

Regulatory role of membrane fluidity in gene expression and physiological functions

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Abstract Plants, algae, and photosynthetic bacteria experience frequent changes in environment. The ability to survive depends on their capacity to acclimate to such changes. In particular, fluctuations in temperature affect the fluidity of cytoplasmic and thylakoid membranes. The molecular mechanisms responsible for the perception of changes in membrane fluidity have not been fully characterized. However, the understanding of the functions of the individual genes for fatty acid desaturases in cyanobacteria and plants led to the directed mutagenesis of such genes that altered the membrane fluidity of cytoplasmic and thylakoid membranes. Characterization of the photosynthetic properties of the transformed cyanobacteria and higher plants revealed that lipid unsaturation is essential for protection of the photosynthetic machinery against environmental stresses, such as strong light, salt stress, and high and low temperatures. The unsaturation of fatty acids enhances the repair of the damaged photosystem II complex under stress conditions. In this review, we summarize the knowledge on the mechanisms that regulate membrane fluidity, on putative sensors that perceive changes in membrane fluidity, on genes that are involved in acclimation to new sets of environmental conditions, and on the influence of membrane properties on photosynthetic functions.

Keywords Cold stress · Cyanobacteria · Membrane fluidity · Fatty acid desaturases · Photosynthesis

Abbreviations

ACP	Acyl carrier protein
DPH	1,6-Diphenyl-1,3,5-hexatriene
FA	Fatty acid
FAD	Fatty acid desaturase
FTIR spectroscopy	Fourier transform infrared spectroscopy
HSP	Heat shock protein
PS I	Photosystem I
PS II	Photosystem II
PUFA	Polyunsaturated fatty acid
UFA	Unsaturated fatty acid
TM	Transmembrane

Introduction

The extent of molecular disorder and molecular motion within a lipid bilayer is referred to as the fluidity of the membrane. Temperature stress causes alterations in the fluidity of the membranes in living cells. Cold causes a decrease in the membrane fluidity (membrane rigidification), which can be compensated by desaturation of membrane lipids by fatty acid desaturases (FADs). Alternatively, heat causes fluidization of the membranes. This is compensated by the synthesis of the membrane-stabilizing proteins and by the replacement of unsaturated fatty acids (UFAs) in membrane lipids by the de novo synthesized saturated FAs. Living organisms are usually capable of synthesizing the saturated FAs (Fig. 1) that are further desaturated by the specific enzymes, the FADs (Los and Murata 1998). Certain exceptions though exist, such as *Escherichia coli*, which produces monounsaturates during

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FA synthesis and elongation (Feng and Cronan 2009). Introduction of double bonds between carbon atoms of the FA chains of membrane lipids alter their conformation and affect physical properties of the membranes (Los and Murata 2004). These changes in membrane properties are perceived by cells via sensory proteins embedded in their membranes (Murata and Los 1997; Los and Zinchenko 2009; Cybulski and de Mendoza 2011). These sensors transfer the signals about the changes in the membranes from the environment (shifts in temperature) to networks of signal-transduction pathways, with the resulting regulation of gene expression (Los and Murata 2004).

Modulation in FA unsaturation is not the only mechanism, which determines the changes in membrane fluidity. Environmentally induced dynamic changes of lipid composition, lipid/protein ratio, and the presence of plant sterols in plant membranes are also important factors controlling membrane dynamics (Hartmann 1998; Espenshade and Hughes 2007; Mullineaux and Kirchhoff 2009).

In some bacteria, *cis-trans* isomerization of UFAs plays an important role in the regulation of membrane fluidity (Okuyama et al. 1991; Heipieper et al. 2003). The conversion of *cis*-UFAs to *trans*-UFAs apparently occurs with considerable efficiency during the adaptation of membrane fluidity to changes in the cellular environment.

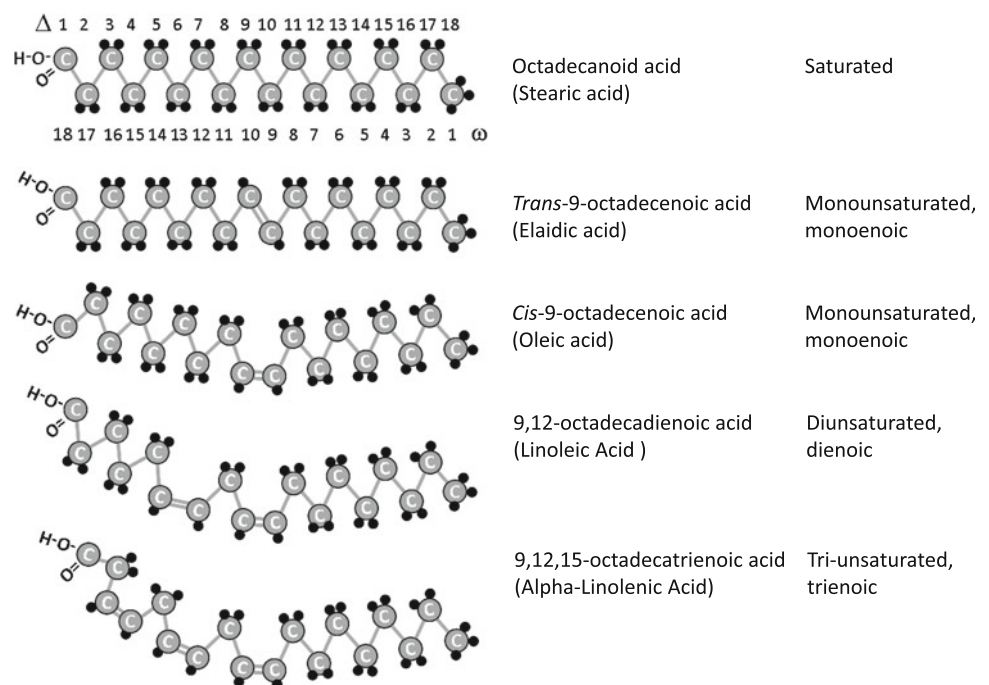
Physical (temperature), chemical (membrane fluidizers, e.g., some drugs and alcohols), and genetic modification (knock-outs of genes for FADs) of the membrane fluidity often have similar effects on the expression of genes that are involved in the acclimation of cells to changing temperature (Los and Murata 2004; Horváth et al. 2012). For a long

time, studies of the influence of membrane fluidity on gene expression have been limited to a small number of known genes that are responsible for the maintenance of the physical properties of membrane lipids. The availability of DNA microarrays that cover the entire genomes of various organisms made it possible to analyze the genome-wide transcription of genes associated with temperature acclimation (Los et al. 2008). It became possible to identify new, earlier unknown, genes, expression of which is affected by changes in membrane fluidity. Since membrane fluidity can directly regulate the activity of membrane-bound proteins, such as various translocators, ion channels, receptor-associated protein kinases (Ruelland and Zachowski 2010; Digel 2011), and sensor proteins (McClung and Davis 2010; Cybulski and de Mendoza 2011), it became the intriguing challenge—to solve a puzzle of temperature and fluidity sensing at the gene level. Many things still remain mysterious. At the same time, a considerable progress has been made in this field. Here, we will summarize recent findings and point to the unsolved questions.

Fatty acids of the membrane lipids

Living organisms, which few exceptions (Feng and Cronan 2009) synthesize the saturated FAs (Fig. 1) that are further desaturated by the FADs (Los and Murata 1998). The cell and photosynthetic membrane lipids are primarily glycerolipids formed by diglycerides, or diacylglycerols (DAGs), consisting of two FA chains covalently bonded to a molecule of tri-carbon alcohol glycerol through ester linkages.

Fig. 1 The example of saturated and unsaturated fatty acids with 18 carbon atoms, which may be esterified to glycerol backbone to form the membrane diacylglycerolipids. The appearance of double bonds in the FA chains is strictly regulated. Carbon atoms are circled, ©; hydrogen atoms are shown by small dark circles. The position of double bonds may be counted from carboxyl-terminus of the fatty acid chain (Δ), or from the methyl-terminus of the chain (ω). Introduction of double bonds appears sequentially: first double bond is formed at position $\Delta 9$, second double bond, at position $\Delta 12(\omega)$, third, at position $\Delta 15(\omega 3)$. See text for further details



