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Minireview

Proteomic analyses of the response of cyanobacteria to different stress conditions

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ABSTRACT

Cyanobacteria are significant contributors to global photosynthetic productivity, thus making it relevant to study how the different environmental stresses can alter their physiological activities. Here, we review the current research work on the response of cyanobacteria to different kinds of stress, mainly focusing on their response to metal stress as studied by using the modern proteomic tools. We also report a proteomic analysis of plastocyanin and cytochrome c_6 deletion mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 grown under copper or iron deprivation, as compared to wild-type cells, so as to get a further understanding of the metal homeostasis in cyanobacteria and their response to changing environmental conditions.

1. Introduction

Environmental stresses influence a plethora of physiological activities in living organisms. To face stress, the functioning of some proteins is inhibited or lost and that of others are enhanced or induced. The genome and proteome of several cyanobacteria, during normal grow or acclimation to different stresses, have been analyzed in deep. In particular, the proteome and transcriptome of *Synechocystis* sp. PCC 6803 (hereinafter referred to as *Synechocystis*) have been studied under a variety of conditions, including heat shock, and salt or metal stress (see a review in [1]). *Synechocystis* is a moderately halotolerant cyanobacterium and has actually become a model system for investigating the environmental stress responses of photosynthetic organisms. Most of these studies are mainly focused on the soluble protein fraction, but some others deal with the membrane protein fraction [2,3].

Metals have a crucial role in cell metabolism by acting as redox cofactors in proteins involved in key metabolic pathways [4]. In particular, iron and copper participate in essential processes, namely photosynthesis and respiration. However, metals at high concentration promote the generation of reactive oxygen species (ROS) and, subsequently, oxidative damage, a process that is responsible for several pathologies and degenerative diseases [5,6]. Thus, the key role of metal homeostasis in maintaining the intracellular concentration of metal ions within a range compatible with cell viability becomes evident.

In this article, we briefly review the research work on the response of cyanobacteria to different kinds of stress and, in particular, to metal stress as studied by using the proteomic approaches. In addition, we describe the functional characterization of two *Synechocystis* knockout mutants for either plastocyanin (Pc) or cytochrome c_6 (Cyt), which are two alternative soluble proteins involved in electron transport—both in photosynthesis and respiration—that can replace each other depending on the relative availabilities of iron and copper in the culture medium. Finally, we present some data on the comparative proteomic analysis of *Synechocystis* wild-type (WT) and mutant cells growing with or without copper, thus yielding relevant information on the metal homeostasis in cyanobacteria and the response of these organisms to changing environmental conditions.

2. Response to salt stress

The understanding of the response of cyanobacteria—which can be used as a simple model for plants—to salt stress is of outmost relevance, as deciphering their adaptive mechanisms will surely

Abbreviations: 2-DE, two-dimensional gel electrophoresis; Cyt, cytochrome c_6; Pc, plastocyanin; PSI, photosystem I; PSII, photosystem II; ROS, reactive oxygen species; WT, wild-type

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contribute not only to understand the plant response but also to design and construct plants resistant to such an environmental limitation. Former studies with two strains of *Anabaena* revealed that salinity-induced modification of protein synthesis occurs in cyanobacteria, like in plants, and that some proteins synthesized during salt stress may be essential for cyanobacterial osmotic adaptation [7].

In the last years, the study of the response of cyanobacteria to environmental stress at the transcriptional level has been potentiated by the development of methods that include reverse transcriptase-polymerase chain reaction (RT-PCR) and DNA microarrays. In fact, genome-wide analysis of gene expression at the transcriptional level with DNA microarrays has allowed identifying almost all genes induced by a particular stress in cyanobacteria [8]. Actually, the transcriptome of *Synechocystis* under salt stress has been analyzed in deep (see [1], for a recent review). Treatment of *Synechocystis* with 0.5 M NaCl for 15 min induces the expression of ca. 360 genes and represses the expression of other ca. 200 genes [9]. Heat shock genes, namely *hspA, htpG, dnaK2, dnaJ* and *groEL2*, as well as genes for proteases, namely *clpB1* and *htrA*, are among the genes whose expression is rapidly and strongly induced by salt stress. Likewise, the expression of genes coding for high light-inducible proteins, superoxide dismutase and RNA polymerase sigma factors is induced by salt stress [9–12].

The MALDI-TOF analysis, along with large two-dimensional gel electrophoresis (2-DE), has become a powerful tool for the automated identification of proteins. In the last years, the proteome of *Synechocystis* under salt stress has been characterized in deep [3,13,14]. In the periplasmic fraction of *Synechocystis* salt-adapted cells, six proteins were greatly enhanced and three proteins were newly induced. Most of the salt-enhanced proteins are enzymes involved in the alteration of the cell wall structure [13] (Fig. 1).

