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Systematic identification of light-regulated cold-responsive proteome in a model cyanobacterium

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ABSTRACT

Differential expression of cold-responsive proteins is necessary for cyanobacteria to acclimate to cold stress frequently occurring in their natural habitats. Accumulating evidence indicates that cold-induced expression of certain proteins is dependent on light illumination, but a systematic identification of light-dependent and/or light-independent cold-responsive proteins in cyanobacteria is still lacking. Herein, we comprehensively identified cold-responsive proteins in a model cyanobacterium *Synechocystis* sp. PCC 6803 (Hereafter *Synechocystis*) that was cold-stressed in light or in dark. In total, 72 proteins were identified as cold-responsive, including 19 and 17 proteins whose cold-responsiveness are light-dependent and light-independent, respectively. Bioinformatic analysis revealed that outer membrane proteins, proteins involved in translation, and proteins involved in divergent types of stress responses were highly enriched in the cold-responsive proteins. Moreover, a protein network responsible for nitrogen assimilation and amino acid biosynthesis, transcription, and translation were upregulated in response to the cold stress. The network contains both light-dependent and light-independent cold-responsive proteins, probably for fine tuning its activity to endow *Synechocystis* the flexibility necessary for cold adaptation in their natural habitats, where days and nights are alternating. Together, our results should serve as an important resource for future study toward understanding the mechanism of cold acclimation in cyanobacteria.

Significance: Photosynthetic cyanobacteria need to acclimate to frequently occurring abiotic stresses such as cold in their natural habitats, and the acclimation process has to be coordinated with photosynthesis, the light-dependent process that provides carbon and energy for propagation of cyanobacteria. It is conceivable that cold-induced differential protein expression can also be regulated by light. Hence it is important to systematically identify cold responsive proteins that are regulated or not regulated by light to better understand the mechanism of cold acclimation in cyanobacteria. In this manuscript, we identified a network involved in protein synthesis that were upregulated by cold. The network contains both light-dependent and light-independent cold-inducible proteins, presumably for fine tuning the activity of the network in natural habitats of cyanobacteria where days and nights are alternating. This finding underscores the significance of proteome reprograming toward enhancing protein synthesis in cold adaptation.

Abbreviations: WT, wild type; LDCRes, light-dependent cold-responsive; LDCRep, light-dependent cold-repressible; LDCInd, light-dependent cold-inducible; LICRes, light-independent cold-responsive; LICRep, light-independent cold-repressible; LICInd, light-independent cold-inducible; CL, cold-stress in light; CD, cold-stress in dark; *Synechocystis*, *Synechocystis* sp. PCC 6803; FASP, filter-aided sample preparation; 2-DE, 2-D electrophoresis; LC-MS, liquid chromatography mass spectrometry; TMT, tandem mass tag; RP-HPLC, reversed phase-HPLC; SD, standard deviation; CV, coefficient of variation

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1. Introduction

Cyanobacteria are a group of photosynthetic gram-negative bacteria that significantly contribute to production of oxygen and biomass in the current biosphere [1–3]. Cyanobacteria distribute in almost every terrestrial and aquatic habitat, and thus have to acclimate to divergent and changing environments. Cold is one of the most-frequently occurring abiotic stresses in the natural habitats of cyanobacteria, a temperature at 22 °C could induce significant cold stress on *Synechocystis*, a freshwater cyanobacterium with optimal growth temperature between 32 and 38 °C [4].

It is conceivable that cyanobacteria have to precisely coordinate light-dependent processes such as photosynthesis with light-independent ones when acclimating to cold. Such a coordination may require differential expression of proteins that are responsive to both light and cold. It was reported cold could induce differential expression of certain genes in a light-dependent mode, including the genes encoding acyl-lipid desaturases (DesA, DesB, DesD), HliB, and NdhD2 [5,6]. The acyl-lipid desaturases are critically important for cold acclimation through desaturating membrane lipids and hence maintaining desired level of membrane fluidity at low temperature [6]. HliB and NdhD2 are also well known stress-inducible proteins, though their exact functions in cold-acclimation remain clarified. In addition to the cold-inducible proteins, cold-repression of some proteins may also depend on light. Unfortunately, scarce information is available regarding the identities and functions of such proteins, despite that a number of proteins have been identified as cold-repressible [7]. Therefore, it is necessary to systematically identify the cold-responsive proteins and to determine the degree of their light dependence.

In the present study, we cold-stressed *Synechocystis* cells in light or in dark, and then quantitatively identified proteins that were differentially expressed between the treated and the non-treated control cells through the tandem mass tag (TMT) labeling-based quantitative proteomics approach [8]. We expected to identify a number of cold-responsive proteins and quantitatively distinguish light-dependent cold-responsive (LDCRes) proteins from light-independent cold-responsive (LICRes) proteins. These could provide important targets for future functional studies toward understanding how light is involved in cold-acclimation of cyanobacterial, and probably of higher plants as well.