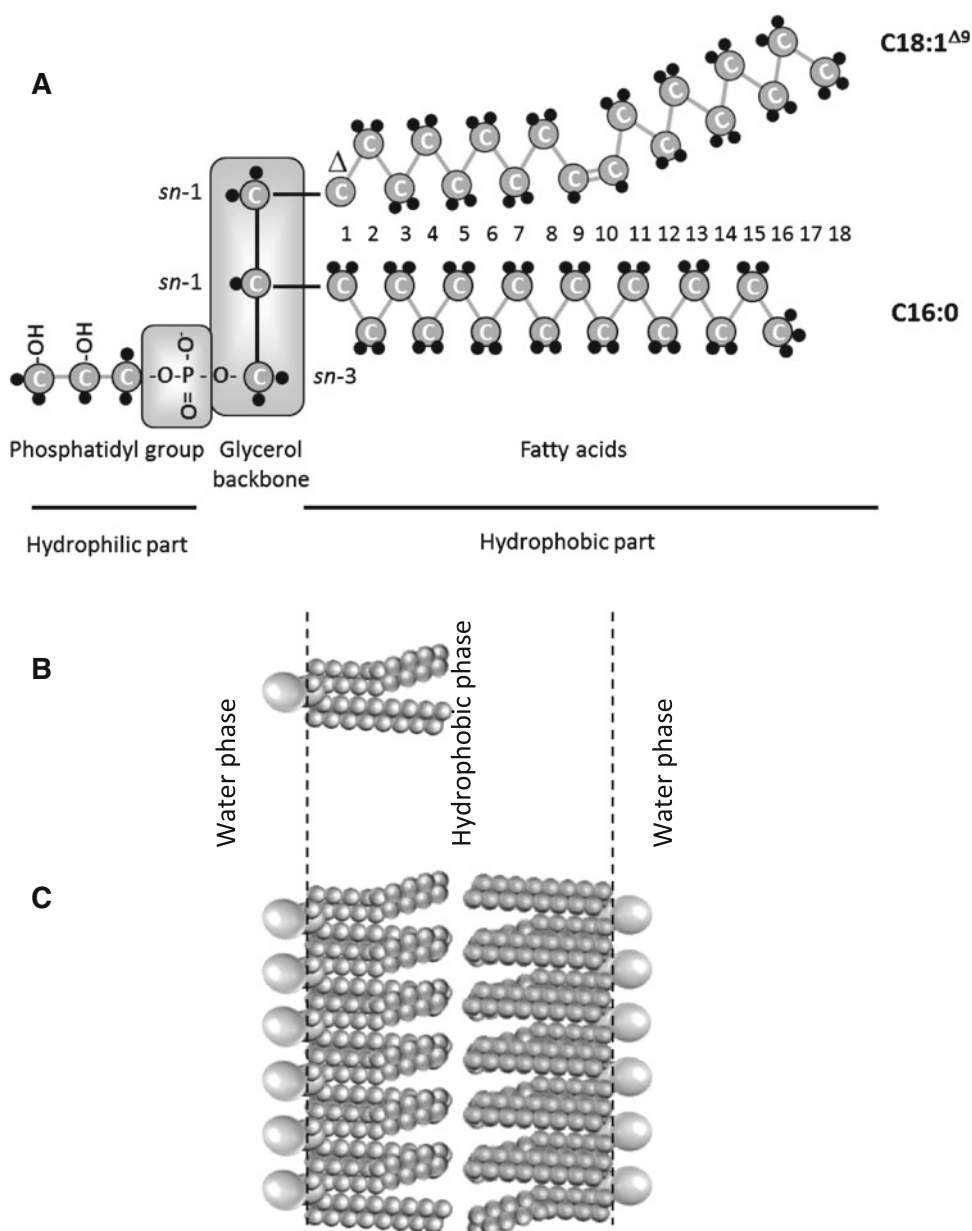
Glycerol has three backbone positions for esterification, thus, stereochemical numbering (*sn*) is usually applied to characterize the structure of membrane lipids. The numbering starts from the *sn*-1 carbon at the top and *sn*-3 carbon at the bottom of the molecule projection. The positions *sn*-1 and *sn*-2 may be occupied by saturated or unsaturated FAs with 14–22 carbon length (C14–C22). The *sn*-3 position is occupied by phosphate group, neutral, or charged molecules (Fig. 2). If all three *sn*-positions are occupied by fatty acyl chains, triglycerides (fats in animals and oils in plants) are formed.

The major lipids in the chloroplast membranes are monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol

(SQDG), phosphatidylglycerol (PG), phosphatidylcholine (PC). Monoglucosyl diacylglycerol (MGIDG) and phosphatidylinositol (PI) are minor components (Shimajima et al. 2009). MGDG and DGDG amount, respectively, to 50 and 25 % of chloroplast or cyanobacterial glycerolipids.

It should be noted that unsaturated double bonds theoretically may be formed in *cis*- or *trans*-positions, which refer to the configuration of a double carbon–carbon bond. These positions determine different spatial structure of the fatty acyl chain (see monounsaturated elaidic and oleic acids in Fig. 1). In nature, *trans* fatty acids are generated by direct enzymatic isomerization of the respective *cis* configuration of the double bond without a shift of its position (Heipieper et al. 2003). *Trans*-FAs are abundantly

Fig. 2 Lipids form double layer of the cellular membrane. **a** One of possible configurations of a phosphatidylglycerol molecule: oleic acid (C18:1 ^{Δ 9cis}) is esterified to the *sn*-1 position of the glycerol backbone, palmitic acid (C16:0) is esterified to the *sn*-2 position of the glycerol backbone, phosphatidyl group is bound to *sn*-3 position of glycerol backbone. Fatty acids are bound to glycerol by their carboxy-termini (Δ -position). The double bond in the oleic acid is at *cis*- Δ 9-position. A phosphate group represents charged hydrophilic “head” of a lipid, whereas fatty acids represent hydrophobic part of a lipid. **b** Schematic molecular model of a lipid molecule represented in (a). Such molecules form a membrane lipid bilayer (c) in aqueous solutions. The definitions and symbols are the same as in Fig. 1



formed during the processing of *cis*-UFAs in food production, such as oil hydrogenation, refining, frying (Martin et al. 2007). With rare exceptions (Gao et al. 2009), FADs produce only *cis*-isomers of UFAs; therefore, we will

further focus only on *cis*-UFAs. The identified FADs of *Arabidopsis* are listed in Table 1.

Among photosynthesizing cells, cyanobacteria possess acyl-lipid FADs that are integrated into both cytoplasmic

Table 1 Fatty acid desaturases of *Arabidopsis thaliana*

Protein family	No	Locus	Gene	Enzyme activity, description	Reference
Acyl-ACP $\Delta 9$ desaturase family	1	AT2g43710	Fab2 (Ssi2)	Stearoyl- and palmytoyl-ACP $\Delta 9$ desaturase, stroma of plastids	Kachroo et al. (2001)
	2	At5g16240	Des1	Stearoyl- and palmytoyl-ACP $\Delta 9$ desaturase, stroma of plastids	Kachroo et al. (2007)
	3	At3g02610	Des2	Stearoyl-ACP $\Delta 9$ desaturase, stroma of plastids	Kachroo et al. (2007)
	4	At5g16230	Des3	Stearoyl- and palmytoyl-ACP $\Delta 9$ desaturase, stroma of plastids	Kachroo et al. (2007)
	5	At3g02620	Des4	Stearoyl- and palmytoyl-ACP $\Delta 9$ desaturase, stroma of plastids	Kachroo et al. (2007)
	6	At3g02630	Des5	Stearoyl- and palmytoyl-ACP $\Delta 9$ desaturase, stroma of plastids	Kachroo et al. (2007)
	7	At1g43800	Des6	Stearoyl-ACP $\Delta 9$ desaturase, stroma of plastids	Kachroo et al. (2007)
Acyl-lipid $\Delta 9$ desaturase family	8	At1g06080	ADS1	$\omega 9$ ($\Delta 9$) desaturase	Smith et al. (2013)
	9	At1g06090	ADS1.2	$\Delta 9$ desaturase	Smith et al. (2013)
	10	At1g06100	ADS1.3	$\omega 7$ desaturase	Smith et al. (2013)
	11	At1g06120	ADS1.4	$\Delta 9$ desaturase	Smith et al. (2013)
	12	At2g31360	ADS2	Homologous to $\Delta 9$ acyl-lipid FADs of cyanobacteria and acyl-CoA FADs of yeast and mammals; transcription is up-regulated by cold	Smith et al. (2013)
	13	AT3g15850	ADS3 (FAD5)	Palmitoyl-monogalactosyl diacylglycerol $\Delta 7$ -desaturase	Heilmann et al. (2004) and Smith et al. (2013)
	14	At1g06350	ADS4	$\Delta 12$ ($\omega 6$) desaturase	Smith et al. (2013)
15	At1g06360	ADS4.2	$\omega 7$ desaturase	Smith et al. (2013)	
16	AT3g15870		Putative $\Delta 9$ desaturase, FAD5 homolog with a chloroplast transit peptide	Smith et al. (2013)	
Acyl-lipid $\Delta 12$ fatty acid desaturases	17	AT3g12120	FAD2	$\Delta 12$ fatty acid desaturase of endoplasmic reticulum.	Okuley et al. (1994)
	18	AT4g30950	FAD6	$\Delta 12(\omega 6)$ fatty acid desaturase, chloroplast synthesis of 16:2 and 18:2 fatty acids from galactolipids, sulpholipids	Falcone et al. (1994)
Acyl-lipid $\omega 3$ fatty acid desaturases	19	At2g29980	FAD3	$\omega 3$ fatty acid desaturase of endoplasmic reticulum. Uses cytochrome b_5 as electron donor.	Aronel et al. (1992)
	20	AT3g11170	FAD7	$\omega 3$ fatty acid desaturase of chloroplast membranes. Uses ferredoxin as electron donor.	Iba et al. (1993) and Gibson et al. (1994)
	21	AT5g05580	FAD8	Temperature-sensitive $\omega 3$ fatty acid desaturase of chloroplast membranes. Uses ferredoxin as electron donor.	Gibson et al. (1994)
Unusual fatty acid desaturases	22	AT4g27030	FAD4	Palmitate desaturase that introduces a $\Delta 3$ trans-double bond at palmitate at the <i>sn</i> -2 position of phosphatidylglycerol	Gao et al. (2009)
	23	AT4g04930	SLD4	$\Delta 4$ sphingolipid desaturase, involved in sphingolipid biosynthesis. Specifically expressed in floral tissues.	Michaelson et al. (2009)
	24	AT3g61580	SLD1	$\Delta 8$ sphingolipid desaturase	Ryan et al. (2007)
	25	At2g46210	SLD2	Putative sphingolipid desaturase, cytochrome b_5 fusion protein	Arabidopsis.org

and thylakoid membranes (Mustardy et al. 1996). These enzymes generate double bonds in the fatty acids esterified to glycerol backbone. Plants possess two types of FADS. 1) soluble acyl–acyl-carrier-protein-FADs (acyl-ACP-desaturases) that introduce first double bonds at position $\Delta 9$ of the fatty acyl chains bound to ACP; 2) membrane-located acyl-lipid desaturases that are similar to cyanobacterial FADs (Table 1). Some of plant FADs are targeted to plastids, and other—to endoplasmic reticulum (Dyer and Mullen 2001; McCartney et al. 2004). In plants, acyl-ACP desaturases with the $\Delta 4$ or $\Delta 6$ positions are known (Guy et al. 2007 and references therein) that participate in oil biosynthesis.

