

## Update on Stress Signaling

# Membrane Fluidity and Temperature Perception<sup>1</sup>

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Plants are incapable of escaping from a changing environment. Therefore, they have developed sophisticated mechanisms for acclimation and survival under unfavorable conditions, such as unfavorable temperatures. Low temperatures induce a number of alterations in cellular components, including the extent of fatty acid unsaturation (Cossins, 1994), the composition of glycerolipids (Lynch and Thompson, 1982), the positional redistribution of saturated and unsaturated fatty acids within lipid molecules (Thompson and Nozawa, 1984), changes in the protein composition (Raison, 1973; Tiku et al., 1996), and activation of ion channels (Knight et al., 1996). Low temperatures activate a number of cold-inducible genes (Jones and Inouye, 1994), such as those that encode dehydrins, lipid transfer proteins, translation elongation factors, and the late-embryogenesis-abundant proteins (for refs., see Nishida and Murata, 1996). Despite extensive research on the mechanisms that regulate the expression of such temperature-induced genes, little is known about the primary sensor that detects a change in temperature or about the transducers of the temperature signal. In this *Update*, we focus on the role of biological membranes in the perception of cold temperatures and the subsequent signal transduction.

### TEMPERATURE-DEPENDENT REGULATION OF UNSATURATION OF FATTY ACIDS OF MEMBRANE LIPIDS IN CYANOBACTERIA AND PLANTS

When poikilothermic organisms such as bacteria (Russel, 1984), fungi (Miller and Baran, 1984), protozoa (Nozawa and Umeki, 1988; Thompson, 1989), plants (Somerville and Browse, 1991; Nishida and Murata, 1996), and animals (Stubbs and Smith, 1984) are exposed to temperatures below those of their normal growth conditions, increases occur in the degree of unsaturation of the fatty acids of their membrane lipids. Details of the desaturation of fatty acids in membrane lipids have been examined in cyanobacteria such as *Anabaena variabilis* (Sato and Murata, 1980)

and *Synechocystis* sp. PCC 6803 (Wada and Murata, 1990). In cyanobacterial cells, the primary products of glycerolipids are esterified only to saturated fatty acids (Sato and Murata, 1982), and all of the desaturation reactions occur after fatty acids have become bound to glycerolipids (Sato et al., 1986).

Upon a downward shift in temperature of 10 to 15°C, the growth of cyanobacterial cells and the synthesis de novo of fatty acids cease for about 10 h. During this period, the fatty acids of membrane lipids are desaturated and, when the degree of unsaturation reaches a certain level, the cells begin to grow again and synthesize fatty acids (Sato and Murata, 1980). Results of RNA gel-blot analysis indicate that the increase in desaturation after a downward shift in temperature is caused by the up-regulation of the expression of the genes for desaturases (Los et al., 1993; Sakamoto and Bryant, 1997).

Upon an upward shift in temperature, the degree of unsaturation of the fatty acids of membrane lipids decreases in cyanobacterial cells. This decrease is caused by suppression of the desaturation of fatty acids and acceleration of the synthesis de novo of saturated fatty acids (Sato and Murata, 1980).

In higher plants the synthesis of unsaturated glycerolipids is more complex. The synthesis of fatty acids occurs exclusively in plastids (Stumpf, 1980). The products of fatty acid synthesis are 16:0-ACP and 18:0-ACP, and the latter is desaturated to 18:1(9)-ACP by stearoyl-ACP desaturase. All of the other desaturation reactions occur after fatty acids are bound to lipids either in the cytoplasm or in plastids (Harwood, 1988). 18:1(9) bound to lipids is desaturated to 18:2(9, 12) and then to 18:3(9, 12, 15) in the cytoplasm and plastids (Somerville and Browse, 1991). 16:0 bound to monogalactosyl diacylglycerol in plastids is sequentially desaturated to 16:1(7), 16:2(7, 10), and 16:3(7, 10, 13) (Somerville and Browse, 1991).

Fatty acids of membrane lipids in higher plants are highly unsaturated; trienoic fatty acids, such as 18:3(9, 12, 15) and 16:3(7, 10, 13), account for about 70% of the total fatty acids. When plants are exposed to low temperatures, the desaturation of fatty acids occurs mainly from 18:2(9, 12) to 18:3(9, 12, 15). Thus, the extent of fatty acid desaturation in higher plants is much less pronounced than that

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Abbreviations: ACP, acyl-carrier protein; X:Y(Z), fatty acid containing X carbons with Y double bonds in the *cis* configuration at the Z position counted from the carboxy terminus.

in cyanobacteria, in which the fatty acids of membrane lipids are not so much desaturated as in higher plants.

