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High Throughput Proteomic Approaches for the Dissection of Light Signal Transduction Pathways in Photosynthetic Cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract

Light is an environmental signal that regulates photomovement and main energy source of photosynthesis in the cyanobacterium *Synechocystis* sp. PCC 6803 (Syn6803). Syn6803 is a popular model system for study of plant functional genomics. In this report, we adopted 2D gel based proteomics study to investigate proteins related with the light absorption and photo-protection in Syn6803. More than 700 proteins were detected on the SDS-gels stained with silver nitrate. Several proteins showing different expression level under various light conditions were identified with MALDI-TOF Mass spectrometry. As a comparison, we also conducted ICAT-based proteome study using WT and *cph1* (cyanobacterial phytochrome 1) mutant. A *cph1* deletion led to changes in the expression of proteins involved in translation, photosynthesis including photosystem and CO2 fixation, and cellular regulation. We are currently involved in TAP-tagging method to study protein-protein interactions in search for the molecular component involved in the light signal transduction of Syn6803 photomovement.

Phytochrome like proteins in *Synechocystis* sp. PCC 6803

Light has important roles, not only as a source for photosynthesis, but also as a stimulus for many developmental processes in photosynthetic organisms. Among them, light takes effects on whole life cycle of photosynthetic microorganisms, such as cyanobacteria. Cyanobacteria have numerous and subtle ways of sensing and responding to light. Therefore cyanobacteria display many photomovement phenomena such as phototaxis, photophobic response, and photokinesis to search optimal environment for photosynthesis (1, 2). It has to be answered what is a photoreceptor of phototaxis, how light signal is transduced to final responses. The field of cyanobacterial light signal transduction was given a tremendous stimulus by the publication in 1996 of the complete genome sequence of Syn6803, the first complete sequence for a photosynthetic organism (3). The sequence revealed at least eight phytochrome-like open reading frames in syn6803. Phytochromes are the well-known photoreceptors of green plants. They have a linear tetrapyrrole chromophore, which typically absorbs red light at about 660nm. After absorbing red light, the pigment was photoconversed to a far-red light-absorbing form. This conformational change of the pigment triggers a conformational switch in the protein, which then initiates a signal transduction cascade. The Syn6803 gene sequence revealed several genes with varying degrees of homology to plant phytochrome (4). Recently, *in vitro* biochemical analysis demonstrated

that two of the eight open reading frames are authentic spectrally functional phytochromes (5, 6), and also revealed that the cyanobacterial phytochrome (named as Cph1) with a downstream open reading frame (slr0474 named as Rcp1) could form a two-component light sensory system (7). Cyanobacterial phytochrome Cph1 has been shown to be light-regulated histidine kinase of two-component system where Cph1 is autophosphorylated when it is exposed to far-red light (7). It was suggested that the Pr-form of Cph1 has higher autophosphorylation activity that the Pfr-form. There have been a few reports dealing with biochemical characteristics of cyanobacterial phytochrome. The two strongest phytochrome homologues (termed cph1 and cph2) were shown to be capable of binding chromophores and undergoing reversible photoconversion *in vitro* (5, 6). But there was not reported about the *in vivo* role of phytochromes in the cyanobacterium until now.

Construction of the *cph1* inactivation mutant

As a first step to elucidate possible *in vivo* functions of the cyanobacterial phytochrome, we constructed a mutant, which had disruption in the phytochrome gene of Syn6803. The putative phytochrome operon, *cph1-rcp1*, was cloned by PCR and ligated in pGEM-T vector, and interposon mutagenized by inserting a spectinomycin/streptomycin gene cartridge into the operon. Then vector containing the *cph1* gene disruption were used to transform Syn6803 cells and antibiotic-resistant transformants were selected. After complete replacement of wild type gene by mutated gene, which was confirmed by southern blot analysis and PCR, we did RT-PCR experiment to find whether *cph1* and *rcp1* was expressed *in vivo*. The size of RT-PCR product using *cph1* primer set is 2.25 kb in wild type, but was not found in *cph1* knockout mutant.

In vivo function study of cyanobacterial phytochrome using proteomic approaches.

To understand the intracellular role of Cph1, differential display two-dimensional gel electrophoresis (2DE) was used to analyze the total extracts derived from the *cph1* knockout mutant and the corresponding wild type. 2DE experiments were repeated at least 3 times with each sample to ensure accuracy, and gels were silver stained using a protocol compatible with subsequent mass spectrometric analysis. Cell preparation of WT and *cph1* mutant resulted in well-defined 2DE profiles containing approximately 700 protein spots. Under the dark condition, more than 30 proteins shown to be differentially regulated were identified by mass spectrometry with peptide finger printing. Of these, the expression of at least 23 proteins was repressed by a factor of 5-fold or greater in the *cph1* mutant. A high proportion (8/23) of proteins identified appears to participate in aspects of photosynthesis which include photosystem and carbon fixation. Three proteins belong to ribosomal proteins, and remaining proteins including cheY subfamily are involved in cellular regulation. Also, the *rcp1* mutant showed the same 2D profile as the *cph1* mutant displayed. Therefore Rcp1 may pair with Cph1 to transduce light signal into the cell. The 2D gel-based approach does not provide a truly comprehensive view of the proteome. Mostly, low copy number proteins could not be observed (<1000 copies/cell). In addition, hydrophobic proteins, basic proteins, and very large proteins (>100 kDa) are not solubilized and brought into focus using the 2D gel methodology (8). To overcome several problems of 2D gel technique, Isotope-Coded

Affinity Tag (ICAT) strategy was conducted (9). ICAT strategy permits the analysis of low-abundance proteins and is compatible with any amount of protein harvested from cells or tissues under any growth conditions. A main strength of proteomics is the ability to analyze the dynamics of biological processes by the systematic analysis of expressed proteins. Combining proteomic technologies with molecular biology and microbial genetics resulted in discovery of *in vivo* protein function. This is the first evidence about *in vivo* function of cyanobacterial phytochrome. Our results are very similar to the recent report that bacteriophytochrome controls photosystem synthesis in anoxygenic bacteria, *Brandyrhizobium* ORS278 (10). We are currently involved in Tandem Affinity Purification (TAP)-tagging method to study protein-protein interactions in searching for the molecular components involved in the light signal transduction of Syn6803.

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