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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

(cyanobacterium/desaturase/temperature adaptation)

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ABSTRACT **One of the well-characterized phenomena associated with the acclimation of organisms to changes in ambient temperature is the regulation of the molecular motion or "fluidity" of membrane lipids via changes in the extent of unsaturation of the fatty acids of membrane lipids. The enzymes responsible for this process when the temperature is decreased are the desaturases, the activities of which are enhanced at low temperature. To examine whether the change in the fluidity of membrane lipids is the first event that signals a change in temperature, we studied the effect of the Pd-catalyzed hydrogenation of membrane lipids on the expression of the *desA* gene, which is responsible for the desaturation of fatty acids of membrane lipids in the cyanobacterium *Synechocystis* PCC6803. The Pd-catalyzed hydrogenation of plasma membrane lipids stimulated the expression of the *desA* gene. We also found that, for unexplained reasons, the hydrogenation was much more specific to a minor phospholipid, phosphatidylglycerol, than to members of other lipid classes. These results suggest that the organism perceives a decrease in the fluidity of plasma membrane lipids when it is exposed to a decrease in temperature.**

Temperature is one of the most important factors in the environment of biological organisms, in particular in the case of plants and algae that are incapable of escaping from suboptimal conditions. The low-temperature-induced desaturation of membrane lipids in cyanobacteria is a well-characterized example of adaptation to a decrease in temperature (1–3).

An organism can maintain the level of molecular motion or "fluidity" of its membrane lipids by regulating the number of double bonds in the fatty acids of these lipids (4). When the fluidity of membrane lipids is reduced by a decrease in temperature, plants and cyanobacteria respond by introducing double bonds into the fatty acids of lipids, so that the membranes return to a more fluid state. Desaturases are responsible for the introduction of these specific double bonds (5, 6). The *desA* gene has been cloned (7) from the cyanobacterium *Synechocystis* PCC6803. This gene encodes an acyl-lipid desaturase that acts at the Δ12 position of fatty acids of membrane lipids and is a responsive element in the temperature-compensation pathway.

We have demonstrated (8) that a decrease in growth temperature caused a marked increase in the level of the transcript of the *desA* gene in *Synechocystis* PCC6803 (8). However, the mechanism for the perception of the change in temperature that stimulates the expression of the *desA* gene has hitherto remained unknown. The decrease in temperature reduces the fluidity of the membrane lipids, but there are also many factors involved in cellular metabolism that might be responsible for an increase in the level of the transcript of the *desA* gene. Therefore, a distinct experimental system is required to enable us to identify the mechanism that is operative in the regulation of expression of the *desA* gene. It is now possible to reduce the molecular motion of membrane lipids *in vivo* under isothermal conditions by homogeneous Pd-catalyzed hydrogenation (9, 10), thereby minimizing the effects that would otherwise be caused by a change in temperature.

In this communication we report that a short period of Pd-catalyzed hydrogenation reduced the double bonds of lipids located in the plasma membrane but not in thylakoid membranes. We also describe how a brief hydrogenation reaction reduced the double bonds of phosphatidylglycerol more specifically than those of other lipids. The specific hydrogenation of lipids in the surface membrane stimulated transcription of the *desA* gene, in a manner similar to that observed upon exposure of the cells to low temperature (8). These findings suggest that a change in the fluidity of lipids in the plasma membrane triggers an increase in the expression of the *desA* gene, which leads subsequently to the desaturation of membrane lipids.

MATERIALS AND METHODS

Culture Conditions and Preparation of Permeaplasts. Cells of *Synechocystis* PCC6803 were grown photoautotrophically at 36°C under illumination from incandescent lamps [70 μE per m_2 per sec; 1 einstein (E) = 1 mol of photons] in BG-11 medium (11) supplemented with 20 mM Hepes·NaOH (pH 7.5), with aeration by 99% sterile air/1% CO_2 (12). To prepare permeaplasts, cells were treated with lysozyme for 20 min as described by Papageorgiou and Lagoyanni (13). As a result of this treatment, cell walls were partially digested and cells were converted to permeaplasts. The permeaplasts were collected by centrifugation at 3000 × *g* for 5 min and suspended in 20 mM Hepes·NaOH (pH 7.5). During these treatments the photosynthetic activity, measured at 36°C, declined from 400 to 300 μmol of O_2 per mg of chlorophyll (Chl) per h.

