Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 4209–4214, April 1998
Biochemistry

Light-induced expression of fatty acid desaturase genes

(gene expression/light regulation/cyanobacterium)

MIHÁLY KIS*, OTTO ZSIROS*, TIBOR FARKAS†, HAJIME WADA‡, FERENC NAGY*, AND ZOLTÁN GOMBOS*§

*Institute of Plant Biology, and †Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary; and ‡Faculty of Science, Kyushu University, Ropponmatsu Fukuoka 810, Japan

Contributed by Tibor Farkas, February 9, 1998

ABSTRACT **In cyanobacterial cells, fatty acid desaturation is one of the crucial steps in the acclimation processes to low-temperature conditions. The expression of all the four acyl lipid desaturase genes of *Synechocystis* PCC 6803 was studied as a function of temperature and separately as a function of light. We used cells grown at 25°C in light-activated heterotrophic growth conditions. In these cells, the production of α-linolenic acid and 18:4 fatty acids was negligible and the synthesis of γ-linolenic acid was remarkably suppressed compared with those of the cells grown photoautotrophically. The cells grown in the light in the presence of glucose showed no difference in fatty acid composition compared with cells grown photoautotrophically. The level of *desC* mRNA for Δ9 desaturase was not affected by either the temperature or the light. It was constitutively expressed at 25°C with and without illumination. The level of *desB* transcripts was negligible in the dark-grown cells and was enhanced about 10-fold by exposure of the cells to light. The maximum level of expression occurred within 15 min. The level of *desA* and *desD* mRNAs was higher in dark-grown cells than that of *desB* mRNA for ω3 desaturase. However, the induction of both *desA* and *desD* mRNAs for Δ12 and Δ6 desaturases, respectively, was enhanced by light about 10-fold. Rifampicin, chloramphenicol, and 3-(3,4-dichlorophenyl)-1,1-dimethylurea completely blocked the induction of the expression of *desA*, *desB*, and *desD*. Consequently, we suggest the regulatory role of light via photosynthetic processes in the induction of the expression of acyl lipid desaturases.**

The physical and biochemical characteristics of membrane lipids depend on the unsaturation level of their fatty acids, whereas the physical state (i.e., phase) of lipids of biological membranes plays an important role in the physiological function of membranes (1). For proper functioning, the membrane constituents require the presence of liquid crystalline state in which rotational transmembrane movements of lipid and protein molecules embedded in the membrane are possible. Accordingly, it has been demonstrated in model membranes that (*i*) the temperature of phase transition between liquid crystalline and solid states depends on the saturation level of membrane glycerolipids (2), (*ii*) the higher the degree of unsaturation of glycerolipids, the lower the phase transition temperature (3), and (*iii*) physiological activities change drastically at phase transition temperature (4, 5). In photosynthetic membranes, phase behavior of glycerolipids is also regulated by the level of unsaturation, a process mediated by the activity of fatty acid desaturases (6) that introduce double bonds directly into fatty acids of glycerolipids. It has been shown that the level of activity of these enzymes indeed plays a key role in regulating responses to changes in ambient temperature, such as tolerance to low-temperature stress, by modulating the temperature of phase transition of the membrane (7, 8).

The cyanobacterium strain *Synechocystis* PCC 6803 is amenable to transformation and has been used extensively as a model for the chloroplasts of higher plants (6). The photosynthetic (chloroplast) membranes of higher plants or cyanobacteria contain high levels of linolenic glycerolipids, suggesting that phase transition of these membranes occurs far below room temperature. However, the photosynthetic membranes of cyanobacteria contain proportionally higher amounts of proteins (9) and this higher protein content results in a higher phase-transition temperature of lipids (10). In addition, Wada *et al.* (11) found that the temperature of the phase transition of cytoplasmic membranes was lower than that of the thylakoid membranes.

