DNA Microarray Analysis of Cyanobacterial Gene Expression during Acclimation to High Light

Yukako Hihara,[a,1] Ayako Kamei,[b] Minoru Kanehisa,[c] Aaron Kaplan,[d] and Masahiko Ikeuchi[b]

[a] Department of Biochemistry and Molecular Biology, Faculty of Science, Saitama University, Urawa, Saitama 338-8570, Japan
[b] Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Tokyo 153-8902, Japan
[c] Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan
[d] Department of Plant Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

DNA microarrays bearing nearly all of the genes of the unicellular cyanobacterium *Synechocystis* sp PCC 6803 were used to examine the temporal program of gene expression during acclimation from low to high light intensity. A complete pattern is provided of gene expression during acclimation of a photosynthetic organism to changing light intensity. More than 160 responsive genes were identified and classified into distinct sets. Genes involved in light absorption and photochemical reactions were downregulated within 15 min of exposure to high light intensity, whereas those associated with CO_2 fixation and protection from photoinhibition were upregulated. Changes in the expression of genes involved in replication, transcription, and translation, which were induced to support cellular proliferation, occurred later. Several unidentified open reading frames were induced or repressed. The possible involvement of these genes in the acclimation to high light conditions is discussed.

INTRODUCTION

Photosynthetic organisms must acclimate to changing light intensity in their environment. The acclimation process includes changes in the photosynthetic apparatus, presumably to balance energy input and consumption (Boardman, 1977). If energy supply (light harvesting and electron transport) exceeds its dissipation (by CO_2 fixation and other energy-demanding processes), particularly under high light (HL) conditions, the photosynthetic electron transport components could become relatively reduced. This may result in excess production of reactive oxygen species (ROS) leading to severe damage to many cellular processes (Asada, 1994). Absorption of excess light energy therefore must be avoided by reduction of both antenna size and photosystem content. Furthermore, under HL conditions the capacity for CO_2 fixation increases (Björkman, 1981; Anderson, 1986) and protection from ROS is enhanced (Grace and Logan, 1996).

Acclimation from low light (LL) to HL conditions can be divided into short-term and long-term processes (Anderson, 1986; Anderson et al., 1995). Short-term acclimation includes state transitions, protective energy dissipation (Campbell et al., 1998; Niyogi, 1999), changes in the efficiency of energy transfer from the harvesting complex to photosystem II (PSII;

Hassidim et al., 1997), and the formation of nonfunctional PSII reaction centers (Chow, 1994; Anderson et al., 1997). These responses occur rather rapidly and usually are completed within several minutes. Conversely, the long-term acclimation to HL is much slower because it involves changes in the composition, function, and structure of the photosynthetic apparatus as well as other photosynthesis-related components. Completion of the long-term processes may take hours or even days.

Physiological responses to changing light intensity have been examined extensively (Foy and Gibson, 1982a, 1982b; Anderson, 1986; Neale and Melis, 1986), and some of the relevant molecular mechanisms have been described (Anderson et al., 1995; Hihara, 1999; Niyogi, 1999). Nevertheless, the mechanisms that enable the cells to acclimate to the changing light field are still poorly understood. By means of DNA microarray analyses, the expression of thousands of genes can be monitored simultaneously (Schena et al., 1995; DeRisi et al., 1996, 1997). In this study, we surveyed time-dependent gene expression in the unicellular cyanobacterium *Synechocystis* sp PCC 6803 as affected by transfer from LL to HL. This organism is well suited for such study because the entire genomic sequence has been determined (Kaneko et al., 1996), and DNA microarrays representing all open reading frames (ORFs) are now available. Mutants impaired in the ability of this organism to acclimate to HL are available in our laboratory including a *pmgA* mutant that

could not modulate the photosystem stoichiometry during acclimation to HL (Hihara and Ikeuchi, 1997; Hihara et al., 1998). We examined the mRNA levels change at 15 min, 1 hr (initial stressed phase), 6 hr, and 15 hr (acclimated phase) after the shift to HL conditions. We found that the expression of 84 ORFs was upregulated and the expression of 80 ORFs was downregulated after these HL exposures.

RESULTS

Light Intensity and Duration of Exposures

Synechocystis cells can grow under a wide range of light intensities, up to approximately 1000 μmol photons m^{-2} sec^{-1} (Mohamed and Jansson, 1989; Yokoyama et al., 1991). However, to avoid excessive damage under very HL intensities (Constant et al., 1997), we examined the response of gene expression to a shift of light intensity from 20 μmol photons m^{-2} sec^{-1} (LL) to 300 μmol photons m^{-2} sec^{-1} (HL). This intensity was sufficient to induce large changes in the gene expression profile. Our earlier studies on the effect of light intensity on the content of chlorophyll and phycocyanin per cell showed that the cells responded in three different phases: (1) drastic reduction to approximately two thirds within 3 hr; (2) moderate decrease at a slower rate for up to 12 hr; and (3) gradual recovery after 12 hr (Hihara et al., 1998). Hence, in the present study, we sampled the cells for RNA isolation after 15 min, 1 hr, 6 hr, and 15 hr of exposure to the HL treatment. The measurements at 15 min and 1 hr represent the initial stressed phase, whereas the 15-hr point represents the acclimated phase. The 6-hr point was selected because it was the doubling time of the culture under the HL regimen and may indicate the change from the stressed phase to the acclimated phase.

