Biochimica et Biophysica Acta 1817 (2012) 1352–1359

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Light-dependent cold-induced fatty acid unsaturation, changes in membrane fluidity, and alterations in gene expression in *Synechocystis* ☆

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ARTICLE INFO

Article history:
Received 20 September 2011
Received in revised form 15 December 2011
Accepted 18 December 2011
Available online 27 December 2011

Keywords:
Synechocystis
Cold stress
Gene expression
Fatty acid desaturase
Membrane fluidity
Unsaturated fatty acid

ABSTRACT

Cold stress causes unsaturation of the membrane lipids. This leads to adjustment of the membrane fluidity, which is necessary for cold acclimation of cells. Here we demonstrate that the cold-induced accumulation of PUFAs in the cyanobacterium *Synechocystis* is light-dependent. The *desA*$^-$/*desD*$^-$ mutant, that lacks the genes for Δ12 and Δ6 desaturases, is still able to adjust the fluidity of its membranes in spite of its inability to synthesize PUFAs and modulate the fatty acid composition of the membrane lipids under cold stress. The expression of cold-induced genes, which are controlled by the cold sensor histidine kinase Hik33, depends on the fluidity of cell membranes and it is regulated by light, though it does not require the activity of the photosynthetic apparatus. The expression of cold-induced genes, which are 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. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

1. Introduction

Poikilothermic organisms desaturate fatty acids of their membrane lipids in response to a decrease in the ambient temperature [1,2]. Insertion of the additional double bonds into the acyl chains of membrane lipids by fatty acid desaturases compensates for a decrease in membrane fluidity caused by a drop in temperature. It was suggested that the process of lipid unsaturation is the major contributor into adjustment of the cold-dependent acclimation of biological membranes, and that the fatty acid desaturases are the key enzymes that participate in the acclimation of cells to cold environment [1–3].

A decade ago, the cold sensor histidine kinase Hik33 had been identified in the mesophilic unicellular freshwater cyanobacterium *Synechocystis* sp. PCC 6803 [4,5]. The analysis of genome-wide gene expression with DNA microarrays in *Synechocystis* revealed about 100 genes that are highly induced by cold [6–8]. Only a part of these genes is controlled by the Hik33 and may be induced due to the changes in membrane fluidity. Another part of cold-inducible genes is regulated by some other cold-sensing system that differs from the Hik33.

Earlier, we demonstrated a feedback between the physical state of the cytoplasmic membrane of *Synechocystis* and the expression of the *desA* gene for the Δ12 fatty acid desaturase by partial catalytic hydrogenation of the acyl chains of the plasma membrane lipids. The latter was induced both by cold and by hydrogenation of double bonds in fatty acyl chains at optimal growth temperature [9]. Recently, we showed similar feedback between the membrane fluidity and the expression of the *desB* gene for the ω3 desaturase [10].

On the other hand, one report stated that the cold-induced expression of the desaturase genes, namely *desA*, *desD*, and *desB* (encode Δ12, Δ6, and ω3 acyl-lipid fatty acid desaturases) in *Synechocystis* is light-dependent and their cold-inducibility may be unrelated to cold-induced changes in membrane fluidity [11].

Here we present the evidence that transcription of a set of cold-induced genes, which are controlled by the sensory histidine kinase Hik33, depends on membrane fluidity and it is regulated in a light-dependent manner. Transcription of another set of cold-inducible Hik33-independent genes does not depend on membrane fluidity and light.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPH, 1,6-diphenyl-1,3,5-hexatriene; FA, fatty acid; PAGE, polyacrylamide gel electrophoresis; PAS domain, the domain that contains PER-ARNT-SIM, (PAS) and phytochrome amino acid features; PUFAs, polyunsaturated fatty acids.

☆ This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

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doi:10.1016/j.bbabio.2011.12.011

2. Materials and methods

2.1. Strains and growth conditions

A glucose-tolerant (GT) strain of *Synechocystis* sp. PCC 6803 was originally obtained from Dr. J.G.K. Williams (Du Pont de Nemours, Wilmington, DE, USA). Cells were cultured at 32 °C in BG11 medium [12] under continuous illumination with light from incandescent lamps at 70 μmol photons m^{-2} s^{-1} and were aerated with air that contained 1% CO_2.

The *desA*$^-$/*desD*$^-$ double mutant strain of *Synechocystis* was constructed as described before [10]. In this mutant, the use of chloramphenicol-resistance gene cassette was avoided, since the latter antibiotic may interfere with photosynthetic electron transport [13].

2.2. Fatty acid analysis

Methyl esters of FAs of the total membrane lipids of *Synechocystis* were obtained by the direct etherification of cells in a mixture of methanol and acetylchloride (10:1, v/v) for 60 min at 80 °C [14]. Methyl esters of FAs have been purified by separation on the Silufol plates (20×20 cm) with a mixture *n*-hexane:diethyl ester (90:10, v/v), visualized under UV light at 254 nm after spraying with a solution of a 0.01% 2′7′-dichlorfluoresceine in ethanol, and eluted with hexane [15]. Fatty acid composition was analyzed by the GS–MS with the Agilent 7890A GC (Agilent Technologies, Inc., США) equipped with the 60 meter capillary column with the diameter of 0.25 mm (DB-23, Ser. US8897617H). The column was packed with the 50% cyanopropylphenyl polysiloxane (0.25 μm film thickness). Methyl esters (10 μg per probe) have been separated under the helium pressure of 245 kPa at 260 °C as described before. The individual peaks were indentified with the program MSD Chem Station G1701EA E.02.00.493 and NIST spectrum library. Unsaturation index was calculated according to Novo and Fonseca [16].