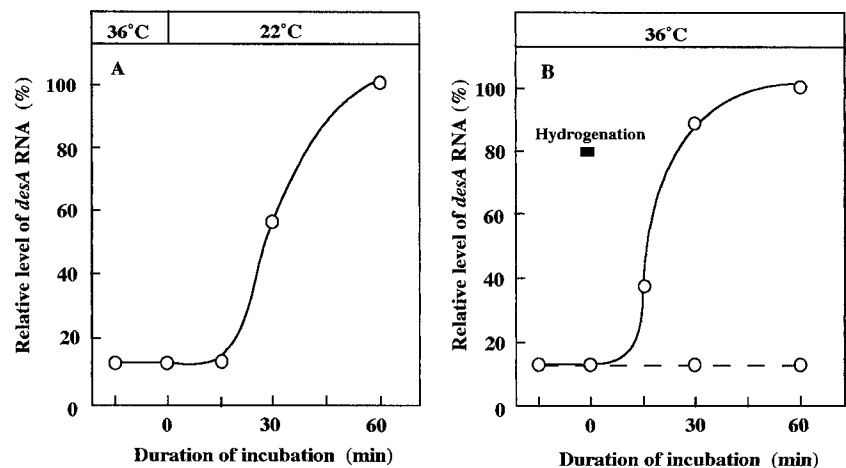
Nonetheless, the importance of the desaturation of fatty acids of membrane lipids in tolerance to low temperature has been demonstrated in transgenic systems. When relative levels of saturated molecular species of phosphatidylglycerol in chloroplasts are reduced by transformation with glycerol-3-phosphate acyltransferase from a chilling-resistant plant, plants become more tolerant to low temperature (Murata et al., 1992; Wolter et al., 1992; Moon et al., 1995). An increase in unsaturated fatty acids in membrane lipids by gene transfer of desaturases results in enhanced tolerance to chilling in cyanobacteria (Wada et al., 1990) and higher plants (Ishizaki-Nishizawa et al., 1996). Mutation in the cytoplasmic  $\Delta 12$  desaturase increases sensitivity of plants to low temperatures (Okuley et al., 1994).

### THE MAINTENANCE OF MEMBRANE FLUIDITY

The term "fluidity" (the reciprocal of viscosity) is used loosely to describe the extent of disorder and the molecular motion within a lipid bilayer (Cossins, 1994). However, no single term adequately covers all the very different dynamic characteristics of a lipid bilayer, such as lateral diffusion of molecules, molecular wobbling, and chain flexing. Nonetheless, membrane fluidity can be estimated by several physical methods, such as fluorescence polarization, electron-spin resonance, and Fourier transform IR spectroscopy.

The extent of unsaturation of membrane lipids is the major factor that influences the fluidity of membrane lipids (Kates et al., 1984; Cossins, 1994). A decrease in temperature leads to a decrease in membrane fluidity, which results in the enhanced expression of the genes for fatty acid desaturases. These enzymes introduce double bonds into the fatty acyl chains of membrane lipids, thereby compensating for the decrease in membrane fluidity (Murata and Wada, 1995). As a result, the physical properties of the membrane are restored to their original state, with the maintenance of appropriate ion gradients across the membranes and the restoration of the functions of membrane-associated enzymes.

**Figure 1.** Changes in the level of the transcript of the *desA* gene, which encodes the  $\Delta 12$  desaturase in the cyanobacterium *Synechocystis* sp. PCC 6803. A, Temperature-induced accumulation of *desA* mRNA. Cells were grown at 36°C and were then transferred to 22°C (adapted from Los et al., 1993). B, Hydrogenation-induced accumulation of *desA* mRNA. Cells were grown at 36°C and hydrogenated at 36°C for 4 min. Under these conditions, only 5% of fatty acids of the glycerolipids of the plasma membrane are hydrogenated and practically no lipids of the thylakoid membrane are hydrogenated (adapted from Vigh et al., 1993).



### FATTY ACID DESATURASES

There are three types of fatty acid desaturase: acyl-CoA desaturases, acyl-ACP desaturases, and acyl-lipid desaturases. These enzymes introduce a double bond into fatty acids bound to CoA, ACP, and glycerolipids, respectively (Murata and Wada, 1995). The acyl-lipid desaturases are the enzymes that most efficiently introduce double bonds into membrane lipids after a downward shift in temperature. All desaturases in cyanobacteria that have been studied to date are of the acyl-lipid type (Murata and Wada, 1995). *Synechocystis* sp. PCC 6803 cells contain four acyl-lipid desaturases. They introduce a double bond specifically at the  $\Delta 6$ ,  $\Delta 9$ ,  $\Delta 12$ , and  $\Delta 15$  ( $\omega 3$ ) positions. Each of these desaturases is encoded by a single-copy gene, designated *desD*, *desC*, *desA*, and *desB*, respectively. These desaturases are located in both the thylakoid and the plasma membranes of the cyanobacterial cells (Mustardy et al., 1996).

