Perception and transduction of low-temperature signals to induce desaturation of fatty acids

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Abstract

When cells of the cyanobacterium *Synechocystis* sp. PCC 6803 are exposed to a low temperature, genes for fatty acid desaturases are expressed with resultant increases in the degree of unsaturation of fatty acids in membrane lipids. However, the sensor and transducers of low-temperature signals had not yet been identified. In order to identify these components we applied to the cyanobacterium *Synechocystis* sp. PCC 6803 the systematic disruption of all 43 putative genes for histidine kinases and random mutagenesis of the whole genome in conjunction with screening by the transcriptional activity of the promoter of the *desB* gene for the ω3 desaturase. This allowed us to identify two histidine kinases and a response regulator as components of the perception and transduction of low-temperature signals for the expression of genes for fatty acid desaturases.

Introduction

Responses of cyanobacterial cells to low-temperature stress are basically of two types. One type involves the cold-induced desaturation of fatty acids in membrane lipids such that the membranes become more fluid to compensate for the decrease in membrane fluidity that would otherwise occur at the temperature [1]. The other type involves the low-temperature-induced synthesis of enzymes that enhance the efficiency of transcription and translation to compensate for the decrease in the efficiency of these processes at low temperature [2]. Both types of response serve to protect the cyanobacterial cells from the detrimental effects of low-temperature stress.

The molecular mechanisms for desaturation of fatty acids have been well characterized in poikilothermic organisms such as cyanobacteria [3,4], *Tetrahymena* [5], *Acanthamoeba* [6] and fish [7]. In these organisms, synthesis of acyl-lipid desaturases is induced at low temperatures and double bonds are introduced to defined positions in the fatty acids in membrane lipids. It could be hypothesized that the low temperature is perceived by an unknown sensor from which low-temperature signals are transduced via unknown components to the low-temperature-inducible genes. The mechanisms of perception and transduction of low-temperature signals have not been clarified in any organisms. Recently we addressed this question by methods of functional genomics [8].

In a previous study [9], we demonstrated that decreases in the degree of unsaturation of fatty acids in the plasma membrane of *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) by catalytic hydrogenation *in vivo* enhanced the expression of the *desA* gene for the Δ12 acyl-lipid desaturase, which is otherwise induced primarily by low temperature. Thus, a change in membrane fluidity appears to be important for the perception of temperature that results in induction of the synthesis of the desaturases [10]. These findings suggest that the hypothesized sensor of low temperature is located in the plasma membrane.

Systematic mutation of putative genes for histidine kinases

In order to monitor the inducibility by low temperature of the *desB* gene for the ω3 desaturase, we generated a strain of *Synechocystis*, designated *pdesB::lux*, in which the promoter region of the *desB* gene was ligated to the coding region of the *luxAB* gene for a bacterial luciferase [10]. Thus, luciferase activity, monitored in terms of luminescence, could be used as an indicator of low-temperature-inducible changes in the activity of the *desB* promoter.

Physical and chemical stimuli that are generated extra- and intra-cellularly are perceived by a group of proteins that includes histidine kinases. These proteins are localized on the plasma membrane or in the cytosol in various prokaryotes [11], yeasts [12] and plants [13,14]. It seems likely, therefore, that temperature-induced changes in membrane fluidity might be mediated by a membrane-bound histidine kinase. Kaneko et al. [15,16] determined the sequence of the genome of

Key words: fatty acid desaturase, signal perception, signal transduction, *Synechocystis* sp. PCC 6803.

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Synechocystis and they identified 43 putative genes for histidine kinases [17]. The products of some of the 43 putative genes for histidine kinases might plausibly be expected to function as sensors or transducers of environmental or intracellular stimuli [11, 17]. We designated the histidine kinases Hik1 to Hik43 and their genes hik1 to hik43.

We inactivated separately each of the genes for histidine kinases in pdesB::lux cells by inserting a spectinomycin-resistance cartridge (Sp'), creating a ‘gene-knockout’ library [8]. We screened members of the library for loss of low-temperature inducibility by monitoring luciferase activity at a low temperature. In the library, only pdesB::lux/ΔHik-19 and pdesB::lux/ΔHik-33, with disruption of genes for Hik-19 and Hik-33, exhibited a reduced capacity for the activation of luciferase by low temperature. These results suggested that Hik-19 and Hik-33 might be involved in the perception and transduction of low-temperature signals. The sites of insertion of the Sp' cassette in hik33 and hik19 genes are shown in Figure 1(A).