Reliability of the DNA Microarray Analysis

Total RNA from LL and HL cells was labeled with fluorescent dye, either Cy3 or Cy5. Generated cDNA probes were mixed and hybridized to CyanoCHIP version 0.8 (see Methods). Figure 1 shows a typical overlay image of Cy3 and Cy5 fluorescence on the microarray after hybridization. In the case presented here, the microarray was hybridized with Cy3-labeled cDNA from LL-grown cells and Cy5-labeled cDNA from cells grown in HL for 6 hr. In this image, some red spots (preferentially expressed in LL) and green spots (preferentially expressed in HL) are seen, in addition to many yellow spots (equally expressed). To evaluate such differences in gene expression, we quantified the fluorescence intensity of each spot in both Cy3 and Cy5 images (see Methods), and the expression of each gene under HL conditions was shown as a percentage of the LL levels.
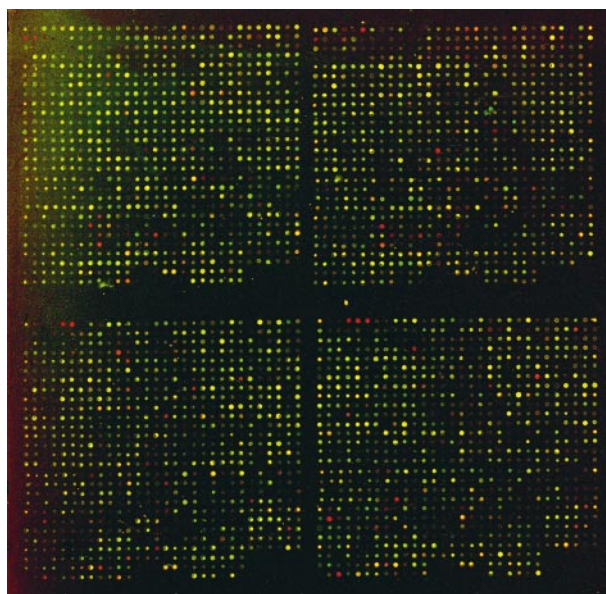

Figure 1. Two-Color Overlaid Fluorescent Image of a DNA Microarray of HL-Induced and HL-Repressed Genes in *Synechocystis* sp PCC 6803.

Two hybridization data sets from the same microarray were converted into pseudocolor images and superimposed to visualize differential gene expression between LL (Cy3, red) and HL of 6 hr (Cy5, green). ORFs unaffected by the HL shift appear as yellow spots. The upper part of the microarray is shown.

To determine the reliability of these data, we compared the expression levels of *psaA* and *psbA2* between the DNA microarray and the RNA gel blot analyses shown in Figure 2. It is clear that relative transcript levels assessed by RNA gel blot analysis correlated well over a wide range with those obtained by the DNA microarray analyses. In some cases, RNA levels deduced by the DNA microarray analyses were higher than those assessed by the RNA gel blot analysis (Figure 2, spot 1), probably because rapidly degrading RNA was being detected by dot blot analysis such as the microarray technique. Furthermore, in the DNA microarray methodology, the ratio of HL to LL signals tended to be lower than in the RNA gel blot analysis. This was most pronounced in the case of "weak spots" due to the masking effects of background hybridization (data not shown). In fact, background fluorescence levels or signal intensities of negative control spots (human transferrin receptor gene) often scored at approximately 2500 of 3079 spots. In the case of genes with expression levels lower than 2000 of 3079, it would be difficult to reproducibly detect changes in the transcript levels even if they were markedly affected by HL. Therefore, in this study, we focused mainly on genes with expression levels that were >2000 of 3079 in both LL and HL conditions.

Changes in the Transcript Abundance of Genes Encoding Thylakoid-Located Complexes

PSII-Related Genes

Among the *psb* genes, which encode subunits of PSII, the transcript abundance of *psbA* genes encoding the D1 protein increased approximately threefold within 15 min and subsequently decreased (Figure 3A). Because the nucleotide sequences of the coding regions of *psbA2* and *psbA3* are almost identical, we could not distinguish their transcripts. Similar induction pattern by HL treatment was observed in the case of *psbX* (Figure 3A), which encodes the small subunit localized to the vicinity of the reaction center (Shi et al., 1999). In contrast, the transcript levels of *psbD1* that encodes reaction center D2, *psbB*-encoding CP47, and *psbC* that encodes CP43 decreased within the first period of HL and reached a minimum value 1 hr after the shift to HL. However, longer exposure to HL resulted in an increased level of transcripts originating from these genes. After 15 hr of HL treatment, their abundance was 50 to 100% of the level observed under LL. Transcripts of genes involved in the oxygen-evolving complex, *psbO*, *psbU*, and *psbV*, were all downregulated to half the level in LL within 15 min. The transcript levels of many other genes that encode small subunits of PSII, including *psbE*, *psbF*, and *psbK*, were not affected by the HL treatment. We did not observe significant induction of the *psbD2* gene, which has been reported to be inducible by HL in *Synechococcus* sp PCC 7942 (Bustos and Golden, 1992; Anandan and Golden, 1997).

Photosystem I–Related Genes

In contrast with the case of genes encoding subunits of PSII, some of which were upregulated and others downregulated (Figure 3A), almost all of the photosystem I (PSI) genes were downregulated by five- to 10-fold within 1 hr of the transfer from LL to HL (Figure 3B). Notably, the level of *psaAB* transcripts for the reaction center of PSI declined by 30-fold of its original value after 1 hr of HL treatment. An exception was one of the two *psaK* genes (Nakamoto and Hasegawa, 1999; Naithani et al., 2000) that was markedly induced within 1 hr and stayed at a high level throughout the exposure to HL (Figure 3B).

Phycobilisome-Related Genes

Figure 3C shows that the transcription of *apc* genes related to allophycocyanin and of *cpc* genes related to phycocyanin is regulated differently. The *apc* genes were downregulated to one-tenth of their original level within 1 hr of HL but then recovered to the initial level within 15 hr. Conversely, the abundance of the *cpc* transcript decreased by 20-fold

