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DNA microarray analysis of *Synechocystis* sp. PCC 6803 cells under PSII and PSI light conditions

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Introduction

Changes in light quality could cause selective excitation of photosystem II (PSII) or photosystem I (PSI) leading to an imbalance in energy distribution between the two photosystems. It is well-known that photosynthetic organisms develop acclimation responses to maintain the photosynthetic efficiency upon the spectral changes in light environment (Fujita et al., 1994). The mechanism known as state transition regulates the distribution of excitation energy between two photosystems to compensate the uneven excitation. Furthermore, photosystem stoichiometry is also modulated upon the changes in light quality. However, information on transcriptional regulation under different light qualities is restricted to only a few photosynthetic genes both in higher plants (Glick et al., 1986; Pfannschmidt et al., 1999) and cyanobacteria (Bissati and Kirilovsky, 2001). In this study, we overviewed the complete profile of gene expression of *Synechocystis* sp. PCC 6803 during the acclimation to different light quality by using DNA microarray technology.

Materials and Methods

Two types of the wild-type strain of *Synechocystis* sp. PCC 6803, PCC (motile, glucose sensitive) and GT (non-motile, glucose resistant) were used in this study. Cells were grown in volumes of 50 ml in test tubes at 25 $\mu E\ m^{-2}\ s^{-1}$. For preparation of the yellow light preferentially absorbed by PSII (PSII light), colored fluorescent lamp (FLR20S·Y-F/M·P; National, Osaka, Japan) was used. The red light preferentially absorbed by PSI (PSI light) was obtained by filtering white light through a red filter (Kyowa paraglass 102k202; Kyowa Gas Chemical, Japan) and a blue filter (SUMIPEX 703; Sumitomo Chemical, Tokyo, Japan). Change in phycocyanin/chlorophyll ratio that is a typical acclimation response to different light qualities was observed under these light conditions within 24 h after the change of light quality. DNA microarray experiments were performed as described in Hihara et al. (2001). RNA gel blot analysis was performed using the DIG system (Roche, Mannheim, Germany) according to the manufacturer's instruction.

Results and Discussion

For DNA microarray experiments, cells grown in white light were transferred to PSII or PSI light conditions. Total RNA was isolated after 30 min, 3 h and 16 h after the shift to either light quality. cDNA derived from cells grown under PSII and PSI light were labeled with

Cy5 and Cy3, respectively, and hybridized with CyanoCHIP ver.0.8 (TaKaRa, Kyoto, Japan). There were only a limited number of genes whose expression levels were influenced by the change of light quality both in GT and PCC strains in contrast to the case of the change of light intensity (Hihara et al., 2001). Table 1 shows the list of genes whose transcript levels were much higher under PSII light conditions than under PSI light conditions.

Table 1. List of genes whose transcript levels were much higher under PSII light conditions than under PSI light conditions.

Duplicate results obtained from upper and lower part of the microarray are shown for each experimental condition. Induction of more than two fold is shown by shadowed box.