2.3. Isolation of cell membranes

Membrane fractions have been isolated in the two-phase system according to Norling et al. [17,18]. Cells of *Synechocystis* have been pelleted by centrifugation from 1.5 l of cell suspension, which had been grown to $OD_{750}=0.6$, washed by 5 mM K-PO_4 buffer pH 7.8 that contained 0.25 M of sucrose (Buffer 1), and disrupted at 70 MPa with French press. The lysate was centrifuged at 3000 *g* for 10 min, and the supernatant was withdrawn and centrifuged at 100,000 *g* for 30 min. The pellet, which contained total cell membranes in 4 ml of Buffer 1 was subjected to two-phase separation. 10 g of the two-phase membrane separation system consisted of polyethylene glycol 3.350 (Sigma-Aldrich, USA) and dextran T-500 (GE Healthcare, UK). Plasma membranes were separated and purified according to Norling et al. [19], and outer membranes have been purified as described by Huang et al. [20].

2.4. Electrophoresis and immunodetection

20 μg of proteins was loaded into each well, separated on 12% SDS-PAGE as described by Laemmli [21], and visualized with Coomassie G-250. Unstained proteins were transferred onto 45 μm nitrocellulose membranes (Hybond-C Extra, GE Healthcare, UK) with the Trans-Blot SD Electrophoretic Semi-Dry Transfer Cell (Bio-Rad, USA) and probed with the primary antibodies raised in rabbits against the D1 protein of *Synechocystis* (Agrisera, Sweden). The D1 protein was visualized with the secondary anti-rabbit antibodies fused to the horseradish peroxidase (GE Healthcare) and chemiluminescence solutions (ECL, GE Healthcare).

2.5. Measurements of steady-state fluorescence anisotropy

The measurements of steady-state fluorescence anisotropy were carried out with 1,6-diphenyl-1,3,5-hexatriene (DPH) using the Fluorescence Spectrophotometer 850 (Hitachi, Japan). The fluorescence anisotropy of the DPH inserted into the isolated membranes reflects their viscosity [22]. Insertion of the DPH into the membranes of *Synechocystis* has been performed in a 3 ml of a phosphate-buffered saline (PBS; Medicago AB, Uppsala, Sweden), which contained a membrane fraction (50 μg of protein) and 0.2 μm of DPH. 3 μl of 0.2 mM DPH in acetone was added to the membrane suspension in PBS with intensive vortexing, and the membranes were incubated with DPH on ice for 30 min. Fluorescence anisotropy was measured with absorbed light at 360 nm and emitted light at 430 nm in a temperature range from 15 °C to 50 °C with the intervals of 5 °C. The values have been calculated as described before [23,24].

2.6. RNA isolation and RT-PCR

Total RNA was isolated with the hot-phenol method [25]. RNA was further purified from DNA with the DNase I (Fermentas) according to the manufacturer's protocols. Reverse transcription was performed with Superscript III (Invitrogen, USA), 2 μg of the isolated total RNA and the gene-specific synthetic oligonucleotides used as primers. PCR was performed with the Hot-Start *Taq* DNA polymerase (Fermentas) with the presence of gene-specific primers (5 pmol each). The amplified DNA fragments were visualized by electrophoresis on a 1% agarose gel and stained with ethidium bromide. The quantitative analysis was done with Quantity One software (Bio-Rad).

3. Results and discussion

3.1. Analysis of temperature- and light-dependent changes in fatty acid composition of membrane lipids

Cells of *Synechocystis* had been grown at 32 °C under continuous illumination (Section 2.1) and then incubated at 22 °C for 12 h under the same light conditions or in the dark. The results of the analysis of fatty acid composition are presented in Tables 1 and 2.

Wild-type cells of *Synechocystis* grown at 32 °C under illumination (optimal growth conditions) mainly accumulate 16:0, 16:1$^{\Delta 9}$, 18:1$^{\Delta 9}$, 18:2$^{\Delta 9,12}$, and γ-18:3 with the unsaturation index of 0.81. Low-temperature treatment of these cells at 22 °C under illumination

Table 1
The fatty acid composition of total lipids in wild-type (WT) and *desA*$^-$/*desD*$^-$ cells of *Synechocystis* sp. PCC 6803, which have been grown at 32 °C in the light and incubated for 12 h at 22 °C in the light.

	Fatty acid (mol%)			
	32 °C		22 °C	
	WT	*desA*$^-$/*desD*$^-$	WT	*desA*$^-$/*desD*$^-$
14:0	0.3±0.1	0.5±0.2	0.3±0.1	0.3±0.1
16:0	56.0±2.5	55.0±1.0	54.0±1.6	56.0±5.1
16:1$^{\Delta 9}$	4.5±0.6	5.0±0.1	5.5±0.4	4.0±0.6
17:0	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1
17:1$^{\Delta 10}$	0.7±0.2	1.0±0.3	0.5±0.1	1.0±0.3
18:0	0.8±0.3	0.8±0.3	0.5±0.1	0.5±0.3
18:1$^{\Delta 9}$	11.5±0.9	37.5±1.1	3.0±0.1	38.0±3.0
18:1$^{\Delta 11}$	–	–	–	–
18:2$^{\Delta 9,12}$	14.0±1.3	–	13.5±0.6	–
γ-18:3$^{\Delta 6,9,12}$	12.0±0.7	–	18.0±0.5	–
α-18:3$^{\Delta 9,12,15}$	–	–	3.0±0.5	–
18:4$^{\Delta 6,9,12,15}$	–	–	1.5±0.3	–
UI	0.8±0.05	0.4±0.01	1.00±0.03	0.4±0.05

UI — unsaturation index.

Table 2
The fatty acid composition of total lipids in wild-type (WT) and *desA*$^{-}$/*desD*$^{-}$ cells of *Synechocystis* sp. PCC 6803, which have been grown at 32 °C in the light and incubated for 12 h at 22 °C in the dark.