In *Synechocystis* sp. PCC 6803, a decrease in temperature leads to enhanced levels of the transcripts of the genes for desaturases (Los et al., 1993; Sakamoto et al., 1994; Sakamoto and Bryant, 1997; Fig. 1A). This finding suggests that the desaturation of fatty acids of membrane lipids after a downward shift in temperature is caused by the up-regulation of the expression of the genes for desaturases.

The temperature-induced up-regulation of the expression of the genes for desaturases depends on the extent of the shift in temperature and not on the absolute temperature (Los et al., 1993). For example, when cells acclimated to 36°C are transferred to various lower temperatures, the accumulation of the *desA* transcript is apparent only below 30°C. However, when cells have been acclimated to 32°C, the accumulation of the *desA* transcript becomes apparent below 26°C.

### MEMBRANE FLUIDITY REGULATES THE EXPRESSION OF GENES FOR DESATURASES: A FEEDBACK LINK

A decrease in temperature induces changes in many metabolic factors that might be responsible for an increase in the level of the transcript of the *desA* gene. However, we

postulated that the primary signal upon a change in temperature might be the change in membrane fluidity. We addressed this question by examining the effects of the catalytic hydrogenation of the fatty acids of membrane lipids with a water-soluble palladium complex as the catalyst. Using this technique, we were able to reduce the degree of unsaturation of the fatty acids of the membrane lipids and, therefore, the fluidity of membrane lipids *in vivo* under isothermal conditions, while minimizing the contribution of phenomena other than changes in membrane fluidity that might be caused by a change in temperature (Vigh et al., 1993).

The hydrogenation of *Synechocystis* cells at 36°C for 4 min converted 5% of unsaturated fatty acids to saturated fatty acids in the glycerolipids of the plasma membrane, but no such conversion occurred in the glycerolipids of the thylakoid membrane. The decrease in the degree of the unsaturation of fatty acids of glycerolipids in the plasma membrane caused an increase in the level of the transcript of the *desA* gene similar to that caused by a decrease in temperature to 22°C (Fig. 1B). Moreover, such hydrogenation of the lipids in the plasma membrane increased the threshold temperature for the expression of the *desA* gene from 28 to 30°C (Vigh et al., 1993). These findings suggest that the primary signal in the perception of a change in temperature is a change in the fluidity of the plasma membrane.

This concept of the contribution of membrane fluidity to the control of gene expression is supported by an observation related to the expression of a gene for a heat-shock protein in *Saccharomyces cerevisiae* (Carratu et al., 1996). Mutant yeast cells that were defective in the  $\Delta 9$  acyl-CoA desaturase were transformed with the gene for the  $\Delta 9$  desaturase under control of promoters of various strengths. In the membrane lipids from such transformed cells there were variations in the relative levels of saturated and unsaturated fatty acids, which were associated with different degrees of membrane fluidity. The expression of a heat-shock gene at 36°C was up-regulated with increases in the level of unsaturated fatty acids. These findings indicate that the expression of the heat-shock gene depends on the degree of unsaturation of the fatty acids of the membrane lipids; in other words, the expression of the heat-shock gene depends on the degree of membrane fluidity.