Yeast and animals possess acyl-coenzyme A FADs (acyl-CoA desaturases), which are also located in membranes and introduce double bonds to the fatty acids bound to CoA. These enzymes have little amino acid sequence homology to acyl-lipid FADs of cyanobacteria and plants, however, their hydrophobic profiles look very similar (Wada et al. 1990).

Galactolipids (MGDG and DGDG) of photosynthetic plant cells usually contain a high proportion of polyunsaturated FAs, up to 95 % of which can be trienoic alpha-linolenic acid (Fig. 1). The most abundant molecular species of mono- and digalactosyldiacylglycerols have 18:3 at both *sn*-1 and *sn*-2 positions of the glycerol backbone. Pea (*Pisum sativum*), which have 18:3 as almost the only FA in the MGDGs, have been termed “18:3 plants.” Other species, including the “model” plant *Arabidopsis thaliana*, contain appreciable amounts of hexadecatrienoic acid (16:3) in the MGDG, and they are termed “16:3 plants.” This acid is located exclusively at the *sn*-2 position of the glycerol backbone. Saturated palmitic acid (16:0) appears only in DGDGs, usually in small amounts. In non-photosynthetic tissues, such as tubers, roots or seeds, C18 FAs are usually more saturated.

On the basis of these structures, galactolipids are classified into two groups. The first has mainly C18 FAs at the *sn*-1 position of the glycerol backbone, and only C16 FAs at the *sn*-2 position. Such lipids are termed as lipids of “prokaryotic” structure, as it is characteristic of cyanobacteria. The second class has C16 or C18 FAs at the *sn*-1 position, but only C18 FAs in the *sn*-2 position. These lipids are of “eukaryotic” structure, as they are present in most glycerolipids, such as the phospholipids, of all eukaryotic cells. The exception is PG, which is synthesized in chloroplasts via the prokaryotic pathway (Li-Beisson et al. 2013).

The “counting” mechanism of FADs, by which they determine the exact place (regiospecificity) of desaturation, was debated for a long time. First double bond is formed at position $\Delta 9$ (after 9th carbon atom counting from the carboxyl-terminus of the chain). In case of C18 FAs, the $\Delta 9$ -position and $\omega 9$ -position (counting from the methyl-

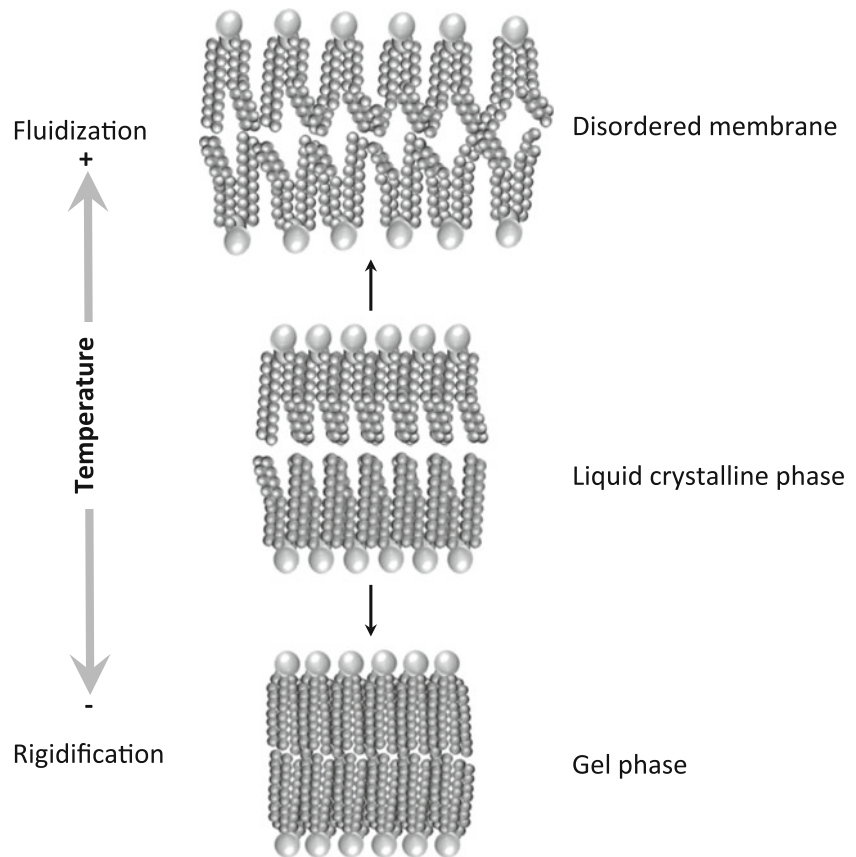
terminus of the acyl chain) coincide. Second double bond is formed at position $\Delta 12(\omega 6)$. Third double bond is formed at position $\Delta 15(\omega 3)$. It was unclear, whether $\Delta 9$ -FAD counts from the methyl (ω) or carboxyl (Δ) end of the fatty acid. Similar question was applied also to $\Delta 12(\omega 6)$ and $\Delta 15(\omega 3)$ -FADs. The available experimental data on desaturation in cyanobacterial cells suggest that the $\Delta 9$ desaturase counts the carbon number from the carboxy terminus, whereas the so-called $\Delta 15$ desaturase is, in fact, the $\omega 3$ -desaturase, which counts the carbon number from the methyl-terminus (Higashi and Murata 1993). Although a significant progress has been made in understanding the molecular basis of regiospecific desaturation by soluble acyl–acyl-carrier-protein desaturases (Cahoon et al. 1997; Guy et al. 2011), the counting order of the acyl-lipid membrane-bound $\Delta 12$ -desaturase is still under question. It is also important to note that the $\omega 3$ -desaturase of the cyanobacterium *Synechocystis* cannot introduce double bonds into $\Delta 9$ monounsaturated fatty acids, and it requires $\Delta 9,12$ di-unsaturated substrate for its activity (Mironov et al. 2012b).

Temperature-dependent changes in membrane fluidity

The effects of changes in temperature on membrane fluidity have been demonstrated by DPH (1,6-diphenyl-1,3,5-hexatriene) fluorescence polarization in fish (Cossins 1977), bacteria (Sinensky 1974), and cyanobacteria (Horváth et al. 1998). DPH is incorporated into membranes in parallel to the acyl chains of membrane lipids. Its fluorescence is weakly depolarized when it interacts stably with rigidified membranes, and it increases in the fluidized membranes. These studies demonstrated low temperatures cause a decrease in membrane fluidity (Fig. 3). Those changes have been confirmed by Fourier transform infrared (FTIR) spectroscopy which monitors 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. Low and high frequencies of the CH_2 stretching mode correspond to the rigidified or fluid states of membrane lipids (Szalontai et al. 2000). The frequency of the CH_2 stretching mode in cytoplasmic membranes and thylakoid membranes isolated from the cyanobacterium *Synechocystis* sp. PCC 6803 (*Synechocystis*) decreases with a decrease in temperature (Szalontai et al. 2000; Inaba et al. 2003).

Heat shock causes the fluidization of membranes (Fig. 3), which at the extremes can lead to disintegration of the lipid bilayer (Vigh et al. 1998; Horváth et al. 2012). Aliphatic alcohols also cause the fluidization of membranes and they are often used in the experiments to mimic heat stress (Horváth et al. 1998, 2008, 2012).

Fig. 3 Schematic representation of changes in membrane structure and in the behavior of lipid bilayers at low and high temperatures. Low temperatures cause an increase in lipid order and *rigidification* of membranes, whereas high temperatures cause a decrease in lipid order and *fluidization* of membranes. (Los and Murata 2004)



Membrane fluidity and fatty acid unsaturation

The dependence of membrane fluidity on the extent of unsaturation of FAs in membrane lipids is a well-characterized phenomenon, which has been demonstrated in animals (Thewke et al. 2003), fish (Tiku et al. 1996), fungi (Maresca and Kobayashi 1993), yeasts (Bossie and Martin 1989; Rodríguez-Vargas et al. 2007), plants (Lyons and Raison 1970; Nishida and Murata 1996), and bacteria (Sinensky 1974; Cybulski et al. 2002), including cyanobacteria (Los and Murata 2004). Cyanobacteria are particularly suitable for studies of such phenomena (Glatz et al. 1998) because the number of unsaturated bonds in their FAs can be altered by targeted mutagenesis of the genes that encode the FADs (Tasaka et al. 1996; Mironov et al. 2012a). Two strains of cyanobacteria, namely, *Synechocystis* and *Synechococcus* sp. PCC 7942 (*Synechococcus*), have been used to study the effects of the unsaturation of FAs on membrane fluidity. *Synechocystis* is characterized by the presence of four genes, designated *desA*, *desB*, *desC*, and *desD*, for FADs and by its ability to synthesize FAs with four double bonds. Thus, its membrane lipids contain high levels of UFAs (Fig. 4).

When the *desA* and *desD* genes were inactivated in *Synechocystis*, a dramatic decrease in membrane fluidity (Szalontai et al. 2000; Inaba et al. 2003; Mironov et al.

2012a) was detectable in the resultant *desA*⁻*desD*⁻ strain, which lost the ability to survive at low temperatures.

Cells of *Synechococcus* naturally possess only one desaturase and they synthesize only monounsaturated 16:1^{Δ9} or 18:1^{Δ9} FAs (Fig. 4). These cells transformed with the *desA* gene (Δ12 desaturase) of *Synechocystis* produced considerable amounts of di-unsaturated 18:2^{Δ9,12} FAs (Wada et al. 1990). They were characterized by a considerable increase in membrane fluidity (Sarcina et al. 2003), which was linked to the newly gained and important feature—the ability to survive at low temperatures.

Thus, an increase in desaturation of FAs fluidizes the membranes, which allows cyanobacterial and plant cells to tolerate chilling and to survive at low temperatures (Tasaka et al. 1996; Ishizaki-Nishizawa et al. 1996; Orlova et al. 2003; Mironov et al. 2012a). Similar effects of engineered membrane fluidization on growth and survival have been recently observed in yeast cells transformed with plant gene for the Δ12 desaturase (Rodríguez-Vargas et al. 2007).

