized phenomenon, which has been demonstrated in animals [23], fish [24,25], fungi [26,27], plants [28,29], bacteria [17,30], and cyanobacteria [2,31,32]. Cyanobacteria are particularly suitable for studies of such phenomena [33] because the number of unsaturated bonds in their fatty acids can be altered by genetic manipulation of the genes that encode the fatty acid desaturases [34,35]. We have used two strains of cyanobacteria, namely, *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) and *Synechococcus* sp. PCC 7942 (hereafter *Synechococcus*), to study the effects of the unsaturation of fatty acids on membrane fluidity. *Synechocystis* is characterized by the presence of four genes, designated *desA*, *desB*, *desC* and *desD*, for fatty acid desaturases and by its ability to synthesize fatty acids with four double bonds. Thus, its membrane lipids contain high levels of unsaturated fatty acids (Fig. 2A).

When the *desA* and *desD* genes were inactivated in *Synechocystis*, a dramatic decrease in membrane fluidity was detectable in the resultant $desA^{-}/desD^{-}$ strain (Fig. 3), which had lost the ability to acclimate to low temperatures [35]. Thus, the unsaturation of fatty acids increases membrane fluidity, and such an increase is necessary if cells are to tolerate chilling and to survive at low temperatures [1,36,37]. It should be noted that the optimal temperature for the growth of these cyanobacterial strains is close to 35 °C, and they experience cold stress when the temperature falls to 20–25 °C.

Unlike *Synechocystis*, *Synechococcus* has only a single Δ9 desaturase and it synthesizes mono-unsaturated fatty acids exclusively (Fig. 2B). When the *desA* gene of *Synechocystis*, which encodes the Δ12 desaturase, was introduced into the genome of *Synechococcus*, the resultant $desA^{+}$ strain produced considerable amounts of di-unsaturated fatty acids [34] (Fig. 4B). This charge resulted in a considerable increase in membrane fluidity (our unpublished results), which was linked to the ability of cells to survive at a low temperature (Fig. 4).

A clear effect of membrane rigidification on cold-induced gene expression was demonstrated recently by genome-wide analysis, using DNA microarrays, of wild-type and $desA^{-}/desD^{-}$ cells of *Synechocystis* [18]. Sixteen genes whose expression was not induced by cold in wild-type cells (for example, several heat-shock genes, such as *hspA*, *clpB1*, *dnaK2*) became strongly cold-inducible upon rigidification of membrane lipids. The level of cold-inducible expression of another group of 17 genes was increased from two- to three-fold upon rigidification of the plasma membrane [18]. These results indicate that expression of a relatively large number of cold-inducible genes is controlled by membrane fluidity.

2.5. Fluidity feedback by desaturation of fatty acids

Three of the four genes for desaturases in *Synechocystis* (*desA*, *desB*, and *desD*) are cold-inducible [32,38,39]. The enhanced synthesis de novo of these three fatty-acid desaturases under cold stress and the subsequent introduction of additional double bonds into the fatty-acyl chains of membrane lipids are involved in the maintenance of membrane fluidity in the liquid-crystalline phase and prevent the membranes from undergoing phase transition to the lethal gel phase [40]. The induction by low temperatures of the expression of genes for desaturases, to compensate for a decrease in membrane fluidity, is a

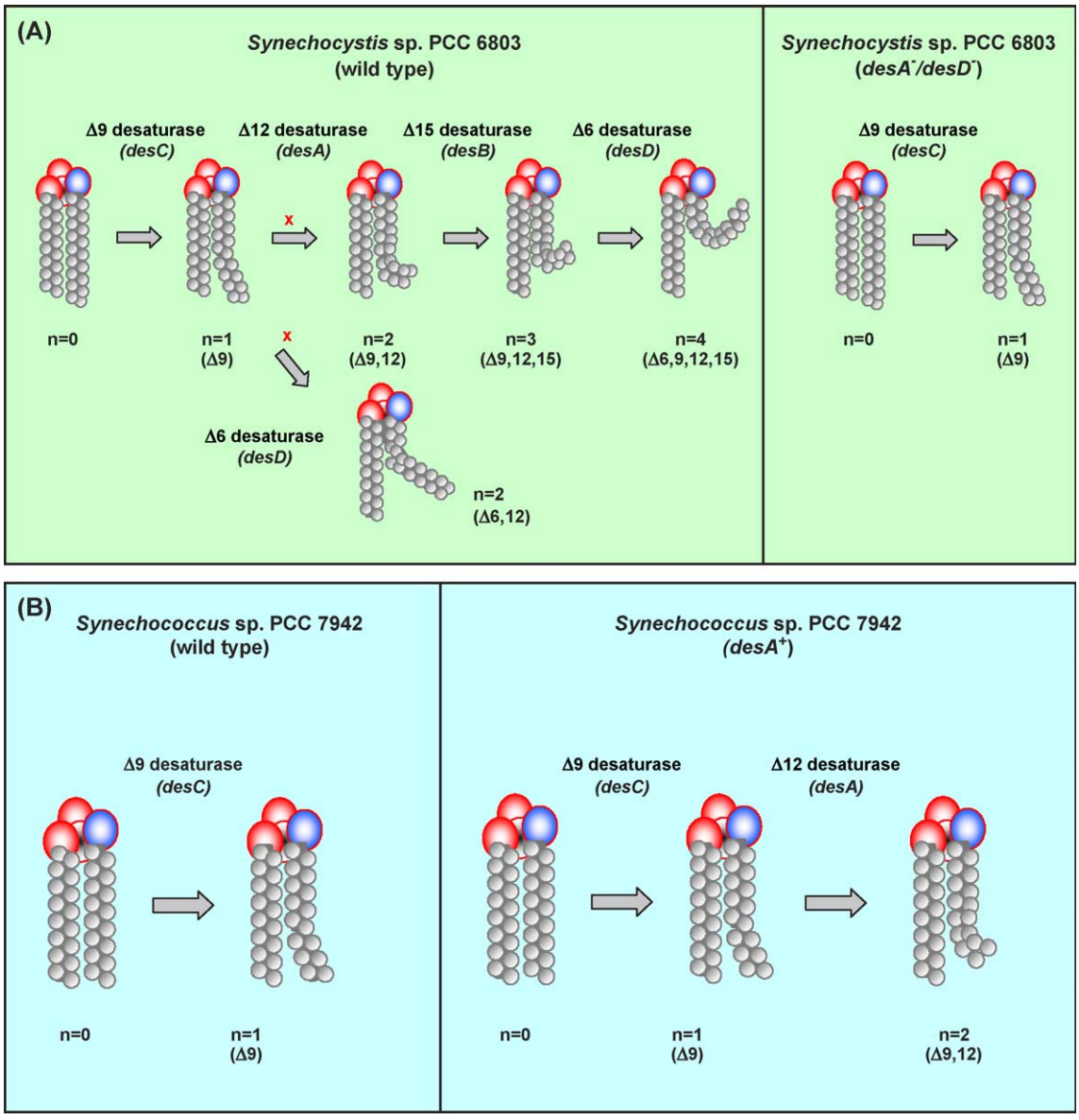

Fig. 2. Desaturation of polar glycerolipids in cyanobacterial cells. (A) Desaturation in wild-type cells of *Synechocystis* is catalyzed by four acyl-lipid desaturases, with formation of C18:4 fatty acid (left panel). Site-directed mutagenesis of the *desA* and *desD* genes (shown by daggers) prevents the synthesis of polyunsaturated fatty acids and only mono-unsaturated fatty acids are produced (right panel). The molecular species of lipids in *Synechocystis* are mainly C18 with minor levels of C16 at the *sn*-1 position and C16:0 at the *sn*-2 position. Desaturation occurs exclusively at the *sn*-1 position. *n*=0, 1, 2, 3, 4 corresponds to the number of double bonds in the fatty-acyl chains. (B) Desaturation in wild-type cells of *Synechococcus* is catalyzed only by the acyl-lipid Δ9-desaturase and only mono-unsaturated fatty acids are produced (left panel). Transformation of this strain with the *desA* gene for the Δ12-desaturase of *Synechocystis* leads to enhanced formation of di-unsaturated fatty acids (right panel). In *Synechococcus*, most of the molecular species are C16 with minor levels of C18 at the *sn*-1 position and C16:0 at the *sn*-2 position. Desaturation occurs at the *sn*-1 and *sn*-2 positions.

widespread phenomenon that can be observed in almost all taxa of poikilothermic organisms, from bacteria to plants, fish, and animals [24,31,32,41,42].

Synthesis of unsaturated fatty acids to compensate for a decrease in membrane fluidity at a low temperature was demonstrated first in *Escherichia coli* [17] and the phenomenon was designated “homeoviscous acclimation”. More recently, the existence of a feedback loop between membrane rigidification and the compensatory expression of genes for desaturases was demonstrated by the chemical hydrogenation of unsaturated fatty acids in the plasma-membrane lipids of *Synechocystis* [1,3,38]. Palladium-catalyzed saturation of a small portion of plasma-membrane lipids at the optimal growth temperature caused the immediate induction of the desaturases that desaturate fatty acids in membrane lipids. These enzymes reversed the chemically induced rigidification of membrane lipids and returned them to their normal fluid state [3].

It is important to note that the cold-induced enhancement of the expression of the genes for desaturases depends on the extent of the shift in temperature and not on the absolute temperature [3,38]. When cells acclimated to 36 °C are

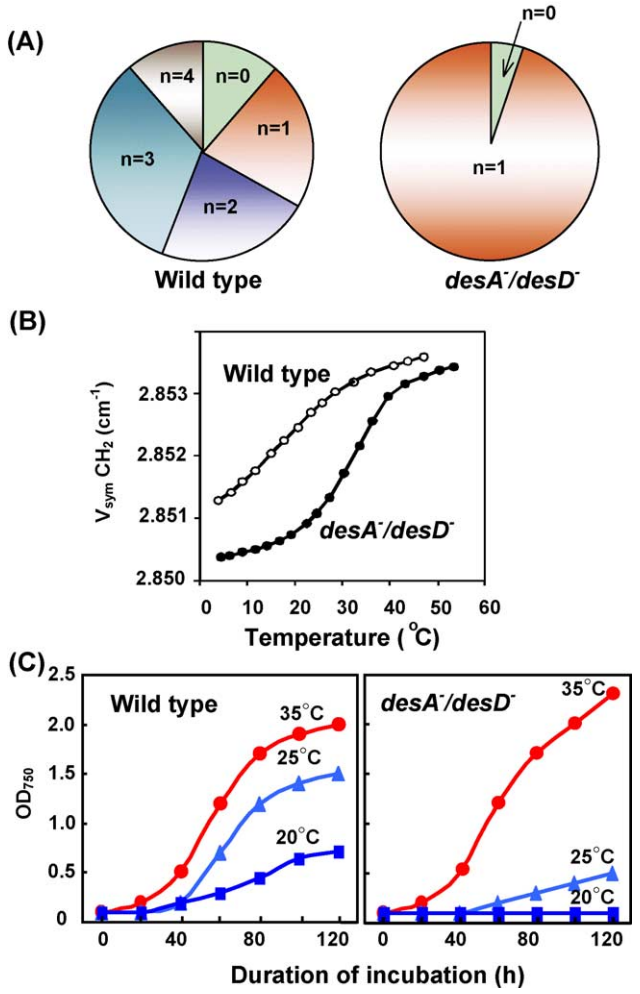

Fig. 3. Site-directed mutagenesis of the *desA* and *desD* genes of *Synechocystis* leads to enrichment of membrane lipids with mono-unsaturated fatty acids (A). As a result, the membrane fluidity decreases, as monitored by FTIR spectroscopy (B). The mutant strain *desA*$^{-}$/*desD*$^{-}$ is unable to acclimate to low temperatures, as demonstrated by growth curves of wild-type and mutant cells at 35, 25 and 20 °C (C). OD_{750}—optical density of cells measured at 750 nm.

transferred to various lower temperatures, the accumulation of transcripts of genes for desaturases becomes apparent only below 30 °C. However, when cells have been acclimated to 32 °C, the accumulation of the transcripts becomes apparent below 26 °C [38].