Various mechanisms have been suggested for the regulation of the unsaturation of the fatty acids of membrane lipids. Murata and Wada (1) proposed that the crucial steps in the regulation of the unsaturation of membrane glycerolipids is the *de novo* synthesis of acyl lipid desaturases in cyanobacterial cells. In this context, it should be noted that the *Synechocystis* PCC 6803 cyanobacterium strain contains four genes, *desA* (12), *desB* (8), *desC* (13), and *desD* (14), encoding acyl lipid desaturases that introduce double bonds specifically at Δ12, ω3, Δ9, and Δ6 positions of C_{18} fatty acids, respectively. Furthermore, it was also demonstrated that the mRNA levels for the Δ12 and ω3 desaturases increased when autotrophically grown *Synechocystis* PCC 6803 cells were transferred from 36°C to 22°C (1, 8), whereas Sakamoto and Bryant (15) showed that in *Synechococcus* PCC 7002 cells grown at 38°C and 22°C, the *desC* gene was expressed at the same level but the accumulation of the *desA* and the *desB* gene transcripts was higher at the lower temperature. An alternative explanation for the elevated level of unsaturated fatty acids at low temperature may be that the desaturase activities may have negative temperature coefficients so the desaturases can be more active at lower temperatures (1).

However, it is interesting to note that, independently of temperature, the expression of the *FAD7* gene encoding an ω3-fatty acid desaturase was proposed to be light responsive in *Arabidopsis* (16). The cooperative effect of light and temperature is an intensively studied phenomenon and it has been shown that higher plants, as well as cyanobacteria, exhibit higher susceptibility to high light exposure at low temperature (17, 18). Moreover, metabolic processes involved in temperature acclimation need large amounts of energy (19–21); therefore, it has been postulated that the photosynthetic apparatus is the machinery that can provide the necessary background for this energy requirement.

In the present work, we studied the effects of light and light-driven photosynthetic processes on the regulation of the expression of genes encoding acyl lipid desaturases. We show

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

§To whom reprint requests should be addressed at: Biological Research Center of the Hungarian Academy of Sciences, P.O. Box 521, H-6701 Szeged, Hungary. e-mail: gombos@everx.szbk.u-szeged.hu.

that the expression of some of these genes, notably that of the *desA*, *desB*, and *desD*, is regulated by light and the reduction state of the cell.

MATERIALS AND METHODS

Cell Culture. A glucose-tolerant strain of *Synechocystis* PCC 6803 was grown photoautotrophically in BG-11 medium as described (22). For light-activated photoheterotrophic growth, the cells were cultivated in BG-11 medium supplemented with 5 mM glucose (23). Liquid cultures (100 ml in 200-ml Erlenmeyer flasks) were maintained in a temperature- and light-regulated box. One 10-min white-light pulse was given per day at an intensity of 40 μmol of photon per m^2 per sec. The cells were grown at 25°C. The growth of liquid cultures was monitored by measuring the optical density at 750 nm (OD_{750}) in a Shimadzu UV/visible light spectrophotometer. For light induction experiments, the cells grown in the dark were exposed to light at the intensity of 70 μmol of photon per m^2 per sec. Fifty-milliliter samples were taken at indicated times of incubation. When indicated, rifampicin (200 μg/ml) and chloramphenicol (40 μg/ml) were added to the cultures and immediately exposed to light. For 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) treatment, DCMU was added to the concentration of 10 μM. The cells were preincubated for 1 min in darkness before the light exposure. In temperature-shift experiments, cells were grown photoautotrophically at 35°C and then incubated at 25°C for 120 min before they were returned to 35°C. Sampling was as described above. Growth of liquid cultures was monitored by measuring the optical density at 750 nm (OD_{750}) in a Shimadzu UV/visible light spectrophotometer.

Analysis of Lipids. Lipids were extracted from the cells by the method of Bligh and Dyer (24). The fatty acid composition was analyzed as described by Wada and Murata (23).

RNA Isolation and Northern Blot Hybridization. Total RNA was isolated according to Nagy *et al*. (25) with the following modification. To prevent RNA degradation before disrupture of cells, aliquots of cultures grown under various conditions were mixed with equal volumes of ice-cold ethanol containing 5% phenol and collected by centrifugation as described by Sato (26). Expression level of the various desaturase genes was determined by Northern blot analysis as follows. Five-microgram total RNA samples were separated by gel electrophoresis on 1.2% agarose gel containing formaldehyde, then blotted, and fixed by UV light treatment onto Hybond-N nylon membranes (Amersham) according to the manufacturer's instructions. Filters were prehybridized for 3 h and then hybridized with radioactively labeled probes in Church buffer (27) at 65°C for 16 h. After washing, the hybridizing RNA species were visualized by autoradiography. The probes used in these Northern blot analysis were prepared as follows. The coding regions of the four desaturase genes and a fragment of 16S rRNA were amplified by PCR using specific synthetic oligonucleotid primers. Isolated DNA fragments were labeled with [α-^{32}P]dCTP by using Megaprime DNA labeling system (Amersham). The size of the hybridizing bands was determined by using RNA molecular weight marker I (Boehringer Mannheim). Northern blots were sequentially probed, stripped, and reprobed. The expression level of the desaturase genes was always normalized to that of the 16S rRNA gene. Quantification was done with a PhosphoImager (type 445SI: software, IMAGE QUANT 4.1) manufactured by Molecular Dynamics.

