Volume 318, number 1, 57–60 FEBS 12148 February 1993

The temperature-dependent expression of the desaturase gene *desA* in *Synechocystis* PCC6803

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Received 6 January 1993

We examined the temperature-dependent regulation of the expression of the *desA* gene, which encodes Δ12 desaturase of *Synechocystis* PCC6803. The level of *desA* transcript increased 10-fold within 1 h upon a decrease in temperature from 36°C to 22°C. This suggests that the low-temperature-induced desaturation of membrane lipid fatty acids is regulated at the level of the expression of the desaturase genes. The accumulation of the *desA* transcript depended on the extent of temperature change over a certain threshold level, but not on the absolute temperature.

Expression of *desA* gene; Desaturase; Desaturation of fatty acid; Low temperature acclimation

1. INTRODUCTION

Living organisms can maintain the molecular motion, or 'fluidity' of their membranes by regulating the level of desaturation of fatty acids in the membrane lipids [1]. For example, cyanobacterial cells respond to a decrease in temperature by introducing double bonds into the fatty acids of membrane lipids [2,3], thus compensating for the temperature-induced decrease in the molecular motion of the membrane lipids. Desaturases are responsible for the introduction of these specific double bonds [4].

Several schemes have been proposed for the mechanism of the regulation of fatty acid desaturation of membrane lipids. On the basis of the findings that low-temperature-induced desaturation in cyanobacteria is inhibited by rifampicin and chloramphenicol, which inhibit prokaryotic transcription and translation [3,5], we previously suggested that the crucial step in this regulation involves de novo synthesis of desaturases [6]. Other reports have suggested that the accelerated desaturation at low temperatures is a result of the negative temperature coefficient of desaturase activities [7,8]. An alternative hypothesis suggests that de novo synthesis of saturated lipids is depressed at low temperatures, while the desaturation of fatty acids is relatively active, leading to an increase in the level of membrane lipid desaturation at low temperatures.

Since we have cloned the *desA* gene from *Synechocystis* PCC6803, which encodes Δ12 desaturase [9], it is now possible to examine the effect of low temperature on the expression of the desaturase gene, and to suggest in more detail the mechanism of the low-temperature-induced desaturation of membrane lipids. The present report describes the effect of low temperature on the level of the *desA* transcript.

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2. MATERIALS AND METHODS

2.1. *Culture conditions*

The cyanobacterial strain *Synechocystis* PCC6803 (Pasteur Culture Collection #6803) was cultivated at 36°C for 3–4 days under photoautotrophic conditions in BG11 medium buffered with HEPES-NaOH (pH 7.5), under illumination by incandescent lamps at 70 $\mu E \cdot m^{-2} \cdot s^{-1}$, as previously described [10]. When cell density reached 4×10^8 cells·ml^{-1}, a portion of the culture was withdrawn and incubated at certain temperatures for designated periods with continuous aeration with air containing 1% CO_2. The light conditions were the same as those described above.

In some conditions, rifampicin at 15 $\mu g \cdot ml^{-1}$, cerulenin at 10 $\mu g \cdot ml^{-1}$, and diuron at 15 μM were added to the cells 20 min before the incubation at various temperatures. This preincubation allowed the inhibitors to penetrate through the cell walls.

2.2. *RNA isolation*

Cells were quickly chilled to 1–5°C in liquid nitrogen and were collected by centrifugation for 3 min at 3 000 × *g* at 2–4°C. The cells were then disrupted with glass beads, as described by Golden et al. [11]. Total nucleic acids were extracted 3 times with phenol–chloroform (1:1, v/v), and were precipitated by ethanol. The RNA was separated from the DNA by LiCl precipitation [12].

2.3. *DNA probe*

The 1.3 kbp DNA fragment carrying the *desA* gene was excised from the plasmid Bluescript/1.5 kbp [9] 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 (NEN) by nick translation.

2.4. *Northern blot hybridization*

The total RNA, amounting to 5 or 10 μg, was electrophoresed in 1.2% agarose gels that contained 6.3% formaldehyde in MOPS buffer [12], and was transferred onto a GeneScreen Plus nylon membrane (Biotechnology Systems, NEN Research Products, Boston, MA, USA). The membrane was baked at 80°C for 2 h to remove traces of formaldehyde. The membrane was prehybridized in a solution of 50% formamide, 1% SDS, 1 M NaCl, and 10% dextrane sulfate for 20 min at 42°C. After the DNA probe (2×10^6 cpm) was added, the membrane was subjected to hybridization for 12 h at 42°C.

The washing procedure was done as recommended by the manufacturer of GeneScreen. The size of *desA* RNA was determined using an RNA molecular weight marker I (Boehringer, Mannheim, Germany).