	Fatty acid (mol%)			
	32 °C		22 °C	
	WT	*desA*$^{-}$/*desD*$^{-}$	WT[a]	*desA*$^{-}$/*desD*$^{-}$[b]
14:0	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
16:0	57.0 ± 3.0	55.0 ± 1.5	55.0 ± 2.0	55.0 ± 2.3
16:1$^{\Delta 9}$	5.0 ± 0.5	4.5 ± 0.9	4.5 ± 0.9	5.0 ± 2.2
17:0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
17:1$^{\Delta 10}$	0.8 ± 0.2	1.0 ± 0.3	0.8 ± 0.2	1.4 ± 0.5
18:0	0.6 ± 0.4	0.7 ± 0.3	1.7 ± 0.7	0.6 ± 0.2
18:1$^{\Delta 9}$	8.0 ± 0.7	38.0 ± 1.7	10.0 ± 0.5	36.0 ± 3.0
18:1$^{\Delta 11}$	–	0.2 ± 0.1	0.6 ± 0.1	1.0 ± 0.1
18:2$^{\Delta 9,12}$	15.0 ± 1.5	–	13.5 ± 0.6	–
γ-18:3$^{\Delta 6,9,12}$	13.0 ± 1.0	–	13.0 ± 0.5	–
α-18:3$^{\Delta 9,12,15}$	–	–	–	–
18:4$^{\Delta 6,9,12,15}$	–	–	–	–
UI	0.8 ± 0.07	0.4 ± 0.02	>1.00 ± 0.03	0.4 ± 0.05

UI — unsaturation index.
[a] In the wild-type cells incubated at 22 °C in the dark, the appearance of 10:0, 12:0, 13:0, and 15:0 was observed. These newly appeared FAs constituted about 1% from a sum of total fatty acids.
[b] In the *desA*$^{-}$/*desD*$^{-}$ mutant cells incubated at 22 °C in the dark, the appearance of 15:0 (0.1%) was detected.

caused more than 4-fold decrease in the amount of the oleic acid (18:1$^{\Delta 9}$), and 1.5-fold increase in the amount of the γ-linolenic acid (Table 1). This is the result of already known activation of the expression of the FA desaturase genes and of the enhanced unsaturation at low temperatures [26,27]. Moreover, cold stress in the light caused accumulation of the ω^3 FAs, namely, α-linolenic (18:3) and octadecatetraenoic acid (18:4). These two FAs have been never detected at normal growth temperature. The unsaturation index of FAs in cold-stressed cells in the light increased up to 1.

Wild-type cells incubated at 32 °C in the dark during 12 h did not demonstrate any significant changes in their fatty acid composition if compared with the illuminated cells (Table 2). Cold stress in the dark had no significant effect on composition of the major FAs of the membrane lipids (Table 2). This observation suggests that light is necessary for the unsaturation of FAs at low temperatures in *Synechocystis*.

Although no major changes in FA composition were observed under cold treatment in the dark, some amounts of FAs, which are rather unusual for *Synechocystis*, have been detected: they were 10:0, 12:0, 15:0, and 18:1$^{\Delta 11}$.

Previously characterized *desA*$^{-}$/*desD*$^{-}$ mutant was constructed by disruption of the *desA* gene with kanamycin-resistance gene, and by disruption of the *desD* gene with the chloramphenicol-resistance gene [28]. Chloramphenicol itself might inhibit photosynthetic electron transport chain playing the role of the electron acceptor from the Photosystem I [13]. Therefore we redesigned the *desA*$^{-}$/*desD*$^{-}$ mutant to avoid the use of chloramphenicol [10]. The spectinomycin-resistance gene was employed to disrupt the *desD* gene in the newly obtained mutant, since spectinomycin does not interfere with photosynthesis in photoautotrophic growth conditions.

Cells of the *desA*$^{-}$/*desD*$^{-}$ mutant of *Synechocystis* were unable to change their fatty acid composition under cold treatment both under illumination and in the dark (Tables 1 and 2). This is reasonable result, since the mutant cells lack essential cold-inducible desaturase genes. The only cold-inducible desaturase gene that remains in the double mutant cells is the *desB*, which encodes the ω3 desaturase. This desaturase, however, is not active in mutant cells, because they completely lack the substrate for this enzyme — linoleic acid (18:2$^{\Delta 9,12}$). Desaturation of stearic acid (18:0) or palmitic acid (16:0) into oleic (18:1$^{\Delta 9}$) and palmitoleic (16:1$^{\Delta 9}$) acid, respectively, is conducted by the Δ9 desaturase, whose expression is constitutive and does not depend on temperature [26] and/or light [11]. Essentially similar results on FA composition have been previously obtained with the *desA*$^{-}$/*desD*$^{-}$ mutant of *Synechocystis* [28]. However, the effect of light and dark incubation on desaturation was not studied in that work.

The amount of linoleic acid (18:2$^{\Delta 9,12}$) slightly decreased in the wild-type cells upon their incubation at 22 °C in the light. At the same time, the amount of the oleic (18:1$^{\Delta 9}$) acid, which serves as a substrate for the Δ12 desaturase, dropped down from about 10 to 3%. Cold treatment of cells in the dark did not cause such dramatic changes in the amount of the oleic acid, indicating that the desaturation at Δ^{12} position is light-dependent.

The amount of γ-linolenic acid (18:3$^{\Delta 6,9,12}$) increased in wild-type cells from 12 up to 18% upon their incubation at 22 °C under illumination, but not in the dark. Thus, desaturation at the Δ^6 position of C18 FAs might be also light-dependent.

The complete absence of the products of the ω3 desaturase (α-linolenic and octadecatetraenoic acids) in cells, which had been treated at 22 °C in the dark, implies that light is obligatory for desaturation at the ω^3 position of C18 FAs of *Synechocystis* (Tables 1 and 2).