Computer-Generated Images. A Hewlett–Packard ScanJet IIcx scanner was used to generate graphic images from autoradiograms. The files were transferred to CORELDRAW 4.00 for lettering and printed on an Epson Stylus Color printer.

RESULTS

Fatty Acid Composition. Table 1 shows the fatty acid composition of total glycerolipids isolated from the cells of *Synechocystis* PCC 6803 grown under photoautotrophic and heterotrophic (dark) conditions at 25°C. In both types of cultures, the most abundant fatty acid was saturated 16:0 (52 and 55%, respectively), most of which is joined by an ester bond to the *sn*-2 position of the glycerol backbone (1), and the level of this fatty acid was unaffected by growth conditions. However, a 2-fold increase in the accumulation of monounsaturated fatty acids was detected on the expense of polyunsaturated fatty acid in dark-grown cultures. In these cells, the level of 18:1 fatty acid increased to 17% as compared with 7% in photoautotrophically grown cells and became the major component of the monounsaturated fatty acids. By contrast, the accumulation level of the polyunsaturated fatty acids including the α-18:3 and 18:4 fatty acid produced by desaturation at the ω3 position of linoleic acid and that of the γ-18:3, respectively, decreased characteristically. Accordingly, in heterotrophic cultures grown in the dark the level of γ-18:3 was significantly lower (13% vs. 17%) and accumulation levels of α-18:3 and 18:4 were reduced below detection limits.

Changes in Fatty Acid Composition in the Light. To monitor the effect of light on the pattern and level of fatty acid unsaturation, heterotrophic dark-grown cells were exposed to light and the changes in the level of polyunsaturated fatty acids were followed by gas chromatography. Results of these experiments are shown in Table 2. We found that exposure of dark-grown cells to light did not effect the levels of 16:0, 16:1, 18:0, or 18:2 fatty acids but led to characteristic changes in the accumulation of the monounsaturated 18:1 and polyunsaturated γ-18:3, α-18:3, and 18:4 fatty acids. A 6-h incubation of dark-grown cells in the light decreased the monounsaturated fatty acid (18:1) content of the cells from 17% to 13% and prolonged light exposure (48 or 72 h) lowered this value to 7%. By contrast, simultaneously with the decrease in 18:1 monounsaturated fatty acid content, levels of polyunsaturated fatty acids increased gradually. Table 2 shows that detectable amounts of α-18:3 and 18:4 fatty acids were synthesized after a 6-h incubation of the cells in the light. Levels of these polyunsaturated fatty acids increased continuously in light and reached their maximum after 48 h of illumination.

To determine the possible effect of light intensity rather than the duration of illumination on the accumulation of fatty acids, *Synechocystis* PCC 6803 cells were grown in a wide range of light intensities, i.e., normal light (70 μmol of photon per m^2 per sec), moderately strong light (500 μmol of photon per m^2 per sec), and strong light (2,000 μmol of photon per m^2 per sec^1). We found that the level of fatty acid unsaturation was not affected by light intensity (data not shown).

Table 1. Fatty acid composition of the total glycerolipids from cells of *Synechocystis* PCC 6803 grown photoautotrophically (PA) or light-activated heterotrophically (LAH)

	% total fatty acids	
Fatty acid	PA	LAH
16:0	52	55
16:1	4	5
18:0	1	1
18:1	7	17
18:2	10	9
γ18:3	17	13
α18:3	5	Tr
18:4	4	Tr

Cells were grown at 25°C. Tr, trace amount (<0.5%). Experiments were repeated three times and deviations were within ±2% of individual values.

Table 2. Time course of the changes in fatty acid composition after the illumination of dark-grown cells at a light intensity of 40 μmol per m^2 per sec

	Fatty acid composition, rel. %							
Time, h	16:0	16:1	18:0	18:1	18:2	γ18:3	α18:3	18:4
0	55	5	1	17	9	13	Tr	Tr
6	53	5	1	13	9	14	2	1
24	53	4	1	10	8	17	4	2
48	53	5	1	7	9	17	6	3
72	52	4	1	7	10	17	5	3

Cells were grown and incubated at 23°C. Rel., relative. Tr, Trace amount (<0.5%). Experiments were repeated three times and deviations were within ±2% of individual values.

