Low-Temperature and Substrate Induction of the Gene for Δ9 Fatty Acid Desaturase in the Thermophilic Cyanobacterium *Synechococcus vulcanus*

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**Abstract**—A decrease in ambient temperature induced the *desC* gene encoding the only acyl-lipid desaturase of thermophilic cyanobacterium *Synechococcus vulcanus*. The addition of the substrate for this enzyme, stearic acid (18:0), to the culture medium of cyanobacterium grown at optimum temperature also induced the *desC* gene. At 55 and 45°C, gene transcription started at the position –59 upstream of the translation initiation codon. After addition of 18:0, two specific pools of transcripts were detected. Some transcripts corresponded in size to transcripts synthesized at 55 and 45°C, and other transcripts were longer. The determination of sites of the transcript synthesis initiation showed that 18:0 additionally induced transcription from positions –163 and –169. These additional initiation sites were positioned within the coding sequence of the *hemN* gene preceding the *desC* gene. Thus, at low-temperature and substrate induction, the expression of the *desC* gene was evidently controlled by different promoter sequences.

**Key words: Synechococcus vulcanus - desaturases - low-temperature regulation - substrate regulation - gene expression**

**INTRODUCTION**

Low-temperature induction of fatty acid (FA) desaturases in mesophilic organisms is a well-documented phenomenon [1–3]. A drop in temperature is believed to reduce membrane fluidity, and FA desaturation in membrane lipids permits a compensation of this process, thus facilitating normal functioning of membrane-bound energetic protein complexes [2–5]. The systems of low-temperature signal transduction resulting in the induction of desaturase genes were identified in mesophilic cyanobacterium *Synechocystis* [6–8] and Gram-positive bacterium *Bacillus subtilis* [9]. They comprise a sensor histidine kinase and the response regulator, which is phosphorylated by this kinase.

The substrate induction of FA desaturases was found only in mammalian cells [10–13] and yeast [14]. In the latter case, the addition of stearic acid to the culture medium accelerated transcription of Δ9-stearoyl-CoA desaturase. This effect was evidently related to the active incorporation of saturated FA in membrane lipids, which interfered the functioning of the respiratory chains [15]; therefore, desaturation of such FA was necessary.

The search for the controlling factors was so far unsuccessful. However, in some organisms, DNA sequences (*cis*-elements) were determined, which might bind the proteins regulating transcription of the desaturase genes [16, 17].

The goal of this work was to study the effects of low temperature and stearic acid, a component of membrane lipids and substrate for the desaturase, on transcription of the *desC* gene encoding the only FA desaturase in cells of the thermophilic cyanobacterium *S. vulcanus*.

**MATERIALS AND METHODS**

Strain *Synechococcus vulcanus* (IPPRAS B-453) was obtained from the Collection of microalgae at the Institute of Plant Physiology RAS. Cells were grown at the optimum temperature of 55°C, continuous illumination from luminescent lamps (50–70 µmol quanta/(m² s)), and bubbling with a gas–air mixture containing 2% CO₂ [18, 19].

RNA isolation and Northern blotting were performed as described earlier [19, 20].

The determination of start points of transcription was performed as described earlier [20] using synthetic oligonucleotides SV1: 5'-AGGTCGAGGTTC-GAAITC and SV2: 5'-AATGGCCTTAGAAAAT-GTCCT as primers for reverse transcription. In experiments with primer extension, 5 µg of total RNA per reaction was used. Reverse transcription was performed using SuperScriptII enzyme (New England Biolabs, United States). Sequencing was performed...
using the same primers and a Sequenase deaza-dGTP kit (US Biochemical, United States). The pUC19 plasmid was used as a matrix for sequencing; this plasmid contained the insert of *S. vulcanus* DNA 6 kb in length and the *desC* gene with flanking sequences [19].

Nucleotide sequences presented in this study were registered in the GenBank, the accession nos. U90417 (*desC*) and AF027176 (*hemN*).

### RESULTS AND DISCUSSION

Earlier it was demonstrated that a decrease in temperature from 55 to 45 or 35°C induced transcription of the *desC* gene in *S. vulcanus* within 12 h [19]. In parallel, the amount of corresponding enzyme increased and then the accumulation of monounsaturated FA, mainly 18:1, occurred [18, 19]. However, in these studies, the amount of specific mRNA, corresponding protein, and FA during 24–48 h at the intervals of 2–12 h were measured. Here, the effects of low temperature on transcription of the *desC* gene within 2 h in order to elucidate the shortest period required for gene induction were studied. As evident from Fig. 1, induction of the *desC* gene was observed as early as 15 min after a drop in temperature from 55 to 45°C. In 30–60 min, the amount of *desC* mRNA increased 8–10-fold, whereas after 2 h, the amount of the transcript reduced, which indicates a transient nature of *desC* gene induction.

Figure 1 demonstrates that all *desC* transcripts have similar length. This implies that transcription of the *desC* gene at 55°C (optimum temperature) and 45°C (low temperature) starts from one and the same promoter. It might be that low temperatures induced another promoter, but this promoter has to be position near the constitutive one, because no difference in the sizes of transcripts synthesized at different temperatures was observed (Fig. 1).

The expression of the *desC* gene is known to be induced not only by the low temperatures but also by factors that cause rigidification of the membrane lipids at the optimum temperature [21, 22].

The enzyme activation at optimum temperature can be induced by the addition of corresponding substrates to the culture medium. Thus, in cells of yeast *Saccharomyces cerevisiae*, which are capable of the synthesis of only monounsaturated FA, the addition of stearic acid activated Δ9-steroyl-CoA desaturase [14]. In contrast, the addition of oleic acid, the product of Δ9 desaturase activity, repressed Δ9 desaturases in yeast cells [14]. Such substrate regulation implies that sodium salts of FA used in experiments were absorbed by the cells and converted into acyl-CoA forms, which are substrates or products of desaturation.

To elucidate whether the excess of the substrate or product affects immediately the expression of the *desC* gene in cells of *S. vulcanus* and which regulatory elements can be putative participants of such regulation, sodium salts of stearic (18:0), oleic (18:1Δ9), or linoleic

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**Fig. 1.** Northern-blot analysis of transcription of the *desC* gene under low temperature. (a) (1) Cells were grown at 55°C with continuous illumination (70 μmol quanta/(m² s)) until *A*₇₅₀ = 0.5; thereafter, cells were exposed to 45°C for (2) 15, (3) 30, (4) 60, and (5) 120 min. Isolated total RNA was separated by electrophoresis in 1.2% agarose gel containing formaldehyde, transferred to nylon membranes, and hybridized with a probe complementary to the *desC* gene of *S. vulcanus* [19]. 10 μg of total RNA was loaded into gel wells. For loading control, after hybridization with *desC* probe, membranes were washed from radioactivity and hybridized again with 16S rRNA probe ((a), lower panel); (b) Quantification of the time-course of transcription of the *desC* gene (average from three independent experiments).