We might expect that the desaturation of fatty acids might also compensate for the rigidification of membrane lipids in cells exposed to hyperosmotic stress. However, genome-wide analysis of transcription in *Synechocystis*, using DNA microarrays, indicated that hyperosmotic stress does not activate the transcription of genes for desaturases [43]. Nevertheless, in *Bacillus subtilis*, hyperosmotic stress decreases the fluidity of cell membranes and subsequently increases the levels of unsaturated fatty acids in membrane lipids [44]. This phenomenon might be due to the enhanced expression of the genes for desaturases, or to the activation of desaturases. This problem requires further careful analysis.

In some bacteria, *cis–trans* isomerization of unsaturated fatty acids plays an important role in the regulation of membrane fluidity [45,46]. The conversion of *cis*-unsaturated fatty acids to *trans*-unsaturated fatty acids apparently occurs with considerable efficiency in the adaptation of membrane fluidity to changes in the cellular environment [47,48].

The transcriptional control of homeostasis in the unsaturation of fatty acids and the fluidity of membrane lipids of warm-blooded animals and humans has been also proposed [23]. It has been suggested that membrane fluidity might be regulated by changes in levels of oleate and polyunsaturated fatty acids relative to the total level of fatty acids, as well as by changes in levels of cholesterol relative to the total level of lipids.

The expression of all the genes for the enzymes involved in the biosynthesis of cholesterol is regulated at the transcriptional level by a common mechanism [49,50]. The promoters of these genes contain sterol-responsive elements (SREs) that bind transcription factors known as SRE-binding proteins (SREBPs), as described in detail elsewhere [23,51]. The promoters of several genes for enzymes involved in fatty acid biosynthesis, such as acetyl-CoA-carboxylase, fatty acid synthase, and stearoyl-CoA-desaturase, also contain SRE-like elements and some SREBPs specifically activate the biosynthesis of fatty acids [23]. Thus, the biosynthetic pathways for the synthesis of

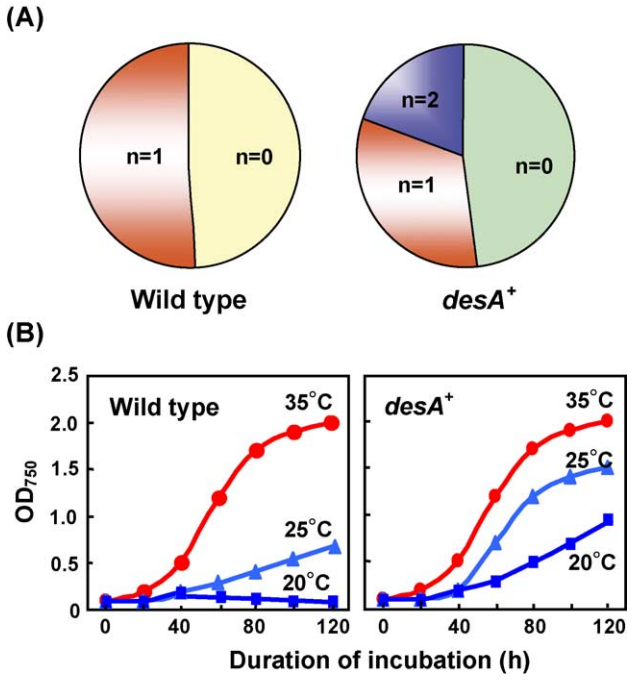

Fig. 4. Transformation of *Synechococcus* sp. PCC 7942 with the *desA* gene for the Δ12 desaturase of *Synechocystis* leads to enrichment of membrane lipids with di-unsaturated fatty acids (A). The transformed cells, *desA*$^{+}$, are able to acclimate to low temperatures, in contrast to wild-type cells which are unable to do so, as demonstrated by growth curves for wild-type and transformed cells at 35, 25 and 20 °C (B). OD_{750}—optical density of cells measured at 750 nm.

Table 1
Genes that are induced by cold in cyanobacteria

Gene	Gene product	Cyanobacterium
Genes for desaturases		
desA	Δ12 desaturase	*Synechocystis* sp. PCC 6803
		Synechocystis sp. PCC 6714
		Synechococcus sp. PCC 7002
		Spirulina platensis
desB	Δ3 desaturase	*Synechocystis* sp. PCC 6803
		Synechococcus sp. PCC 7002
desC	Δ9 desaturase	*Synechococcus* sp. PCC 6301
		Synechococcus sp. PCC 7002
		Synechococcus vulcanus
desD	Δ6 desaturase	*Synechocystis* sp. PCC 6803
		Spirulina platensis
Genes for RNA-binding proteins		
rbpAl	RNA-binding protein (Rbpl)	*Synechocystis* sp. PCC 6803
		Anabaena variabilis M3
rbpA2	RNA-binding protein (Rbp2)	*Anabaena variabilis* M3
rbpA3	RNA-binding protein (Rbp3)	*Anabaena variabilis* M3
rbpB	RNA-binding protein (RbpB)	*Anabaena variabilis* M3
rbpC	RNA-binding protein (RbpC)	*Anabaena variabilis* M3
rbpF	RNA-binding protein (RbpE)	*Anabaena variabilis* M3
rbpF	RNA-binding protein (RbpF)	*Anabaena variabilis* M3
crhB	RNA helicase (CrhB)	*Anabaena* sp. PCC 7120
crhC	RNA helicase (CrhC)	*Anabaena* sp. PCC 7120
deaD	RNA helicase (DeaD)	*Synechocystis* sp. PCC 6803
Genes for caseinolytic proteases		
clpB	Molecular chaperone (ClpB)	*Synechococcus* sp. PCC 7942
clpPl	Protease (ClpP)	*Synechococcus* sp. PCC 7942
clpX	Unknown	*Synechococcus* sp. PCC 7942
Genes for ribosomal proteins		
rpsU	30S ribosomal subunit (S21)	*Anabaena variabilis* M3
rpsl2	30S ribosomal subunit (S12)	*Synechocystis* sp. PCC 6803
rps13	30S ribosomal subunit (S13)	*Synechocystis* sp. PCC 6803
rpl1	50S ribosomal subunit (L1)	*Synechocystis* sp. PCC 6803
rpl3	50S ribosomal subunit (L3)	*Synechocystis* sp. PCC 6803
rpl4	SOS ribosomal subunit (L4)	*Synechocystis* sp. PCC 6803
rpl11	SOS ribosomal subunit (L11)	*Synechocystis* sp. PCC 6803
rpl2(j	50S ribosomal subunit (L20)	*Synechocystis* sp. PCC 6803
rpttl	50S ribosomal subunit (L2I)	*Synechocystis* sp. PCC 6803
rpl23	50S ribosomal subunit (L23)	*Synechocystis* sp. PCC 6803
fus	Elongation factor EF-G	*Synechocystis* sp. PCC 6803
Genes for other proteins		
rpoA	α subunit of RNA polymerase	*Synechocystis* sp. PCC 6803
rpoD	σ^{70} factor of RNA polymerase	*Synechocystis* sp. PCC 6803
cbiM	Cobalamin biosynthetic prot.	*Synechocystis* sp. PCC 6803
cytM	Cytochrome c_M	*Synechocystis* sp. PCC 6803
ndhC	NADH dehydrogenase subunit 3	*Synechocystis* sp. PCC 6803
ndhD2	NADH dehydrogenase subunit 4	*Synechocystis* sp. PCC 6803
ndhD6	NADH dehydrogenase subunit 6	*Synechocystis* sp. PCC 6803
folE	GTP cyclohydrolase I	*Synechocystis* sp. PCC 6803
Ssl1633	CAB/ELIP/FLIP superfamily	*Synechocystis* sp. PCC 6803
hliA	High light-inducible protein	*Synechocystis* sp. PCC 6803
Genes for proteins of as yet unknown function		
slr1544		*Synechocystis* sp. PCC 6803
slr0082		*Synechocystis* sp. PCC 6803
sll0551		*Synechocystis* sp. PCC 6803
sll0668		*Synechocystis* sp. PCC 6803
slr1974		*Synechocystis* sp. PCC 6803
slr0955		*Synechocystis* sp. PCC 6803

The complete list of genes is available on the internet at http://www.genome.ad.jp/keg/expression.

cholesterol (a membrane rigidifier) and oleate (a membrane fluidizer) are interrelated. In mammalian cells, chemical fluidizers of membranes induce the synthesis of cholesterol [52,53], as predicted by the hypothesis of "homeoviscous acclimation" which was proposed initially for bacteria and plants. The existence of a sensor of the fluidity of plasma membranes in animals has been predicted [23], but such a sensor has not yet been identified. Nevertheless, in animals, changes in membrane fluidity do affect the activities of G proteins, which are components of signal-transduction systems, such as those involved in photoreception, taste, and olfaction [21], and are linked to transmembrane receptors. It has been demonstrated that the fluidization of membrane lipids can activate heterotrimeric G proteins directly on the cytosolic face of the plasma membrane.

3. Perception of low temperature

3.1. Cold-inducible genes in cyanobacteria

There are several comprehensive reviews of the responses of cyanobacteria to cold stress [1,2,31,32,54]. It has been proposed that cold-inducible genes can be grouped into six categories, as follows: (1) genes for fatty acid desaturases that are responsible for adjustments in membrane fluidity; (2) genes for RNA-binding proteins (Rbps) that, probably, serve as RNA chaperones similarly to the Csp proteins of *E. coli* and *B. subtilis*; (3) genes for RNA helicases that destabilize the secondary structures of mRNAs, thereby overcoming inhibition of the initiation of translation at low temperatures; (4) genes for ribosomal proteins, an excess of which is necessary for acclimation of the translational machinery to cold; (5) genes for caseinolytic proteases that participate in the renewal of Photosystem II; and (6) various other genes that do not fall in any of the other five categories. The availability of genome-wide DNA microarrays of *Synechocystis* has provided new opportunities for studies of responses of the entire genome to cold stress [55]. The expression of close to 50 genes is strongly induced in *Synechocystis* under cold stress (Table 1). In addition to the abovementioned groups, some other important genes appear to be induced at low temperatures, namely, the *rpoA* gene for RNA polymerase; the *sigD* gene for sigma factor D; the *fus* gene for elongation factor EF-G; the *hliA*, *hliB*, and *hliC* genes that encode high light-

inducible proteins, which are involved in the regulation of photosynthesis; the *ndhD2* gene for subunit 4 of NADH dehydrogenase; the *cytM* gene for an alternative form of cytochrome *c*; several genes that are expressed in response to oxidative stress; and several genes for proteins of as yet unknown function (Table 1). Thus, it is evident that cold stress enhances the expression of many genes whose products control membrane fluidity, transcription, translation, and the energy status of the cell.