Hydrogenation. The permeaplasts were suspended in 20 mM Hepes·NaOH (pH 7.5), at a concentration that corresponded to Chl at 2 μg/ml, and hydrogenated under atmospheric hydrogen, as described (9), in the presence of the hydride form of palladium (sodium alizarinesulfonate)$_2$, prepared by the method of Joó *et al.* (10). After one wash with 20 mM Hepes·NaOH (pH 7.5), the treated permeaplasts were

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Abbreviations: Chl, chlorophyll; PG, phosphatidylglycerol.

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harvested for further studies. All the above procedures were performed at 36°C.

Separation of Thylakoid Membranes and Plasma Membranes. Permeaplasts were harvested by centrifugation at 5000 × *g* for 10 min. They were washed twice with 10 mM Tes·NaOH buffer (pH 7.0) that contained 600 mM sucrose, 20 mM $CaCl_2$, and 10 mM $MgCl_2$ (buffer A) and were then suspended in this buffer. The suspension was mixed with the same volume of glass beads (0.10–0.11 mm in diameter; G-8893; Sigma), and the mixture was agitated for three 3-min periods on a Vortex mixer, with 2-min intervals of cooling in an ice-water bath. Unbroken cells, cell debris, and glass beads were removed by centrifugation at 1000 × *g* for 10 min, and the supernatant was subjected to separation of plasma membranes and thylakoid membranes by the method of Murata and Omata (14). All the procedures for preparation of membranes were carried out at 0–4°C.

Analysis of Fatty Acids. Lipids were extracted from the permeaplasts by the method of Bligh and Dyer (15). The extracted lipids were subjected to methanolysis with 5% (vol/vol) HCl in methanol at 85°C for 2.5 h. The esterified fatty acids were analyzed with a gas–liquid chromatographic system (GC-7A; Shimadzu) equipped with a hydrogen flame ionization detector and a capillary column (16). Relative amounts of fatty acid methyl esters were calculated by comparing areas under chromatographic peaks with a data processor (C-R 2AX; Shimadzu).

Measurement of Photosynthetic Activities. Photosynthetic evolution of oxygen by permeaplasts, suspended in 20 mM Hepes·NaOH (pH 7.5), was monitored by means of oxygen exchange with a Clark-type oxygen electrode. The oxygen-evolving activity of permeaplasts due to the activity of photosystem II was measured with 1.0 mM 1,4-benzoquinone supplemented with 1.0 mM $K_3Fe(CN)_6$ as the electron acceptor. Light at 3.5 mE per m^2 per sec was provided from an incandescent lamp combined with a red optical filter (VR620; Hoya Glass, Tokyo) and a far-red-absorbing optical filter (HA 50; Hoya Glass). The concentration of Chl was determined by the method of Arnon *et al.* (17).

Isolation of RNA. Cells were quickly chilled to 3°C with liquid nitrogen and collected by centrifugation for 3 min at 3000 × *g* at 3°C. The cells were disrupted with glass beads as described by Golden *et al.* (18). Total nucleic acids were extracted three times with phenol/chloroform, 1:1 (vol/vol), and were precipitated in ethanol. RNA was separated from DNA by LiCl precipitation (19).

DNA Probe. A DNA fragment of 1.3-kbp that carried the *desA* gene was excised from the plasmid Bluescript/1.5 kbp, as described (7), by digestion with *Hin*cII and *Sac* I restriction endonucleases. The DNA probe was produced by labeling the 1.3-kbp fragment with [α-^{32}P]dCTP by nick translation.

Northern Blot Hybridization. Total RNA (10 μg) was subjected to electrophoresis in a 1.2% agarose gel that contained 6.3% (vol/vol) formaldehyde (19) and then the fractionated RNA was transferred onto a nylon membrane (GeneScreen*Plus*; Biotechnology Systems, NEN). The membrane was baked at 80°C for 2 h to remove traces of formaldehyde. The membrane was prehybridized in 50% (vol/vol) formamide/1.0% SDS/1.0 M NaCl/10% (wt/vol) dextran sulfate for 20 min at 42°C. The DNA probe (2 × 10^6 cpm) was added, and the RNA on the membrane was allowed to hybridize with the probe for 12 h at 42°C. The subsequent washing procedure was performed as recommended by the manufacturer of GeneScreen*Plus*.