Feedback of desaturation on membrane fluidity

Three of the four genes for desaturases in *Synechocystis* (*desA*, *desB*, and *desD*) are cold-inducible (Los et al. 1997).

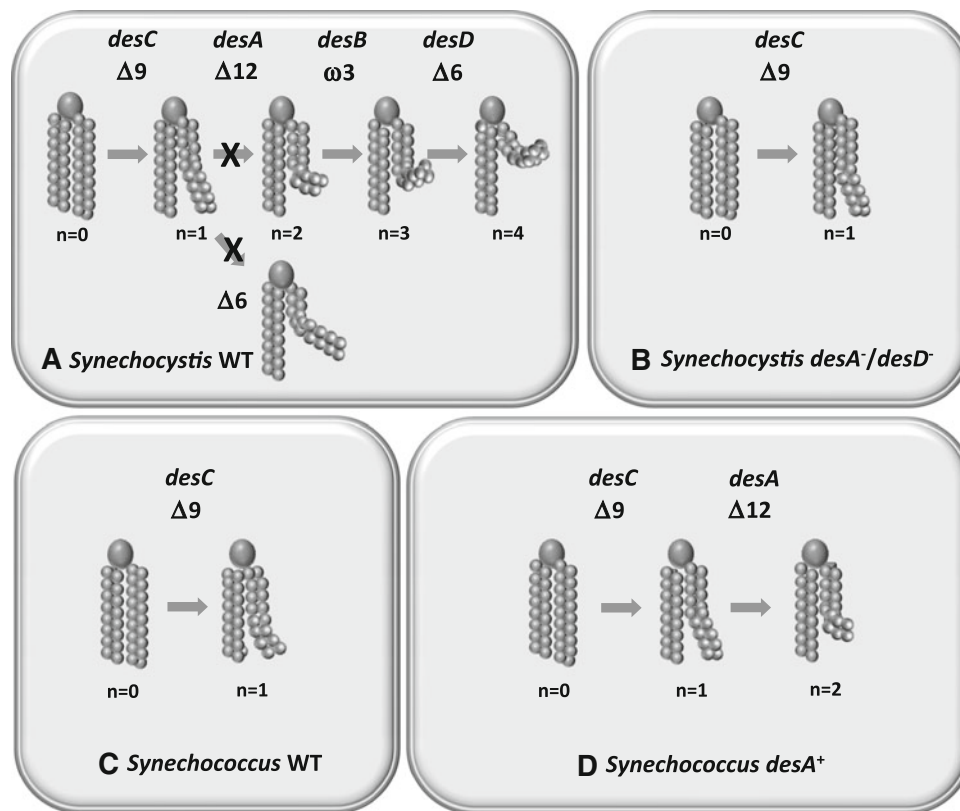


Fig. 4 Desaturation of polar glycerolipids in cyanobacterial cells. **a** Desaturation in wild-type cells of *Synechocystis* (WT) is catalyzed by four acyl-lipid desaturases ($\Delta 6$, $\Delta 9$, $\Delta 12$, $\omega 3$) with formation of C18:4 fatty acid. Site-directed mutagenesis of the *desA* and *desD* genes (shown by crosses) prevents the synthesis of polyunsaturated fatty acids and only monounsaturated fatty acids are produced in the *desA*⁻/*desD*⁻ mutant cells (**b**). 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. **c** Desaturation in wild-type cells of

Synechococcus (WT) is catalyzed only by the acyl-lipid $\Delta 9$ -desaturase and only monounsaturated fatty acids are produced. Transformation of this strain with the *desA* gene for the $\Delta 12$ -desaturase of *Synechocystis* (*desA*⁺) leads to enhanced formation of di-unsaturated fatty acids (**d**). 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. *n* = 0, 1, 2, 3, 4 corresponds to the number of double bonds in the fatty acyl chains

The enhanced synthesis de novo of these three FADs 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 (Hazel 1995). The cold-induced expression of desaturase genes to compensate for a decrease in membrane fluidity, is a widespread phenomenon that can be observed in almost all taxa of poikilothermic organisms, from bacteria to plants, fish, and animals (Macartney et al. 1994; Los and Murata 2004; Aguilar and de Mendoza 2006).

Synthesis of UFAs as a compensatory response to a decrease in membrane fluidity at low temperatures was demonstrated first in *E. coli*, and the phenomenon was designated “homeoviscous acclimation” (Sinensky 1974). Later on, the existence of a feedback loop between membrane rigidification and the compensatory expression of

genes for FADs was demonstrated by the chemical hydrogenation of UFAs in the plasma-membrane lipids of *Synechocystis* (Vigh et al. 1993), or by the use of genetically engineered mutants with decreased unsaturation of membrane lipids (Tasaka et al. 1996; Mironov et al. 2012a, b). Palladium-catalyzed saturation of a small portion of plasma-membrane lipids at the optimal growth temperature caused rapid induction in transcription of the *desA* gene for the $\Delta 12$ desaturase, which is responsible for the synthesis of dienoic FAs. This enzyme compensated the chemically induced rigidification of membrane lipids and returned them to their optimal fluid state (Vigh et al. 1993).

The *desB* gene for the $\omega 3$ desaturase is responsible for the generation of the trienoic FAs. Genetically engineered rigidification of the membranes (the *desA*⁻/*desD*⁻ cells of *Synechocystis*) resulted in the induction of the *desB* at 32 °C, whereas its induction in wild-type cells was evident only at 26 °C (Fig. 5). Thus, the existence of the feedback

link between the fluidity of biological membranes and the expression of genes responsible for the maintenance of the optimal physical state of membranes during a change of the ambient temperature has been confirmed using two independent approaches (Vigh et al. 1993; Mironov et al. 2012a).

It is important that the cold-induced enhancement of the expression of the genes for desaturases depends on the extent of the shift in temperature but not on the absolute temperature (Mironov et al. 2012a). Similar conclusions have been made from the studies of non-photosynthesizing bacteria (Porta et al. 2010a, b). In plants, however, experimental evidence suggests the existence of two types of the thermometers: one type may detect absolute temperature, and the other—a rate of change in temperature (Knight and Knight 2012).

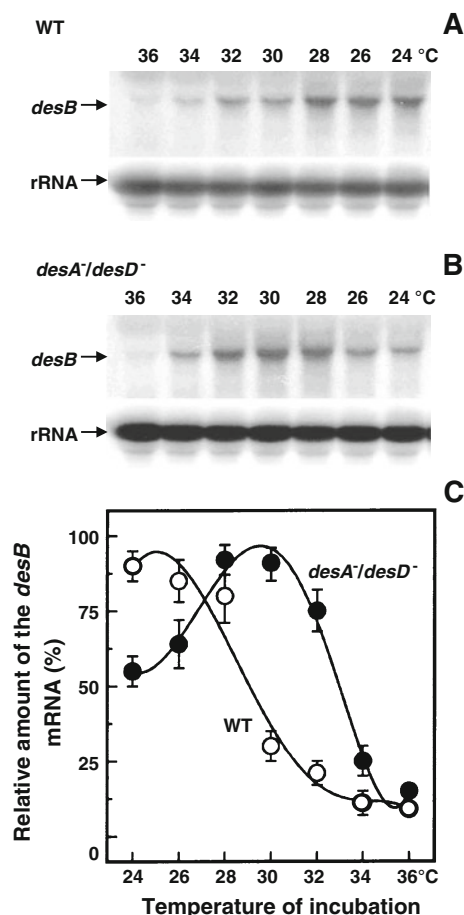


Fig. 5 Analysis of the *desB* gene transcription in the *Synechocystis* wild-type (a) and the *desA*⁻/*desD*⁻ mutant (b) cells (northern blotting). Cells were grown at 36 °C and incubated at different temperatures (from 34 to 24 °C) for 20 min. Then, RNA was isolated from cells for northern blotting with the *desB* and 16S rRNA gene fragments as probes. c Data for the *desB* expression are graphically presented as a dependence of the level of expression (in % from the maximal level) on the temperature of the incubation of wild-type (open symbols) and the *desA*⁻/*desD*⁻ (dark symbols) cells

Membrane rigidification and cold sensing

Cold-inducible genes have been grouped into several categories, as follows: (1) genes for FADs that are responsible for adjustments in membrane fluidity; (2) genes for RNA-binding proteins—RNA chaperones and RNA helicases (*rbpA*, *crhR*) that destabilize the secondary structures of mRNAs, thereby overcoming inhibition of the initiation of translation at low temperatures; (3) genes for ribosomal proteins and translation factors (*rps*, *rpl*, *fus*) an excess of which is necessary for acclimation of the translational machinery to cold; (4) genes for caseinolytic proteases that participate in the renewal of photosystem II; (5) genes for subunits of RNA polymerase (*rpoA*, *sigD*); several stress-activated genes (*cytM*, *hli*-family) (Los et al. 2008). In total, cold strongly induces more than 50 genes in *Synechocystis* (Suzuki et al. 2001; Inaba et al. 2003).

The cold sensor Hik33 (histidine kinase 33) regulates the expression of about a half of cold-inducible genes (Suzuki et al. 2000, 2001). The expression of these Hik33-regulated genes depends on the fluidity of cell membranes, and it is regulated by light, though it does not require the activity of the photosynthetic apparatus (Mironov et al. 2012b). The expression of another group of cold-induced genes, which is not controlled by Hik33, does not depend on the membrane fluidity or light. Thus, membrane fluidity determines the temperature dependence of the expression of cold-induced genes that are under control of the Hik33, which might be the sensor of changes in the membrane fluidity.

It might be that Hik33 perceives cold-induced membrane rigidification as the primary signal of cold stress. The transmembrane sensor protein Hik33 contains several conserved domains, namely, a type-P linker, a leucine zipper and a PAS domain (Fig. 6). The type-P linker might be responsible for intermolecular dimerization of the protein under cold stress, when rigidification of the membranes takes place. The PAS domain senses oxidative stress, which might accompany cold stress shortly after a drop in temperature (Los and Murata 2004).