Analyzing the total soluble fraction of *Synechocystis* cell extracts, 55 soluble proteins were found with either an expression level enhanced by salt shock (18 proteins) or even accumulated at high concentration after long-term salt acclimation (37 proteins) [14]. A small number of the proteins are salt stress-specific, such as enzymes involved in the synthesis of glucosylglycerol, but most of them are involved in general stress acclimation [14]. In particular, a number of proteins against heat shock (DnaK2, GroEL1, GroEL2, HspA and GroES) or against lesions by ROS (superoxide dismutase and peroxiredoxin) were induced by salt stress. Even some changes in enzymes involved in basic carbohydrate metabolism were detected [14]. A comparison of the proteome and transcriptome analyses of salt-stressed cells reveals an increase in mRNA concentration for most of the 18 short-term-induced proteins [11]. However, for 40% of the proteins that were accumulated in salt-acclimated *Synechocystis* cells, gene induction was not observed in the DNA microarray experiments, thereby indicating the involvement of posttranscriptional regulation in salt acclimation [14].

A complete proteomic analysis of salt-stress proteins in plasma membranes of *Synechocystis* cells resulted in the identification of 109 proteins. The expression of 20 of these proteins was enhanced, and that of 5 was reduced during salt stress [3]. Seven of the salt-enhanced proteins were periplasmic-binding proteins of ABC-transporters. Within this group, the proteins that exhibited the highest expression enhancement included FutA1 (iron-binding protein) and Vipp1 (vesicle-inducing protein in plastids 1), which have been suggested to be involved in protection of photosystem II (PSII) and in thylakoid membrane formation, respectively. Other proteins induced by salt-stress were regulatory proteins [3]. The increase in salinity, on the other hand, does not induce any noticeable change in the large integral membrane protein complexes, as compared with the cells cultured in the absence of added NaCl [2]. However, the development of new methods to analyze the proteome of integral membrane proteins will allow a more detailed study [15].

The salt-stress induced proteins cited above seem to be characteristic for all photosynthetic organisms since similar proteins were identified in the proteome of salt-stressed algae and plants, thus validating the use of cyanobacteria as a simple model for this kind of studies in plants [14].

3. Proteomics of the heat shock response

Cyanobacteria must respond to different temperature ranges and heat exposure. Actually, thermophilic cyanobacteria grow in hot springs and deserts, whereas mesophilic cyanobacteria can tolerate temperatures up to 50 °C [16]. Heat shock response has been already studied in cyanobacteria both at the transcription/expression level of specific genes and proteins and at the transcriptomic/proteomic level of the whole cell (see Ref. [1], for a review).

Heat-exposed cyanobacteria exhibit differential increased expression of two heat shock proteins encoded by the *groEL1* and *groEL2* genes (chaperones) [17–19], whose regulation involves the CIRCE/HrcA system [20]. Inactivation of several other genes, such as *clpB* (protease), *hspA*, *htpG*, *dnaK2* (chaperones), *sigB* and *sigC* (sigma factors), induces thermal sensitivity [21–24]. In addition, the Hik34 histidine kinase is believed to down regulate the

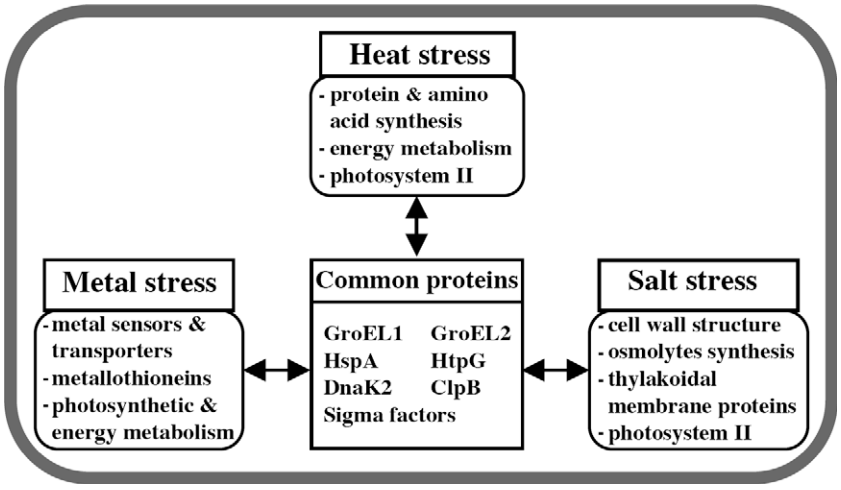

Fig. 1. Proteins whose expression level is increased and metabolic processes altered by heat, metal and salt stress in cyanobacterial cells. The 'Common proteins' box contains proteins whose expression is enhanced by either type of stress. See text for details.