RESULTS

Preparation of Permeaplasts. During the treatment of intact cells of *Synechocystis* PCC6803 with lysozyme, the rate of the photosynthetic evolution of oxygen in the presence of 1.0 mM $K_3Fe(CN)_6$ increased transiently with a maximum rate at ≈20 min and then it gradually declined to ≈50% of the maximum rate after a 120-min treatment. According to the characteristics of permeaplasts, as defined by Papageorgiou and Lagoyanni (13), the cells that had been treated with lysozyme for 20 min and retained such activity had all been converted to permeaplasts. Moreover, there was no detectable difference between the permeaplasts and intact cells in terms of the level of the *desA* transcript after a decrease in temperature (see below).

Hydrogenation of Permeaplasts. We attempted initially to apply the technique of Pd-catalytic hydrogenation directly to intact cells. In contrast to the hydrogenation of the membrane lipids of intact cells of *Anacystis nidulans* (20), Pd-catalyzed hydrogenation of intact cells of *Synechocystis* PCC6803 appeared to be very inefficient. Accordingly, no changes in fatty acid composition were observed after hydrogenation for 15 min (Fig. 1*A*). However, when the permeaplasts were subjected to the conditions for hydrogenation, the membrane lipids were reduced to an appreciable extent. During hydrogenation for 15 min, the level of stearic acid (18:0) increased from 1% to 10%, whereas levels of linoleic acid (18:2) and γ-linolenic acid (18:3) decreased from 23% to 13% and from 13% to 7%, respectively. The level of oleic acid (18:1) remained rather constant at ≈14%. Stereochemical and positional isomers, such as Δ^9-*trans*-hexadecenoic acid and Δ^{12}-*cis*-hexadecenoic acid, did not exceed 0.3% of the total fatty acids. These findings suggest that the double bonds of unsaturated fatty acids of membrane lipids were reduced by the catalytic hydrogenation of permeaplasts.

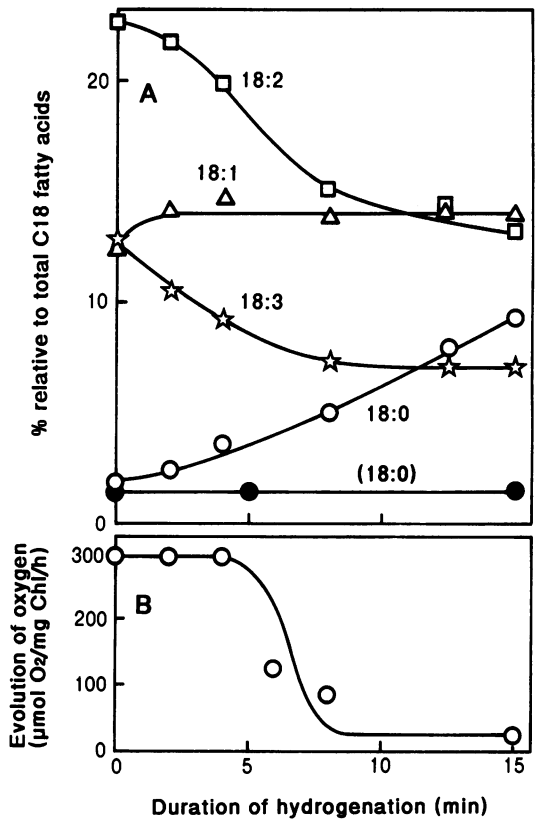

FIG. 1. Changes in fatty acid composition and in photosynthetic activity during Pd-catalyzed hydrogenation of permeaplasts of *Synechocystis* PCC6803. (*A*) Changes in relative levels of fatty acids in permeaplasts and intact cells. ○, 18:0 in permeaplasts; △, 18:1 in permeaplasts; □, 18:2 in permeaplasts; ☆, 18:3 in permeaplasts; ●, 18:0 in intact cells. (*B*) Changes in the rate of photosynthetic evolution of oxygen.