2. Materials and methods

2.1. Cell culture

The wild type strain of *Synechocystis* were grown photoautotrophically to exponential phase (O.D. 730 nm of ~0.8) in liquid BG11 medium at 30 °C with a photosynthetic photon flux density of 50 μmol m^{-2} s^{-1}. For cold treatment, the cells were incubated at 22 °C under the same light conditions or in the dark for 3 h (referred to as cold-stress in light: CL and cold-stress in dark: CD, respectively). For the control (Ctrl), the cells were incubated at 30 °C in light for 3 h. All cells were then harvested by centrifugation.

2.2. RNA isolation and RT-PCR

Total RNA was isolated with phenol–chloroform extraction method as described previously [9]. The extracted RNA solution was then treated with RNase-free DNase (TIANGEN BIOTECH, Beijing, China) at 37 °C for 1.5 h to remove contaminating genomic DNA. The reverse transcription reaction was performed with the ThermoScript RT–PCR System (Promega, Madison, WI). The nucleotide sequences of the forward and reverse primers for the RT–PCR are listed in Supplemental Table 1. The gene *rnpB*, which encodes a subunit of ribonuclease P and is not differentially expressed under different growth conditions [10], was used as the internal control. All PCR experiments were performed in triplicate.

2.3. Protein preparation

Harvested cells were lysed in a buffer containing 0.4 M sucrose, 50 mM 3-(*N*-morpholino) propanesulfonic acid, pH 7.0, 10 mM NaCl, 5 mM EDTA, and 0.5 mM PMSF with a bead beater. Insoluble debris was removed from the lysates by centrifugation for 30 min at 5000 × *g* at 4 °C. Total proteins were then precipitated with ice-cold 10% trichloroacetic acid in acetone at −20 °C. The precipitated proteins were washed with acetone to remove lipids, pigments, and residual trichloroacetic acid and then air dried. The dried proteins were then resolubilized with 4% sodium dodecyl sulfate (SDS) in 0.1 M Tris-HCl, pH 7.6. The protein concentration was determined using a BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL).

2.4. Protein digestion and TMT labeling

Protein digestion and TMT labeling were performed in the same way as we recently reported [8]. Briefly, the filter-aided sample preparation (FASP) method and sequencing grade trypsin (Promega, Madison, WI) were used for protein digestion [11]. The resultant tryptic peptides from each sample were then labeled with an individual TMT of the 6-plex TMT-reagents (Thermo Scientific, Rockford, IL). The labeled samples were mixed together with an equal molar ratio before prefractionation.

2.5. Peptide prefractionation

The sample of the TMT-labeled peptide mixture was separated by off-line reversed phase (RP)-high performance liquid chromatography (HPLC) using a Waters e2695 separations HPLC system. The separation was performed on a phenomenex gemini-NX 5u C18 column (250 × 3.0 mm, 110 Å) (Torrance, CA, USA). The LC separation was performed as previously described using a 97 min basic RP-LC gradient with a flow rate of 0.4 mL/min [12]. The separated samples were combined into 14 fractions and then dried with a Speed-Vac concentrator and frozen at −20 °C. Immediately before use, the dried peptides were resuspended in 0.1% formic acid (FA), and the final concentration was adjusted to 1 μg/μL.

2.6. Liquid chromatography (LC)-tandem mass spectrometry (MS/MS) analysis

A LTQ Orbitrap Elite mass spectrometer (Thermo Scientific, Rockford, IL Waltham, MA) coupled online to an Easy-nLC 1000 was used for the LC-MS/MS analysis, which was run in the data-dependent mode. Each sample (2 μL) was injected into a capillary analytic column with 25 cm in length and 75 μm in inner diameter, which was packed with C18 particles with 5 μm diameters. The mobile phases for the LC contain buffer A (0.1% FA) and buffer B (100% ACN, 0.1% FA). The peptides were separated by a 90-min non-linear LC gradient, which is composed of 3%–8% B for 10 min, 8%–20% B for 60 min, 20%–30% B for 8 min, 30%–100% B for 2 min, and 100% B for 10 min. The flow rate of the LC separation was set at 300 nL/min. The source voltage was set at 2.5 KV and current was set at 100 μA. The MS measurement was performed with positive ion mode, and a mass range of 300–1800 *m/z* was set for spectra acquisition. Each full MS scan was performed with a resolution 240,000 at m/z 400, and fifteen most abundant ions was fragmented by Higher-energy collisional dissociation (HCD) for MS^2 spectra acquisition. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD006111 (http://www.proteomexchange.org) [13].

2.7. Database search

All raw MS files were searched against the *Synechocystis* proteome