3.2. A sensor of cold stress in cyanobacteria

The cold sensor histidine kinase 33 (Hik33) was originally identified in *Synechocystis* as a regulator of the cold-inducible expression of the *desB* gene, which encodes the ω3 desaturase, after random mutagenesis of a strain that carried a reporter gene in which the *desB* promoter had been fused to the gene for a bacterial luciferase and targeted mutagenesis of almost all the genes for histidine kinases in this organism [56,57]. 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 [55].

The amino acid sequence of the Hik33 sensory histidine kinase contains several conserved domains, namely, a type-P linker [58], a leucine zipper and a PAS domain [59] (Fig. 5A). The type-P linker consists of two helical regions, in tandem, that transduce stress signals via intramolecular structural changes, which occur as a result of interactions between the two helical regions and lead to intermolecular dimerization of the protein [58,60,61]. The PAS domain senses oxidative stress [59], which might accompany cold stress shortly after a drop in temperature [62,63]. Several of the genes that are induced by oxidative stress are also induced by cold stress in *Synechocystis* (Table 1). This observation suggests that cells might experience oxidative stress at the early stages of cold stress. However, our results with DNA microarrays indicate that *Synechocystis* perceives oxidative stress and cold stress as two distinctly different signals (our unpublished results). The structural features of Hik33 suggest that cold stress might promote a conformational change in the type-P linker, with the subsequent activation of Hik33 as a result of dimerization of the protein (Fig. 5B) [2]. Thus, cold stress might trigger the dimerization of Hik33.

There are two transmembrane domains in the amino-terminal region of Hik33 (Fig. 5) [53]. The results of analysis by FTIR spectroscopy [14,18] strongly suggest that Hik33 might recognize a change in membrane fluidity at low temperatures [2]. This possibility was tested by mutation of the *hik33* gene in *desA*$^-$/*desD*$^-$ cells. In the resultant *desA*$^-$/*desD*$^-$/*hik33*$^-$ mutant cells, the expression of Hik33-regulated genes (*hliA*, *hliB*, and *sigD*) was no longer inducible by cold [18]. Thus, it appears that Hik33 perceives a decrease in membrane fluidity as the primary signal of cold stress. Membrane fluidity depends, in its turn, on the extent of fatty acid unsaturation. It is possible that the cold-induced rigidification of the plasma membrane might lead to dimerization and the auto-phosphorylation of Hik33. However, the exact way in which Hik33 perceives changes in membrane fluidity at the submolecular level remains to be clarified.

In *B. subtilis*, the histidine kinase DesK was identified as a cold sensor that regulates the cold-inducible expression of the *des* gene for the Δ5 desaturase (Fig. 5C) [64]. Resembling Hik33, DesK is a membrane-bound sensor. Each monomer of DesK has four transmembrane domains and a histidine kinase domain. However, in contrast to Hik33, DesK lacks PAS and leucine zipper domains.

The *desK* gene forms an operon with the *desR* gene, which encodes a response regulator that binds specifically to the promoter region of the gene for the DesK desaturase. Induction of expression of the desaturase in *B. subtilis* by the DesK–DesR two-component system is inhibited by the addition of exogenous unsaturated fatty acids or isoleucine [30,64]. This observation suggests the presence of a feedback loop between the function of the sensor and the extent of fatty acid unsaturation. However, the actual signal that is perceived by DesK upon cold shock remains to be identified. It should be noted that the Desk–DesR two-component system regulates the cold-inducible expression of the *des* gene for the Δ5 desaturase while, by contrast, the cyanobacterial Hik33 sensor regulates the expression of approximately 30 cold-inducible genes.

3.3. Cold-inducible gene expression in plants

Large numbers of cold-inducible genes have been identified in plants [62,63,65–79]. These genes include genes for fatty acid desaturases [36,42,80], confirming the key role of the desaturation of fatty acids in membrane lipids in the regulation of membrane fluidity and the acclimation of plants to low temperatures, as postulated above [1,29,31,81,82].

In *Arabidopsis thaliana*, DNA microarray analysis of the expression of 8000 genes revealed that the expression of 218 genes is induced by cold [63]. These genes could be classified into several groups, such as genes for transcription factors, signal transducers, transporters, enzymes involved in the synthesis of cell walls and enzymes involved in the response to oxidative stress.

3.4. Cold sensing in plants

The discovery of the CBF cold-regulation pathway in plants led to further progress in the characterization of transduction of the low-temperature signal [70,71,79,83–88]. An analysis of the transcriptional control of two cold-inducible genes (*rd29A* and *cor15a*) in *A. thaliana* led to the identification of a cold-responsive element, the CRT/DRE [(C-repeat)/(dehydration-responsive element)], in their promoters [71]. Members of a family of AP2-domain tran-

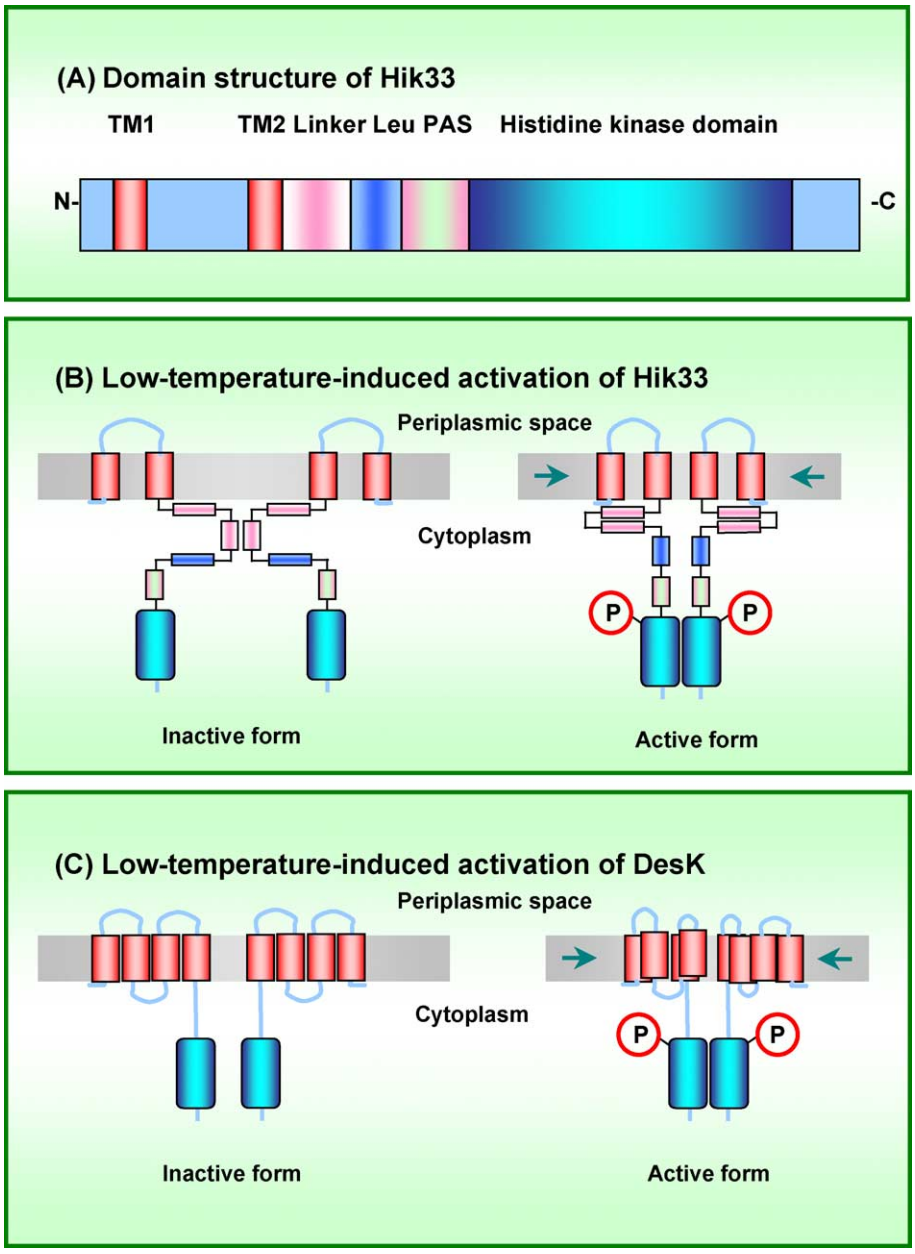

Fig. 5. A hypothetical scheme for the structure and activation of cold-sensing histidine kinases. (A) Domain structure of Hik33 of *Synechocystis*. TM1 and TM2, transmembrane domains 1 and 2; Linker, type-P linker; Leu, leucine zipper; PAS, PAS domain. (B) Hypothetical scheme for the activation of Hik33 upon a decrease in temperature. A decrease in temperature rigidifies the membrane, leading to compression of the lipid bilayer (shown by arrows). This compression forces the membrane-spanning domains to move closer together, changes the linker conformation, and leads finally to dimerization and auto-phosphorylation of the histidine kinase domains. (C) The activation of DesK of *B. subtilis* at low temperatures. Each monomeric subunit of DesK has four distinct transmembrane domains. Phosphorylation might occur upon rigidification of the cytoplasmic membrane and movement of the transmembrane domains closer together, as in the case of Hik33.

scription factors, namely, DREB1 (DRE-binding protein) and CBF (CRT-binding factor), bind to the CRT/DRE element and activate transcription [70–72,89]. Expression of the genes for these transcription factors was induced rapidly upon cold treatment of plants. Moreover, at normal temperatures, the overexpression of CBF1, CBF2, and CBF3 in transgenic *Arabidopsis* enhanced the expression of 41 genes, 30 of which had been identified as cold-inducible genes in wild-type plants [63]. Thus, CBF transcription factors might regulate the expression not only of cold-inducible genes but also of genes whose expression is induced by other signals. By contrast, some of the cold-inducible genes did not appear to be under the control of the CBF pathway, suggesting that some other regulatory system might exist for the cold-inducible regulation of approximately 60 genes [63].