within 1 hr of HL and recovered after 15 hr of HL to a level 50% of the initial. The *cpc* genes may be downregulated more strictly than *apc* genes during exposure to HL because phycocyanin, which is located at the terminal regions of the phycobilisome rods, is the primary target for the reduction of antenna size. It is interesting that *cpcD*, which is part of the *cpcBACCD* operon, apparently was not downregulated as much as the other *cpc* genes. It is possible that this was due to the relatively weak signal of the *cpcD* spot. As indicated above, changes in transcript levels of weakly expressed genes tend to be underestimated because of background hybridization. Furthermore, *cpcD* may be transcribed not only polycistronically but also monocistronically.
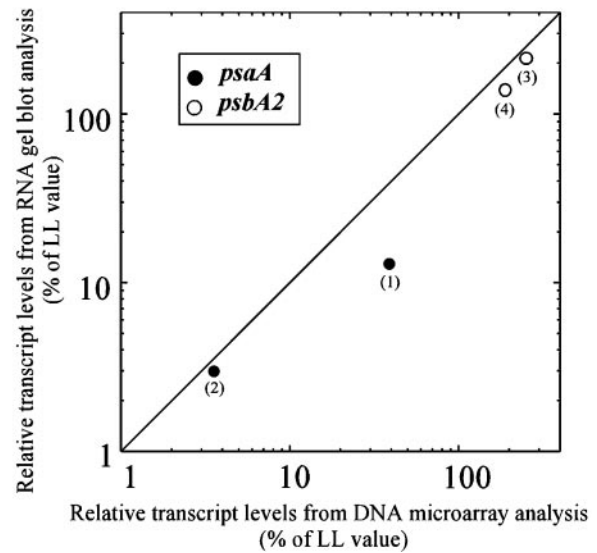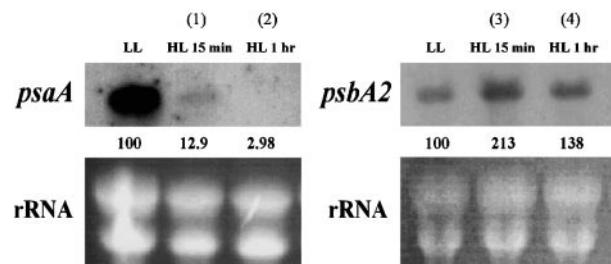

Figure 2. Comparison of the Results Obtained by DNA Microarray Analysis and RNA Gel Blot Analysis.

The top panel shows the RNA gel blot analysis of *psaA* and *psbA2* transcripts under LL and HL conditions. Values below the blots show the relative levels of transcripts expressed as a percentage of values under LL conditions. The profile of rRNAs stained with ethidium bromide is shown as a control for equal RNA loading. The bottom panel shows the correlation between the relative transcript levels obtained by DNA microarray analysis and RNA gel blot analysis. The same RNA sample was used for the DNA microarray analysis and the RNA gel blot analysis.
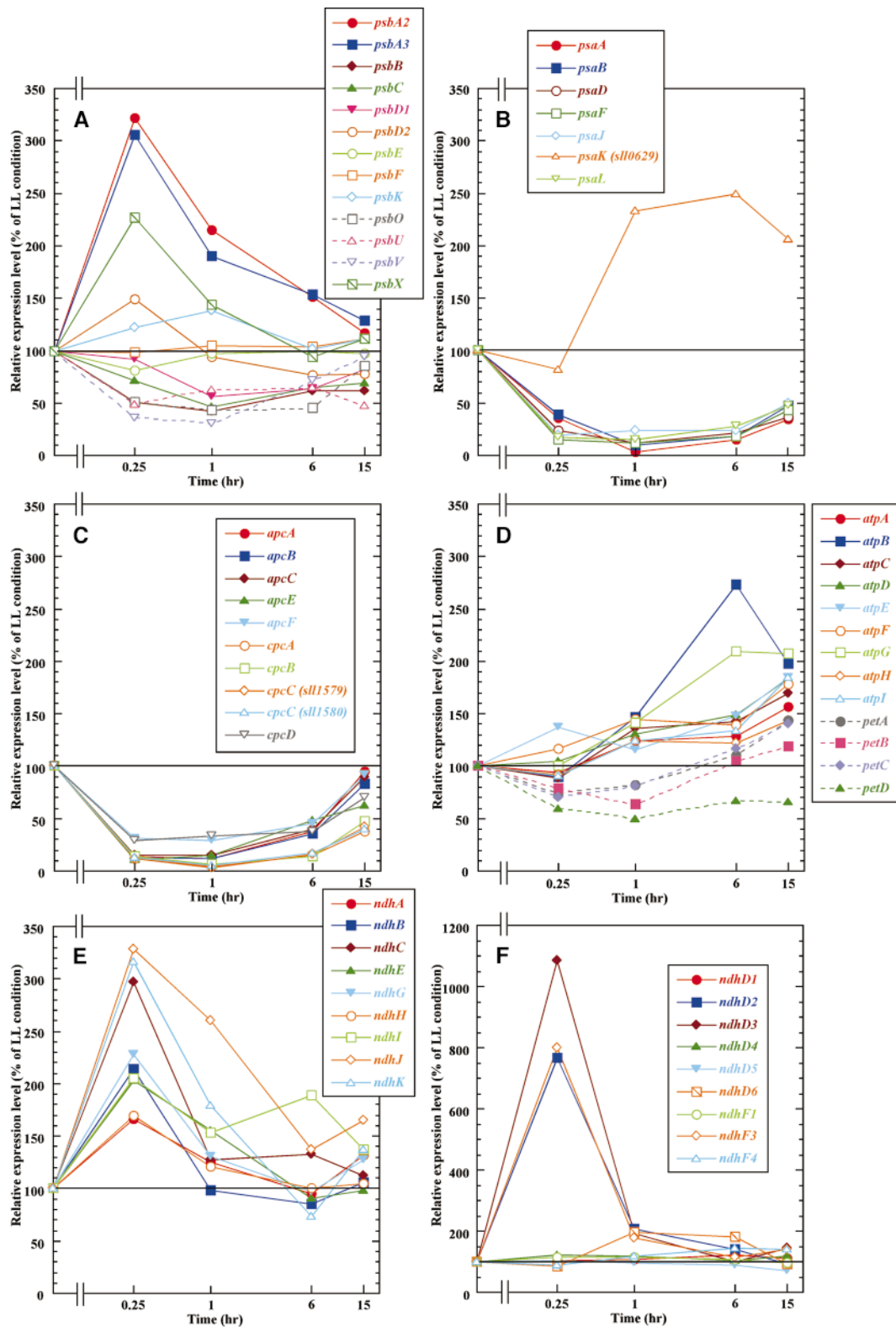
Figure 3. Accumulation Profile of Transcripts for Protein Complexes in Thylakoid Membranes upon the Shift to HL.