Changes in the Level of the mRNAs for Desaturases. To characterize the light regulation of the expression of the four desaturase genes *desA, desB, desC*, and *desD,* we determined the steady-state mRNA levels for these genes in *Synechocystis* PCC 6803 cells grown at 25°C in the dark and subsequently exposed to light. To this end, total RNAs were prepared from samples taken at indicated times and subjected to Northern blot analysis.

Fig. 1*A* shows that the probes used are specific for *desA, desB*, and *desD* genes and detected single bands corresponding to 1.2-, 1.4-, and 1.4-kb transcripts, respectively. We note that for *desC* mRNA two hybridizing bands of 1.1 and 1.7 kb appear. The primary mRNA product might be cleaved post-transcriptionally. Notwithstanding, Fig. 1*B* shows that the *desC* gene is expressed constitutively, and the mRNA levels of the other three enzymes increase rapidly and dramatically in dark-grown cells shifted to light. The accumulation of the latter three transcripts was already evident after only 10 min, and these increased levels were maintained when the duration of the light treatment was extended to 120 min. Fig. 1*B* also illustrates that these increased levels of *desA* and *desD* transcripts declined rapidly, within 15 min, in cells transferred back to dark, but the reduction in the level of *desB* mRNA was slower and required longer time. We compared this light-shift-induced increase of desaturase gene transcripts to that induced by temperature shift, as described (8, 28). To this end, *Synechocystis* PCC 6803 cells were grown autotrophically at 35°C, then incubated at 25°C for 120 min, and subsequently shifted back to 35°C; total RNA isolated from these cells was analyzed by Northern blot hybridization as described above. Results shown in Fig. 2*A* illustrate that the pattern and level of transcript accumulation for the four desaturase genes were nearly identical to those shown in Fig. 1*A*. Again, the expression of *desC* was stable, whereas low basic levels of the other three desaturase gene transcripts increased rapidly upon exposure to low temperature and decreased again to low levels in cells shifted back to high temperature (Fig. 2*B*). Therefore, we conclude that transcript levels of desaturase genes are modulated similarly by temperature shifts in autotrophic cells and by light activation in dark-grown heterotrophic cells.

Fig. 3 demonstrates the induction of desaturase genes when the *Synechocystis* PCC 6803 cells grown at 35°C photoheterotrophically were transferred to 25°C in the dark. The Northern blot analysis shows that the transcription level of the fatty acid desaturase genes was not induced. The low-temperature induction of desaturase genes was blocked in the dark.

To further characterize the possible signal transduction pathways involved in regulating the expression of desaturase genes, we performed two lines of experiments. To determine whether light-activated transcription of the *desA*, *desB*, and *desD* genes is affected by photosynthetic electron transport and the redox state of intermediate carriers, we preincubated dark-grown cultures with DCMU before light exposure. Fig. 4 shows that preincubation of cells with DCMU completely inhibits accumulation of transcripts for *desA*, *desB*, and *desD* genes. To test whether the light-induced increase of these