Despite the accumulation of important data about the transduction of cold signals, little is known about cold

sensors in plants. The experiments that demonstrated the induction of the expression of genes for desaturases (see above) and the experiments with fluidizers and rigidifiers of membrane lipids [66,69,90,91] suggest that membrane rigidification might be involved in the perception of cold signals. However, the actual sensors of low temperature in plants remain to be identified.

In the mammalian nervous system, Ca^{2+}-permeable channels have been identified as cold sensors [92–96]. Calcium ions play important regulatory roles as second messengers in many signal-transduction pathways [97–99]. The rapid influx of Ca^{2+} ions into plant cells under cold stress [100,101] suggests that Ca^{2+} channels or some non-specific ion channels might function as cold sensors in plants [101,102]. However, direct experimental evidence is required to confirm this hypothesis.

4. Perception of high temperature

Heat stress increases membrane fluidity [5,19]. If a cold sensor is activated by rigidification of the plasma membrane [2,3,56], it is reasonable to predict that a putative heat sensor in the membrane might be activated by membrane fluidization. However, no membrane-located candidate for a heat sensor has been identified to date. Nevertheless, it has been demonstrated that, in *Saccharomyces cerevisiae*, genetic enhancement of the extent of saturation of fatty acids in plasma-membrane lipids enhances the expression of the gene for heat-shock protein 90 (Hsp90) [4]. It appears that, in the yeast cells, the expression of the heat-shock gene depends on the fluidity of the membrane [4,19]. Evidence to support this possibility was obtained in studies of the responses of *Synechocystis* cells to heat shock [5,103]. At the normal growth temperature, the membrane fluidizer benzyl alcohol activated transcription of the *hspA* gene as efficiently as heat stress. However, other studies do not support the putative involvement of membrane fluidity in heat-induced gene expression. Replacement of polyunsaturated fatty acids by mono-unsaturated fatty acids in the membrane lipids of *Synechocystis* by site-directed mutagenesis [35] (Fig. 3) did not affect the heat-induced expression of genes [18]. Moreover, we observed that benzyl alcohol and heat treatment each induced an essentially different set of genes. Genes whose expression was induced by both the fluidizer and heat treatment comprised a group that included genes whose expression is induced by various kinds of stress, such as heat, salt, and hyperosmotic stress. These genes are so-called heat-shock genes, namely, *hspA*, *cplB*, *htpG*, *dnaJ*, *dnaK2*, *groESL* and *cpn60* (representing a group of chaperonins), *sodB*, *sigB*, and some other genes that are affiliated with different classes of metabolic and regulatory genes. These findings suggest that treatment with benzylalcohol is not a substitute for heat shock. Thus, sensing of heat via the detection of increases in membrane fluidity remains a controversial issue.

In *E. coli*, the heat signal is transduced, in part, via the CpxA–CpxR two-component system [104–107]. CpxA is a histidine kinase that contains two transmembrane regions and CpxR is a response regulator of heat-inducible genes [108–110]. The activity of CpxA is influenced by the composition of membrane lipids [111], suggesting that this sensor might perceive changes in membrane fluidity. This regulatory system has, however, been found only in *E. coli*, *Salmonella typhi* and *Yersinia pestis* and it has not been found in any other bacteria examined to date [112]. Thus, these organisms may have heat sensors that differ from CpxA in terms of primary structure and, possibly, in terms of the nature of signal perception.

A histidine kinase, HsfA, and a response regulator, HsfB, were recently identified in *Myxococcus xanthus* as components of a heat-transducing system [113]. HsfA appears to be a soluble protein with a phosphate-receiver domain and, thus, it is likely to be a transducer rather than a heat sensor [113]. A heat-sensitive transient receptor potential channel has been represented as a heat sensor in mammalian keratinocytes [96,114], but no heat sensors have yet been identified in any photosynthetic organisms.

5. Perception of osmotic stress

5.1. Hyperosmotic stress-induced gene expression

An increase in extracellular osmolarity causes the outward movement of water from cells, decreasing the cytoplasmic volume and cell turgor and, ultimately, inducing plasmolysis [7]. Studies of the expression of genes in response to hypertonic stress have focused mainly on "defense genes" that ensure the synthesis of compatible solutes, such as proline, glycine betaine, trehalose, and sucrose [7,8].

Our recent analysis of genome-wide patterns of transcription indicated that the expression of 257 genes is induced upon exposure of *Synechocystis* cells to hyperosmotic stress [43]. The products of these genes are responsible for the synthesis of components of cell walls and membranes and the phosphate-transport system. The gene products also include regulators of photosynthesis, signal transduction, gene expression and protein turnover, and they catalyze the synthesis of glucosylglycerol, the main compatible solute in *Synechocystis* [115,116]. Similar phenomena have been observed in studies of genome-wide transcriptional responses to hyperosmotic stress in yeast [117–120]. Moreover, in both *Synechocystis* [43] and yeast [8], a relatively small number of genes is induced by hyperosmotic stress specifically. Most of the genes induced by hyperosmotic stress are also induced by other kinds of stress, such as cold, heat, salt, and strong light [18,43,55,121–124]. Among the entire complement of hyperosmotic stress-inducible genes, quite a substantial number of genes encode proteins of as yet unknown function. At present, it is

difficult to conclude anything about the roles of these genes in acclimation to hypertonic stress. Further investigations with genetically engineered mutants are necessary if we are to elucidate the roles of these genes in the responses of cells to hyperosmotic stress.

5.2. Sensors of hyperosmotic stress

In *E. coli*, a membrane-integrated histidine kinase, EnvZ, is a putative osmosensor that perceives hyperosmotic stress and transmits the signal to induce the expression of hyperosmotic stress-inducible genes [10,125]. EnvZ regulates the expression of the *omp* genes that encode outer-membrane porins [126].

Application of membrane fluidizers, such as procaine and chlorpromazine, stimulates the phosphorylation of EnvZ and the transfer of a phosphate group to the response regulator OmpR [9,127–129]. These findings suggest that the activity of the osmosensor might be regulated by changes in the physical state of membrane lipids.

In *Lactococcus lactis*, OpuA, an osmoregulated transporter of quaternary ammonium compounds, has the properties of an osmosensor and an osmoregulator, and it plays a key role in the protection of *L. lactis* from hyperosmotic stress [130,131]. Reconstitution of an active OpuA system in artificial membrane vesicles composed of phospholipids demonstrated that OpuA is both essential and sufficient for the hyperosmotic activation of the uptake of glycine betaine by bacterial cells. The application of cationic (tetracaine and chloropromazine) and anionic (dipyrimidole) amphipathic molecules, which interact with the charged head groups of phospholipid molecules and alter the physical properties of phospholipid membranes, activated OpuA under iso-osmotic conditions to the same extent as the activation observed under hyperosmotic conditions, suggesting strongly that changes in the fluidity of membrane lipids lead to the activation of OpuA [130].

The osmosensing systems and transduction of osmotic signals in yeast were reviewed by Hohmann in 2003 [8]. Cells of *S. cerevisiae* have two membrane-integrated proteins, Sho1p and Sln1p, which are putative hyperosmosensors. Sho1p has four transmembrane domains and an SH3 domain for protein–protein interactions at its carboxyl terminus but it lacks kinase and phosphatase regulatory domains [132]. Thus, it is unlikely that Sho1p itself is a sensor of hyperosmolarity. However, it might operate in association with some other sensing protein(s), which has not yet been identified. Sln1p is a histidine kinase with a response-regulator domain and two transmembrane domains, and its role as a sensor of hyperosmolarity is well-documented [133,134]. This sensor activates the high osmolarity glycerol (HOG) MAP kinase pathway for the expression of genes for the synthesis of glycerol, which is a major compatible solute in *S. cerevisiae* [8,135]. It remains to be determined whether sensing of hyperosmolarity by Sln1p is modulated by membrane fluidity. At present, we can only postulate that this transmembrane sensory protein might perceive membrane rigidification or mechanical stress during the cell shrinkage that occurs as a result of the efflux of water under hyperosmotic stress.

One problem associated with studies of Sln1p in yeast is that cells were exposed to hyperosmotic stress via addition of NaCl to the culture medium [8,119,136]. However, NaCl has two kinds of effect on cells: it can induce hyperosmotic stress and it can induce ionic stress [137]. In *Synechocystis*, salt stress due to 0.5 M NaCl and hyperosmotic stress due to 0.5 M sorbitol induce the expression of very different sets of genes [43]. These observations imply that salt stress and osmotic stress might be sensed by different sensor molecules, although the existence of common sensors cannot yet be excluded.

Genome-wide analysis of transcription in *Synechocystis* with DNA microarrays indicated that Hik33, which was identified originally as a cold sensor (see above), controls the inducibility by hyperosmotic stress of nearly 60% of hyperosmotic stress-inducible genes [122]. Hik33 controls the expression of genes whose products are involved in the synthesis and maintenance of cell walls and membranes, in formation of a phosphate-transport system, in protection of the photosynthetic apparatus, and in certain other phenomena that are important for acclimation to hyperosmotic stress. In particular, Hik33 controls the osmostress-inducible expression of the *ndpK* gene for nucleoside-diphosphate kinase, which catalyzes the synthesis of nucleoside triphosphates and acts as a regulator of signal transduction via the histidine-specific phosphorylation of the osmosensors EnvZ and CheA [138–140]. It is very likely that Hik33 recognizes changes in membrane fluidity as the primary signal of both cold stress and hyperosmotic stress. However, details of the molecular mechanism of signal perception remain to be clarified.

The histidine kinase AtHK1 of *Arabidopsis* can complement mutations in the osmosensor Sln1p in *S. cerevisiae*. Therefore, it is likely that AtHK1 is a plant hyperosmosensor [141]. Such complementation implies that this histidine kinase from *Arabidopsis* can substitute for the osmosensor in yeast and can efficiently transduce a signal to a downstream MAP-kinase cascade. At present, AtHK1 is the only putative osmosensor identified in plants. The molecular mechanisms of osmosensing in plants remain unknown and further detailed investigations at the sub-molecular level are required in view of the importance to agriculture of plants that can withstand environmental stress.

5.3. Sensors of hypoosmotic stress

The molecular mechanisms by which cells perceive hypoosmotic stress are poorly understood. Research in this field has focused mainly on volume-regulated or stretch-activated channels, which were identified many years ago in biophysical and physiological studies of mammalian and bacterial cells. Such channels are thought to play a role in

the regulation of cell volume by controlling the export of solutes and ions from the cell. The best-studied system is the bacterial MscL (*m*echano*s*ensitive *c*hannel of *l*arge conductance) [6,142,143]. This protein mediates the non-selective export of solutes from bacterial cells upon their exposure to severe hypoosmotic shock [144,145]. Modeling based on the crystal structure of MscL [146], as well as results of cross-linking and mutagenesis experiments, supports a two-stage model of channel opening, which leads to an increase in pore diameter of 1–13 Å. In this model, membrane tension leads to a conformational change from a closed conformation to a closed-expanded and eventually open conformation, in which certain transmembrane helices are twisted dramatically within the membrane [6,144,145,147].