3. RESULTS AND DISCUSSION

3.1. *Regulation of the level of* desA *transcript by temperature*

The change in the level of the mRNA for the *desA* gene upon the temperature shift from 36°C to 22°C was examined using Northern blot analysis (Fig. 1). Only one hybridizable band appeared at 1.2 kb under the various conditions. This suggests that the *desA* gene of *Synechocystis* PCC6803 is transcribed as a monocistronic operon that produces a single transcript with a size corresponding to the putative RNA length calculated from the nucleotide sequence of the *desA* gene [9]. The *desA* transcript began to accumulate 15 min after the temperature shift, and increased 10-fold to reach its maximum level 1 h after the shift (Fig. 2). When the temperature was shifted back to 36°C, the level of *desA* transcript decreased almost to its original level within 30 min (Fig. 2). These observations suggest that the decrease in ambient temperature regulates the level of *desA* transcript which can accelerate the de novo synthesis of desaturase. The increase in the level of *desA* transcript at low temperatures can be explained either by acceleration of transcription, by the increase in RNA stability, or by a combination of both. Further investigation will be necessary to confirm the actual cause of this increase.

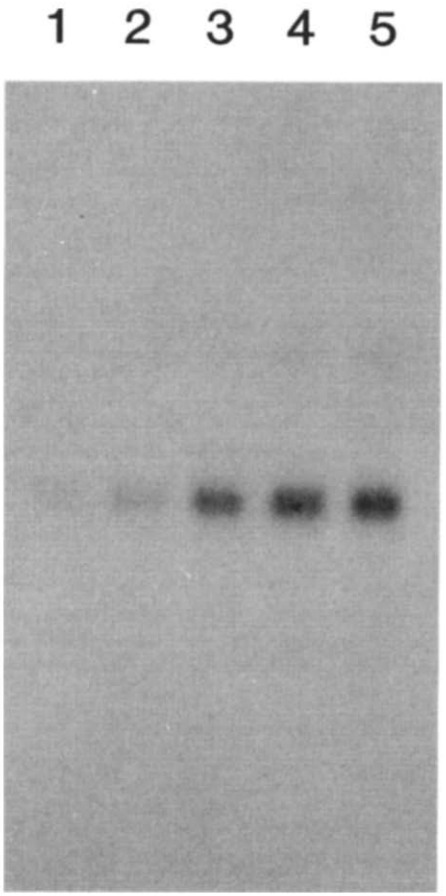

Fig. 1. Northern blot analysis of changes in the level of *desA* transcript upon a temperature shift from 36°C to 22°C. Cells grown at 36°C (control, lane 1) were incubated at 22°C as follows: for 15 min (lane 2), 30 min (lane 3) 60 min (lane 4) and 120 min (lane 5).

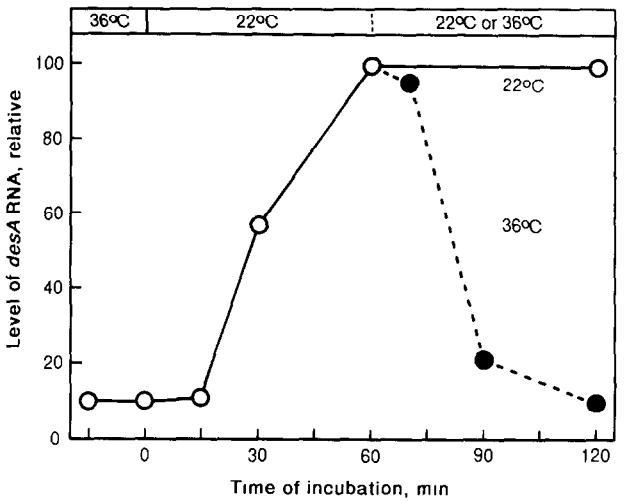

Fig. 2. The change in the level of *desA* transcript after the shift of temperature from 36°C to 22°C (○ ○), and back to 36°C (● - ●). The level of *desA* mRNA was determined by densitometry of the radioautogram of the Northern hybridization patterns.

3.2. *Effect of inhibitors on the level of* desA *transcript*

Fig. 3 shows the effect of rifampicin, cerulenin, and diuron on the low-temperature-induced change in the level of *desA* transcript. Rifampicin, an inhibitor of prokaryotic transcription, prevented the low-temperature-induced increase in the level of *desA* transcript (Fig. 3, lane 3). This is compatible with our previous findings [5], which demonstrated that this antibiotic inhibited the low-temperature-induced desaturation of fatty acids. In contrast, cerulenin, an inhibitor of fatty acid synthesis [5], had no effect on the level of *desA* transcript at low temperatures (Fig. 3, lane 4). This suggests that de novo synthesis of fatty acids does not affect the level of *desA* transcript.