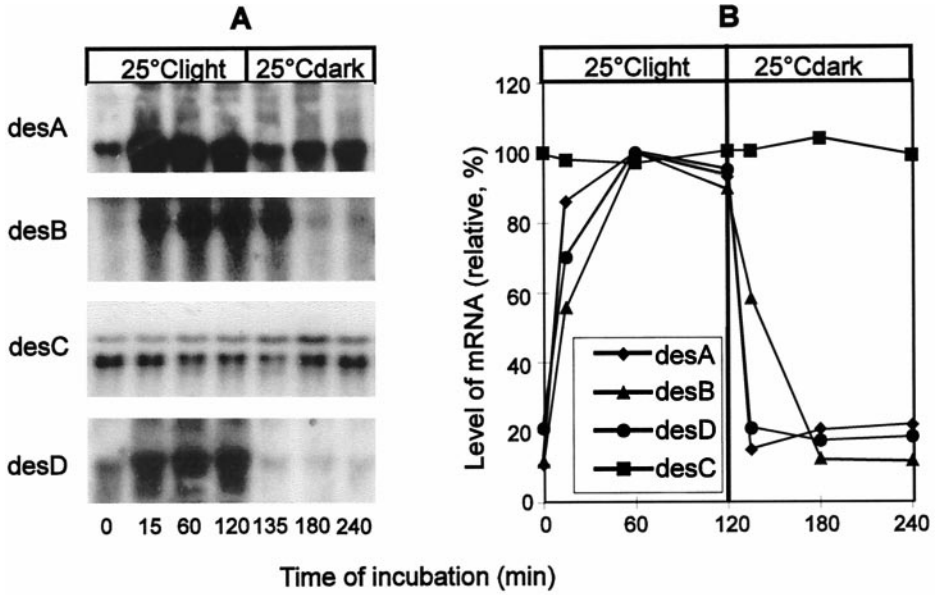

FIG. 1. Expression level of *Synechocystis* PCC 6803 desaturase genes is regulated differentially by light. Cells were grown heterotrophically in the dark at 25°C, then transferred to light, incubated for 120 min, and subsequently transferred back to dark for an additional 120 min. The first sample (0 min) was taken immediately after transferring the culture from dark to light and additional samples were collected as indicated. Each lane contains 10 μg of total RNA. (*A*) mRNA levels of the four desaturase genes were determined by Northern blot analyses. (*B*) Relative changes of mRNA levels are shown in percentage and were obtained after quantification with a PhosphorImager.

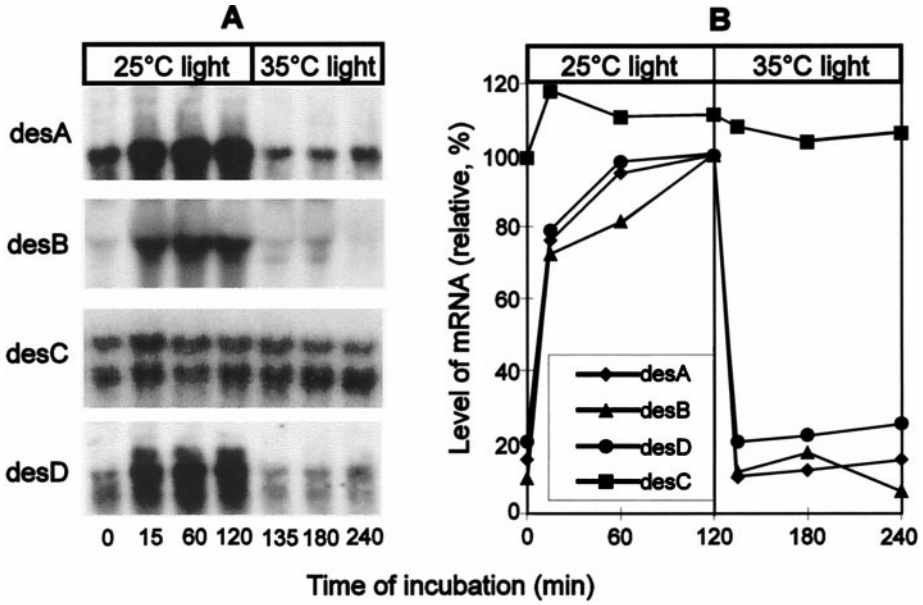

FIG. 2. Expression level of *Synechocystis* PCC 6803 desaturase genes is regulated differentially by changes in the ambient temperature. Cells were grown autotrophically at 35°C, then transferred to 25°C for 120 min, and subsequently transferred back to 35°C for an additional 120 min. The first sample (0 min) was taken immediately after transferring the culture from 35°C to 25°C, and additional samples were collected as indicated. Each lane contains 10 μg of total RNA. (*A*) mRNA levels of the four desaturase genes were determined by Northern blot analysis. (*B*) Relative changes of mRNA levels are shown in percentage and were obtained after quantification with a PhosphoImager.

transcripts requires *de novo* synthesis of macromolecules, a light-shift experiment was performed in the presence of bacterial transcription and translation inhibitors, rifampicin and chloramphenicol, respectively. Fig. 4 shows that similarly to DCMU, both of these inhibitors completely blocked the light-induced increases in the desaturase transcript levels.