Belknap and Haselkorn (1987) reported that, in *Anabaena* sp PCC 7120, the *cpcBACDE* operon was downregulated by HL, whereas the level of the transcript that encodes the last two ORFs, *cpcDE*, was relatively insensitive to light intensity.

The ndh Genes

Synechocystis sp PCC 6803 possesses many *ndh* genes encoding subunits of NADPH dehydrogenase. Some of the *ndh* genes are present in single copy (Figure 3E), and some are present in multiple copies (Figure 3F). The transcript abundance of *ndhC*, *ndhK*, and *ndhJ*, which constitutes an operon structure (*slr1279* to *slr1281*), increased by threefold within 15 min of exposure to HL (Figure 3E), whereas the extent of induction of *ndhA*, *ndhI*, *ndhG*, and *ndhE* (which constitutes another operon, *sll0519* to *sll0522*) was smaller (Figure 3E). There are six copies of the *ndhD* gene and three copies of the *ndhF* gene in *Synechocystis* sp PCC 6803 (Price et al., 1998). Surprisingly, *ndhD2*, *ndhD3*, and *ndhF3* showed transient and prominent induction by eightfold or even more within 15 min of HL, whereas transcription of the other genes did not respond to the HL treatment (Figure 3F). *ndhF3*, *ndhD3*, and an ORF encoded by *sll1734* form an operon thought to be involved in CO_2 uptake in *Synechocystis* sp PCC 6803 (Ohkawa et al., 1998, 2000). The putative protein encoded by *sll1734* also was induced by HL (Table 1). Ohkawa et al. (1998) observed that *ndhD2*, which is transcribed monocistronically, and the *ndhF3* operon also are induced by low CO_2. These *ndh* genes may be induced by common signals shared by low CO_2 and HL (Kaplan and Reinhold, 1999).

Other Thylakoid-Related Genes

The genes encoding subunits of ATP synthase, *atp*, and cytochrome *b6/f* complex, *pet*, were little affected by HL. The level of transcripts originating from *atp* increased gradually by 1.5- to twofold, whereas those from *pet* decreased slightly at the beginning of exposure to HL and then returned to the original level (Figure 3D). We could not reproducibly detect changes in transcript levels of *cta* genes, which encode subunits of cytochrome *c* oxidase (data not shown).

Changes in the Transcript Abundance of the Other Genes

The expression of many genes not directly related to light harvesting or thylakoid structure also was affected by the light treatment. Although our microarray analysis included all 3079 ORFs of *Synechocystis* PCC 6803, we present in Table 1 only those for which the transcript levels were altered by at least twofold during the exposure to HL (15-min, 1-hr, 6-hr, and 15-hr time points). In view of these criteria, 84 ORFs were induced by HL, and 80 ORFs were repressed in the genome of *Synechocystis* sp PCC 6803. For simplicity, we subdivided these ORFs into six groups according to the changing patterns of their transcription after the shift from LL to HL: type 1, induction observed within 15 min, then decreased; type 2, induced at a high level throughout the HL period; type 3, induction observed after 1 hr; type 4, transcript level decreased within 15 min, then increased; type 5, repressed throughout the HL period; and type 6, repressed at approximately 1 hr. Below, we mention some of the ORFs that were strongly up- or downregulated by HL.

Many genes homologous with heat shock proteins from other organisms have been identified in *Synechocystis* sp PCC 6803, including *clpB*, *htpG*, three *dnaK*s, *groES*, *groEL*, *groEL-2*, four *dnaJ*s, *grpE*, and *hsp17* (Kaneko et al., 1996; Glatz et al., 1999). Notably, many of them were markedly induced by the shift to HL. For example, *clpB*, *htpG*, *dnaK* (*sll0170*), *groES*, *groEL*, *groEL-2*, and *hsp17* were

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Figure 3. (continued).

The transcript abundance as affected by time after transfer of *Synechocystis* sp PCC 6803 from LL to HL. Transcript levels for subunits of PSII **(A)**, subunits of PSI **(B)**, phycobiliproteins **(C)**, subunits of ATP synthase and the cytochrome *b6/f* complex **(D)**, subunits of NADPH dehydrogenase in which genes exist as a single copy in the genome **(E)**, and subunits of NADPH dehydrogenase that are encoded by multiple genes **(F)** are shown. Each time point is shown logarithmically on the horizontal axis. Accumulation levels of transcripts are expressed on the vertical axis as a percentage of values under LL conditions. Values at 15 min and 1 hr are averages of duplicate results for three independent experiments (six data points in total), and values at 6 and 15 hr are averages of duplicate results for two experiments (four data points in total). The weakly expressed genes encoding thylakoid proteins are omitted from the figures. Identification numbers of the depicted genes are as follows: *psbA2*, *slr1311*; *psbA3*, *sll1867*; *psbB*, *slr0906*; *psbC*, *sll0851*; *psbD1*, *sll0849*; *psbD2*, *slr0927*; *psbE*, *ssr3451*; *psbF*, *smr0006*; *psbK*, *sml0005*; *psbO*, *sll0427*; *psbU*, *sll1194*; *psbV*, *sll0258*; *psbX*, *sml0002*; *psaA*, *slr1834*; *psaB*, *slr1835*; *psaD*, *slr0737*; *psaF*, *sll0819*; *psaJ*, *sml0008*; *psaK*, *sll0629*; *psaL*, *slr1655*; *apcA*, *slr2067*; *apcB*, *slr1986*; *apcC*, *ssr3383*; *apcE*, *slr0335*; *apcF*, *slr1459*; *cpcA*, *sll1578*; *cpcB*, *sll1577*; *cpcC*, *sll1579* and *sll1580*; *cpcD*, *ssl3093*; *atpA*, *sll1326*; *atpB*, *slr1329*; *atpC*, *sll1327*; *atpD*, *sll1325*; *atpE*, *slr1330*; *atpF*, *sll1324*; *atpG*, *sll1323*; *atpH*, *ssl2615*; *atpI*, *sll1322*; *ndhA*, *sll0519*; *ndhB*, *sll0223*; *ndhC*, *slr1279*; *ndhE*, *sll0522*; *ndhG*, *sll0521*; *ndhH*, *slr0261*; *ndhI*, *sll0520*; *ndhJ*, *slr1281*; *ndhK*, *slr1280*; *ndhD1*, *slr0331*; *ndhD2*, *slr1291*; *ndhD3*, *sll1733*; *ndhD4*, *sll0027*; *ndhD5*, *slr2007*; *ndhD6*, *slr2009*; *ndhF1*, *slr0844*; *ndhF3*, *sll1732*; and *ndhF4*, *sll0026*.