Our recent studies of the MscL of the freshwater cyanobacterium *Synechocystis* revealed that this protein might sense not only membrane hypertension but also the membrane depolarization that occurs under cold stress [148]. In addition to the role of MscL in the regulation of cell volume, as postulated in *E. coli* [149–151], the cyanobacterial MscL functions as an outward Ca^{2+} channel in response to cold stress [148].

A functional (as distinct from structural) homolog of the bacterial MscL in yeast is the channel protein Fps1p [152,153]. Several possibilities for the functions of Fps1p have been proposed. The regulatory domain of Fps1p might fold so that it closes the channel, as in the case for the gated K^+ channels in animal cells [93,154]. Alternatively, extension of the protein might help to orient the Fps1p transmembrane domains in the membrane such that they are specifically sensitive to membrane stretching, as is the case for MscL [144,155]. To date, all attempts to identify proteins that interact with Fps1p have failed [8]. Further studies of possible sensors will require defined systems in vitro, composed of purified proteins. The power of such systems became evident recently, when it was demonstrated that the activity of BetP, an osmoregulated betaine-carrier in *Corynebacterium glutamicum*, was regulated by the internal concentration of K^+ ions rather than by mechanical stimuli. Hence, this protein functions as a chemosensor, with K^+ ions acting as the concentration-dependent second messenger that transduces the signal of cell shrinkage [156]. A similar mode of regulation has been proposed for KdpD in the two-component sensor of K^+ status in *E. coli* [7].

At present, there is no direct evidence of changes in membrane fluidity upon exposure of cells to hypotonic stress. Therefore, the involvement of membrane fluidity in the regulation of a cell's response to hypotonic stress remains the focus of assumptions and speculation.

6. Multifunctional sensors

There appears to be substantial cross-talk among regulatory pathways that control the responses to various kinds of stress and hormonally regulated cascades [2,62,122,157–164]. It is now clear that different environmental stimuli can be perceived by common sensory proteins. For example, the histidine kinase Hik33 of *Synechocystis* was first identified as a component of a system that endows resistance to drugs that inhibit photosynthesis [165]. We subsequently characterized Hik33 as a cold sensor that appears to perceive membrane rigidification under cold stress [56,57]. Then Hik33 was found to be involved in the sensing of hyperosmotic stress [122] and of salt stress [124]. Studies with DNA microarrays indicate that Hik33 controls different sets of genes under different kinds of stress. The mechanism(s) by which Hik33 recognizes cold stress, salt stress, and osmotic stress are still in question. It is likely that Hik33 perceives changes in membrane fluidity that occur under cold stress and hyperosmotic conditions [2,18,164]. It remains unclear whether changes in the physical motion of fatty acids in membrane lipids or changes in the surface charges of membrane lipids alter the conformation of Hik33, with its resultant activation [2]. Our recent observation that cold stress causes depolarization of the plasma membrane [148] does not exclude the possibility of the involvement of surface charge. Sln1p in yeast, which was characterized initially as an osmosensor, is also involved in perception of oxidative stress and heat stress [117,118,166–168].

The existence of multifunctional sensors has been discussed in plants [62]. Cold, drought, and salinity induce a transient influx of Ca^{2+} ions into the cytoplasm of plant cells [169,170]. Therefore, it has been suggested that Ca^{2+} channels might be multifunctional sensors that sense stress-induced perturbations in plasma membranes, which might include changes in the fluidity of membrane lipids [69,91].

The existence of sensors that perceive a variety of signals related to environmental conditions is an intriguing discovery. Considering membrane fluidity as the key parameter that allows cells to perceive environmental signals appropriately, we can conclude that there might be sensors that perceive changes in the physical state of the membrane, no matter what the nature of the stress that is responsible for the perturbations within the membrane. Such sensors should be bound to membranes or, at least, associated with membranes as are, for example, Hik33, Sln1p, and mechanosensitive Ca^{2+} and K^+ channels.

7. Conclusions and perspectives

It has been postulated that a change in membrane fluidity might be the primary signal in the perception of cold stress and, possibly, of osmotic stress. However, the molecular mechanisms that control perception and transduction of these signals via membranes have not been fully characterized. The availability of the complete genomic sequences of many organisms and DNA microarrays provide powerful new tools for the study of genome-wide expression of

individual genes and for the identification of groups of genes that are induced specifically and non-specifically under certain stress conditions. Such analyses also allow us to identify the sensors and transducers of various types of environmental stress.

It is very likely that the rigidification of membrane lipids at low temperatures and under hyperosmotic stress is the primary trigger for the corresponding acclimatory responses in cells. In cyanobacteria, Hik33 perceives these changes. However, an additional cold sensor probably exists since not all of the cold-induced genes in cyanobacteria are controlled by Hik33 and, therefore, by membrane fluidity [55]. The involvement of membrane fluidity in the perception of heat stress remains controversial [5,18]. More precise investigations are necessary if we are to understand how membrane fluidity contributes to the perception of temperature and other environmental signals.

It is possible that some common sensor(s) (such as Hik33) recognizes the rigidification of membrane lipids irrespective of the nature of the stimulus (for example, cold stress, hyperosmotic stress, or salt stress). We do not yet know how sensory transmembrane proteins recognize a change in the membrane's physical state, nor do we know what distinct domains and amino acids are involved in signal perception. It is essential that efforts now be made to identify specific lipids or lipid domains that interact with each sensor and that participate in the modulation of its conformation and/or activity. When we understand how organisms respond to environmental stresses, we shall be in a better position to protect economically important plants and animals from such stresses.

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References

[1] N. Murata, D.A. Los, Membrane fluidity and temperature perception, Plant Physiol. 115 (1997) 875–879.
[2] D.A. Los, N. Murata, Regulation of enzymatic activity and gene expression by membrane fluidity, Sci. Signal Transduct. Knowl. Environ. (2000) (http://www.stke.org/cgi/content/full/OC_sigtrans;2000/62/pe1).
[3] L. Vigh, D.A. Los, I. Horvath, N. Murata, The primary signal in the biological perception of temperature: Pd-catalyzed hydrogenation of membrane lipids stimulated the expression of the desA gene in *Synechocystis* PCC6803, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 9090–9094.
[4] L. Carratu, S. Franceschelli, C.L. Pardini, G.S. Kobayashi, I. Horvath, L. Vigh, B. Maresca, Membrane lipid perturbation modifies the set point of the temperature of heat shock response in yeast, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 3870–3875.
[5] I. Horvath, A. Glatz, V. Varvasovszki, Z. Torok, T. Pali, G. Balogh, E. Kovacs, L. Nadasdi, S. Benko, F. Joo, L. Vigh, Membrane physical state controls the signaling mechanism of the heat shock response in *Synechocystis* PCC 6803: identification of hsp17 as a "fluidity gene", Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 3513–3518.
[6] S. Sukharev, Mechanosensitive channels in bacteria as membrane tension reporters, FASEB J. 13 (1999) S55–S61 (Suppl.).
[7] J.M. Wood, Osmosensing by bacteria: signals and membrane-based sensors, Microbiol. Mol. Biol. Rev. 63 (1999) 230–262.
[8] S. Hohmann, Osmotic stress signaling and osmoadaptation in yeasts, Microbiol. Mol. Biol. Rev. 66 (2003) 300–372.
[9] S. Tokishita, T. Mizuno, Transmembrane signal transduction by the *Escherichia coli* osmotic sensor, EnvZ: intermolecular complementation of transmembrane signaling, Mol. Microbiol. 13 (1994) 435–444.
[10] A. Sugiura, K. Hirokawa, K. Nakashima, T. Mizuno, Signal-sensing mechanisms of the putative osmosensor KdpD in *Escherichia coli*, Mol. Microbiol. 14 (1994) 929–938.
[11] D. Grunberger, R. Haimovitz, M. Shinitzky, Resolution of plasma membrane lipid fluidity in intact cells labeled with diphenylhexatriene, Biochim. Biophys. Acta 688 (1982) 764–774.
[12] B.R. Lentz, Use of fluorescent probes to monitor molecular order and motions within liposome bilayers, Chem. Phys. Lipids 64 (1993) 99–116.
[13] C. Laroche, L. Beney, P.A. Marechal, P. Gervais, The effect of osmotic pressure on the membrane fluidity of *Saccharomyces cerevisiae* at different physiological temperatures, Appl. Microbiol. Biotechnol. 56 (2001) 249–254.
[14] B. Szalontai, Y. Nishiyama, Z. Gombos, N. Murata, 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 (2000) 409–419.
[15] A.R. Cossins, J. Christiansen, C.L. Prosser, Adaptation of biological membranes to temperature. The lack of homeoviscous adaptation in the sarcoplasmic reticulum, Biochim. Biophys. Acta 511 (1978) 442–452.
[16] D.J. Pehowich, P.M. Macdonald, R.N. McElhaney, A.R. Cossins, L.C. Wang, Calorimetric and spectroscopic studies of lipid thermotropic phase behavior in liver inner mitochondrial membranes from a mammalian hibernator, Biochemistry 27 (1988) 4632–4638.
[17] M. Sinensky, Homeoviscous adaptation—a homeostatic process that regulates viscosity of membrane lipids in *Escherichia coli*, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 522–525.
[18] M. Inaba, I. Suzuki, B. Szalontai, Y. Kanesaki, D.A. Los, H. Hayashi, N. Murata, Gene-engineered rigidification of membrane lipids enhances the cold inducibility of gene expression in *Synechocystis*, J. Biol. Chem. 278 (2003) 12191–12198.
[19] L. Vigh, B. Maresca, J.L. Harwood, Does the membrane's physical state control the expression of heat shock and other genes? Trends Biochem. Sci. 23 (1998) 369–374.
[20] M. Yamazaki, S. Ohnishi, T. Ito, Osmoelastic coupling in biological structures: decrease in membrane fluidity and osmophobic association of phospholipid vesicles in response to osmotic stress, Biochemistry 28 (1989) 3710–3715.
[21] S. Gudi, J.P. Nolan, J.A. Frangos, Modulation of GTPase activity of G proteins by fluid shear stress and phospholipid composition, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 2515–2519.
[22] N. Kabelitz, P.M. Santos, H.J. Heipieper, Effect of aliphatic alcohols on growth and degree of saturation of membrane lipids in *Acinetobacter calcoaceticus*, FEMS Microbiol. Lett. 220 (2003) 223–227.