3.2. Temperature- and light-dependent changes in the membrane fluidity

To characterize the fluidity/viscosity of the membranes of *Synechocystis*, cells of wild-type and the *desA*$^{-}$/*desD*$^{-}$ mutant had been grown at 32 °C and incubated at 22°C for 18 h under illumination or in the dark. From these normally grown and cold-treated cells the outer, cytoplasmic and thylakoid membranes have been isolated [17–20]. The purity of the membranes has been checked by western blotting of the membrane proteins from each membrane fraction.

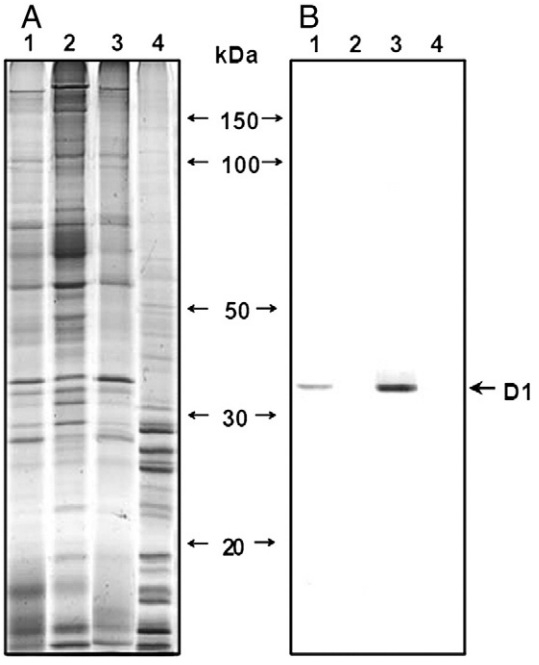

Fig. 1. SDS-PAGE separation of the membrane proteins of *Synechocystis* (A) and their western-blotting with the antibodies against D1 protein (B). Membrane fractions have been isolated in the two-phase system according to Norling et al. [17,18]. Outer membranes have been purified as described by Huang et al. [20]. 20 μg of proteins was loaded into each well, separated by SDS-PAGE, and visualized with Coomassie G-250 (A). Unstained proteins were transferred onto 45 μm nitrocellulose membranes (Hybond-C Extra, GE Healthcare, UK) with the Trans-Blot SD Electrophoretic Semi-Dry Transfer Cell (Bio-Rad, USA) and probed with the primary antibodies raised in rabbits against the D1 protein of *Synechocystis* (Agrisera, Sweden) (B). The D1 protein was visualized with the secondary anti-rabbit antibodies fused to the horseradish peroxidase (GE Healthcare, UK). 1 — total membrane proteins; 2 — plasma membrane proteins; 3 — thylakoid membrane proteins; 4 — outer membrane proteins.

Membrane proteins had been separated by SDS-PAGE and probed with the antibodies against D1, the major protein of the Photosystem II, which is a marker of the thylakoid membranes (Fig. 1). The absence of D1-specific signals in preparations of the outer and cytoplasmic membranes implies their purity in terms of the absence of contamination with the thylakoid membranes.

The measurements of steady-state fluorescence anisotropy were carried out with isolated outer, cytoplasmic, and thylakoid membranes. DPH was incorporated into the cytoplasmic membranes most successfully and displayed the background level of fluorescence at <5% of its possible maximum. The outer and thylakoid membranes were characterized by much modest yield of fluorescence (Fig. 2).

The membranes (outer, cytoplasmic, and thylakoid) of wild-type and the *desA*$^{-}$/*desD*$^{-}$ mutant cells exposed to cold stress in the light and in the dark, were characterized by lower viscosity if compared to the membranes isolated from cells grown at optimal growth conditions. This feature is more pronounced and visible in preparations of the outer membranes (Fig. 2A, B).

All membranes isolated from the *desA*$^{-}$/*desD*$^{-}$ mutant cells were more rigid if compared to the membranes of the wild-type cells (Fig. 2A, D). Similar characteristics have been demonstrated earlier by the application of the Fourier transform infrared (FTIR) spectroscopy to cytoplasmic membranes of *Synechocystis* [7,29]. These similarities in the physical properties of the membranes measured