Table 1. Complete List of the HL-Induced and HL-Repressed Genes

Gene and Description	HL incubation (% of LL) [a]			
	15 min	1 hr	6 hr	15 hr
Type 1, induced within 15 min, then decreased				
Photosystem II reaction center D1				
slr1311 psbA2	322.2 ± 78.7	214.9 ± 108.0	151.0 ± 20.3	116.3 ± 47.2
sll1867 psbA3	305.6 ± 43.2	190.3 ± 25.4	153.3 ± 18.9	128.5 ± 83.2
NAD(P)H dehydrogenase				
sll1732 ndhF3	801.2 ± 237.5	178.3 ± 47.2	116.0 ± 24.7	141.8 ± 18.8
sll1733 ndhD3	1084.4 ± 161.9	192.3 ± 62.2	101.6 ± 25.1	145.8 ± 33.3
sll1734	454.7 ± 135.8	134.4 ± 20.1	107.2 ± 27.6	128.0 ± 12.0
slr1280 ndhK	319.3 ± 54.5	178.8 ± 60.6	97.4 ± 34.0	137.6 ± 13.5
slr1291 ndhD2	766.1 ± 233.1	209.3 ± 59.6	139.5 ± 11.1	93.9 ± 12.1
Heat shock protein				
sll0170 dnaK	638.1 ± 316.1	206.0 ± 85.0	89.9 ± 8.5	105.7 ± 8.6
sll0430 htpG	624.4 ± 181.2	196.4 ± 38.3	131.6 ± 34.1	141.2 ± 5.1
sll1514 hsp17	1221.3 ± 919.5	125.8 ± 58.4	114.9 ± 45.0	67.5 ± 9.8
slr1641 clpB	815.2 ± 289.7	158.1 ± 62.7	96.9 ± 25.3	97.3 ± 19.0
Fatty acid desaturase				
slr1350 desA	268.7 ± 44.0	135.2 ± 56.0	118.7 ± 22.0	160.9 ± 65.9
sll1441 desB	260.8 ± 85.5	260.5 ± 42.2	205.8 ± 24.9	161.2 ± 30.2
Scavenging enzyme for reactive oxygen species				
slr1516 sodB	420.9 ± 200.0	167.4 ± 81.7	207.5 ± 65.5	180.9 ± 79.2
slr1992 glutathione peroxidase	296.7 ± 95.3	321.0 ± 47.6	269.9 ± 96.1	172.8 ± 5.6
FtsH				
slr0228 ftsH	552.2 ± 96.4	243.4 ± 150.0	196.3 ± 51.5	95.8 ± 47.6
slr1604 ftsH	457.0 ± 126.2	178.7 ± 45.5	113.6 ± 26.2	110.9 ± 33.5
Hydrogenase				
slr1675 hypA	519.6 ± 287.3	183.1 ± 61.2	116.9 ± 29.4	83.5 ± 11.0
ssl3044 hydrogenase component	577.7 ± 229.2	279.6 ± 79.1	235.2 ± 22.9	186.8 ± 40.6
Others				
sll1483 transforming growth factor–induced protein	756.8 ± 433.5	617.0 ± 211.5	312.6 ± 162.8	153.5 ± 44.2
sll1541 "lignostilbene–α,β–dioxygenase"	443.2 ± 101.1	284.3 ± 59.9	207.1 ± 90.1	100.7 ± 29.4
sll2012 rpoD	394.6 ± 209.5	309.6 ± 258.6	275.0 ± 55.6	79.1 ± 22.9
slr0394 pgk phosphoglycerate kinase	285.8 ± 69.3	161.2 ± 57.0	135.2 ± 36.4	129.2 ± 13.2
slr0426 folE or *gchL* GTP cyclohydrolase I	268.5 ± 55.7	299.5 ± 88.3	253.1 ± 62.5	196.9 ± 36.1
slr1105 fus elongation factor EF-G	324.8 ± 69.2	330.6 ± 43.7	190.3 ± 37.2	136.7 ± 42.4
slr1351 murF	332.2 ± 81.5	213.9 ± 73.3	153.2 ± 26.9	140.0 ± 20.1
slr1594 patA	390.5 ± 153.4	154.5 ± 18.4	77.3 ± 17.0	66.5 ± 12.3
sll0217 potential FMN protein	598.4 ± 223.2	167.9 ± 39.4	138.4 ± 42.3	107.2 ± 11.0
sll0218	1134.5 ± 456.2	136.7 ± 57.4	112.9 ± 64.8	76.8 ± 15.5
sll0219 potential FMN protein	901.2 ± 459.1	142.8 ± 74.5	76.1 ± 22.4	65.5 ± 9.8
sll0528	1562.1 ± 497.4	160.6 ± 24.3	110.2 ± 19.8	81.2 ± 14.6
sll0668	245.5 ± 40.4	157.6 ± 48.8	112.7 ± 35.4	149.5 ± 23.7
sll0814	381.7 ± 105.0	215.6 ± 68.4	172.8 ± 32.3	117.7 ± 14.5
sll0846	559.7 ± 221.0	107.5 ± 26.2	92.8 ± 32.7	67.0 ± 8.8
sll1884	719.4 ± 175.1	214.2 ± 56.7	141.0 ± 44.9	139.3 ± 23.4
slr0007	400.7 ± 52.3	281.5 ± 96.4	149.9 ± 23.1	166.3 ± 20.9
slr0023	286.0 ± 61.9	136.4 ± 52.0	98.6 ± 18.6	101.2 ± 10.7
slr0476	294.4 ± 43.0	129.7 ± 63.4	100.3 ± 27.4	110.0 ± 18.4
slr0740	364.2 ± 240.3	183.6 ± 66.5	219.3 ± 9.5	194.3 ± 15.2
slr0959	501.3 ± 163.5	202.5 ± 52.5	160.7 ± 20.3	134.7 ± 15.4
slr1544	924.0 ± 685.4	750.8 ± 698.5	372.4 ± 177.5	94.1 ± 46.8
slr1674	479.2 ± 122.3	126.2 ± 29.6	83.9 ± 10.8	60.0 ± 18.0
slr1686	387.8 ± 128.1	180.4 ± 74.1	134.4 ± 46.6	109.6 ± 13.2
slr1963	614.9 ± 223.0	419.7 ± 132.0	210.1 ± 28.5	183.4 ± 32.8
Type 2, induced continuously at high levels				
CO$_2$–concentrating mechanism protein				
sll1028 ccmK	621.8 ± 97.8	294.7 ± 194.4	229.0 ± 142.9	282.5 ± 86.6
sll1029 ccmK	458.0 ± 217.1	240.3 ± 16.7	298.1 ± 52.9	289.4 ± 30.7
sll1031 ccmM	468.7 ± 90.2	275.8 ± 91.0	245.4 ± 93.4	255.4 ± 52.9
sll1032 ccmN	564.6 ± 261.6	460.2 ± 93.9	250.6 ± 69.3	266.2 ± 37.2
RuBP carboxylase				
slr0009 rbcL	352.0 ± 15.4	194.1 ± 127.6	153.4 ± 66.7	215.0 ± 46.4
slr0011 rbcX	362.1 ± 85.3	192.8 ± 123.4	152.3 ± 52.2	199.9 ± 42.7
slr0012 rbcS	304.6 ± 111.7	192.1 ± 95.9	182.7 ± 79.5	209.7 ± 60.9
GroESL				
slr2075 groES	534.0 ± 205.3	591.8 ± 238.3	345.6 ± 137.3	311.3 ± 50.6
slr2076 groEL	542.2 ± 260.6	461.0 ± 209.8	261.0 ± 45.3	316.9 ± 112.9
sll0416 groEL-2	471.8 ± 118.5	367.5 ± 28.3	188.9 ± 26.0	207.7 ± 26.2