The molecular mechanism of this sensing is still unclear. It was suggested that lateral diffusion of the membrane-spanning domains of the Hik33 may lead to a drift of its subunits, to dimerization and to autophosphorylation (Los and Murata 2004; Los and Zinchenko 2009). Dimerization of the PAS domain of Hik33 in vivo was recently confirmed by bacterial two-hybrid assay (Shimura et al. 2012).

As a member of the two-component regulatory system, Hik33 should operate via its cognate response regulator (Rre) to transduce a signal of membrane rigidification and to induce the responsive genes. Recently, such transducer, Rre26, has been identified (Kappell and van Waasbergen 2007).

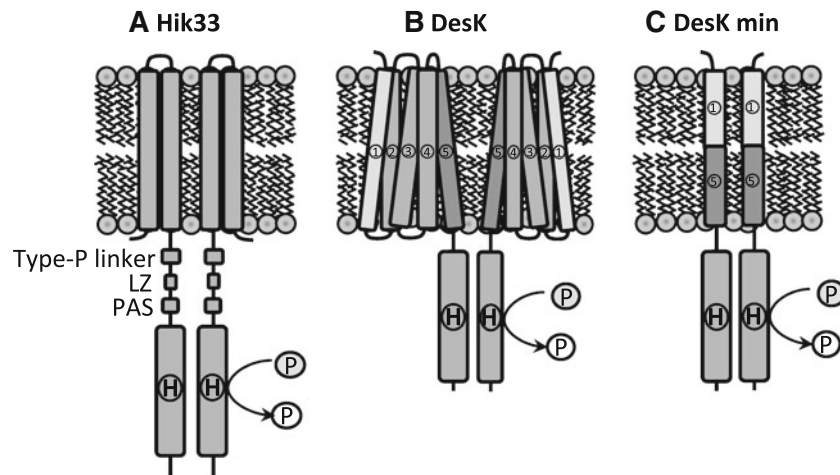


Fig. 6 Cold sensors. **a** The histidine kinase Hik33 of *Synechocystis* consists of two transmembrane domains, type-P linker, leucine zipper (LZ), and PAS domain (Murata and Los 2006). **b** The histidine kinase DesK of *Bacillus subtilis* includes five transmembrane segments. The rigidification of the membrane promotes autophosphorylation and transfer the phosphate group to the response regulator DesR, which

activates expression of the *des* gene coding for a $\Delta 5$ -desaturase. **c** A truncated chimera of DesK represents the “minimum sensor” (DesK min), in which the N-terminal domain of TM1 is fused to the C-terminal domain of TM5. This engineered sensor allows near-normal control of membrane-mediated DesK signaling (Cybulski and de Mendoza 2011)

A typical prokaryotic two-component system consists of a Hik and a Rre. The Hik perceives changes in the environment via its sensor domain and then a histidine residue within the conserved histidine kinase domain is autophosphorylated, with ATP as the donor of the phosphate group (Fig. 6). The phosphate group is transferred from the Hik to an aspartate residue in the conserved receiver domain of the cognate Rre. Upon phosphorylation, the Rre changes its conformation, and this change allows the Rre to bind to the promoter regions of genes that are located further downstream in the signal-transduction pathway. The Hiks in *Synechocystis* appear to be involved in the perception and transduction of environmental signals due to low temperature and high temperature stress, hyperosmolarity and high NaCl concentrations, phosphate and manganese deprivation, excess nickel ions, etc. (for reviews, see Los et al. 2008, 2010).

Recent findings on the temperature sensor histidine kinase DesK of Gram-positive *Bacillus subtilis* provide another clue for understanding the molecular mechanisms of sensing the changes in membrane properties (Cybulski et al. 2010; Cybulski and de Mendoza 2011). The DesK sensor kinase includes five TM-spanning segments that sense an increase in the order of membrane lipids promoting a kinase dominant state of DesK, which undergoes autophosphorylation and transfer of the phosphate group to the response regulator DesR (Aguilar et al. 2001; Cybulski et al. 2002). Phospho-DesR activates expression of the *des* gene (Albanesi et al. 2004, 2009; Najle et al. 2009) for the $\Delta 5$ -desaturase, the only FAD in *B. subtilis* that is involved

in the maintenance of the membrane fluidity (Mansilla et al. 2004; Mansilla and de Mendoza 2005).

A truncated DesK, in which the N-terminal domain of TM1 is fused to the C-terminal domain of TM5, still allows near-normal control of membrane-mediated DesK signaling state (Fig. 7). The temperature-dependent signaling of the engineered sensor protein with just one transmembrane domain implies that lateral movements of the transmembrane domains are not so important for sensing the membrane physical state. It is now thought that the most important parameters of sensing is the match between hydrophobic thickness of the membrane and the hydrophobic thickness of transmembrane segments of integral membrane protein. Any mismatch between the thicknesses of the lipid bilayer and the protein may lead to modification in the structure of the protein (Cybulski et al. 2010), which may trigger the low temperature response (Cybulski and de Mendoza 2011).

The DesK of *Bacillus* does not have PAS domain or type-P linker. It should be noted that the DesK was unable to substitute the inactivated cold sensor Hik33 in *Synechocystis*. On the other hand, the Hik33-homolog from *Synechococcus* sp. PCC 7942 could successfully complement the knock-out of Hik33 in *Synechocystis* (L. Kiseleva, I. Suzuki, D.A. Los, N. Murata—the unpublished observations). This may reflect the differences in membrane properties of cyanobacteria and *Bacillus*. Thus, the additional experiments on truncation of transmembrane domains of cyanobacterial Hik33 (Shimura et al. 2012) are necessary to confirm the importance of membrane thickness for fluidity and temperature sensing.

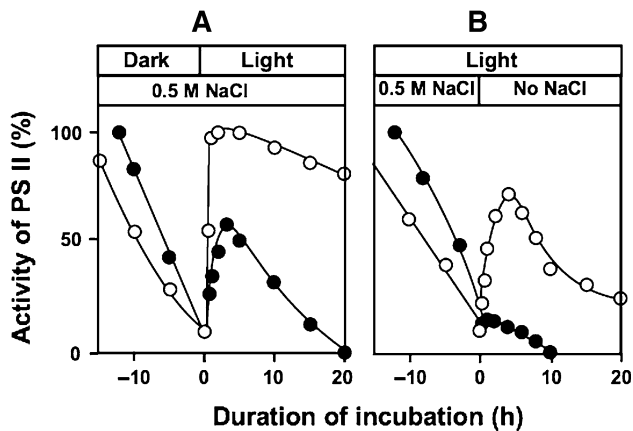


Fig. 7 The defects in polyunsaturated fatty acids in the *desA⁻/desD⁻* mutant cells of *Synechocystis* accelerated the NaCl-induced inactivation of PS II and reduced the light-dependent recovery of PS II activity. **a** Wild-type and *desA⁻/desD⁻* cells were incubated for 12 h in darkness in the presence of 0.5 M NaCl. Then, cells were exposed to light at 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 20 h. **b** Wild-type and *desA⁻/desD⁻* cells were incubated with 0.5 M NaCl in light at 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 12 h. Then, cells were collected by centrifugation, resuspended in fresh medium with no added NaCl, and incubated in light at 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 20 h. Activity of PS II was monitored by means of the light-dependent evolution of oxygen in the presence of 1.0 mM 1,4-benzoquinone. Open circles: wild-type cells, dark circles: *desA⁻/desD⁻* cells (Allakhverdiev et al. 1999)

Engineered rigidification of membranes in *Synechocystis*

As mentioned above, a reduction in the extent of unsaturation of FAs was achieved by targeted mutagenesis of the FADs in the cyanobacterium *Synechocystis* (Fig. 4). The effects of the unsaturation of FAs on the thermotropic behavior of thylakoid membranes from wild-type and mutant cells was examined first by differential scanning calorimetry, DPH fluorescence polarization, FTIR and Raman spectrometry (Tasaka et al. 1996; Szalontai et al. 2000; Mironov et al. 2012a, b). The replacement of PUFAs in membrane lipids by a monounsaturated FAs rigidified cytoplasmic and thylakoid membranes and raised the temperature for the phase transition of the lipids in thylakoids.

The effects of the rigidification of membranes on the growth of cells were examined at various temperatures (Tasaka et al. 1996; Mironov et al. 2012a). Maximum growth of wild-type cells was observed at $\sim 30\text{--}35$ °C and the cells were able to grow even at 20 °C. By contrast, *desA⁻/desD⁻* mutant cells could not grow at 20 °C. These results indicated that replacement of PUFAs by a monounsaturated species had a significant effect on the proliferation of cells at low temperature.

Since membrane-bound protein complexes, such as PSI, PSII, the cytochrome *b₆f* complex and ATP synthase, are

associated directly with lipids in thylakoid membranes, it is reasonable to predict that their activities are affected by the properties of membrane lipids. In this context, the effects of membrane rigidification on the electron transport (from water to ferricyanide), on the uptake of protons, and on the synthesis of ATP have been examined (Dilley et al. 2001). These activities in wild-type cells were reduced with a decrease in temperature but were retained at certain low levels at temperatures close to freezing. By contrast, *desA⁻/desD⁻* mutant cells lost the ability to synthesize ATP but not the ability to transport electrons and take up protons at ~ 5 °C. These findings suggest that a decrease in the membrane fluidity might block the ability of thylakoid membranes to couple a bioenergetically competent proton-motive force to the synthesis of ATP at low temperatures.