DISCUSSION

In this study we provide evidence that in *Synechocystis* PCC 6803 cells altered expression levels of genes encoding acyl lipid desaturases and specific changes in the fatty acid composition of membranes, resembling modulations caused by exposure to low temperature, can be brought about by light treatment independent of changes in the ambient temperature of the environment. Vigh *et al.* (29) proposed that a specific sensor of low temperature located in the cytoplasmic membrane was involved in signaling and in the regulation of desaturase genes. Nevertheless, the mechanism by which low-temperature signals are perceived and transduced into specific biochemical responses has remained largely unknown. By using heterotrophically grown *Synechocystis* PCC 6803 cells, we were able to investigate the effect of light exposure separately from the

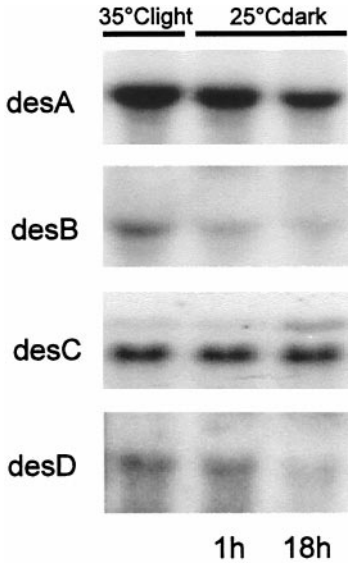

FIG. 3. Effect of temperature down-shift in the dark on the mRNA level of the desaturase genes in *Synechocystis* PCC6803 cells as demonstrated by Northern blot analyses. Cells were grown photoheterotrophically at 35°C under light and then incubated in the dark at 25°C. The first sample (0 min) was taken immediately after transferring the culture from 35°C to 25°C and additional samples were collected as indicated. Each lane contains 10 μg of total RNA.

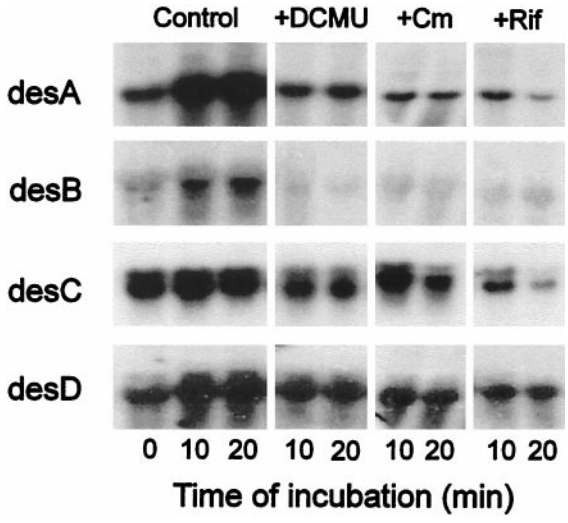

FIG. 4. Effects of transcription (Rif), translation (Cm), and photosynthesis (DCMU) inhibitors on the expression of desaturase genes. mRNA levels were determined by Northern blot analysis. Each lane contains 5 μg of total RNA. Cells were grown heterotrophically at 25°C in dark and then transferred to light in the absence (control) or presence (+) of the inhibitors. When DCMU was used, the cells were preincubated for 1 min in darkness. The first sample (0 min) was taken immediately before transferring the cultures to light, and additional samples were collected as indicated. Rif, rifampicin, 200 μg/ml; Cm, chloramphenicol, 40 μg/ml; DCMU, 10 μM.

effect of temperature on fatty acid desaturation, which is one of the most important processes in cold acclimation. As a result of the data obtained, we postulate that the initial signaling of low-temperature stress might be regulated by factors dependent on the metabolic and energy state rather than a specialized temperature sensor in the membrane or by a combination of these two. Furthermore, we propose that any factor that chronically affects photosynthesis may ultimately influence the induction of adaptation to an environmental stress such as low temperature. This hypothesis is in good agreement with previous observations indicating that (*i*) the biochemical efficiency of the cells is largely reduced and (*ii*) the photosynthetic activity, the ultimate energy source, is strongly decreased by low temperature and shows a strong temperature susceptibility in cyanobacteria, similar to other photosynthetic organisms (30). The mechanism by which organisms compensate for this reduction in photosynthetic activity is apparently the ability of cells to manipulate the glycerolipid unsaturation level of their thylakoid membranes. It has been shown that the photosystem II excitation pressure that can be affected by the level of glycerolipid unsaturation (31) can be modulated by changing the temperature. The adaptation to low temperature manifests itself in elevated levels of unsaturated fatty acids (32). In the dark, where the photosynthetic activity of the cells is suppressed, this adaptation process is blocked and fatty acid desaturase activity is reduced. Therefore, in the absence of the proper signaling mechanism for inducing desaturation, we consistently found that the cells grown in the dark at 25°C possessed a desaturation pattern similar to that of the cells grown photoautotrophically at 35°C rather than to that of the cells grown photoautotrophically at 25°C (33). In contrast, shifting of dark-grown cells to light under isothermal conditions increases both photosynthetic activity and desaturation, and these cells exhibit desaturation patterns reminiscent of that detected in cells grown photoautotrophically but shifted from 35°C to 25°C. Although these data strongly suggest that the light-driven photosynthesis is the primary process that regulates desaturation of photosynthetic membranes, most likely via photosynthesis-derived factors, this observation does not fit the theory of homeoviscous adaptation of membranes (34).