Table 1. (continued)

Others				
sll0322 hypF transcriptional regulatory protein	329.9 ± 186.5	236.6 ± 90.4	301.5 ± 36.2	238.3 ± 34.5
sll0927 metX S-adenosylmethionine synthetase	289.5 ± 75.4	258.0 ± 140.8	191.1 ± 79.3	273.2 ± 47.0
sll1096 rps12	228.7 ± 44.5	401.3 ± 140.4	322.3 ± 30.9	358.6 ± 122.6

Type 3, induced at approximately 1 hr

Ribosomal protein				
sll1097 rps7	156.2 ± 19.3	313.1 ± 66.1	241.5 ± 53.8	271.9 ± 66.2
sll1101 rps10	189.0 ± 45.3	289.4 ± 138.5	222.6 ± 66.6	250.5 ± 22.6
sll1743 rpl11	101.4 ± 29.8	320.0 ± 78.2	253.8 ± 47.6	321.2 ± 31.6
sll1744 rpl1	111.0 ± 11.9	326.7 ± 140.3	217.1 ± 38.7	275.5 ± 18.9
sll1745 rpl10	74.6 ± 18.3	269.3 ± 88.7	205.3 ± 59.4	280.4 ± 40.1
sll1799 rpl3	176.3 ± 47.9	253.4 ± 82.0	304.1 ± 66.4	331.6 ± 34.5
sll1800 rpl4	117.3 ± 62.2	269.6 ± 59.6	312.9 ± 24.4	275.8 ± 38.2
sll1801 rpl23	141.9 ± 55.9	327.6 ± 47.6	272.8 ± 82.1	299.4 ± 47.6
sll1802 rpl2	156.6 ± 41.0	282.0 ± 137.2	296.8 ± 45.6	363.4 ± 59.6
sll1804 rps3	92.2 ± 46.0	246.4 ± 99.2	370.5 ± 84.9	289.4 ± 20.7
sll1807 rpl24	99.6 ± 10.5	327.6 ± 90.7	288.8 ± 95.8	297.5 ± 53.9
sll1809 rps8	98.8 ± 7.7	249.4 ± 48.9	226.4 ± 44.1	226.1 ± 40.3
sll1810 rpl6	63.2 ± 32.1	251.3 ± 77.3	272.6 ± 59.2	235.1 ± 14.2
sll1816 rps13	111.3 ± 16.9	280.0 ± 106.5	223.9 ± 18.5	261.6 ± 42.3
ssl3437 rps17	102.0 ± 16.7	318.4 ± 136.3	235.5 ± 111.0	294.8 ± 93.6
Others				
sll0533 tig trigger factor	140.8 ± 25.9	279.0 ± 152.3	212.5 ± 8.0	262.7 ± 74.1
sll1261 tsf elongation factor TS	139.5 ± 20.9	210.6 ± 90.4	212.3 ± 42.6	241.3 ± 16.7
sll1742 nusG transcription antitermination protein	146.1 ± 29.7	381.4 ± 136.3	217.7 ± 46.6	266.8 ± 38.3
sll1818 rpoA RNA polymerase α subunit	84.5 ± 42.0	216.1 ± 84.8	459.0 ± 237.9	273.4 ± 87.9
slr0743 nusA N utilization substance protein	163.1 ± 15.7	327.3 ± 46.4	262.0 ± 24.8	240.1 ± 16.3
slr1251 cyp peptidyl-prolyl cis-trans isomerase	149.4 ± 29.7	226.6 ± 87.1	245.0 ± 22.0	227.9 ± 27.4
slr0364	182.6 ± 92.0	278.3 ± 128.4	404.2 ± 87.0	353.8 ± 84.5
slr0742	188.1 ± 70.2	249.3 ± 93.8	217.0 ± 88.8	215.9 ± 8.1
slr0923	143.9 ± 41.3	256.4 ± 55.5	253.3 ± 61.4	293.5 ± 75.2
slr0955	103.8 ± 13.2	251.7 ± 50.3	226.4 ± 96.8	169.1 ± 36.5
sll1043	122.2 ± 18.3	180.0 ± 81.0	227.5 ± 23.6	237.0 ± 19.4
slr0554	145.9 ± 41.9	177.7 ± 54.2	302.1 ± 39.6	251.0 ± 26.6