	GT30min		GT3h		GT16h		PCC16h	
induced transiently								
sll0108 ammonium/methylammonium permease (*amt1*)	2.14	2.30	1.62	1.69	1.35	1.54	1.10	1.11
slr2075 10kD chaperonin (*groES*)	2.00	1.87	2.00	1.91	0.87	1.09	1.23	1.11
slr2076 60kD chaperonin 1 (*groEL*)	1.62	1.98	1.94	1.78	0.92	0.82	0.94	0.95
sll1816 30S ribosomal protein S13 (*rps13*)	1.57	1.32	2.02	1.75	1.00	1.43	1.41	1.93
ssl3437 30S ribosomal protein S17 (*rps17*)	1.95	1.65	1.92	2.37	1.28	1.34	1.35	1.47
slr0319 β-lactamase (*blaOXA-3*)	1.00	1.14	2.32	2.64	1.41	1.70	0.69	0.91
induced continuously								
sll1471 cpcG	2.44	3.11	2.69	3.06	1.61	1.91	2.10	2.36
sll1472	1.56	1.14	1.87	1.90	1.16	1.16	2.08	1.99
sll1799 50S ribosomal protein L3 (*rpl3*)	1.82	1.67	1.75	1.49	1.36	1.79	2.55	2.95
sll1801 50S ribosomal protein L23 (*rpl23*)	1.69	1.72	1.77	1.50	1.88	1.84	2.32	2.40
sll1802 50S ribosomal protein L2 (*rpl2*)	1.83	1.49	1.61	1.49	1.43	1.69	2.08	2.32
sll1804 30S ribosomal protein S3 (*rps3*)	1.71	1.88	1.22	1.42	1.70	1.43	2.51	2.25
sll1805 50S ribosomal protein L16 (*rpl16*)	1.71	1.54	1.91	2.05	1.41	1.46	1.76	1.95
sll1807 50S ribosomal protein L24 (*rpl24*)	1.85	1.86	1.92	2.20	1.40	1.57	1.89	1.90
sll1808 50S ribosomal protein L5 (*rpl5*)	1.89	1.66	1.10	1.15	1.49	1.84	2.09	2.45
induced later								
sll0227 peptidyl-prolyl cis-trans isomerase B (*ppiB*)	1.20	1.45	1.96	1.81	1.81	1.56	2.09	1.54
sll0533 trigger factor (*tig*)	1.24	1.29	1.82	1.87	1.52	1.52	2.39	2.57
sll0927 S-adenosylmethionine synthetase (*metX*)	1.00	1.04	1.79	2.18	2.17	1.96	2.44	2.50
sll1069 β-ketoacyl-acyl carrier protein synthase (*fabF*)	1.20	1.20	1.61	1.60	1.81	1.66	2.06	1.68
sll1096 30S ribosomal protein S12 (*rps12*)	1.34	1.30	2.02	1.72	2.10	2.14	3.17	3.04
sll1809 30S ribosomal protein S8 (*rps8*)	1.46	1.52	1.59	1.15	1.82	1.72	2.18	2.04
sll1818 RNA polymerase alpha subunit (*rpoA*)	1.43	1.44	1.61	1.39	1.72	1.36	2.60	2.61
sll1099 protein synthesis elongation factor Tu (*tufA*)	1.26	1.28	1.41	1.06	1.92	1.88	2.25	2.11
sll1406 ferrichrome-iron receptor (*fhuA*)	0.60	0.77	0.77	0.66	3.31	3.82	2.37	1.83
slr0513 periplasmic iron-binding protein	0.86	1.05	1.21	1.02	2.79	2.64	2.11	2.39
slr1295 iron transport protein (*sufA*)	1.08	1.01	1.45	1.45	2.21	2.06	1.65	2.49
slr1392 ferrous iron transport protein B (*feoB*)	0.87	0.84	0.80	0.73	2.40	1.52	2.32	2.41
slr1237 cytosine deaminase (*codA*)	1.13	1.25	1.38	1.17	1.80	1.83	1.81	2.01
sll1830	1.12	1.20	1.19	1.05	1.87	1.66	2.11	2.29
slr1484	0.75	0.82	0.65	0.75	2.02	1.95	2.31	1.65
slr1854	1.19	1.20	1.53	1.29	2.15	2.11	1.87	1.82
induced only in PCC strain								
sll0017 glutamate-1-semialdehyde aminomutase(*hemL*)	1.08	1.08	1.29	0.99	1.50	1.27	2.20	2.40
slr1030 Mg chelatase subunit ChlI (*chlI*)	1.03	0.97	1.01	0.92	1.23	0.94	2.27	2.03
sll0629 psaK	1.19	1.26	1.63	1.37	1.47	1.55	2.03	1.90
sll1743 50S ribosomal protein L11 (*rplI1*)	1.53	1.51	1.24	1.22	1.63	1.68	2.68	2.54
sll1810 50S ribosomal protein L6 (*rpl6*)	1.53	1.55	1.08	1.43	1.44	1.26	2.08	1.87
sll0577	1.16	1.17	1.28	1.08	1.66	1.42	2.00	2.10