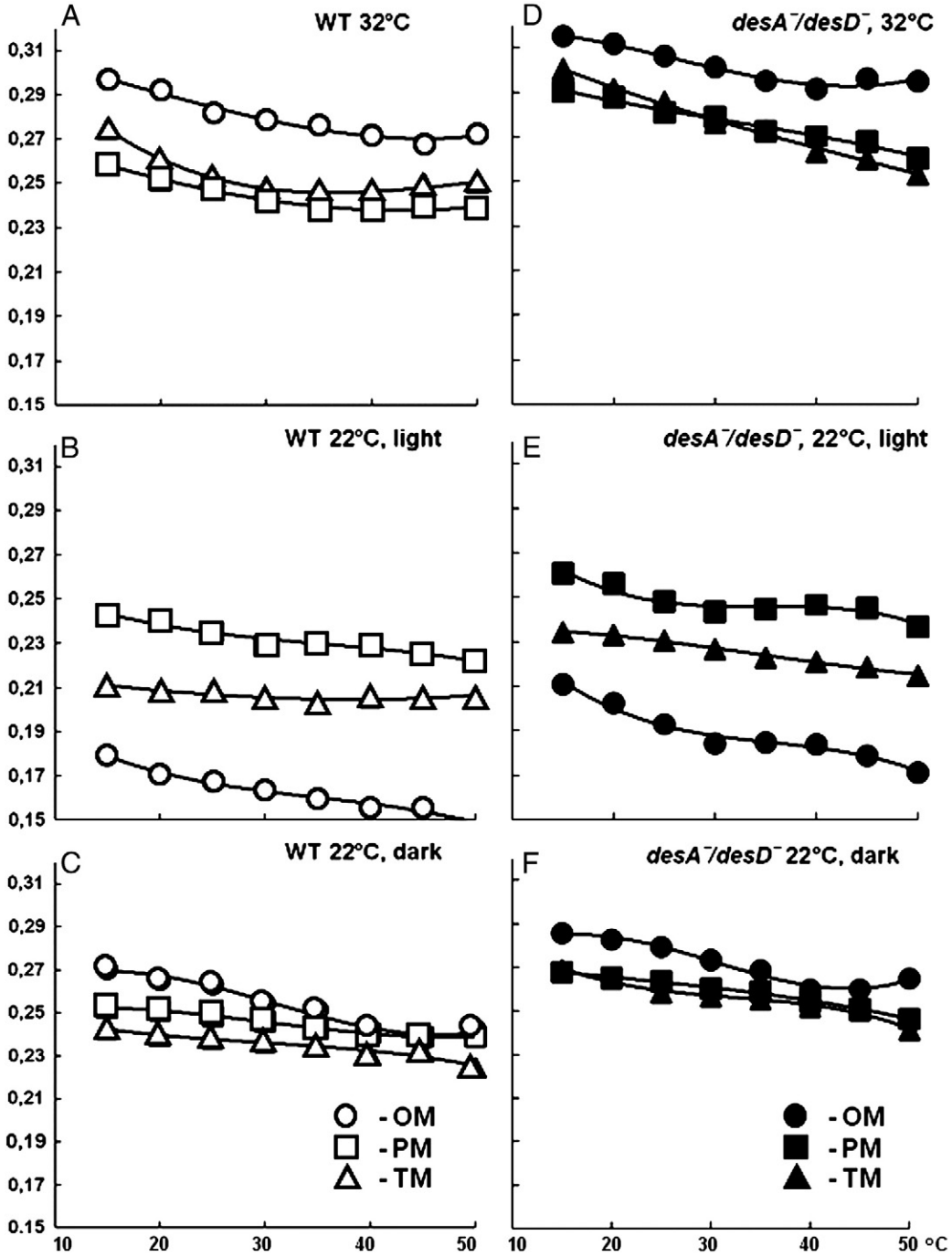

Fig. 2. Fluorescence anisotropy of DPH (0.2 μm) in outer (circles), cytoplasmic (squares), and thylakoid (triangles) membranes, isolated from *Synechocystis* cells grown under illumination at 32 °C (upper part) and incubated for 18 h at 22 °C under illumination (middle part), or in the dark (lower part). WT — wild type cells are shown with open symbols. The *desA*$^{-}$/*desD*$^{-}$ mutant cells are shown with dark symbols.

by different methods are important for correct estimation of the membrane properties. FTIR spectroscopy monitors the disorder of the acyl chains as well as the interactions between lipids and membrane proteins in terms of the frequency of the symmetric CH_2 stretching mode [29], where low and high frequencies of the CH_2 stretching mode correspond to the rigidified and fluid states of membrane lipids. Fluorescence anisotropy of DPH applied in this study reflects the total sum of molecular motion in a lipid bilayer including any constrains to that motion. Moreover, since DPH is not completely free to rotate within membranes, the extent of the interactions between DPH and membrane lipids is restricted. Although the latter method may be less suitable for quantitative measurements of membrane fluidity than FTIR spectroscopy, it can be applied for comparative analysis of changes in membrane fluidity, which is presented in Fig. 2.

Cold-induced regulation of the fluidity/viscosity of the membranes is light-dependent in wild-type (Fig. 2B, C) and in the *desA*$^-$/*desD*$^-$ mutant (Fig. 2E, F) cells. Despite of their inability to generate PUFAs, the *desA*$^-$/*desD*$^-$ cells are able to modulate the degree of their membrane fluidity under cold treatment, although not to such high extent as the wild-type cells. The latter implies that the ability to desaturate fatty acids of membrane lipid and to produce PUFAs is important, but not the only one mechanism to regulate the membrane fluidity under cold stress.

It is known that membrane fluidity may be regulated through the changes in polar heads of phospholipids [30]. It this study, we did not measure the changes in lipid composition in wild-type cells of *Synechocystis* and in its *desA*$^-$/*desD*$^-$ mutant. Nevertheless, it is known that *Synechocystis* contains less than 10% of phospholipids in their membranes. Earlier studies of the Fad12 mutant of *Synechocystis*, which had similar FA composition to the *desA*$^-$/*desD*$^-$ strain, did not reveal any major temperature-dependent changes in composition of the lipid species [31]. Thus, some other mechanism that differs from changes in FA composition or in lipid molecular species should be considered for temperature-dependent adjustment of the membrane fluidity in the *desA*$^-$/*desD*$^-$ mutant cells.

3.3. Temperature- and light-dependent changes in cold-inducible gene expression

One of the aim of this study was to establish a link, if exists, between the cold-inducibility of genes, which are controlled by the membrane fluidity [7], and light regulation.

We exposed the wild-type and the *desA*$^-$/*desD*$^-$ mutant cells with different fluidity of their membranes to different temperatures. Cells, which had been grown at 32 °C, were treated for 30 min at designated temperatures with the interval of 2 °C (30, 28, 26, 24, 22, 20 or 18 °C) in the light or in the dark. The changes in transcription of cold-induced genes were followed by the RT-PCR (Figs. 3–6). In addition to wild-type and *desA*$^-$/*desD*$^-$ mutant cells, we also employed the mutant defective in the cold sensor histidine kinase Hik33. DCMU was applied to the cells in the light as the inhibitor of the photosynthetic electron chain.