Beside the effect on the desaturation pattern of fatty acids, we also determined the effect of light treatments on the expression level of the four desaturase genes of *Synechocystis* PCC 6803. In good agreement with the desaturation patterns observed, we found that the expression of the *desC* gene (responsible for desaturation at the Δ9 position) was not significantly affected by light treatments. However, transcript levels of *desA, desB,* and *desD* genes (introducing double bonds at positions Δ12, ω3, and Δ6, respectively) were strongly induced by light (Fig. 5). Moreover, we found that the expression patterns of these desaturase genes, including the degree of accumulation, the rapidity of transcript accumulation, and the decrease during the recovery period, are nearly identical with those detected in cells exposed to low temperature reported in this article and in *Synechocystis* PCC 6803 (33) and

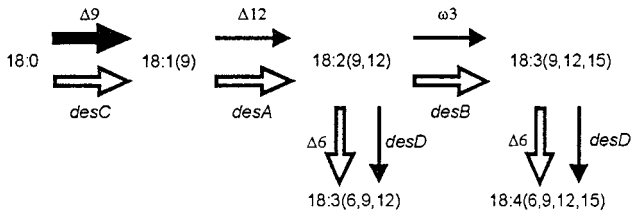

FIG. 5. Scheme for the expression of fatty acid desaturase genes in *Synechocystis* PCC 6803 cells at 25°C in the light (open arrows) and in the dark (solid arrows). The difference in width of the arrows represents the activity of the expression of the individual genes.

Synechococcus 7002 (15) cells. Maximum induction of the mRNAs of acyl lipid desaturases occurred relatively faster than the appearance of polyunsaturated glycerolipids containing a double bond at the ω3 position. The cells can apparently survive a short period at low temperature without a perfect acclimation in case of an extended time spent at reduced temperatures; however, cells need to adjust the glycerolipid composition of their membranes. This newly constructed membrane structure can provide an optimum background for important processes restricted to membranes such as photosynthesis. We note that in *Arabidopsis thaliana* the expression of ω3 fatty acid desaturase was proposed to be light responsive. The expression of the *FAD7* gene was studied and shown to be regulated by light (16). However, in this report we compare the expression patterns of desaturase genes induced by light and low temperature in the same organism *Synechocystis* PCC 6803. Thus, these results suggest that the desaturation level of photosynthetic membranes is primarily regulated via modulated expression of desaturases by a light-dependent signaling pathway rather than by changes in the ambient temperature of the environment.

To gain more information about this pathway, we showed that light-induced mRNA accumulation for these desaturase genes can be prevented by inhibitors of transcription, translation, or photosynthesis. These observations clearly suggest that light-induced expression of desaturase genes (*i*) requires *de novo* protein synthesis, (*ii*) is likely to be regulated at the level of transcription, and (*iii*) is mediated by a signaling pathway that includes factors generated by photosynthetic electron transport. We also demonstrated that the light-induced fatty acid desaturation of *Synechocystis* PCC 6803 does not depend on the intensity of light exposure as reported by Sakamoto and Bryant (15) in *Synechococcus* PCC 7002, and we provided evidence that in *Synechocystis* PCC 6803 light induces specific changes in the expression of desaturase genes and alterations in the fatty acid composition. These data could make feasible the isolation of cis/trans-acting elements and other components of this signaling pathway mediating and/or mutants impaired in these responses.

We are indebted to Drs. Miklós Szekeres and Erzsébet Fejes, Institute of Plant Biology Biological Research Center, Szeged, Hungary, for their helpful discussion and correcting the manuscript. We also thank Erika Zukic for her excellent technical assistance. This work was supported, in part, by grants from the Hungarian Science Foundation (OTKA; Grants T 020293 and F 023794) to Z.G. and O.Z. It was also supported, in part, by OTKA Grants T 016167 and T 025804, and Howard Hughes HHMI Grant 75195–542401 to F.N.