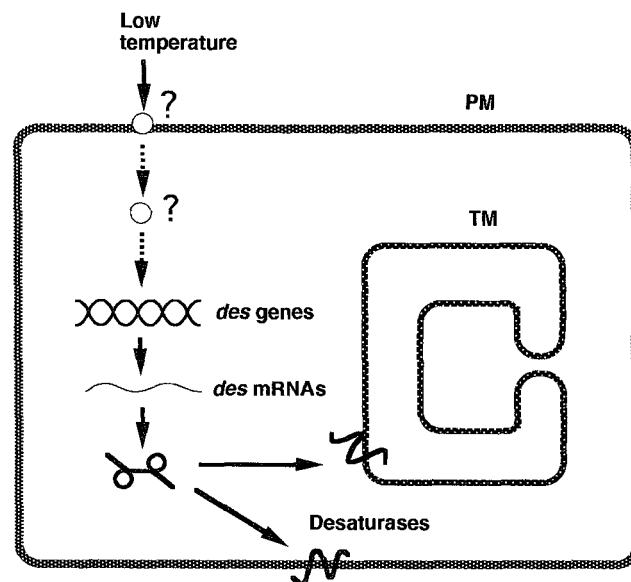
#### A PUTATIVE SENSOR FOR PERCEPTION OF CHANGES IN TEMPERATURE

The results of the catalytic hydrogenation experiment suggest that a sensor for perception of temperature might be located in the plasma membrane and might detect the changes in membrane fluidity (Vigh et al., 1993). However, a downward shift in temperature of 10°C results in only a 3% change, at most, in the molecular motion of lipid molecules since the molecular motion is proportional to the absolute temperature, unless transitions of the physical phase occur. The physical phase transition is not predicted to occur in the plasma membrane in the range of temperatures in such experiments (Murata, 1989; Tasaka et al., 1996). It is unlikely that the putative sensor detects such a small change in the molecular motion of membrane lipids.

Therefore, we speculate that a physical phase transition occurs in microdomains of the plasma membrane upon a downward shift in temperature and that the putative sensor detects a dramatic conformational change in such microdomains, e.g. the transition of the physical phase from the liquid-crystalline to the gel state. The hypothetical sensor protein might undergo a conformational change or a cycle of phosphorylation and dephosphorylation as the primary event in transduction of the temperature signal.

The hypothetical sensor protein might resemble a His kinase, which has been identified as a sensor of changes in osmolarity in *S. cerevisiae*. This His kinase is phosphorylated in response to an increase in ambient osmolarity and transduces this signal to the mitogen-activated protein kinase cascade (Maeda et al., 1994). Another possibility, proposed by Monroy and Dhindsa (1995), is that in higher plants the putative sensor is a  $\text{Ca}^{2+}$  channel, but to our knowledge, such a channel has not yet been characterized. According to this hypothesis, the  $\text{Ca}^{2+}$  channel opens at low temperatures upon a decrease in membrane fluidity, and the entering  $\text{Ca}^{2+}$  ions activate a signal transduction pathway for up-regulation of the expression of low-temperature-inducible genes.

The events from the downward shift in temperature to the induced synthesis of desaturases are summarized in Figure 2. The pathway from the perception of the shift in temperature to the induction of the genes for desaturases is unknown. However, subsequent reactions have been rather well characterized. After the downward shift in temperature or a decrease in membrane fluidity, the genes for desaturases are up-regulated and the desaturases are synthesized *de novo* (Los et al., 1997) and targeted to both the plasma and the thylakoid membranes (Mustardy et al.,



**Figure 2.** Proposed model of temperature perception and transduction of the signal during the low-temperature-induced expression of the genes for desaturases in cyanobacterial cells. Putative sensor and signal-transducing components are indicated by question marks. PM, Plasma membrane; TM, thylakoid membrane; *des*, desaturase.

1996). These enzymes catalyze the desaturation of the fatty acids of the membrane lipids to compensate for the decrease in membrane fluidity that has been caused by a decrease in temperature.

### CONTRIBUTION OF MEMBRANES TO SIGNAL TRANSDUCTION IN OTHER ORGANISMS

The direct involvement of the physical state of the membrane in the regulation of transcription has been demonstrated only in the case of genes for desaturases and heat-shock proteins (see above). However, several research groups have demonstrated that membranes are involved in various signal transduction pathways. de Jonge et al. (1996) reported that alterations in membrane fluidity were associated with modulation of the activity of receptor-mediated phospholipase C in cultured cardiomyocytes from rats. Kamada et al. (1995) reported that membrane fluidity regulates the activity of protein kinase C, which is a key enzyme in signal transduction during the thermoacclimation of *S. cerevisiae*. It has been suggested that membrane fluidity is also linked to regulation of the influx or mobilization of  $Ca^{2+}$  ions and the change in the concentration of cAMP in ciliary cells from the frog palate (Schootemeijer et al., 1995) and in human cultured cells (Alfahel et al., 1996).

The regulation of membrane fluidity in higher plants and cyanobacteria under heat and cold stress has been studied intensively (Nishida and Murata, 1996). However, the molecular mechanism of the relationship between changes in membrane fluidity and signal transduction remains unclear.

### FUTURE PERSPECTIVE

The role of membrane fluidity in temperature perception and in transduction of the signal seems now to be indisputable. There is strong evidence for a sensory role of membranes in a variety of living organisms. Questions that remain to be answered mainly involve interactions between membranes and proteins: What is the nature of the sensor proteins that perceive changes in membrane fluidity? How are the sensor proteins modified upon perception of the signal? Do conformational changes or phosphorylation/dephosphorylation cycles occur? What is the nature of the secondary messengers that transduce the signal after it has been recognized by the sensor protein? These are some of the unanswered questions in the field of temperature biology.

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