3.3.1. Cold-induced expression of the desB gene for the terminal ω3 desaturase

In wild-type cells that contained PUFAs in their membrane lipids, the level of the mRNA for the *desB* gene gradually increased with the decrease in temperature up to 18 °C (Fig. 3A, open circles) in the light. In the *desA*$^-$/*desD*$^-$ cells, that contain only monounsaturated fatty acids in their membrane lipids, the level of the mRNA for the *desB* gene was initially higher at 32 °C. It increased with the maximum at 24–22 °C and then it declined (Fig. 3A, open squares). These observations demonstrated that in the light, the response of the *desB* gene to low temperature in the *desA*$^-$/*desD*$^-$ cells shifted toward high temperatures as compared with that in wild-type cells.

Similar experiments performed in the dark (Fig. 3A, dark symbols), demonstrated that the *desB* gene was not induced in the dark by low temperatures in both, wild-type and *desA*$^-$/*desD*$^-$ mutant cells, in spite of differences in the fluidity of their membranes. Thus, the regulation of transcription of the *desB* gene depends on the membrane fluidity: rigid membranes of the *desA*$^-$/*desD*$^-$ cells determine the induction of the gene at higher temperatures than the fluid membranes of the wild-type cells. Moreover, this cold induction of transcription appears as a light-dependent process (Fig. 3).

It is known that the *desB* gene is under partial control of the cold sensor Hik33 [4,6], which may sense the cold-induced changes in the membrane fluidity [2,32,33]. To clarify the existence of a link between fluidity and light regulation of the cold-induced *desB* gene, we employed the Δ*hik33* mutant defective in the Hik33 [6,7]. The inhibitor of the photosynthetic electron flow, DCMU, was also applied to verify the involvement of the photosynthetic electron flow into light-dependent regulation of the *desB* gene (Fig. 3B).

Fig. 3B demonstrates that transcription of the *desB* gene is induced by cold in the light in the wild-type cells. It also shows that transcription of the *desB* depends on the activity of Hik33 and on

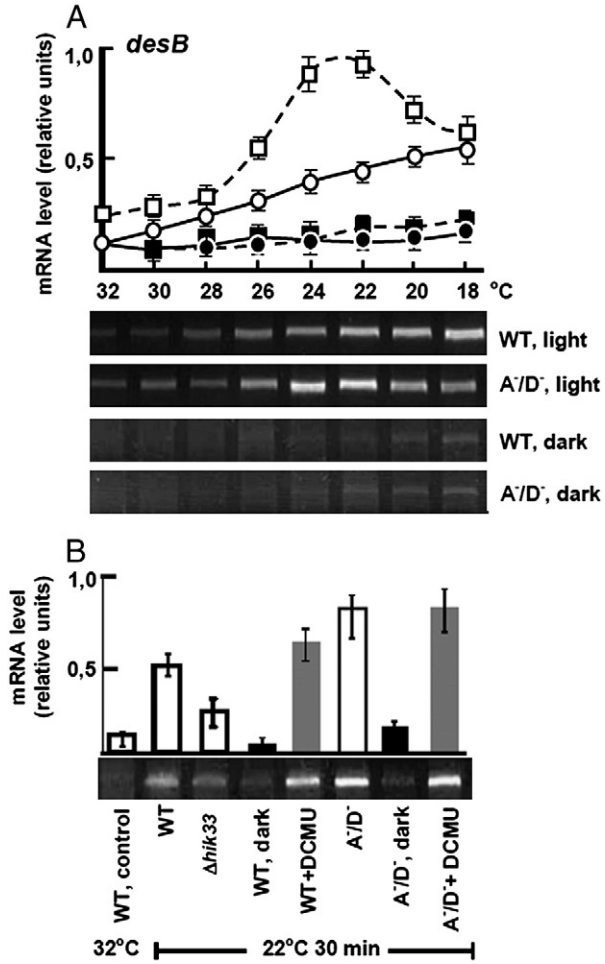

Fig. 3. Cold-induced expression of the *desB* gene for the ω3 desaturase. (A) Cells of wild-type (circles) or *desA*$^-$/*desD*$^-$ mutant (squares) had been grown at 32 °C under continuous illumination and incubated at 30, 28, 26, 24, 22, 20 or 18 °C for 30 min in the light (open symbols) or in the darkness (dark symbols). The changes in the levels of the specific mRNAs were determined by the RT-PCR method. The results of RT-PCR are presented in lower panels. The quantified results are presented in the upper graphical panel. (B) Cells of wild-type (WT, control) had been grown at 32 °C under continuous illumination. Cells of wild-type (WT), or of the mutant defective in the cold sensor Hik33 (Δ*hik33*), or of the *desA*$^-$/*desD*$^-$ mutant (A$^-$/D$^-$), have been incubated at 22 °C for 30 min in the light (white bars) or in the darkness (dark; black bars), or in the presence of DCMU (+DCMU) in the light (gray bars). Quantified results of RT-PCR are presented by bars.

the membrane fluidity (A^-/D^-) in the light, but not in darkness. Inhibition of the photosynthetic electron flow in the light by DCMU could not inhibit the induction of the *desB* by cold in both wild-type and *desA*$^-$/*desD*$^-$ mutant cells (Fig. 3B). This result suggests that the cold induction of the *desB* is light-dependent, however, it does not require the activity of the photosynthetic machinery.

Kis et al. [11], however, reported that DCMU inhibited transcription of the *desB* gene. This contradicts with our results that demonstrate that the addition of DCMU to *Synechocystis* cells does not alter or even slightly enhances transcription of the *desB* gene. This contradiction might be explained by the differences in the set-up of the experiments. Kis with coworkers studied the expression of the desaturase genes in cell cultures grown heterotrophically on glucose in darkness [11], while our experiments have been performed with cultures grown and exposed to low temperatures in photoautotrophic conditions.