It may be noted that ribosomal operons were induced. Genes involved in some metabolic pathways and iron transport were also up-regulated. Although transcript levels of many photosynthesis-related genes were maintained nearly constant, *sll1471*(*cpcG2*) that encodes rod-core linker protein of phycobilisome showed remarkable induction. Table 2 shows the list of genes preferentially expressed under PSI light conditions. Induction of *chlN* and some unidentified ORFs was prominent. psbA genes were also induced, which is consistent with the

Table 2. List of genes whose transcript levels were much higher under PSI light conditions than under PSII light conditions.

Duplicate results obtained from upper and lower part of the microarray are shown for each experimental condition. Induction of more than two fold is shown by shadowed box.

	GT30min		GT3h		GT16h		PCC16h	
induced transiently								
sll0525	2.12	2.12	2.01	1.49	1.74	1.55	1.47	1.39
sll1834	2.20	1.54	2.11	2.63	1.43	1.41	1.23	1.46
sll0815	1.35	1.47	2.61	2.19	1.66	1.76	1.79	1.38
sll1702	1.56	1.26	2.27	2.39	1.67	1.76	1.21	1.15
slr0474 CheY subfamily	1.56	1.43	2.13	2.56	1.29	1.53	1.80	1.83
slr1437	1.46	1.23	2.13	2.19	1.67	1.68	1.48	1.41
induced continuously								
sll0283	2.54	1.70	3.17	2.46	2.48	2.16	1.83	2.26
sll0501	4.28	2.60	2.04	2.35	1.85	2.27	1.34	1.80
sll0872	1.72	2.10	1.87	2.13	2.38	2.27	2.16	2.32
sll1514 16.6 kDa small heat shock protein (*hsp17*)	1.77	1.59	1.69	1.95	2.50	2.46	1.41	1.30
slr0656	1.60	2.00	2.46	3.16	1.73	2.14	2.01	2.00
slr0750 protochlorophillide reductase subunit (*chlN*)	2.08	2.42	2.28	2.10	2.33	2.20	2.62	2.15
slr1634	1.98	1.84	3.68	4.10	2.21	1.93	2.05	1.80
induced later								
sll1222	1.41	1.77	1.96	2.10	1.57	1.73	2.04	1.73
sll1224 hydrogenase small subunit (*hoxY*)	1.18	1.44	2.14	2.35	1.41	1.86	1.71	2.17
sll1515	1.30	1.54	1.83	2.15	2.55	2.46	2.24	2.22
sll1796 cytochrome c553 (*petJ*)	1.41	1.49	1.44	1.46	2.22	2.11	1.72	2.01
sll1862	1.13	1.18	1.19	1.46	1.75	1.92	2.06	2.08
sll1864 chloride channel protein	1.68	1.66	1.87	2.13	2.04	1.89	1.63	1.67
sll1867 photosystem II D1 protein (*psbA3*)	0.98	1.03	1.37	1.27	2.37	2.16	2.01	1.84
slr0373	1.52	1.41	1.75	1.84	1.79	1.79	2.20	2.37
slr0376	1.37	1.27	1.47	1.44	2.20	1.49	2.22	2.07
slr0885	1.19	1.33	1.45	2.40	1.99	2.22	2.93	1.81
slr1068	1.61	1.76	1.74	2.06	3.35	2.49	2.09	2.81
slr1311 photosystem II D1 protein (*psbA2*)	1.22	1.06	1.29	1.37	2.08	2.15	1.85	1.79
slr1397	1.32	1.32	2.34	2.61	1.75	1.96	2.25	2.07
slr1928	0.93	0.89	2.44	2.08	3.26	2.60	2.03	1.70
slr1929	1.43	1.07	2.08	2.16	2.55	2.47	2.25	1.96
slr2010	1.83	1.54	2.35	2.07	1.67	1.97	1.71	1.33
ssl1911	1.23	1.35	1.45	1.41	2.23	2.11	1.98	1.78
induced only in PCC strain								
slr1667	1.25	1.19	1.83	1.36	1.46	1.89	2.49	2.69
slr1668	1.11	1.12	0.97	0.94	1.52	1.67	2.90	2.29