The low-temperature-induced increase in the level of *desA* transcript was also inhibited by diuron (Fig. 3, lane 5). No enhancement of *desA* transcript was observed when cells were incubated in the dark at 22°C (data not shown). These observations imply that photosynthetically produced energy is essential for the increase in the level of *desA* transcript at low temperature. All of these findings are compatible with our previous studies on the low-temperature-induced desaturation of membrane lipids [3,5].

3.3. *The temperature profile of the level of the* desA *transcript*

We also investigated the means by which the level of *desA* transcript is regulated by temperature. Fig. 4 shows the relationship between the extent of temperature shift and the level of *desA* transcript in cells grown at 36°C or 32°C. In cells grown at 36°C, the accumulation of *desA* transcript began to appear at 28°C. In the cells grown at 32°C, the accumulation began to appear at 26°C. These observations indicate that the organism actually perceived the change in temperature, but not the absolute temperature, and further, that the organism sensed the change in temperature only when it exceeded a threshold of 6°C.

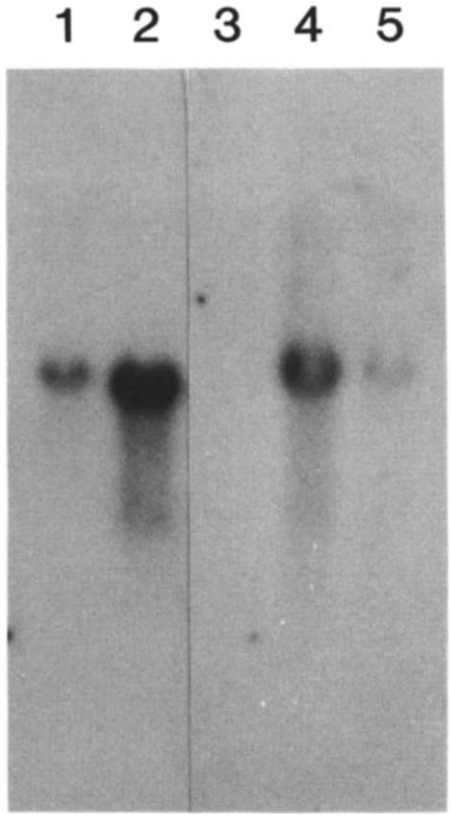

Fig. 3. The effect of the inhibitors on the level of *desA* transcript after the temperature shift from 36°C to 22°C. Cells grown at 36°C (control, lane 1) were incubated at 22°C for 30 min as follows: in the absence of inhibitors (lane 2), or in the presence of (lane 3) rifampicin, (lane 4) cerulenin, or (lane 5) diuron.

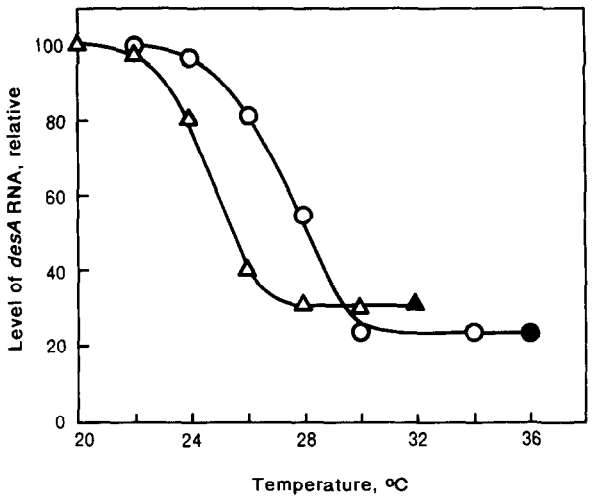

Fig. 4. The temperature profile of the temperature-shift-induced change in the level of *desA* transcript. Cells grown isothermally at 32°C (△-△) or 36°C (○-○) for 3 days were incubated for 30 min at designated temperatures. The level of *desA* mRNA was determined by densitometry of the radioautogram of the Northern hybridization patterns.

In terms of physical response this biological perception of temperature can be considered quite sensitive, since a 6°C change in temperature represents a very small reduction in molecular motion. In fact, a 6°C change in temperature corresponds to only a 2% change in molecular motion. It would be interesting to explore the mechanism by which the organism can convert such a small change in a physical parameter into a biological signal.

4. CONCLUSION

The low-temperature-induced desaturation of membrane lipid fatty acids in *Synechocystis* PCC6803 is regulated at the level of the expression of the desaturase gene. The organism perceives the extent of temperature change, but not the absolute temperature.

Acknowledgements: This work was supported, in part, by Grants-in-Aid for Scientific Research on the Priority Areas (04273102 and 04273103) from the Ministry of Education, Science and Culture, Japan, to N.M., and by the Japan Society for the Promotion of Science Post-Doctoral Fellowship to D.L.

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