Membrane rigidification stimulates photoinhibition at low temperatures

The inactivation of photosystem II (PS II) by strong light is a phenomenon known as photoinhibition, which is due to an imbalance between the rate of photodamage to PS II and the rate of repair of the damaged PS II. Photodamage is a result of the direct inactivation of the oxygen-evolving complex and the photochemical reaction center by light (Ohnishi et al. 2005; Hakala et al. 2005). In addition, strong light induces the production of reactive oxygen species, which inhibit the repair of photodamaged PS II by suppressing, primarily, the synthesis of proteins de novo (Murata et al. 2007; Takahashi and Murata 2008; Nishiyama et al. 2011). The effects of environmental stress on damage to and repair of PS II can be examined separately and it appears that environmental stresses, such as low temperature, act primarily by inhibiting the repair of PS II (Nishiyama et al. 2011).

A comparison of the turnover of the D1 protein, which is an important component of the photochemical reaction center of PS II, in wild-type and *desA⁻/desD⁻* cells of *Synechocystis* revealed that post-translational carboxy-terminal processing of the precursor to the D1 protein was extremely sensitive to low temperature and was also dependent on the fluidity of thylakoid membranes (Kanervo et al. 1995, 1997). A decrease in temperature from 33 to 18 °C caused a failure to recover from photodamage in *desA⁻/desD⁻* mutant cells due to their inability to process the newly synthesized precursor to the D1 protein (Kanervo et al. 1997). Thus, membrane fluidity helps to mitigate photoinhibition by counteracting the inhibitory effects of low temperature on the processing of the precursor to D1 (Takahashi and Murata 2008; Nishiyama et al. 2011).

Membrane rigidification raises sensitivity to salt stress

The ability of the photosynthetic machinery to tolerate salt stress has been examined in detail in wild-type and *desA⁻/desD⁻* mutant cells of *Synechocystis*. The disappearance of the oxygen-evolving activity of PS II under salt stress due to 0.5 M NaCl, both in darkness and in light, was much more rapid in *desA⁻/desD⁻* cells with rigid membranes than in wild-type cells with fluid membranes (Fig. 4, Allakhverdiev et al. 1999). Moreover, the photosynthetic activity of wild-type cells recovered more rapidly than that of the mutant cells after removal of NaCl from the growth medium (Fig. 7). Recovery in the presence of NaCl depended on light and ATP was required for the synthesis de novo of the proteins necessary for reactivation of the oxygen-evolving machinery (Allakhverdiev et al. 1999).

The recovery of the oxygen-evolving activity under salt stress depends on the activity of the Na⁺/H⁺ antiporter system, which, in its turn, is regulated by the membrane fluidity. Incubation of wild-type and *desA⁻/desD⁻* cells in the presence of 1.0 M NaCl in darkness resulted in total inactivation of the Na⁺/H⁺ antiporter system. Upon exposure to low light, the activity of the Na⁺/H⁺ antiporter system in wild-type cells returned to 30 % of the original level while, in *desA⁻/desD⁻* cells, it returned to only to 5 % of the original level. The NaCl-induced inactivation of the oxygen-evolving complex in thylakoid membranes isolated from *desA⁻/desD⁻* cells also occurred much more rapidly than in membranes from wild-type cells (Allakhverdiev et al. 1999). In general, *desA⁻/desD⁻* cells were characterized by enhanced sensitivity to NaCl and a reduced ability to recover from salt stress, as compared to wild-type cells.

This may be explained by the following: (a) the membrane fluidity might act directly to protect the oxygen-evolving machinery against salt-induced inactivation; (b) the activity of the Na⁺/H⁺ antiporter system might be regulated by membrane fluidity; (c) the recovery of Na⁺/H⁺ antiporter activity is more efficient in unsaturated (fluid) membranes (Allakhverdiev et al. 1999; Allakhverdiev and Murata 2008).

Recent results obtained with the mutants of *Arabidopsis* defective in $\Delta 12$ fatty acid desaturase FAD2 confirmed the importance of unsaturation for the maintenance of the Na⁺/H⁺ antiporter activity and for tolerance to salt stress (Zhang et al. 2012).

Engineered fluidization of membranes in *Synechococcus*

The increase in unsaturation of FAs by genetic engineering was achieved by transformation of *Synechococcus* cells

with the gene for the $\Delta 12$ desaturase of *Synechocystis* (Wada et al. 1990). *Synechococcus* synthesizes only monounsaturated FAs, mainly 16:1 ^{$\Delta 9$} and 18:1 ^{$\Delta 9$} . It cannot grow and propagate at low temperatures. The *desA⁺*-transformed *Synechococcus* cells were able to introduce a double bond at the $\Delta 12$ position of the FA that was esterified to the *sn*-1 position of the glycerol moiety (Fig. 4). The resulting change in the FA composition was recognized as an increase in the level of di-unsaturated FAs at the expense of monounsaturated FA (Wada et al. 1990): the transformed cells contained almost equal amounts of monounsaturated and di-unsaturated FAs.

Membrane fluidization mitigates photoinhibition at low temperatures

As mentioned above, photoinhibition in vivo reflects the effects of photodamage to PS II and subsequent repair (Murata et al. 2007; Nishiyama et al. 2011). The damage corresponds to the light-induced inactivation of PS II due to a damage to the D1 protein, while repair consists of a series of steps, namely, degradation of the D1 protein in the photodamaged PS II, synthesis of the precursor to the D1 protein de novo, incorporation of the precursor into the PS II complex and, finally, processing of the precursor to yield the mature D1 protein.

The *desA⁺*-transformed *Synechococcus* cells, glycerolipids of which included di-unsaturated FAs, were more resistant to photoinhibition at low temperatures than were wild-type cells, which contained monounsaturated FAs exclusively (Gombos et al. 1997). Studies of the light-induced inactivation and recovery of the activity of the PS II complex revealed that the recovery process was markedly accelerated in di-unsaturated membranes. The synthesis of the D1 protein de novo at low temperature was much faster in the *desA⁺*-transformed cells than in the wild-type cells. Thus, increased unsaturation of membrane lipids enhanced the ability of cells to withstand strong light by accelerating the de novo synthesis and turnover of the D1 protein (Gombos et al. 1997).

Three isoforms of the D1 protein are encoded by three *psbA* genes in *Synechococcus*. The *psbAI* gene encodes D1 form I (D1:1). The *psbAII* and *psbAIII* genes are identical and they encode the transiently expressed D1 form II (D1:2). Transfer of cultures of *Synechococcus* cells from 32 to 25 °C under light at 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ resulted in replacement of D1:1, the prevailing form at the higher temperature, by D1:2 in both wild-type cells and the *desA⁺*-transformed cells, with no loss of PS II activity (Sippola et al. 1998). A further downward shift in temperature to 18 °C caused a dramatic decrease in PS II activity in wild-type cells, while the

desA⁺-transformed cells were affected to a much smaller extent. It appeared that wild-type cells were incapable of accumulating D1:2 to compensate for the loss of D1:1, with resultant disassembly of PS II at the low temperatures (Sippola et al. 1998). These results suggested a crucial role of the UFAs and membrane fluidity in promotion of the exchange of the prevailing forms of the D1 protein and, thus, in the maintenance of the activity of PS II at low temperature (Takahashi and Murata 2008; Nishiyama et al. 2011).

Fluidization of membranes enhances tolerance to salt stress

In *Synechococcus*, the photoinactivation of PS II under salt stress was more prominent in wild-type cells than in *desA*⁺-transformed cells (Allakhverdiev et al. 2001). A similar effect was also observed when the activity of PS I was examined: the reduction of P700⁺ in the PS I complex was more resistant to the damaging action of a high concentration of NaCl in *desA*⁺-transformed cells than in wild-type cells.

The NaCl-induced inactivation of PS I and PS II, as well as that of the Na⁺/H⁺ antiporter system, consists of a rapid phase and a slow phase (Allakhverdiev et al. 2000; Allakhverdiev and Murata 2008). The rapid phase is reversible and is induced by the osmotic effect of NaCl, which reduces the amount of water in the cytosol via the efflux of water through water channels. The slow phase is irreversible and is induced by ionic effects that are due to the influx of Na⁺ ions through K⁺/Na⁺ channels.

Unsaturation of FAs in membrane lipids protects PS I and PS II against both rapid phase and slow phase of NaCl-induced inactivation (Allakhverdiev et al. 2001). Experiments with isolated thylakoid membranes demonstrated that the NaCl-induced inactivation occurred more slowly in membranes isolated from *desA*⁺-transformed cells than in those from wild-type cells.

Recovery of the activities of PS I and PS II was alleviated in *desA*⁺-transformed cells than in wild-type cells. The available evidence suggests that the unsaturation of FAs has two effects: (a) it mitigates the NaCl-induced damage to PS I and PS II both in vivo and in vitro; (b) it enhances the repair of damaged PS I and PS II in vivo. These phenomena might be due to the activities of the Na⁺/H⁺ antiporter system and H⁺-ATPase(s), as well as to water channels and K⁺(Na⁺) channels, which might be influenced by the fluidity of membranes. Thus, there is the direct evidence that membrane fluidity plays important role in the maintenance of the photosynthetic machinery under salt stress.

Modulation of lipid unsaturation in higher plants

Genetics of fatty acid unsaturation

Large numbers of cold-inducible genes have been identified in plants (Seki et al. 2001; Fowler and Thomashow 2002; Benedict et al. 2006; Zeller et al. 2009; Matsui et al. 2010). 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, FADs (Maruyama et al. 2009). The level of unsaturation of membrane FAs in higher plants correlates with growth temperature. Thus, desaturation plays the key role in the regulation of membrane fluidity and the acclimation of plants to low temperatures (Orvar et al. 2000; Vaultier et al. 2006; Knight and Knight 2012). Although the induction of the expression of genes for desaturases and the experiments with fluidizers and rigidifiers of membrane lipids (Sangwan et al. 2002) suggest that membrane rigidification might also participate in cold signaling, the distinct knowledge about cold sensors in plants is still missing (for updates on the topic, please, see Thomashow 2010; Knight and Knight 2012). Future efforts will be required to identify the sensor proteins that act as thermosensors in plants and linking the perception of temperature to response.