During hydrogenation experiments, the physiological activity, measured in terms of the photosynthetic evolution of oxygen, remained constant for ≈5 min and then began to decline (Fig. 1*B*). The hydrogenation for 4 min did not affect the ability to recover the photosynthetic activity; i.e., when the control permeaplasts and hydrogenated permeaplasts were incubated in the culture medium at 36°C in the light, the oxygen-evolving activity recovered from a level of ≈300 μmol per mg of Chl per h to that of ≈400 μmol per mg of Chl per h with half-recovery times of ≈50 min. Therefore, for most further experiments, hydrogenation for 4 min was adopted to obtain the maximum effect without disturbing the physiological activities of the permeaplasts.

Hydrogenation of Membrane Lipids. Table 1 shows the changes in fatty acid composition of lipids of the plasma membranes and thylakoid membranes of permeaplasts, isolated after a 4-min hydrogenation. It is clear that the hydrogenation of lipids occurred only in the plasma membranes, and no statistically significant changes were observed in the thylakoid membranes. The most marked changes in the plasma membrane were an increase in the level of 18:0 and a decrease in that of 18:2.

Table 2 shows the changes in the fatty acid composition of individual lipid classes upon hydrogenation. In phosphatidylglycerol (PG) the level of 18:0 increased and the level of 18:2 decreased. The saturation of fatty acids in the other lipid classes was insignificant when compared with that in PG. These results together with data from the isolated plasma membranes demonstrate that hydrogenation for 4 min reduced the double bonds of PG in plasma membranes much more significantly than those in the other classes of lipids.

Regulation of Expression of the *desA* Gene. Northern blot hybridization demonstrated that the level of the *desA* transcript was not affected by the presence of either hydrogen or the Pd catalyst when the effects of the presence of each were examined individually. However, when the catalyst was activated by hydrogen before addition to the suspension of permeaplasts, the level of the *desA* transcript reached a maximum after hydrogenation for 4 min and declined to a low level after hydrogenation for 6 min. The decline in the level of the transcript corresponded closely to the timing of the inactivation of photosynthesis (Fig. 1*B*). These observations suggest that the level of the *desA* transcript is enhanced when the extent of the decrease in fluidity of the plasma membrane due to hydrogenation remains within certain limits and that, when the decrease exceeds a threshold level, the level of the transcript is no longer enhanced.

Fig. 2 shows the changes in levels of the *desA* transcript after Pd-catalyzed hydrogenation for 4 min. There was a short lag after the hydrogenation, and then the *desA* transcript increased to reach a maximum level 30 min after the hydrogenation. The lifetime of the *desA* mRNA was determined by following the decay of the mRNA after addition of rifampicin at 15 μg/ml. The half-decay time at 36°C was 2 min and was not affected by hydrogenation for 4 min. These findings suggest that the Pd-catalyzed hydrogenation *in vivo* accelerates the transcription of the *desA* gene without any effect on the rate of degradation of the transcript.

Fig. 3 shows the effects of inhibitors on the level of the *desA* transcript 30 min after the 4-min hydrogenation of permeaplasts. Rifampicin, an inhibitor of transcription, entirely eliminated the hydrogenation-induced accumulation of the *desA* transcript, whereas cerulenin, an inhibitor of fatty acid synthesis, had no effect on the level of transcript.

Table 1. Changes in compositions of fatty acids in plasma and thylakoid membranes upon hydrogenation of permeaplasts

	% of total fatty acids					
Hydrogenation	16:0	16:1	18:0	18:1	18:2	18:3
Plasma membrane						
Control	50.3	1.8	14.6	18.3	10.3	4.7
Hydrogenated	50.6	1.0	19.3	19.5	6.0	3.6
Difference	+0.3	−0.8	+4.7	+1.2	−4.3	−1.1
Thylakoid membrane						
Control	52.0	2.4	1.3	13.0	21.6	9.7
Hydrogenated	52.0	2.5	1.4	13.1	21.6	9.4
Difference	0.0	+0.1	+0.1	+0.1	0.0	−0.3

Permeaplasts derived from cells grown 36°C were hydrogenated for 4 min at 36°C. Then, the plasma membrane and thylakoid membrane were isolated from the control and hydrogenated cells. Data are averages of three experiments. The range of experimental deviations was within 0.5%.

