Biochimica et Biophysica Acta 1860 (2016) 2391–2403

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

New insights in cyanobacterial cold stress responses: Genes, sensors, and molecular triggers

Maria A. Sinetova, Dmitry A. Los *

Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, 127276 Moscow, Russian Federation

ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 16 June 2016
Accepted 9 July 2016
Available online 12 July 2016

Keywords:
Cold stress
Cyanobacteria
Protein kinases
Red light
ROS
Universal triggers

ABSTRACT

Background: Cold stress strongly induces the expression of ~100 genes in cyanobacteria. Some of these genes are necessary to protect cellular functions by adjustment of membranes, as well as transcriptional and translational machineries. About a half of cold-induced genes are not functionally characterized. A part of cold-induced genes is under control of a two-component regulatory system, consisting of histidine kinase Hik33 and response regulator Rre26. The mechanism(s) that control another part of cold-inducible genes are still unknown.
Scope of review: The aim of this review is to summarise the latest findings in cyanobacterial cold-stress responses including transcriptomics, cold sensing, and molecular triggers.
Major conclusions: A feedback loop between the membrane fluidity and transcription of genes for fatty acid desaturases operates via the transmembrane red-light-activated cold sensor Hik33, which perceives cold-induced membrane rigidification as a change in its thickness. The cold-induced kinase activity of Hik33 is facilitated by interaction with a small protein, Ssl3451 – the third contributor to a canonical two-component regulatory system, which may explain the ability of some cyanobacterial histidine kinases to interact with different response regulators under different stress conditions. Other regulatory systems that control cold-stress responses operate via Ser/Thr protein kinase, SpkE, and via temperature-dependent changes in DNA supercoiling. Transcriptomic analysis shows that universal triggers of stress responses are reactive oxygen species and changes in redox status of plastoquinone pool.
General significance: Deeper understanding of molecular mechanisms of temperature sensing and regulation of cold-stress responses in photosynthetic cells provide a background for generation of cold-resistant crops.

1. Introduction

Cyanobacteria belong to a unique group of ancient prokaryotic organisms that perform oxygenic photosynthesis [1]. Studies of fossil microorganisms in Precambrian rocks (3.5–0.5 billion years ago) indicated the temporal morphological changes in fossil cyanobacterial communities caused by the irreversible changes of physicochemical conditions on Earth [2]. Cyanobacteria adapted to all global changes through several billion years, including reversal changes in temperature associated with cold glacial epochs separated by warmer interglacial periods. Studies on modern extremophilic cyanobacteria demonstrated that they are very conservative and have changed insignificantly both morphologically and physiologically during the past, at least, 2 billion years [3]. Therefore, studies on modern model [4] and relict [3] cyanobacteria are important for understanding the principles of temperature acclimation and stress resistance.

Cyanobacterial species are widely distributed in nature and they inhabit almost all the environments – from Antarctica, where temperature exceeds −20 °C [5], to hot springs, where temperature reaches +70 °C [6]. Cyanobacteria of Arctic and Antarctic ice, where temperature is always below 0 °C, are metabolically active and perform oxygenic photosynthesis [7]. Some thermophilic species perform active photosynthesis at 55-60 °C and experience cold stress at 35-45 °C [8].

The complete nucleotide sequences of the genomes of hundreds cyanobacterial species have been determined. These genomic data provide a molecular background for studies of the stress sensing and transduction mechanisms. Random or targeted mutagenesis with transposons or with antibiotic-resistant cartridges can be applied to many cyanobacterial species: random or targeted knockout mutants can be generated and the function of the corresponding genes can be determined. DNA-microarray [9] and RNA-Seq [10] based transcriptomic analysis of gene expression has been developed. These powerful techniques allow a whole-genome-scale examination of the expression of each individual gene under stress conditions and the effect of mutations on gene expression. The

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPH, 1,6-diphenyl-1,3,5-hexatriene; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; FA, fatty acid; FAD, fatty acid desaturase; MV, methylviologen; PAS domain, the domain that contains PER-ARNT-SIM (PAS) and phytochrome amino acid features; PQ, plastoquinone.

* Corresponding author at: Institute of Plant Physiology, Russian Academy of Science, Botanicheskaya Street 35, Moscow 127276, Russian Federation.
E-mail address: losda@ippras.ru (D.A. Los).

http://dx.doi.org/10.1016/j.bbagen.2016.07.006
0304-4165/

results generated by modern experimental approaches are now handled with the tools of bioinformatics that allow systematic and cluster analysis of massive sets of expression data, identification of the putative regulatory circuits, and setup for metabolic re-modeling [11,12].

2. Cold-induced changes in transcriptome and cellular responses

The genes that are induced by cold stress were identified by whole-genome scale transcriptomic analysis [13–16]. Cold stress was applied by exposing the cells of motile [16] or non-motile [13–15] *Synechocystis* strains grown at 30–34 °C to 22 °C [13–15] or 28 °C to 10 °C [16] for 20–30 min. Short exposure to low temperatures allows to focus on the primary events in patterns of gene transcription under cold stress. The microarray expression data shows that more than 100 genes are induced by cold with the induction factor 2 or higher. These genes may be grouped in functional categories (Table 1), such as

1) signal perception and transduction;
2) transcription and translation;
3) cell wall and membrane maintenance;
4) photosynthesis and respiration;
5) various cellular functions (cofactor biosynthesis, nucleotide metabolism);
6) unknown functions

2.1. Cold-induced genes involved in signal perception and transduction

Cold-induced regulatory genes are limited to two genes for the protein kinases (*hik3* and *hik31*) and two genes for the DNA-binding transcriptional regulators (*rre5* and *sfsA*).