In parallel with the development of techniques for the genetic engineering of various aspects of plant metabolism, including the unsaturation of FAs, extensive efforts have been made to isolate and characterize mutant plants with defects in the unsaturation of FAs by a classical genetic approach (Wallis and Browse 2002). The *Arabidopsis* mutant with reduced activity of the $\Delta 12$ desaturases accumulated high levels of 16:1 and 18:1 in lipids. This mutant was characterized by abnormal chloroplast ultrastructure, the protein and chlorophyll contents of thylakoid membranes, electron-transport activity, and the thermal stability of photosynthetic membranes (Hugly et al. 1989). These observations pointed to the central role for diunsaturated FAs in lipids in determining chloroplast structure and maintaining normal photosynthetic functions. These studies demonstrated that the unsaturation of FAs has a direct effect on the thermal stability of photosynthetic membranes.

In unicellular prokaryotic *Synechocystis* (Tasaka et al. 1996) and in eukaryotic protozoan *Acanthamoeba castellanii* (Harwood 2007), the $\Delta 12$ -desaturase also plays the crucial role in cold acclimation and survival at low temperatures.

The complete absence of trienoic FAs in thylakoid lipids of *Arabidopsis* due to triple mutation of genes for $\omega 3$ de-

saturases (*fad3-2/fad7-2/fad8*) stipulated hypersensitivity of such plants to photoinhibition (Vijayan and Browse 2002). The rate of photodamage to PS II was similar in wild-type and triple-mutant plants. However, the recovery of photodamaged PS II was much slower in triple-mutants. These results support the proposed importance of PUFAs in the recovery of PS II activity at low temperatures (Moon et al. 1995). Similar conclusions have been drawn from the lessons learned from the studies of cyanobacteria (Tasaka et al. 1996; Allakhverdiev et al. 1999, 2000).

It should be mentioned that cold-induced desaturation in cyanobacteria occurs mainly due to transcriptional induction and due to the increase in desaturase mRNA stability (Los and Murata 2004). In plants, as well as in fish (Tiku et al. 1996) desaturases, in addition to transcriptional level, are regulated at the post-transcriptional and post-translational levels including phosphorylation and modulation of protein stability (Matsuda et al. 2005; Tang et al. 2005).

The effect of changes in membrane fluidity on the oxygen-evolving capability of isolated thylakoids was recently studied. Membranes were rigidified by incorporation of the plant sterol stigmaterol. Such rigidification was accompanied by a reduction of the activity of PSII and by even more pronounced inhibition of PSI-mediated electron transport (Popova et al. 2007; Velitchkova et al. 2009).

For the assembly and function of photosynthetic machinery, the translocation of proteins through the thylakoid membranes is very important. This transport across membranes occurs via one of three distinct pathways (Gutensohn et al. 2006). A study of three mutant lines of *Arabidopsis* with depressed unsaturation, namely, *fad6*, *fad5*, and *fad3/fad7/fad8*, has provided useful information relevant to the characterization of these three translocation pathways (Ma and Browse 2006). The mutations in desaturases reduced, by up to 50 %, the rate of transport of the 18-kDa extrinsic protein of PS II via the Tat pathway, which depends on a pH gradient across membranes. By contrast, the mutations substantially increased the transport of the 33-kDa protein of PS II via the Sec pathway. This increased capacity for protein import might compensate partially for the reduced capacity for thylakoid transport via the Tat pathway. It means that the transport of proteins might depend on the properties of the chloroplast membranes. Impaired transport of proteins in the mutants might contribute to their low-temperature-sensitive phenotype (Ma and Browse 2006).

Engineered fatty acid unsaturation in plants

Another approach to modulate the unsaturation of FAs involves transformation of tobacco plants with the *desC* gene for $\Delta 9$ desaturase from *Synechococcus* (Ishizaki-

Nishizawa et al. 1996). The expression of this acyl-lipid desaturase increased the relative level of monounsaturated FAs in the PG of chloroplasts in tobacco leaves. The excess of monounsaturated FAs served as substrates for further desaturation. As a result, such transformation of tobacco plants dramatically increased levels of PUFAs and decreased levels of saturated FAs from 44 to 24 %. These changes dramatically enhanced the ability of the transgenic tobacco plants to tolerate low temperatures.

The tobacco plants have also been transformed with the *desC* gene from the thermophilic cyanobacterium *Synechococcus vulcanus* (Orlova et al. 2003). The lipid content and extent of unsaturation of FAs were significantly higher in leaves of transgenic plants if compared to parental plants. The chilling tolerance of the *desC*⁺ plants also increased, as estimated by measurements of the leakage of electrolytes from tissues upon exposure to low temperatures (Fig. 8a). Seeds of the transgenic plants exhibited greater chilling tolerance than the control seeds. Strong light at low temperature caused leaf bleaching in wild-type plants, but not in the *desC*⁺ transformants (Fig. 8b, c), indicating that unsaturation of lipids protects photosynthetic machinery under strong light at low temperatures.

Overexpression in tomato plants of the *fad7* gene for $\omega 3$ fatty acid desaturase from tomato increased the level of α -linolenic acid (18:3) in the glycerolipids of thylakoid membranes (Liu et al. 2008). Upon exposure to chilling stress at 4 °C under light at 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the *fad7*⁺-transgenic plants maintained greater oxygen-evolving activity and greater photochemical efficiency than wild-type plants.

Together with the mutants with defects at certain steps in desaturation pathway, these transgenic plants should serve as useful models for direct assessment of the roles of FA unsaturation in photosynthesis and related physiological activities in higher plants.

Changes in fatty acids unsaturation by acyltransferases

Glycerol-3-phosphate acyltransferase (GPAT), encoded by the *ATSI* gene (Nishida et al. 1993) and localized in plastids, catalyzes the transfer of the acyl group of acyl-ACP to the *sn*-1 position of glycerol 3-phosphate (Nishida and Murata 1996). In chilling-resistant plants, such as spinach (*Spinacea oleracea*) and *Arabidopsis*, this enzyme uses 18:1-ACP specifically as its substrate. By contrast, in chilling-sensitive squash (*Cucurbita moschata*), the enzyme utilizes both 18:1-ACP and 16:0-ACP (Frentzen et al. 1987; Nishida et al. 1987).

The extent of unsaturation of FAs was altered in transgenic tobacco (*Nicotiana tabacum*) that expressed GPAT from plants that differed in terms of chilling sensitivity (Murata et al. 1992). In tobacco that expressed GPAT

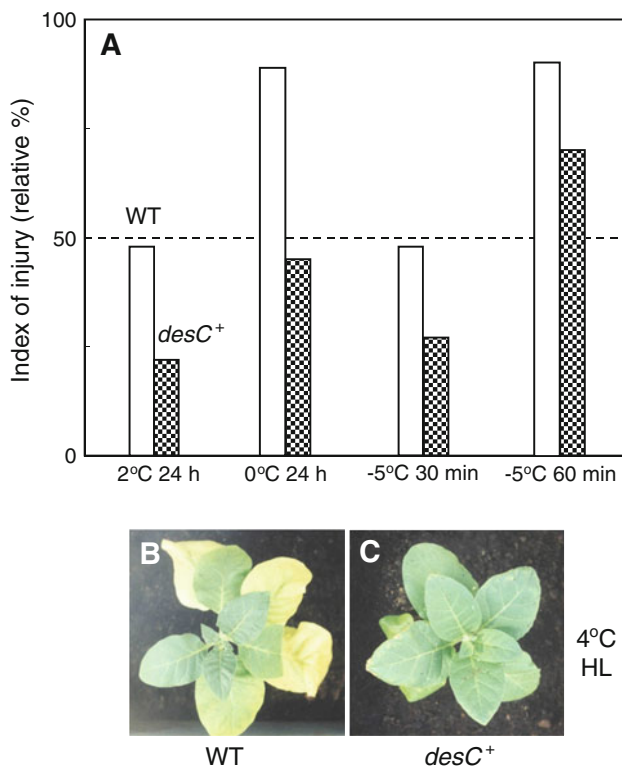


Fig. 8 Tests for cold sensitivity of wild-type and *desC*⁺-transgenic tobacco. **a** Wild-type tobacco plants (white bars) and the *desC*⁺ tobacco transformants (shaded bars) have been tested for chilling sensitivity by measuring the electric conductivity of water extracts of plant tissues. Plants have been exposed at 2 or 0 °C for 24 h; at –5 °C for 30 min (no ice nucleation) or for 60 min (with ice nucleation). The index of injury represented leakage of electrolytes from the damaged tissues as percent of the leakage from the tissues completely destroyed by boiling (Orlova et al. 2003). Higher index of injury reflects higher cold sensitivity of plants. *Lower panel*: tobacco plants of wild-type (**b**) and *desC*⁺ transformant (**c**) were exposed to high light intensity (HL—1,000 μmol photons m⁻² s⁻¹) at 4 °C for 3 days

derived from squash, the fluidity of thylakoid membranes decreased in response to the increase in the proportion of saturated plus *trans*-monounsaturated molecular species of PG from 24 to 65 % (Szalontai et al. 2003). These plants displayed more pronounced sensitivity to photoinhibition at low temperatures and lower rates of PSII recovery than non-transformed plants. These results suggested that the unsaturation of FAs of PG in thylakoid membranes might stabilize the photosynthetic machinery against photoinhibition at low temperature by accelerating the repair of PS II (Moon et al. 1995).

Chilling stress also damaged inflorescences: the abscission of flower buds and inflorescence meristems occurred more frequently in transgenic plants than wild-type plants. Thus, a decrease in the proportion of *cis*-unsaturated PG provoked the sensitivity of reproductive organs to chilling (Sakamoto et al. 2003).