Table 2. Changes in fatty acid composition of individual lipid classes upon the hydrogenation of permeaplasts

	% of total fatty acids					
Class	16:0	16:1	18:0	18:1	18:2	18:3
MGDG (52%)						
Control	52.5	3.1	0.5	10.3	22.2	11.4
Hydrogenated	52.0	3.1	1.9	11.2	20.6	11.3
(Difference)	(−0.5)	(0.0)	(+1.4)	(+0.9)	(−1.6)	(−0.1)
DGDG (13%)						
Control	50.2	3.2	0.9	7.3	21.3	17.1
Hydrogenated	50.4	3.2	1.9	8.6	19.3	16.5
(Difference)	(+0.2)	(0.0)	(+1.0)	(+1.3)	(−2.0)	(−0.6)
SQDG (25%)						
Control	66.1	4.1	1.8	17.6	9.3	0.9
Hydrogenated	66.8	4.1	3.7	16.9	7.5	0.9
(Difference)	(+0.7)	(0.0)	(+1.9)	(−0.7)	(−1.8)	(0.0)
PG (10%)						
Control	51.2	1.0	4.7	20.0	22.1	1.0
Hydrogenated	51.5	1.1	13.6	18.2	14.6	1.0
(Difference)	(+0.3)	(+0.1)	(+8.9)	(−1.8)	(−7.5)	(0.0)

Permeaplasts derived from cells grown at 36°C were hydrogenated for 4 min at 36°C. The percentages in parentheses next to the lipid classes represent the contributions of individual lipids to the total glycerolipids. Data are averages of three experiments. The range of experimental deviations was within 0.3%. MGDG, monogalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; DGDG, digalactosyldiacylglycerol.

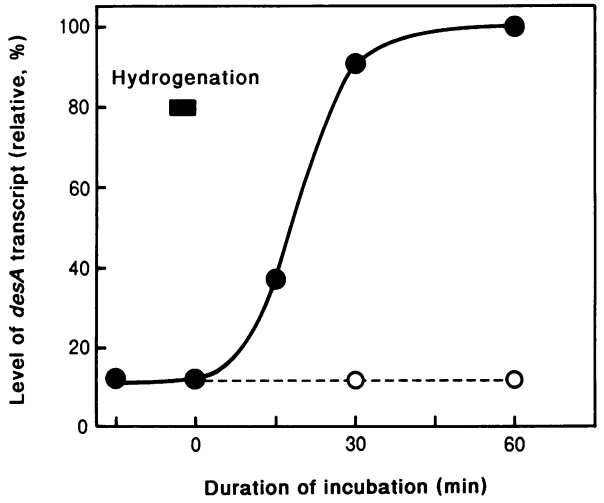

FIG. 2. Changes in the level of the *desA* transcript after hydrogenation. After the permeaplasts had been hydrogenated at 36°C for 4 min as indicated by the bar, they were incubated at 36°C in the light under aerobic conditions for the designated periods of time. The level of the *desA* transcript was determined by Northern blot analysis and is expressed in units relative to the level at 60 min after hydrogenation. ○, Permeaplasts without hydrogenation; ●, permeaplasts hydrogenated for 4 min.

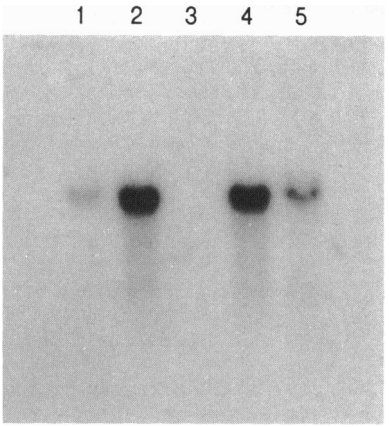

FIG. 3. Effect of inhibitors on the level of the *desA* transcript after hydrogenation. After permeaplasts were hydrogenated at 36°C for 4 min and after inhibitors were added, they were incubated at 36°C in the light for 30 min under aerobic conditions. Total RNA was extracted and subjected to Northern blot analysis. Lanes: 1, without hydrogenation (control); 2, hydrogenation, no addition; 3, hydrogenation, rifampicin at 15 μg/ml; 4, hydrogenation, cerulenin at 10 μg/ml; 5, hydrogenation, diuron at 20 μM.

Diuron, an inhibitor of the photosynthetic electron transport, markedly depressed the level of the *desA* transcript. These effects of inhibitors on the level of the transcript were essentially the same as those observed upon exposure of cells to low temperature (8), indicating a close similarity between the effects of low temperature and the effects of hydrogenation in the regulation of the transcription of the *desA* gene.