3.3.2. Cold-induced expression of other genes regulated via Hik33

The *ndhD2* gene, which is under tight control of the cold sensor Hik33 [7], behaved similarly to the *desB* gene. The *ndhD2* was induced by cold only under illumination but not in the darkness. DCMU did not block its inducibility by cold. Its induction depended on the presence of Hik33 in cells and on the membrane fluidity (Fig. 4).

Essentially similar observations have been obtained with the cold inducibility of the *hliB* gene for the high-light inducible protein (Fig. 5), which is also tightly controlled by the Hik33.

Altogether these results support our previous suggestion that the genes, whose cold-inducibility depends on the fluidity of cell membranes, are controlled by the sensor histidine kinase Hik33 [7]. Thus, Hik33 might be, indeed, the sensor of the membrane fluidity.

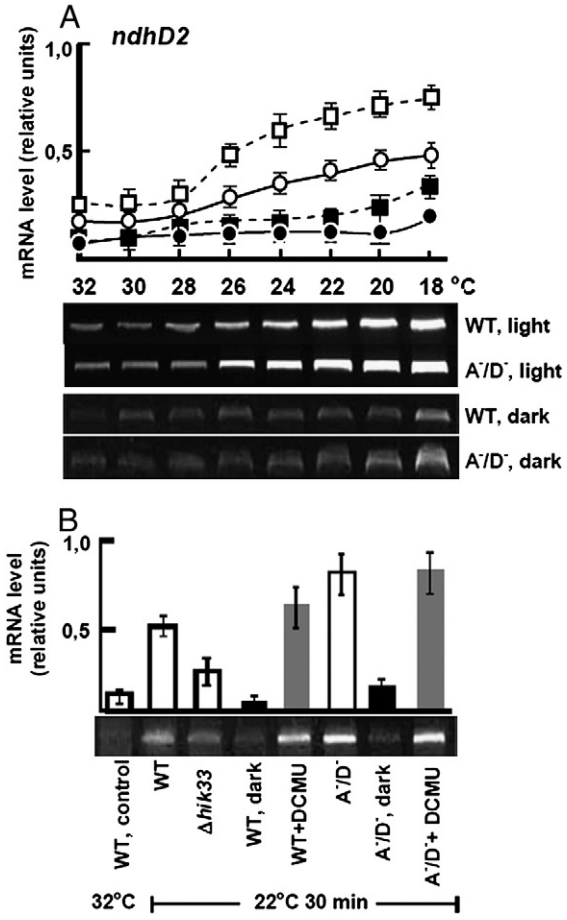

Fig. 4. Cold-induced expression of the *ndhD2* gene. All definitions and marks are the same as in Fig. 3.

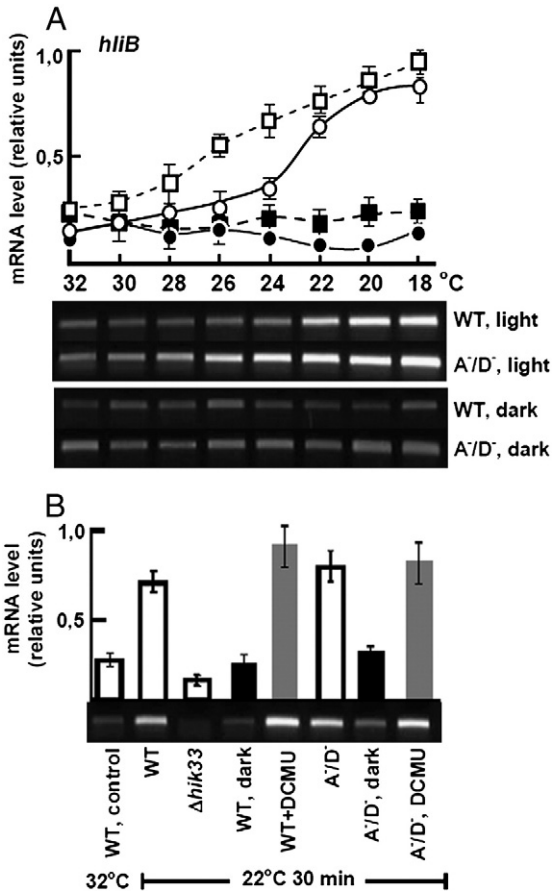

Fig. 5. Cold-induced expression of the *hliB* gene. All definitions and marks are the same as in Fig. 3.

Moreover, cold induction of genes regulated through Hik33 is light dependent, though, with no relation to the activity of the photosynthetic machinery.

Hik33 carries the PAS-domain [34]. The latter is attributed to regulatory proteins that participate in regulation of circadian clock in various organisms and in coordination of metabolism in photosynthetic cells [34,35]. In *Synechococcus*, the homolog of the Hik33, the histidine-kinase NblS, was shown to regulate light-dependent expression of the *hli* genes [36]. Thus, light-dependence of the induction of cold-stress genes through the Hik33 might be related to the PAS domain of this sensory kinase.

3.3.3. Cold-induced expression of genes whose inducibility is not under control of the Hik33

The genome-wide analysis of cold-inducible genes in *Synechocystis* revealed that about 2/3 of 100 highly cold-inducible genes are under control of Hik33 and another 1/3 is not [6,7,32]. Among those genes that are not controlled by the Hik33, there were the *crhR* gene for the cold-induced RNA-helicase, and the *rbpA1* gene for the cold-inducible RNA-binding protein, which plays a role of the RNA chaperon under cold stress in cyanobacteria, similarly to the *cspA* and other *csp* genes of *Escherichia coli* [37].