[23] D. Thewke, M. Kramer, M.S. Sinensky, Transcriptional homeostatic control of membrane lipid composition, Biochem. Biophys. Res. Commun. 273 (2003) 1–4.
[24] P.E. Tiku, A.Y. Gracey, A.I. Macartney, R.J. Beynon, A.R. Cossins, Cold-induced expression of Δ9-desaturase in carp by transcriptional and posttranslational mechanisms, Science 271 (1996) 815–818.
[25] A.I. Macartney, P.E. Tiku, A.R. Cossins, An isothermal induction of Δ9-desaturase in cultured carp hepatocytes, Biochim. Biophys. Acta 1302 (1996) 207–216.
[26] B. Maresca, G. Kobayashi, Changes in membrane fluidity modulate heat shock gene expression and produced attenuated strains in the dimorphic fungus *Histoplasma capsulatum*, Arch. Med. Res. 24 (1993) 247–249.
[27] M.A. Bossie, C.E. Martin, Nutritional regulation of yeast Δ9 fatty acid desaturase activity, J. Bacteriol. 171 (1989) 6409–6413.
[28] J.M. Lyons, J.K. Raison, Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury, Plant Physiol. 45 (1970) 386–389.
[29] I. Nishida, N. Murata, Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 541–568.
[30] L.E. Cybulski, M.C. Mansilla, P.S. Aguilar, D. de Mendoza, Mechanism of membrane fluidity optimization: isothermal control of the *Bacillus subtilis* acyl-lipid desaturase, Mol. Microbiol. 45 (2002) 1379–1388.
[31] D.A. Los, N. Murata, Structure and expression of fatty acid desaturases, Biochim. Biophys. Acta 1394 (1998) 3–15.
[32] D.A. Los, N. Murata, Responses to cold shock in cyanobacteria, J. Mol. Microbiol. Biotechnol. 1 (1999) 221–230.
[33] A. Glatz, I. Vass, D.A. Los, L. Vigh, The *Synechocystis* model of stress: from molecular chaperones to membranes, Plant Physiol. Biochem. 37 (1999) 1–12.
[34] H. Wada, Z. Gombos, N. Murata, Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation, Nature 347 (1990) 200–203.
[35] Y. Tasaka, Z. Gombos, Y. Nishiyama, P. Mohanty, T. Ohba, K. Ohki, N. Murata, Targeted mutagenesis of acyl-lipid desaturases in *Synechocystis*: evidence for the important roles of polyunsaturated membrane lipids in growth, respiration and photosynthesis, EMBO J. 15 (1996) 6416–6425.
[36] O. Ishizaki-Nishizawa, T. Fujii, M. Azuma, K. Sekiguchi, N. Murata, T. Ohtani, T. Toguri, Low-temperature resistance of higher plants is significantly enhanced by a nonspecific cyanobacterial desaturase, Nat. Biotechnol. 14 (1996) 1003–1006.
[37] I.V. Orlova, T.S. Serebriiskaya, V. Popov, N. Merkulova, A.M. Nosov, T.I. Trunova, V.D. Tsydendambaev, D.A. Los, Transformation of tobacco with a gene for the thermophilic acyl-lipid desaturase enhances the chilling tolerance of plants, Plant Cell Physiol. 44 (2003) 447–450.
[38] D. Los, I. Horvath, L. Vigh, N. Murata, The temperature-dependent expression of the desaturase gene desA in *Synechocystis* PCC6803, FEBS Lett. 318 (1993) 57–60.
[39] D.A. Los, M.K. Ray, N. Murata, Differences in the control of the temperature-dependent expression of four genes for desaturases in *Synechocystis* sp., PCC 6803, Mol. Microbiol. 25 (1997) 1167–1175.
[40] J.R. Hazel, Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? Annu. Rev. Physiol. 57 (1995) 19–42.
[41] A.I. Macartney, B. Maresca, A.R. Cossins, Acyl-CoA desaturases and the adaptive regulation of membrane lipid composition, in: A.R. Cossins (Ed.), Temperature Adaptation of Biological Membranes, Portland Press, London, 1994, pp. 129–139.
[42] E. Wodtke, A.R. Cossins, Rapid cold-induced changes of membrane order and Δ9-desaturase activity in endoplasmic reticulum of carp liver: a time-course study of thermal acclimation, Biochim. Biophys. Acta 1064 (1991) 342–350.
[43] Y. Kanesaki, I. Suzuki, S.I. Allakhverdiev, K. Mikami, N. Murata, Salt stress and hyperosmotic stress regulate the expression of different sets of genes in *Synechocystis* sp., PCC 6803, Biochem. Biophys. Res. Commun. 290 (2002) 339–348.
[44] C.S. Lopez, H. Heras, H. Garda, S. Rusal, C. Sanches-Rivas, E. Rivas, Biochemical and biophysical studies of *Bacillus subtilis* envelopes under hyperosmotic stress, Int. J. Food Microbiol. 55 (2000) 137–142.
[45] H. Okuyama, S. Sasaki, S. Higashi, N. Murata, A trans-unsaturated fatty acid in a psychrophilic bacterium, *Vibrio* sp. strain ABE-1, J. Bacteriol. 172 (1990) 3515–3518.
[46] H. Okuyama, N. Okajima, S. Sasaki, S. Higashi, N. Murata, The *cis*/*trans* isomerization of the double bond of a fatty acid as a strategy for adaptation to changes in ambient temperature in the psychrophilic bacterium, *Vibrio* sp. strain ABE-1, Biochim. Biophys. Acta 1084 (1991) 13–20.
[47] A. von Wallbrunn, H.H. Richnow, G. Neumann, F. Meinhardt, H.J. Heipieper, Mechanism of *cis–trans* isomerization of unsaturated fatty acids in *Pseudomonas putida*, J. Bacteriol. 185 (2003) 1730–1733.
[48] H.J. Heipieper, F. Meinhardt, A. Segura, The *cis–trans* isomerase of unsaturated fatty acids in *Pseudomonas* and *Vibrio*: biochemistry, molecular biology and physiological function of a unique stress-adaptive mechanism, FEMS Microbiol. Lett. 229 (2003) 1–7.
[49] P.A. Edwards, J. Ericsson, Sterols and isoprenoids: signaling molecules derived from the cholesterol biosynthetic pathway, Annu. Rev. Biochem. 68 (1999) 157–185.
[50] M.S. Brown, J.L. Goldstein, A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 11041–11048.
[51] J.D. Horton, I. Shimomura, Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis, Curr. Opin. Lipidol. 10 (1999) 143–150.
[52] J.L. Goldstein, R.B. Rawson, M.S. Brown, Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis, Arch. Biochem. Biophys. 397 (2002) 139–148.
[53] C.M. Adams, J.L. Goldstein, M.S. Brown, Cholesterol-induced conformational change in SCAP enhanced by Insig proteins and mimicked by cationic amphiphiles, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10647–10652.
[54] N. Murata, H. Wada, Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria, Biochem. J. 308 (1995) 1–8.
[55] I. Suzuki, Y. Kanesaki, K. Mikami, M. Kanehisa, N. Murata, Cold-regulated genes under control of the cold sensor Hik33 in *Synechocystis*, Mol. Microbiol. 40 (2001) 235–244.
[56] I. Suzuki, D.A. Los, Y. Kanesaki, K. Mikami, N. Murata, The pathway for perception and transduction of low-temperature signals in *Synechocystis*, EMBO J. 19 (2000) 1327–1334.
[57] I. Suzuki, D.A. Los, N. Murata, Perception and transduction of low-temperature signals to induce desaturation of fatty acids, Biochem. Soc. Trans. 28 (2000) 628–630.
[58] S.B. Williams, V. Stewart, Functional similarities among two-component sensors and methyl-accepting chemotaxis proteins suggest a role for linker region amphipathic helices in transmembrane signal transduction, Mol. Microbiol. 33 (1999) 1093–1102.
[59] B.L. Taylor, I.B. Zhulin, PAS domains: internal sensors of oxygen, redox potential, and light, Microbiol. Mol. Biol. Rev. 63 (1999) 479–506.
[60] L. Aravind, C.P. Ponting, The cytoplasmic helical linker domain of receptor histidine kinase and methyl-accepting proteins is common to many prokaryotic signalling proteins, FEMS Microbiol. Lett. 176 (1999) 111–116.
[61] L. Aravind, V. Anantharaman, L.M. Iyer, Evolutionary connections between bacterial and eukaryotic signaling systems: a genomic perspective, Curr. Opin. Microbiol. 6 (2003) 490–507.