The characteristics of the perception of a decrease in temperature were further studied by monitoring increases in levels of the *desA* transcript after specific decreases in temperature (Fig. 4). In control permeaplasts, elevation of the level of the *desA* transcript was evident when the shift in temperature was >5°C. Thus, the response of the organism to a change in temperature occurs only when the change exceeds a certain threshold. This observation is essentially the same as that made with intact cells (8). When similar

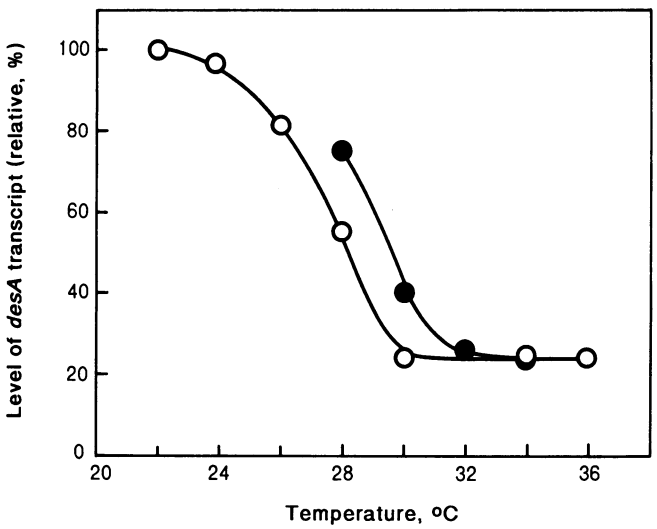

FIG. 4. Temperature profile of the temperature-shift-induced change in the level of the *desA* transcript. Permeaplasts and the hydrogenated permeaplasts prepared from the cells that had been grown at 36°C were incubated in the light for 30 min at designated temperatures. The level of the *desA* transcript was determined by Northern blot analysis and is expressed in units relative to the level in control permeaplasts treated at 22°C. ○, Permeaplasts without hydrogenation; ●, permeaplasts hydrogenated for 2 min.

experiments were performed on partially hydrogenated permeaplasts, the threshold became smaller (Fig. 4). These findings indicate that the effects of a decrease in temperature and Pd-catalyzed hydrogenation are additive and interchangeable in terms of the regulation of the level of the transcript of the *desA* gene.

DISCUSSION

Specific Hydrogenation of Plasma Membranes. Two physiologically important membranes in the cyanobacterial cells are the plasma membrane and the thylakoid membrane; the former is a boundary membrane responsible for controlling the influx and the efflux of ions and metabolites, whereas the latter is the site of energy-related metabolism, such as photosynthesis, respiration, and the synthesis of ATP. In the present study we found that only the lipids in the plasma membrane were saturated after hydrogenation for 4 min. These findings are compatible with the characteristics of the action of the Pd catalyst, as described in reports of a wide range of experiments (21).

Hydrogenation of Individual Classes of Lipids. Cyanobacterial cells contain four major glycerolipids: monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol, and PG (1). In *Synechocystis* PCC6803, monogalactosyldiacylglycerol accounts for half of the total glycerolipids, whereas PG accounts for only 10%. However, the Pd-catalyzed hydrogenation of membrane lipids saturated PG in a very specific manner. The other lipids were also saturated but to a much lower extent. Since hydrogenation occurred only in the plasma membrane, these findings suggest that a large fraction of the PG in the plasma membrane is saturated under the conditions of hydrogenation. Whether the saturation of PG is essential for the regulation of expression of *desA* and for the subsequent desaturation remains an open question. It is also unclear why PG is particularly susceptible to Pd-catalyzed hydrogenation.

Regulation of the Expression of the *desA* Gene by the Fluidity of Membrane Lipid. As demonstrated in our study (8), the level of the *desA* transcript is enhanced with decreases in temperature. Since hydrogenation of the plasma membrane can effectively regulate the expression of the *desA* gene, it is most likely that the low-temperature-induced change in the level of the *desA* transcript is also regulated by the fluidity of the plasma membrane. This possibility suggests that the first signal in the perception of a change in temperature is a change in the fluidity of the plasma membrane in *Synechocystis* PCC6803. This hypothesis is supported by the data in Fig. 4, which demonstrate that the effects of hydrogenation and a decrease in temperature are additive in increasing the level of the *desA* transcript.