Hik3 (or PlpA – phytochrome-like protein) is a histidine kinase (hik), which is equipped with PAS/PAC, and GAF sensory domains [17,18].

Hik31 (or CopS) is a transmembrane multisensory histidine kinase that regulates autotrophic growth [19], responses to light [20], and resistance to cations, Cd^{2+}, Cu^{2+}, and Zn^{2+} [21]. Hik31 controls genes that are involved in photosynthesis and genes for ribosomal proteins. Fluctuations in photosynthetic electron transport (addition of DBMIB, nitrogen limitation, etc.) oxidize plastocyanin and promote the release of Cu^{2+}, which binds to Hik31 and trigger the regulatory cascade [22,23].

Rre5 belongs to a PatA subfamily of two component response regulators, which is involved in CO_2 uptake and associated pH homeostasis. SfsA-like protein is involved in regulation of sugar catabolism, but its exact function in cyanobacteria is unknown.

2.2. Cold-induced genes involved in transcription and translation

The sigma (σ) factors of RNA polymerase play central roles when bacteria adapt to different environmental conditions. Replacement of one σ factor in the RNA polymerase holoenzyme by another one changes the transcription pattern. In *Synechocystis*, there are nine σ-factors of RNA-polymerase that are divided into three groups [24]. SigA is an indispensable primary σ-factor, which maintains transcription of housekeeping genes, and belongs to Group 1. Group 2 is represented by the homologs of SigA (SigB, SigC, SigD, and SigE), although their functions are not crucial under regular growth conditions, and the corresponding genes can be deleted without any consequences [25]. Group 3 is represented by the alternative σ factors that are variable in their amino acid sequences (SigF, SigG, SigH, and SigI), and that control transcription of the specific regulons under certain stress conditions [24, 25].

Cold-induced *sigD* (*rpoD*) encodes a Group 2 RNA polymerase σ factor, which is the only σ factor abundant in the dark [26]. Transcription of *sigD* is induced by strong light [27], by the inhibitor of photosynthetic electron transfer chain, DCMU [26] and by H_2O_2 [28]. These data suggest that SigD may be involved in light-dependent redox regulation of transcription.

The function of NusG, a cofactor of Rho transcriptional terminator, was not studied in cyanobacteria. It is known, however, that in eubacteria NusG operates in combination with histone-like nucleoid-structuring protein H-NS and Rho-dependent transcriptional terminators to diminish genome-wide antisense transcription [29]. Under normal growth conditions, the contribution of Rho-NusG modulation of sense and antisense transcription should be limited. Under cold stress, however, antisense transcription may cause a serious additional problem together with difficulties in the maintenance of a proper RNA secondary structure(s), and loss of speed, efficiency, and fidelity of transcription and translation. Thus, the activation of NusG expression may help cells to silence the global antisense transcription under cold stress.

Liu et al. [16] listed 36 genes for tRNAs and 15 genes for ribosomal proteins that were up-regulated by cold stress. However, in those experiments, cold treatment was provided by placing *Synechocystis* cells grown at 28 °C to 10 °C or 4 °C [16]. It is therefore unlikely, that an increase in the amounts of the specific mRNAs reflects physiological cellular responses at such low temperatures [30]. Such increase might rather result from the arrest of all physiological processes, including mRNA degradation, caused by severe cold shock.

Prokaryotic-type cyanobacterial ribosomes are similar to those in *E. coli* [31]. The *rpsU* gene for 30S ribosomal subunit protein S21 is induced 10-fold by cold stress in *Anabaena* [32] and *Synechocystis* [33]. In ribosomes isolated from cold-treated cells, the S21 protein was present at an equimolar level relative to other ribosomal proteins, but its level decreases with an increase in ambient temperature [33]. The changes in the level of S21 protein in cyanobacterial ribosomes with changes in temperature raise an interesting (but still unanswered) question about the role of this protein in the acclimation of the translational apparatus to cold stress. It is known that variation in the amounts of some ribosomal proteins may contribute to a fine tuning of ribosome function and, in particular, ribosome selectivity for distinct transcripts. Unlike cyanobacteria, non-photosynthetic *E. coli* expressed *rpsU* constitutively, and S21 protein is always present in ribosomes at an appropriate stoichiometric level [34].

Low temperature also induces genes for 50S ribosomal proteins L20 and L11, 30S protein S12, and ribosome chaperone trigger factor (Tig). The latter supports early folding events and prevents misfolding and aggregation of proteins.

The *smpB* gene encodes the protein that is required for rescue of ribosomes stalled on defective messages. SmpB binds to SsrA RNA to mimic tRNA that mediates the addition of a short peptide tag to the C-terminus of the partially synthesized nascent polypeptide chain [35]. The SsrA-tagged proteins are then degraded by C-terminal proteases.

Among cold-induced genes there are genes for tRNA- and ribosomal protein methyltransferases, translation elongation factor, and peptide chain release factor 2 that participates in termination of protein synthesis at UAA and UGA stop-codons.

The effect of low temperatures on cyanobacteria translational machinery was noticed earlier on scattered examples [36,37]. The transcriptomics approach, however, revealed the complete set of genes that are affected by cold.

2.3. Cold-induced RNA helicases

RNA structural transitions are important in processes such as translation, pre-mRNA splicing, RNA processing, chromosome end maintenance, and the regulation of gene expression. Such transitions imply folding to specific three-dimensional structures that include secondary structure (RNA helices), tertiary structure, and formation of ribonucleoprotein complexes [38]. RNA helicases are responsible for modifying the secondary structure of mRNAs, which is a critical factor in the regulation