[62] L. Xiong, K.S. Schumaker, J.K. Zhu, Cell signaling during cold, drought, and salt stress, Plant Cell (2002) S165–S183 (Suppl.).
[63] S. Fowler, M.F. Thomashow, Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold-response pathway, Plant Cell 14 (2003) 1675–1690.
[64] P.S. Aguilar, A.M. Hernandez-Arriaga, L.E. Cybulski, A.C. Erazo, D. de Mendoza, Molecular basis of thermosensing: a two-component signal transduction thermometer in *Bacillus subtilis*, EMBO J. 20 (2001) 1681–1691.
[65] L.A. Wolfraim, R.S. Dhindsa, Cloning and sequencing of the cDNA for cas17, a cold acclimation-specific gene of alfalfa, Plant Physiol. 103 (1993) 667–668.
[66] A.F. Monroy, R.S. Dhindsa, Low-temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25 °C, Plant Cell 7 (1995) 321–331.
[67] A.F. Monroy, E. Labbe, R.S. Dhindsa, Low-temperature perception in plants: effects of cold on protein phosphorylation in cell-free extracts, FEBS Lett. 410 (1997) 206–209.
[68] S. Tahtiharju, V. Sangwan, A.F. Monroy, R.S. Dhindsa, M. Borg, The induction of kin genes in cold-acclimating *Arabidopsis thaliana*, evidence of a role for calcium, Planta 203 (1997) 442–447.
[69] B.L. Orvar, V. Sangwan, F. Omann, R.S. Dhindsa, Early steps in cold sensing by plant cells: the role of actin cytoskeleton and membrane fluidity, Plant J. 23 (2000) 785–794.
[70] Q. Liu, M. Kasuga, Y. Sakuma, H. Abe, S. Miura, K. Yamaguchi-Shinozaki, K. Shinozaki, Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA-binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*, Plant Cell 10 (1998) 1391–1406.
[71] Z.K. Shinwari, K. Nakashima, S. Miura, M. Kasuga, M. Seki, K. Yamaguchi-Shinozaki, K. Shinozaki, An *Arabidopsis* gene family encoding DRE/CRT-binding proteins involved in low-temperature-responsive gene expression, Biochem. Biophys. Res. Commun. 250 (1998) 161–170.
[72] K. Shinozaki, K. Yamaguchi-Shinozaki, Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways, Curr. Opin. Plant Biol. 3 (2000) 217–223.
[73] M. Seki, M. Narusaka, H. Abe, M. Kasuga, K. Yamaguchi-Shinozaki, P. Carninci, Y. Hayashizaki, K. Shinozaki, Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray, Plant Cell 13 (2001) 61–72.
[74] J.K. Zhu, Cell signaling under salt, water and cold stresses, Curr. Opin. Plant Biol. 4 (2001) 401–406.
[75] S.J. Gilmour, N.N. Artus, M.F. Thomashow, cDNA sequence analysis and expression of two cold-regulated genes of *Arabidopsis thaliana*, Plant Mol. Biol. 18 (1992) 13–21.
[76] C. Lin, M.F. Thomashow, A cold-regulated *Arabidopsis* gene encodes a polypeptide having potent cryoprotective activity, Biochem. Biophys. Res. Commun. 183 (1992) 1103–1108.
[77] K.S. Wilhelm, M.F. Thomashow, *Arabidopsis thaliana cor15b*, an apparent homologue of *cor15a*, is strongly responsive to cold and ABA, but not drought, Plant Mol. Biol. 23 (1993) 1073–1077.
[78] M.S. Webb, S.J. Gilmour, M.F. Thomashow, P.L. Steponkus, Effects of COR6.6 and COR15am polypeptides encoded by COR (cold-regulated) genes of *Arabidopsis thaliana* on dehydration-induced phase transitions of phospholipid membranes, Plant Physiol. 111 (1996) 301–312.
[79] M.F. Thomashow, Role of cold-responsive genes in plant freezing tolerance, Plant Physiol. 118 (1998) 1–8.
[80] T. Hamada, H. Kodama, M. Nishimura, K. Iba, Cloning of a cDNA encoding tobacco ω3 fatty acid desaturase, Gene 147 (1994) 293–294.
[81] J.K. Raison, J.M. Lyons, R.J. Mehlhorn, A.D. Keith, Temperature-induced phase changes in mitochondrial membranes detected by spin labeling, J. Biol. Chem. 246 (1971) 4036–4040.
[82] J.K. Raison, Temperature-induced phase changes in membrane lipids and their influence on metabolic regulation, Symp. Soc. Exp. Biol. 27 (1973) 485–512.
[83] K. Yamaguchi-Shinozaki, K. Shinozaki, A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress, Plant Cell 6 (1994) 251–264.
[84] T. Taji, M. Seki, K. Yamaguchi-Shinozaki, H. Kamada, J. Giraudat, K. Shinozaki, Mapping of 25 drought-inducible genes, RD and ERD, in *Arabidopsis thaliana*, Plant Cell Physiol. 40 (1999) 119–123.
[85] E.J. Stockinger, S.J. Gilmour, M.F. Thomashow, *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 1035–1040.
[86] K.R. Jaglo-Ottosen, S.J. Gilmour, D.G. Zarka, O. Schabenberger, M.F. Thomashow, *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance, Science 280 (1998) 104–106.
[87] S.J. Gilmour, D.G. Zarka, E.J. Stockinger, M.P. Salazar, J.M. Houghton, M.F. Thomashow, Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression, Plant J. 16 (1998) 433–442.
[88] S.J. Gilmour, A.M. Sebolt, M.P. Salazar, J.D. Everard, M.F. Thomashow, Overexpression of the *Arabidopsis* CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation, Plant Physiol. 124 (2000) 1854–1865.
[89] K. Nakashima, Z.K. Shinwari, Y. Sakuma, M. Seki, S. Miura, K. Shinozaki, K. Yamaguchi-Shinozaki, Organization and expression of two *Arabidopsis* DREB2 genes encoding DRE-binding proteins involved in dehydration- and high-salinity-responsive gene expression, Plant Mol. Biol. 42 (2000) 657–665.
[90] V. Sangwan, I. Foulds, J. Singh, R.S. Dhindsa, Cold-activation of *Brassica napus* BN115 promoter is mediated by structural changes in membranes and cytoskeleton, and requires Ca^{2+} influx, Plant J. 27 (2001) 1–12.
[91] V. Sangwan, B.L. Orvar, J. Beyerly, H. Hirt, R.S. Dhindsa, Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways, Plant J. 31 (2002) 629–638.
[92] D.D. McKemy, W.M. Neuhausser, D. Julius, Identification of a cold receptor reveals a general role for TRP channels in thermosensation, Nature 416 (2002) 52–58.
[93] S.E. Jordt, D.D. McKemy, D. Julius, Lessons from peppers and peppermint: the molecular logic of thermosensation, Curr. Opin. Neurobiol. 13 (2003) 487–492.
[94] A.M. Peier, A. Moqrich, A.C. Hergarden, A.J. Reeve, D.A. Andersson, G.M. Story, T.J. Earley, I. Dragoni, P. McIntyre, S. Bevan, A. Patapoutian, A TRP channel that senses cold stimuli and menthol, Cell 108 (2002) 705–715.
[95] G.M. Story, A.M. Peier, A.J. Reeve, S.R. Eid, J. Mosbacher, T.R. Hricik, T.J. Earley, A.C. Hergarden, D.A. Andersson, S.W. Hwang, P. McIntyre, T. Jegla, S. Bevan, A. Patapoutian, ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures, Cell 112 (2003) 819–829.
[96] A. Patapoutian, A.M. Peier, G.M. Story, V. Viswanath, ThermoTRP channels and beyond: mechanisms of temperature sensation, Nat. Rev., Neurosci. 4 (2003) 529–539.
[97] A.J. Trewavas, R. Malho, Ca^{2+} signalling in plant cells: the big network!, Curr. Opin. Plant Biol. 1 (1998) 428–433.
[98] M.J. Chrispeels, L. Holuigue, R. Latorre, S. Luan, A. Orellana, H. Pena-Cortes, N.V. Raikhel, P.C. Ronald, A. Trewavas, Signal transduction networks and the biology of plant cells, Biol. Res. 32 (1999) 35–60.

[99] S. Gilroy, A. Trewavas, Signal processing and transduction in plant cells: the end of the beginning? Nat. Rev., Mol. Cell Biol. 2 (2001) 307–314.
[100] H. Knight, A.J. Trewavas, M.R. Knight, Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signature after acclimation, Plant Cell 8 (1996) 489–503.
[101] A. Trewavas, N. Read, A.K. Campbell, M. Knight, Transduction of Ca^{2+} signals in plant cells and compartmentalization of the Ca^{2+} signal, Biochem. Soc. Trans. 24 (1996) 971–974.
[102] A.F. Monroy, F. Sarhan, R.S. Dhindsa, Cold-induced changes in freezing tolerance, protein phosphorylation, and gene expression (evidence for a role of calcium), Plant Physiol. 102 (1993) 1227–1235.
[103] Z. Torok, P. Goloubinoff, I. Horvath, N.M. Tsvetkova, A. Glatz, G. Balogh, V. Varvasovszki, D.A. Los, E. Vierling, J.H. Crowe, L. Vigh, *Synechocystis* HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone-mediated refolding, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3098–3103.
[104] P.N. Danese, W.B. Snyder, C.L. Cosma, L.J. Davis, T.J. Silhavy, The Cpx two-component signal transduction pathway of *Escherichia coli* regulates transcription of the gene specifying the stress-inducible periplasmic protease, DegP, Genes Dev. 9 (1995) 387–398.
[105] P.N. Danese, T.J. Silhavy, CpxP, a stress-combative member of the Cpx regulon, J. Bacteriol. 180 (1998) 831–839.
[106] T.L. Raivio, T.J. Silhavy, Periplasmic stress and ECF sigma factors, Annu. Rev. Microbiol. 55 (2001) 591–624.
[107] P.A. DiGiuseppe, T.J. Silhavy, Signal detection and target gene induction by the CpxRA two-component system, J. Bacteriol. 185 (2003) 2432–2440.
[108] P.N. Danese, T.J. Silhavy, The sigma(E) and the Cpx signal transduction systems control the synthesis of periplasmic protein-folding enzymes in *Escherichia coli*, Genes Dev. 11 (1997) 1183–1193.
[109] L. Connolly, A. De Las Penas, B.M. Alba, C.A. Gross, The response to extracytoplasmic stress in *Escherichia coli* is controlled by partially overlapping pathways, Genes Dev. 11 (1997) 2012–2021.
[110] J. Pogliano, A.S. Lynch, D. Belin, E.C. Lin, J. Beckwith, Regulation of *Escherichia coli* cell envelope proteins involved in protein folding and degradation by the Cpx two-component system, Genes Dev. 11 (1997) 1169–1182.
[111] E. Mileykovskaya, W. Dowhan, The Cpx two-component signal transduction pathway is activated in *Escherichia coli* mutant strains lacking phosphatidylethanolamine, J. Bacteriol. 179 (1997) 1029–1034.
[112] P. De Wulf, B.J. Akerley, E.C. Lin, Presence of the Cpx system in bacteria, Microbiology 146 (2000) 247–248.
[113] T. Ueki, S. Inouye, Transcriptional activation of a heat-shock gene, lonD, of *Myxococcus xanthus* by a two-component histidine-aspartate phosphorelay system, J. Biol. Chem. 277 (2002) 6170–6177.
[114] A.M. Peier, A.J. Reeve, D.A. Andersson, A. Moqrich, T.J. Earley, A.C. Hergarden, G.M. Story, S. Colley, J.B. Hogenesch, P. McIntyre, S. Bevan, A. Patapoutian, A heat-sensitive TRP channel expressed in keratinocytes, Science (2002) 2046–2049.
[115] M. Hagemann, A. Schoor, R. Jeanjean, E. Zuther, F. Joset, The stpA gene from *Synechocystis* sp. strain PCC 6803 encodes the glucosylglycerol-phosphate phosphatase involved in cyanobacterial osmotic response to salt shock, J. Bacteriol. 179 (1997) 1727–1733.
[116] F. Engelbrecht, K. Marin, M. Hagemann, Expression of the ggpS gene, involved in osmolyte synthesis in the marine cyanobacterium *Synechococcus* sp. strain PCC 7002, revealed regulatory differences between this strain and the freshwater strain Synechocystis sp. strain PCC 6803, Appl. Environ. Microbiol. 65 (1999) 4822–4829.
[117] A.P. Gasch, P.T. Spelman, C.M. Kao, O. Carmel-Harel, M.B. Eisen, G. Storz, D. Botstein, P.O. Brown, Genomic expression programs in the response of yeast cells to environmental changes, Mol. Biol. Cell 11 (2000) 4241–4257.
[118] H.C. Causton, B. Ren, S.S. Koh, C.T. Harbison, E. Kanin, E.G. Jennings, T.I. Lee, H.L. True, E.S. Lander, R.A. Young, Remodeling of yeast genome expression in response to environmental changes, Mol. Biol. Cell 12 (2001) 323–337.
[119] M. Rep, M. Krantz, J.M. Thevelein, S. Hohmann, The transcriptional response of *Saccharomyces cerevisiae* to osmotic shock. Hot1p and Msn2p/Msn4p are required for the induction of subsets of high-osmolarity glycerol pathway-dependent genes, J. Biol. Chem. 275 (2000) 8290–8300.
[120] J. Yale, H.J. Bohnert, Transcript expression in *Saccharomyces cerevisiae* at high salinity, J. Biol. Chem. 276 (2001) 15996–16007.
[121] Y. Hihara, A. Kamei, M. Kanehisa, A. Kaplan, M. Ikeuchi, DNA microarray analysis of cyanobacterial gene expression during acclimation to high light, Plant Cell 13 (2001) 793–806.
[122] K. Mikami, Y. Kanesaki, I. Suzuki, N. Murata, The histidine kinase Hik33 perceives osmotic stress and cold stress in *Synechocystis* sp., PCC 6803, Mol. Microbiol. 46 (2003) 905–915.
[123] Y. Hihara, K. Sonoike, M. Kanehisa, M. Ikeuchi, DNA microarray analysis of redox-responsive genes in the genome of the cyanobacterium *Synechocystis* sp. strain PCC 6803, J. Bacteriol. 185 (2003) 1719–1725.
[124] K. Marin, I. Suzuki, K. Yamaguchi, K. Ribbeck, H. Yamamoto, Y. Kanesaki, M. Hagemann, N. Murata, Identification of histidine kinases that act as sensors in the perception of salt stress in *Synechocystis* sp., PCC 6803, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9061–9066.
[125] K. Nakashima, A. Sugiura, T. Mizuno, Functional reconstitution of the putative *Escherichia coli* osmosensor, KdpD, into liposomes, J. Biochem. 114 (1993) 615–621.
[126] L. Qin, R. Dutta, H. Kurokawa, M. Ikura, M. Inouye, A monomeric histidine kinase derived from EnvZ, an *Escherichia coli* osmosensor, Mol. Microbiol. 36 (2000) 24–32.
[127] S. Tokishita, A. Kojima, H. Aiba, T. Mizuno, Transmembrane signal transduction and osmoregulation in *Escherichia coli*. Functional importance of the periplasmic domain of the membrane-located protein kinase, EnvZ, J. Biol. Chem. 266 (1991) 6780–6785.
[128] K. Nakashima, A. Sugiura, K. Kanamaru, T. Mizuno, Signal transduction between the two regulatory components involved in the regulation of the kdpABC operon in *Escherichia coli*: phosphorylation-dependent functioning of the positive regulator, KdpE, Mol. Microbiol. 7 (1993) 109–116.
[129] K. Nakashima, K. Kanamaru, H. Aiba, T. Mizuno, Osmoregulatory expression of the porin genes in *Escherichia coli*: evidence for signal titration in the signal transduction through EnvZ–OmpR phosphotransfer, FEMS Microbiol. Lett. 66 (1991) 43–47.
[130] T. van der Heide, B. Poolman, Osmoregulated ABC-transport system of *Lactococcus lactis* senses water stress via changes in the physical state of the membrane, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 7102–7106.
[131] T. van der Heide, M.C. Stuart, B. Poolman, On the osmotic signal and osmosensing mechanism of an ABC transport system for glycine betaine, EMBO J. 20 (2001) 7022–7032.
[132] D.C. Raitt, F. Posas, H. Saito, Yeast Cdc42 GTPase and Ste20 PAK-like kinase regulate Sho1-dependent activation of the Hog1 MAP kinase pathway, EMBO J. 19 (2000) 4623–4631.
[133] I.M. Ota, A. Varshavsky, A yeast protein similar to bacterial two-component regulators, Science 262 (1993) 566–569.
[134] T. Maeda, S.M. Wurgler-Murphy, H. Saito, A two-component system that regulates an osmosensing MAP kinase cascade in yeast, Nature 369 (1994) 242–245.
[135] F. Posas, S.M. Wurgler-Murphy, T. Maeda, E.A. Witten, T.C. Thai, H. Saito, Yeast HOG1 MAP kinase cascade is regulated by a multistep phosphorelay mechanism in the SLN1–YPD1–SSK1 "two-component" osmosensor, Cell 86 (1996) 865–875.
[136] M. Rep, V. Reiser, U. Gartner, J.M. Thevelein, S. Hohmann, G. Ammerer, H. Ruis, Osmotic stress-induced gene expression in