It was demonstrated that the mRNA level of stearoyl-CoA desaturase in yeast is depressed by feeding of unsaturated fatty acids (22, 23). Since these fatty acids are incorporated into the membrane lipids, it is likely that the fluidity of membrane lipids regulates the expression of the yeast desaturase. This is compatible with our finding that the decrease in the membrane fluidity stimulates the transcription of the desaturase genes.

Effects of Inhibitors. An inhibitor of prokaryotic transcription, rifampicin, totally blocked any accumulation of the *desA* transcript as a result of hydrogenation. It is of great interest that diuron, an inhibitor of photosynthetic electron transport, depressed the increase in the level of the *desA* transcript caused by hydrogenation. It appears that the energy produced by photosynthesis is necessary for active transcription of the *desA* gene. This inference is consistent with our previous observation that the low-temperature-induced desaturation of membrane lipids occurs in the light but not in the dark (24). The effects of the inhibitors described

herein on the transcription of the *desA* gene that is stimulated by hydrogenation *in vivo* are the same as those found (24) upon low-temperature treatment. This parity again confirms that a common mechanism operates in the regulation of the level of the transcript of the *desA* gene that is induced either by Pd-catalyzed hydrogenation of specific lipids or by a decrease in temperature in *Synechocystis* PCC6803.

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1. Murata, N. & Nishida, I. (1987) in *The Biochemistry of Plants*, ed. Stumpf, P. K. (Academic, New York), Vol. 9, pp. 315–347.
2. Murata, N. (1989) *J. Bioenerg. Biomembr.* **21**, 61–75.
3. Sato, N. & Murata, N. (1981) *Plant Cell Physiol.* **22**, 1043–1050.
4. Russel, N. J. (1984) *Trends Biochem. Sci.* **9**, 108–112.
5. Jaworski, J. G. (1987) in *The Biochemistry of Plants*, ed. Stumpf, P. K. (Academic, New York), Vol. 9, pp. 159–174.
6. Harwood, J. L. (1988) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 101–138.
7. Wada, H., Gombos, Z. & Murata, N. (1990) *Nature (London)* **347**, 200–203.
8. Los, D., Horváth, I., Vigh, L. & Murata, N. (1993) *FEBS Lett.* **318**, 57–60.
9. Vigh, L., Horváth, I. & Thompson, G. A., Jr. (1988) *Biochim. Biophys. Acta* **937**, 42–50.
10. Joó, F., Balogh, N., Horváth, L. I., Filep, G., Horváth, I. & Vigh, L. (1991) *Anal. Biochem.* **194**, 34–40.
11. Stanier, R. Y., Kunisawa, R., Mandel, M. & Cohen-Bazire, G. (1971) *Bacteriol. Rev.* **35**, 171–205.
12. Ono, T. & Murata, N. (1981) *Plant Physiol.* **67**, 176–181.
13. Papageorgiou, G. C. & Lagoyanni, T. (1985) *Biochim. Biophys. Acta* **807**, 230–237.
14. Murata, N. & Omata, T. (1988) *Methods Enzymol.* **167**, 245–251.
15. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917.
16. Sato, N. & Murata, N. (1988) *Methods Enzymol.* **167**, 251–259.
17. Arnon, D. I., McSwain, B. D., Tsujimoto, H. Y. & Wada, K. (1974) *Biochim. Biophys. Acta* **357**, 231–245.
18. Golden, S. S., Brusslan, J. & Haselkorn, R. (1987) *Methods Enzymol.* **153**, 215–231.
19. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1987) *Current Protocols in Molecular Biology* (Wiley, New York).
20. Vigh, L. & Joó, F. (1983) *FEBS Lett.* **162**, 423–427.
21. Quinn, P. J., Joó, F. & Vigh, L. (1989) *Prog. Biophys. Mol. Biol.* **53**, 71–103.
22. Bossie, M. A. & Martin, C. E. (1989) *J. Bacteriol.* **171**, 6409–6413.
23. McDonough, V. M., Stukey, J. E. & Martin, C. E. (1992) *J. Biol. Chem.* **267**, 5931–5936.
24. Wada, H. & Murata, N. (1990) *Plant Physiol.* **92**, 1062–1069.