observation of Bissati and Kirilovski (2001). The gene expression profiles of PCC and GT strains were similar, although they showed quite different physiological responses to changing light qualities. For instance, phycocyanin/chlorophyll ratio is much higher in PCC strain. Moreover, PCC strain, but not GT strain, modulates PSII/PSI ratio under different light qualities (not shown). These differences in PCC and GT strains should be due to other factors than transcriptional regulation.

We further examined transcript level of *sll1471* (*cpcG2*) that were preferentially induced under PSII conditions by RNA gel blot analysis. As shown in Fig. 1A,

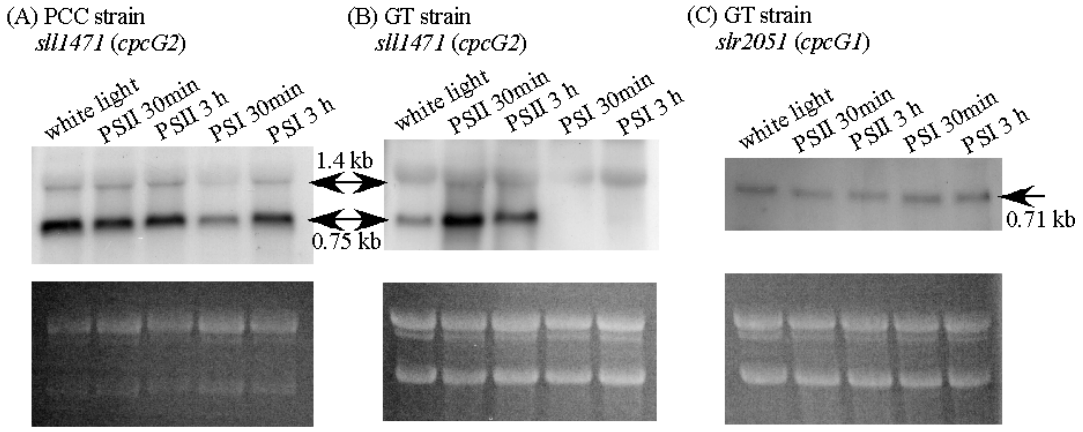

Fig.1 Transcript levels of cpcG genes under different light qualities. The top panel shows the RNA gel blot analysis of *cpcG1* and *cpcG2* transcripts under different light qualities. Sizes of transcripts are shown by arrows. The profile of rRNA stained with ethidium bromide is shown as a control for equal RNA loading.

PCC strain only transiently reduced the transcript level under PSI light conditions. On the other hand, in GT strain, the transcript was remarkably up-regulated under PSII light conditions and totally disappeared under PSI light conditions (Fig. 1B). About 1.4 kb of *sll1471-1472* transcript was also observed in addition to 0.75 kb of *sll1471* transcript. In contrast to *cpcG2*, gene expression of *cpcG1* (*slr2051*) was not influenced by light quality (Fig. 1C). *cpcG* genes are known to exist as a multigene family in cyanobacteria (Glauser et al., 1992). However, the physiological role of each copy has not been determined. In *Synechocystis* sp. PCC 6803, *cpcG2* gene may have some special role in acclimation to different light qualities such as energy transfer to PSI. Accumulation of *cpcG2* transcript was largely repressed by addition of both DCMU and DBMIB (not shown), indicating that its expression is modulated by the redox state of cytochromeb_6/f complex. Further characterization of this gene is now in progress.

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