As shown in Fig. 6, cold-induction of the *rbpA1* does not depend on the membrane fluidity. The temperature-dependent profiles of the induction of this gene are similar in wild-type cells and in the *desA*$^-$/*desD*$^-$ mutant (Fig. 6A). Transcription of the *rbpA1* does not depend on light. It is equally induced by a decrease in ambient temperature both in the light and in the darkness. Its induction does not depend on the activity of the Hik33 and on the activity of the photosynthetic apparatus inhibited by DCMU (Fig. 6B).

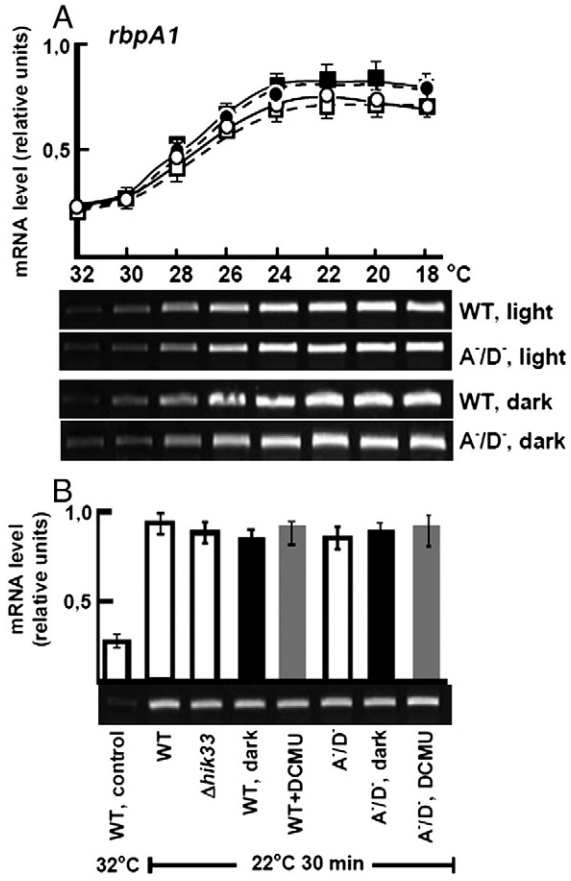

Fig. 6. Cold-induced expression of the *rbpA1* gene. All definitions and marks are the same as in Fig. 3.

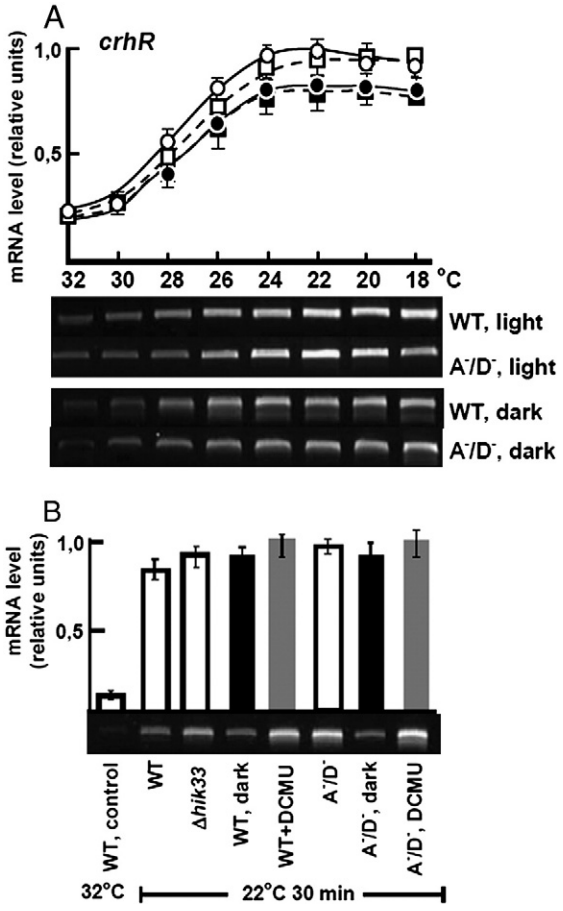

Fig. 7. Cold-induced expression of the *crhR* gene. All definitions and marks are the same as in Fig. 3.

Very similar behavior was observed for the *crhR* gene. It was not regulated either by the membrane fluidity, or light (Fig. 7), or DCMU.

Thus, the expression of genes, which are not controlled by the cold sensor Hik33, is not regulated by the membrane fluidity and/or light. Their cold-inducibility was solely dependent on temperature.

4. Conclusion

The cold-induced accumulation of PUFAs in *Synechocystis* is light-dependent. Cells of *Synechocystis* regulate the fluidity of their membranes upon decrease in temperature in a light-dependent manner. The *desA*$^{-}$/*desD*$^{-}$ mutant is able to adjust the fluidity of its membranes in spite of complete inability to synthesize PUFAs and modulate the fatty acid composition of the membrane lipids. Thus, some mechanism exists that regulates the membrane fluidity apart from the changes in FA composition of membrane lipids. The expression of cold-induced genes, which are controlled by the cold sensor histidine kinase Hik33 (*desB*, *hliB*, *ndhD2*), depends on the fluidity of cell membranes and it is regulated by light. The expression of cold-induced genes, which are not controlled by Hik33 (*crhR*, *rbpA1*), does not depend on the membrane fluidity or light. Membrane fluidity determines the temperature dependence of the expression of cold-induced genes, which are under control of the Hik33. This expression is light-dependent, though it does not depend on the activity of the photosynthetic machinery.

Acknowledgements

The work was supported by grants (nos. 11-04-01389 and 12-04-00473) from the Russian Foundation for Basic Research to D.A.L. and S.I.A., and by a grant from the "Molecular and Cell Biology Program" of the Russian Academy of Sciences, and by a grant from Russian Ministry of Education and Science (no. 16.740.11.0176) to D.A.L.

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