1. Murata, N. & Wada, H. (1995) *Biochem. J.* **308,** 1–8.
2. Coolbear, K. P., Berde, C. B. & Keough, K. M. W. (1983) *Biochemistry* **22,** 1466–1473.
3. Chapman, D. (1975) *Q. Rev. Biophys.* **8,** 185–235.
4. Murata, N. (1989) *J. Bioenerg. Biomembr.* **21,** 61–75.
5. Omata, T. & Murata, N. (1985) *Biochim. Biophys. Acta* **810,** 354–361.
6. Nishida, I. & Murata, N. (1996) *Annu. Rev. Plant Mol. Biol.* **47,** 541–568.
7. Los, D. A., Horvath, I., Vigh, L. & Murata, N. (1993) *FEBS Lett.* **318,** 57–60.
8. Sakamoto, T., Los, D. A., Higashi, S., Wada, H., Nishida, I., Ohmori, M. & Murata, N. (1993) *Plant Mol. Biol.* **26,** 249–263.
9. Omata, T. & Murata, N. (1983) *Plant Cell Physiol.* **24,** 1101–1112.
10. Ono, T. & Murata, N. (1982) *Plant Physiol.* **69,** 125–129.
11. Wada, H., Hirasawa, R., Omata, T. & Murata, N. (1984) *Plant Cell Physiol.* **25,** 909–911.
12. Wada, H., Gombos, Z. & Murata, N. (1990) *Nature (London)* **347,** 200–203.
13. Sakamoto, T., Wada, H., Nishida, I., Ohmori, M. & Murata, N. (1994) *J. Biol. Chem.* **269,** 25576–25580.
14. Reddy, A. S., Nuccio, M. I., Gross, L. M. & Thomas, T. L. (1993) *Plant Mol. Biol.* **27,** 283–300.

15. Sakamoto, T. & Bryant, D. A. (1997) *Mol. Microbiol.* **23,** 1281–1292.
16. Nishiuchi, T., Nakamura, T., Abe, T., Kodama, H, Nishimura, M. & Iba, K. (1995) *Plant Mol. Biol.* **29,** 599–609.
17. Gray, G. R., Chauvin, L.-P., Sarhan, F. & Huner, N. P. A. (1997) *Plant Physiol.* **114,** 467–474.
18. Gombos, Z., Wada, H. & Murata, N. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8787–8791.
19. Melis, A., Mandori, A., Glick, R. E., Ghirardi, M. L., McCanley, S. W. & Neale, P. J. (1985) *Physiol. Veg.* **23,** 757–765.
20. Anderson, J. M., Chow, W. S. & Park, Y.-I. (1995) *Photosynth. Res.* **46,** 129–139.
21. Huner, N. P. A., Maxwell, D. P., Gray, G. R., Savitch, L. V., Krol, M., Ivanov, A. G. & Falk, S. (1996) *Physiol. Plant* **98,** 358–364.
22. Wada, H. & Murata, N. (1989) *Plant Cell Physiol.* **30,** 971–978.
23. Anderson, S. L. & McIntosh, L. (1991) *J. Bacteriol.* **173,** 2761–2767.
24. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37,** 911–917.
25. Nagy, F., Kay, S. A., Chua, N.-H. (1988) in *Plant Gene Research Manual*, eds. Gelvin, S. B. & Schilperoort, A. R. (Kluwer, Dordrecht, the Netherlands), pp. 1–29.
26. Sato, N. (1995) *Nucleic Acids Res.* **23,** 2161–2167.
27. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
28. Wada, H., Avelange-Macharel, M.-H. & Murata, N. (1993) *J. Bacteriol.* **175,** 6056–6058.
29. Vigh, L., Los, D. A., Horvath, I. & Murata, N. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9090–9094.
30. Gombos, Z., Wada, H. & Murata, N. (1991) *Plant Cell Physiol.* **32,** 205–211.
31. Ono, T. & Murata, N. (1981) *Plant Physiol.* **67,** 176–181.
32. Sato, N. & Murata, N. (1980) *Biochim. Biophys. Acta* **619,** 353–366.
33. Wada, H. & Murata, N. (1990) *Plant Physiol.* **92,** 1062–1069.
34. Cossins, A. R. (1994) *Temperature Adaptation of Biological Membranes*, ed. Cossins, A. R. (Portland, London), pp. 73–76.