Sui et al. (2007a) overexpressed cDNA for GPAT of tomato in tomato plants under the control of the strong 35S promoter of cauliflower mosaic virus (CaMV) and observed an increase in relative level of *cis*-unsaturated PG. This enhanced the tolerance of plants to cold stress and accelerated the recovery of PS II after photodamage during cold-induced photoinhibition. When the GPAT was repressed in tomato by the antisense method, an increase in the relative level of saturated FAs in PG from leaves was observed. A decrease in unsaturation reduced male fertility, increased the size of tapetal cells, and mitigated photoinhibition of PS II under mild-heat conditions (Sui et al. 2007b).

In rice leaves (*Oryza sativa* L.), the level of UFAs in PG have been genetically raised by introduction of cDNA for GPAT from spinach (Yokoi et al. 1998; Ariizumi et al. 2002). At 25 °C, transformed and non-transformed plants revealed similar activities of photosynthesis. However, at chilling temperature (14 °C), the photosynthetic activity of transgenic plants was higher than that of wild-type plants. Moreover, growth at chilling temperature of transgenic plants was greater than that of wild-type plants (Ariizumi et al. 2002). These results demonstrate the strict dependence of photosynthetic reactions on the lipid environment.

Recently, the leaves of transgenic tobacco plants with decreased levels of FA unsaturation in PG (Murata et al. 1992) have been investigated in terms of function and stability of the PSI reaction center P700 (Ivanov et al. 2012). The PSI photochemistry of wild-type plants was only slightly affected by high light treatments, whereas transgenic lines exhibited much higher susceptibility to photoinhibition of PSI. Thus, FA unsaturation and membrane fluidity play important roles in the function of both PSII and PSI in plant cells.

Freezing-induced lipid remodeling in chloroplast membranes

Moderate *cold or chilling stress*, which does not imply intracellular ice nucleation, causes metabolic rearrangements that result in activation of multiple pathways leading to acclimation: increases in intracellular solutes, the accumulation of cryoprotective metabolites and proteins, adjustments of membrane fluidity, etc. (Uemura et al. 1995; Nishida and Murata 1996). This process is known as cold acclimation. However, recent studies of plants exposed to freezing temperatures indicate a distinct mechanism required for freezing tolerance. Freezing destroys cellular and photosynthetic membranes due to ice formation, dehydration, the formation of non-bilayer lipid structures, their transition to gel phase. Thus, stabilization of membranes within the dehydrated plant cells is a key factor that determines the survival of plants under freezing.

Thylakoid membranes of plants and cyanobacteria contain high amounts of galactolipids (~50 % of MGDG and ~25 % of DGDG of total chloroplast glycerolipids) that are indispensable for the efficiency of photosynthetic light reactions. The stability of the thylakoid membrane bilayer is determined by the ratio of MGDG to DGDG (Shimajima and Ohta 2011). Under freezing, a certain proportion of MGDG is converted into DGDG, tri- or tetragalactolipids by the galactolipid:galactolipid galactosyl-transferase (GGGT) enzyme (Moellering and Benning 2011). The reaction of such transgalactosylation is accomplished by the transfer of the galactosyl moiety from one MGDG or DGDG to another. The GGGT-encoding gene was recently identified to be associated with the SENSITIVE TO FREEZING 2 (SFR2) phenotype of *Arabidopsis* (Moellering et al. 2010). It was proposed that a decrease in MGDG content accompanied by the proportional increase in oligogalactolipids leads to the remodeling of the thylakoids that prevents fissions and fusions of chloroplast membranes provoked by freezing-induced dehydration (Moellering and Benning 2011).

This hypothesis, for the involvement of GGGT in freezing tolerance, needs to be further tested experimentally. So far, the location of GGGT associated with the SFR phenotype has been restricted to the plastid envelope membranes. If the scenario of lipid remodeling is extrapolated to the whole cell and organism, one should expect the existence of lipid-transferase-like enzyme(s) associated with cytoplasmic and/or mitochondrial or nuclear membranes.

Membrane fluidization and heat sensing

Heat-induced gene expression has been studied in *Synechocystis* on a genome-wide level (Inaba et al. 2003; Suzuki et al. 2005). 59 genes were induced more than threefold in *Synechocystis* under heat stress (Suzuki et al. 2005). Mostly the genes were characterized by the transient mode of induction: they were highly induced by heat stress within 10–20 min, but 1 h after the onset of stress their transcription levels significantly declined. These genes encode proteins that belong mostly to the chaperone class (*hspA*, *groESL*, *groEL2*, *dnaJ*, *hspG*, *dnaK2*). Heat stress also induced the expression of *htrA* and *clpB1* genes for proteases, *sigB* for RNA polymerase sigma factor and *hik34* for histidine kinase 34, as well as *sodB* for superoxide dismutase and *hliC* for high light-inducible protein C. The expression of most genes involved in energy and lipid metabolism, pigment biosynthesis and photosynthesis decreased under heat stress conditions (Suzuki et al. 2005).

Proteomic studies of heat shock response in *Synechocystis* revealed over 100 spots, corresponding to 65 different proteins alter heat shock (Slabas et al. 2006).

Changes occurred not only in the classical heat shock proteins but also in the protein biosynthetic machinery, amino acid biosynthetic enzymes, components of the light and dark acts of photosynthesis and energy metabolism. In particular, following heat shock treatment, there was a twofold increase in the level of the manganese-stabilizing protein, PsbO. Phycobilisome proteins decrease in abundance upon heat shock, and RUBISCO large subunit RbcL decreased over twofold (Slabas et al. 2006).

The majority of the heat-induced genes and proteins, the so-called HSPs, are not specific for heat stress, but they are also induced by osmotic, salt, as well as by oxidative stress, strong visible light and UV-B light (Los et al. 2008, 2010). Thus, these proteins should be rather considered as general stress proteins, GSPs, but not HSPs. In *Synechocystis*, the genes that are specifically induced by heat are limited to a short list of open reading frames with unknown function: *sll0441*, *sll0688*, *sll1106*, *sll1884*, *slr0852*, *slr0095*, and *slr1597*.

Heat stress causes membrane fluidization (Horváth et al. 2008). If we consider that the cold sensor is activated by rigidification of the plasma-membrane (Los and Murata 2004; Cybulski and de Mendoza 2011), one would suggest that a putative heat sensor in the membrane might be activated by its fluidization (Vigh et al. 1998; Mittler et al. 2012). However, no membrane-located candidate for a heat sensor has been identified in photosynthetic cells to date.

In *S. cerevisiae*, the engineered rigidification of the cytoplasmic membrane activated transcription of the *hsp90* gene (Carratu et al. 1996). This demonstrates the dependence of the expression of HSPs on membrane fluidity. The supporting evidence was obtained in studies of the responses of *Synechocystis* cells to heat shock and to chemical membrane fluidizers. At normal growth temperatures, the membrane fluidizer benzyl alcohol activated transcription of the *hspA* gene as efficiently as heat stress (Horváth et al. 1998; Török et al. 2001). However, engineered rigidification of membranes in *Synechocystis* did not affect the heat-induced transcription of genes (Inaba et al. 2003). It should be taken into account that the membrane fluidity in the engineered strain with defective genes for the FADs may be compensated by some unknown mechanism, which differs from FA unsaturation (Mironov et al. 2012b). Thus, the consensus on heat sensing via membrane fluidization, at least, in cyanobacteria, still require additional studies.

The identified heat sensor of *Synechocystis*, the histidine kinase Hik34, is a soluble protein that negatively controls transcription of several GSP genes (Suzuki et al. 2005). Overexpressed and purified recombinant Hik34 was autophosphorylated in vitro at physiological temperatures, but not at elevated temperatures, such as 44 °C. Unlike wild-type cells, the knock-out mutant cells deficient in Hik34

survived incubation at 48 °C for 3 h. The Hik34-deficient mutant displayed the enhanced levels of transcripts of a number of GSP genes, including *hspG*, *groESL1*, and *groEL2*. Alternatively, overexpression of the Hik34 repressed transcription of these GSP genes (*hspA*, *hspG*, *dnaK2*, *groESL1*, and *groEL2*).

Two transcription factors, Sll1670 (HrcA) and Sll1130, negatively control transcription of GSP genes in *Synechocystis* (Nakamoto et al. 2003; Krishna et al. 2013). HrcA represses nearly the same set of genes (*groESL* operon and the *groEL2* gene), which are under control of Hik34 (Kojima and Nakamoto 2007). Sll1130 represses *hspG*, *hspA*, *isiA*, *isiB*, *frpC*, *rre5*, and several hypothetical genes. Thus, the Hik34 and two transcription factors control the full set of GSP genes in *Synechocystis*. It is still unknown whether Hik34 and transcription factors HrcA and/or Sll1617 interact with each other in vivo.

Conclusions and perspectives

It has been postulated that a change in membrane fluidity might be the primary signal in the perception of cold stress that triggers the acclimatory responses in cells. Now, we have the example of how the sensory transmembrane histidine kinase may perceive the changes in the physical state of the biological membranes (Cybulski and de Mendoza 2011). The whole-genome and proteome studies in combination with the targeted mutagenesis and modifications of certain signal-transduction and biochemical pathways allow identification of stress sensors, transducers, individual genes, and groups of genes that are induced specifically and non-specifically under certain stress conditions.

The sensor proteins, such as DesK of *B. subtilis* or Hik33 of *Synechocystis*, may recognize the rigidification of membranes due to decrease in ambient temperature. Although, other mechanisms of cold signaling, which differ from classical two-component regulatory systems, may operate in cyanobacterial cells (Prakash et al. 2009), we still do not know the whole picture of this network. We do not know whether membrane-associated heat sensors exist in photosynthetic cells, and how such sensors are controlled by the membrane fluidity. Nothing is known about cold and heat sensors in higher plants. It is essential that efforts now be made to identify these molecules and the complete pathways of temperature perception and transduction in photosynthetic organisms.

Temperature sensors of plant cells remain to be identified. According to our knowledge on “cell thermometers,” the successful candidates might be found among protein kinases, ion (Ca^{2+}) channels, small RNAs, proteins that regulate the structure of chromatin (McClung and Davis 2010; Knight and Knight 2012).

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