Type 4, repressed within 15 min, then increased

Photosystem II oxygen-evolving complex				
sll0258 psbV cytochrome c550	35.6 ± 5.5	30.9 ± 11.4	71.5 ± 10.1	93.7 ± 11.4
sll0427 psbO	51.1 ± 9.2	43.5 ± 20.4	45.2 ± 3.5	85.2 ± 17.0
Allophycocyanin				
slr2067 apcA	13.6 ± 3.2	12.2 ± 4.1	37.9 ± 13.2	95.3 ± 16.1
slr1986 apcB	12.6 ± 2.7	11.8 ± 5.4	35.5 ± 7.7	82.8 ± 6.3
ssr3383 apcC	15.2 ± 2.4	15.1 ± 5.3	39.9 ± 13.8	90.1 ± 16.0
slr0335 apcE	10.5 ± 6.4	14.9 ± 7.4	48.7 ± 11.5	62.6 ± 5.9
slr1459 apcF	31.8 ± 6.1	29.6 ± 11.9	45.6 ± 10.3	92.1 ± 11.2
Phycocyanin				
ssl3093 cpcD	29.5 ± 4.5	33.0 ± 9.4	37.5 ± 9.1	70.3 ± 11.2
slr2051 cpcG	18.2 ± 3.2	15.0 ± 8.1	29.2 ± 10.4	53.9 ± 6.9
Biosynthetic enzyme of photosynthetic pigments				
slr1808 hemA tRNA-Gln reductase	25.6 ± 11.0	39.4 ± 6.9	95.3 ± 11.7	180.6 ± 38.3
sll1185 hemF coproporphyrinogen III oxidase	17.9 ± 6.1	21.7 ± 13.6	39.1 ± 8.5	130.0 ± 63.7
sll1184 ho heme oxygenase	42.3 ± 20.0	27.3 ± 3.2	54.5 ± 23.1	70.5 ± 27.7
slr0506 pcr protochlorophyllide oxidoreductase	32.3 ± 8.9	53.7 ± 26.6	88.5 ± 20.3	107.0 ± 12.8
slr0750 chlN protochlorophyllide reductase ChlN	33.2 ± 12.0	27.5 ± 12.6	47.3 ± 12.8	52.0 ± 5.3
slr0772 chlB protochlorophyllide reductase ChlB	16.4 ± 1.8	23.0 ± 14.4	97.4 ± 68.9	71.8 ± 20.6
sll1091 391-amino acid bacteriochlorophyll synthase	21.9 ± 5.3	24.2 ± 10.2	49.4 ± 9.3	108.4 ± 39.4
Motility related				
sll1533 pilT	38.8 ± 8.7	44.7 ± 16.6	66.6 ± 4.5	75.6 ± 10.1
slr0162 gspF (pilC)	52.5 ± 17.2	37.9 ± 7.9	62.6 ± 7.2	58.3 ± 6.1
Others				
sll0947 lrtA light-repressed protein	27.2 ± 10.7	26.9 ± 4.5	75.1 ± 19.6	71.5 ± 8.1
sll1009 frpC iron-regulated protein	25.9 ± 7.8	40.8 ± 25.0	84.1 ± 28.4	40.8 ± 12.1
sll1214 PNIL 34 phytochrome-regulated gene	33.4 ± 19.5	34.1 ± 10.7	43.7 ± 9.0	95.4 ± 37.2
sll1583 lig or ligA DNA ligase	29.1 ± 18.4	54.9 ± 46.8	151.9 ± 123.8	82.1 ± 28.3
sll1626 lexA	43.4 ± 9.6	41.7 ± 8.3	138.5 ± 51.5	146.4 ± 7.5
sll1713 hisC histidinol-phosphate aminotransferase	44.0 ± 14.5	25.6 ± 11.3	52.8 ± 18.1	64.2 ± 18.6
slr0623 trxA thioredoxin	44.3 ± 4.5	57.8 ± 11.3	83.9 ± 22.1	77.5 ± 17.3
slr0513 periplasmic iron-binding protein	50.1 ± 15.4	49.8 ± 28.8	26.4 ± 17.4	94.6 ± 72.2
slr1295 sufA iron transport protein	50.1 ± 29.0	44.8 ± 13.7	24.6 ± 16.4	50.5 ± 33.0
sll0327	43.8 ± 14.7	44.7 ± 4.9	63.2 ± 5.5	53.2 ± 15.4
sll0944	31.8 ± 11.9	25.3 ± 7.6	46.6 ± 8.4	63.5 ± 24.0
sll1304	25.5 ± 7.7	39.8 ± 5.5	47.7 ± 11.4	83.5 ± 20.0