Figure 1
Sites of insertion of Sp' cassette into the components of low-temperature signal transduction

(A) Sites of insertion of the Sp' cassette in pdesB::lux/ΔHik33 and pdesB::lux/ΔHik19 cells. Open rectangles indicate open reading frames. (B) The site of insertion of the Sp' cassette in the chromosome (hatched rectangle) and the sites of insertion of the Sp' cassette to assay the activity of individual genes (triangles) are shown. The open reading frame sll0038 corresponds to the response regulator Rer1.

Random mutagenesis
In order to find other components in the pathway for low-temperature signalling, we introduced the Sp' cassette randomly into the chromosome of pdesB::lux cells by cassette mutagenesis [8]. From among approx. 20000 spectinomycin-resistant mutants, we isolated 18 mutants in which the response of luciferase activity to a downward shift in temperature was different from that in parental pdesB::lux cells. We found that, in two of the 18 mutants, the hik19 gene for Hik19 had been inactivated by insertion of the Sp' cassette.

In another of these mutant lines in which the desB promoter was not activated by low temperature, the Sp' cassette had been inserted in the upstream region of two putative operons, as shown in Figure 1(B). In order to identify the gene responsible for the elimination of the transcriptional activity, we inactivated separately five putative genes (sll0037, sll0032, sll0031, sll0038, sll0040) in pdesB::lux by inserting an Sp' cassette. Inactivation of gene sll0038, but not of any of the other genes, depressed the low-temperature-induced increase in luciferase activity in the same way as observed in the mutant cells. The amino acid sequence deduced from the nucleotide sequence of this gene indicated that its product was one of the 38 response regulators identified in Synechocystis [17]. We designated the gene rer1 and its product Rer1.

Characteristics of Hik33, Hik19 and Rer1
The amino acid sequence deduced from the hik33 gene indicates that Hik33 contains 663 amino acid residues and that the strongly conserved histidine kinase domain is located near the C-terminus and two hydrophobic helices that might span the membrane. This last property of Hik33 appears to be consistent with that of a sensor that can detect a decrease in membrane fluidity.

Hik19 contains 1014 amino acid residues and might be a soluble protein in the cytosol. A strongly conserved histidine kinase domain is located in a central region of the protein. One signal-receiver domain is localized at the N-terminus and another is near the C-terminal region. Furthermore, a histidine phospho-transfer (Hpt) domain is located at the C-terminus. Thus, Hik19 is a hybrid-type histidine kinase [17], and is more likely than Hik33 to be a transducer of the low-temperature signal.
Rer1 contains 402 amino acid residues. Unlike most response regulators that have a signal-receiver domain at the N-terminus, Rer1 has a signal-receiver domain at the C-terminus. However, the N-terminal region is homologous with the DNA-binding domain, known as an HMG box, found in regulators of transcription in vertebrates. The central part of Rer1 is similar to the transcriptional activation domain of the aryl hydrocarbon receptor nuclear translocator (Arnt).

Expression of the des gene for fatty acid desaturases

We next inactivated, separately, the hik33, hik19 and rer1 genes in wild-type cells to examine, by Northern blotting analysis, the effects of these genes on the expression of des genes for fatty acid desaturases. Inactivation of either hik33 or hik19 significantly depressed the low-temperature inducibility of the desB gene and desD for the Δ⁶ desaturase. However, the low-temperature-induced enhancement of the expression of the desA gene was unaffected by the inactivation of hik33 and hik19. These results indicated that inactivation of hik33 and hik19 suppressed the low-temperature-induced expression of the desB and desD genes but not of the desA gene.

To examine the role of Rer1 in the regulation of expression of genes for fatty acid desaturases, we inactivated the rer1 gene in wild-type cells by inserting the Sp' cassette. Although the extent of the low-temperature-dependent induction of the desB transcript was reduced, the inducibility by low temperature of the desD and desA genes was unaffected by the mutation. These results indicated that Rer1 might specifically regulate the expression of the desB gene but not that of the other genes examined.

A hypothetical pathway for perception and transduction of low-temperature signals

Figure 2 shows a hypothetical scheme for the transduction of low-temperature signals. When the temperature is decreased or the fatty acids are more saturated, the histidine residue in the histidine kinase domain of Hik33 may be phosphorylated. A phosphate group is then transferred to Hik19, and finally to Rer1, which regulates the expression of the desB gene.

References

Received 26 June 2000