Saccharomyces cerevisiae requires Msn1p and the novel nuclear factor Hot1p, Mol. Cell. Biol. 19 (1999) 5474–5485.
[137] S.I. Allakhverdiev, A. Sakamoto, Y. Nishiyama, M. Inaba, N. Murata, Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp., Plant Physiol. 123 (2000) 1047–1056.
[138] T. Yoshida, L. Qin, M. Inouye, Formation of the stoichiometric complex of EnvZ, a histidine kinase, with its response regulator, OmpR, Mol. Microbiol. 46 (2002) 1273–1282.
[139] L. Qin, S. Cai, Y. Zhu, M. Inouye, Cysteine-scanning analysis of the dimerization domain of EnvZ, an osmosensing histidine kinase, J. Bacteriol. 185 (2003) 3429–3435.
[140] Y. Zhu, M. Inouye, Analysis of the role of the EnvZ linker region in signal transduction using a chimeric Tar/EnvZ receptor protein, Tez1, J. Biol. Chem. 278 (2003) 22812–22819.
[141] T. Urao, B. Yakubov, R. Satoh, K. Yamaguchi-Shinozaki, M. Seki, T. Hirayama, K. Shinozaki, A transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor, Plant Cell 11 (1999) 1743–1754.
[142] S.I. Sukharev, P. Blount, B. Martinac, F.R. Blattner, C. Kung, A large-conductance mechanosensitive channel in *E. coli* encoded by mscL alone, Nature 368 (1994) 265–268.
[143] S.I. Sukharev, P. Blount, B. Martinac, H.R. Guy, C. Kung, MscL: a mechanosensitive channel in *Escherichia coli*, Soc. Gen. Physiol. Ser. 51 (1996) 133–141.
[144] S.I. Sukharev, M. Betanzos, C.S. Chiang, H.R. Guy, The gating mechanism of the large mechanosensitive channel MscL, Nature 409 (2001) 720–724.
[145] S. Sukharev, S.R. Durell, H.R. Guy, Structural models of the MscL gating mechanism, Biophys. J. 81 (2001) 917–936.
[146] G. Chang, R.H. Spencer, A.T. Lee, M.T. Barclay, D.C. Rees, Structure of the MscL homolog from *Mycobacterium tuberculosis*: a gated mechanosensitive ion channel, Science 282 (1998) 2220–2226.
[147] P. Blount, S.I. Sukharev, P.C. Moe, B. Martinac, C. Kung, Mechanosensitive channels of bacteria, Methods Enzymol. 294 (1999) 458–482.
[148] L.V. Nazarenko, I.M. Andreev, A.A. Lyukevich, T.V. Pisareva, D.A. Los, Calcium release from *Synechocystis* cells induced by depolarization of the plasma membrane: MscL as an outward Ca^{2+} channel, Microbiol. GSM 149 (2003) 1147–1153.
[149] P. Blount, P.C. Moe, Bacterial mechanosensitive channels: integrating physiology, structure and function, Trends Microbiol. 7 (1999) 420–424.
[150] P.C. Moe, G. Levin, P. Blount, Correlating a protein structure with function of a bacterial mechanosensitive channel, J. Biol. Chem. 275 (2000) 31121–31127.
[151] B. Poolman, P. Blount, J.H. Folgering, R.H. Friesen, P.C. Moe, T. van der Heide, How do membrane proteins sense water stress? Mol. Microbiol. 44 (2002) 889–902.
[152] E.K. Hoffmann, P.B. Dunham, Membrane mechanisms and intracellular signalling in cell volume regulation, Int. Rev. Cyt. 161 (1995) 173–262.
[153] S.I. Sukharev, P. Blount, B. Martinac, C. Kung, Mechanosensitive channels of *Escherichia coli*: the MscL gene, protein, and activities, Annu. Rev. Physiol. 59 (1997) 633–657.
[154] S.-E. Jordt, T.J. Jentsch, Molecular dissection of gating in the ClC-2 chloride channel, EMBO J. 16 (1997) 1582–1592.
[155] P.C. Biggin, M.S. Sansom, Channel gating: twist to open, Curr. Biol. 11 (2001) R364–R366.
[156] R. Rubenhagen, S. Morbach, R. Kramer, The osmoreactive betaine carrier BetP from *Corynebacterium glutamicum* is a sensor for cytoplasmic K, EMBO J. 20 (2001) 5412–5420.
[157] M. Ishitani, L. Xiong, B. Stevenson, J.K. Zhu, Genetic analysis of osmotic and cold stress signal transduction in *Arabidopsis*: interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways, Plant Cell 9 (1997) 1935–1949.
[158] L. Xiong, M. Ishitani, J.K. Zhu, Interaction of osmotic stress, temperature, and abscisic acid in the regulation of gene expression in *Arabidopsis*, Plant Physiol. 119 (1999) 205–221.
[159] K. Shinozaki, K. Yamaguchi-Shinozaki, Molecular responses to drought and cold stress, Curr. Opin. Biotechnol. 7 (1996) 161–167.
[160] M. Kasuga, Q. Liu, S. Miura, K. Yamaguchi-Shinozaki, K. Shinozaki, Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor, Nat. Biotechnol. 17 (1999) 287–291.
[161] T. Mizoguchi, K. Ichimura, R. Yoshida, K. Shinozaki, MAP kinase cascades in *Arabidopsis*: their roles in stress and hormone responses, Results Probl. Cell Differ. 27 (2000) 29–38.
[162] K. Ichimura, T. Mizoguchi, R. Yoshida, T. Yuasa, K. Shinozaki, Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases ATMPK4 and ATMPK6, Plant J. 24 (2000) 655–665.
[163] K. Yamaguchi-Shinozaki, K. Shinozaki, Improving plant drought, salt and freezing tolerance by gene transfer of a single stress-inducible transcription factor, Novartis Found. Symp. 236 (2001) 176–186.
[164] K. Mikami, I. Suzuki, N. Murata, Sensors of abiotic stress in *Synechocystis*, in: H. Hirt, K. Shinozaki (Eds.), Topics in Current Genetics, vol. 4, Plant Responses to Abiotic Stress, Springer-Verlag, Berlin, 2003, pp. 103–119.
[165] V.V. Bartsevich, S.V. Shestakov, The dspA gene product of the cyanobacterium *Synechocystis* sp. strain PCC 6803 influences sensitivity to chemically different growth inhibitors and has amino acid similarity to histidine protein kinases, Microbiology 141 (1995) 2915–2920.
[166] H. Ruis, C. Schuller, Stress signaling in yeast, BioEssays 17 (1995) 959–965.
[167] O. van Wuytswinkel, V. Reiser, M. Siderius, M.C. Kelders, G. Ammerer, H. Ruis, W.H. Mager, Response of *Saccharomyces cerevisiae* to severe osmotic stress: evidence for a novel activation mechanism of the HOG MAP kinase pathway, Mol. Microbiol. 37 (2000) 382–397.
[168] C. Godon, G. Lagniel, J. Lee, J.M. Buhler, S. Kieffer, M. Perrot, H. Boucherie, M.B. Toledano, J. Labarre, The H_2O_2 stimulon in *Saccharomyces cerevisiae*, J. Biol. Chem. 273 (1998) 22480–22489.
[169] E. Kiegle, C.A. Moore, J. Haseloff, M.A. Tester, M.R. Knight, Cell-type-specific calcium responses to drought, salt and cold in the *Arabidopsis* root, Plant J. 23 (2000) 267–278.
[170] H. Knight, M.R. Knight, Abiotic stress signalling pathways: specificity and cross-talk, Trends Plant Sci. 6 (2001) 262–267.