Continued

Table 1. (continued)

sll1305	29.1 ± 10.4	37.4 ± 7.6	44.9 ± 10.7	77.4 ± 14.0
sll1306	34.0 ± 12.3	43.3 ± 7.8	50.2 ± 18.1	79.9 ± 28.2
sll1307	29.3 ± 17.7	29.0 ± 8.3	37.4 ± 10.7	68.4 ± 23.8
sll1783	19.3 ± 4.8	28.5 ± 10.6	44.3 ± 4.6	100.2 ± 26.0
sll1784	14.4 ± 7.9	14.5 ± 4.4	25.4 ± 1.5	76.5 ± 15.1
sll1785	7.5 ± 5.3	15.7 ± 11.5	54.0 ± 28.9	69.0 ± 22.6
sll1830	28.6 ± 9.4	77.0 ± 57.9	116.3 ± 66.8	105.1 ± 33.1
slr0144	21.6 ± 6.4	30.2 ± 10.0	110.0 ± 57.5	77.9 ± 16.6
slr0145	20.2 ± 2.5	18.1 ± 7.7	50.6 ± 6.1	58.5 ± 19.0
slr0148	39.3 ± 8.6	45.1 ± 12.5	103.7 ± 13.7	79.8 ± 29.6
slr0149	35.5 ± 11.4	34.5 ± 12.5	51.7 ± 17.8	65.0 ± 28.0
slr0244	41.0 ± 15.1	33.2 ± 14.2	81.1 ± 28.2	87.3 ± 31.7
slr0888	42.4 ± 5.9	55.2 ± 9.3	78.0 ± 26.3	55.5 ± 15.2
slr1161	27.7 ± 4.8	39.1 ± 7.5	40.1 ± 6.0	57.0 ± 14.6
slr1173	34.6 ± 6.9	44.6 ± 16.5	43.7 ± 6.1	51.9 ± 11.9
slr1533	35.4 ± 17.9	30.7 ± 11.5	40.6 ± 12.9	52.8 ± 14.6
slr1535	31.8 ± 5.4	27.7 ± 6.2	67.1 ± 24.7	49.9 ± 11.0
slr1852	25.8 ± 12.0	31.9 ± 7.9	66.0 ± 6.5	136.4 ± 22.8
slr1854	33.7 ± 17.9	34.1 ± 12.9	58.5 ± 11.1	174.4 ± 24.7
slr1855	14.9 ± 7.3	20.9 ± 9.9	60.1 ± 8.7	92.4 ± 9.3
slr1856	36.3 ± 1.2	51.2 ± 24.7	56.8 ± 11.6	103.2 ± 12.2
ssl0483	35.9 ± 3.4	38.4 ± 9.1	69.7 ± 9.8	92.6 ± 3.6
ssl2384	41.3 ± 6.5	47.6 ± 9.3	58.3 ± 11.7	68.6 ± 7.6
ssl3692	37.5 ± 18.8	30.7 ± 8.8	46.6 ± 2.2	86.8 ± 2.1
ssl1533	54.5 ± 9.2	36.4 ± 14.6	33.5 ± 4.2	56.4 ± 11.9
Type 5, repressed continuously at low levels				
Photosystem I				
slr1834 psaA	35.4 ± 6.0	2.8 ± 1.0	15.3 ± 2.3	34.1 ± 2.1
slr1835 psaB	38.7 ± 7.1	9.5 ± 3.6	18.7 ± 5.8	47.4 ± 3.0
slr0737 psaD	23.7 ± 5.3	11.7 ± 4.7	21.9 ± 3.7	36.4 ± 8.3
sll0819 psaF	15.7 ± 0.9	11.5 ± 6.4	18.8 ± 3.0	43.7 ± 4.7
sml0008 psaJ	19.5 ± 2.5	23.8 ± 9.7	23.8 ± 5.2	50.2 ± 3.6
slr1655 psaL	17.1 ± 5.0	14.9 ± 7.2	27.8 ± 1.5	47.5 ± 6.3
Phycocyanin				
sll1577 cpcB	14.5 ± 3.1	6.3 ± 2.0	14.5 ± 5.7	47.5 ± 3.9
sll1578 cpcA	12.1 ± 2.2	4.7 ± 2.5	14.9 ± 3.7	37.5 ± 2.3
sll1579 cpcC	11.6 ± 1.7	3.7 ± 1.2	15.9 ± 6.5	42.1 ± 5.3
sll1580 cpcC	13.1 ± 3.0	5.4 ± 2.6	17.2 ± 1.8	40.1 ± 9.1
Others				
sll0051	28.2 ± 9.3	29.2 ± 9.5	38.2 ± 4.9	39.1 ± 5.7
sll0543	23.7 ± 6.8	17.8 ± 8.0	20.0 ± 2.6	21.9 ± 5.6
sll1358	30.0 ± 8.7	29.3 ± 4.7	43.5 ± 7.5	36.5 ± 8.8
slr0146	17.1 ± 7.0	15.0 ± 4.5	33.2 ± 9.1	43.9 ± 5.5
slr0147	17.2 ± 7.0	18.7 ± 10.3	33.1 ± 3.5	40.9 ± 13.7
slr0373	22.7 ± 8.3	7.2 ± 2.9	9.3 ± 1.6	4.5 ± 2.7
slr0374 cell division cycle protein	62.2 ± 14.4	46.4 ± 18.0	40.0 ± 3.2	37.6 ± 17.7
slr0376	23.5 ± 5.1	14.4 ± 3.3	17.6 ± 4.1	9.9 ± 3.0
slr0572	31.3 ± 11.9	27.0 ± 5.6	40.7 ± 12.3	41.3 ± 7.0
slr1634	13.1 ± 8.1	8.1 ± 1.0	7.2 ± 0.9	8.9 ± 2.2
Type 6, repressed at approximately 1 hr				
sll1688 thrC threonine synthase	266.8 ± 114.2	37.4 ± 13.8	84.7 ± 13.0	117.0 ± 44.5
slr0885	91.4 ± 68.4	31.9 ± 11.0	112.1 ± 65.4	57.3 ± 8.4
slr1348 cysE serine acetyltransferase	79.3 ± 24.1	45.2 ± 17.8	42.8 ± 4.7	64.9 ± 2.9
sll1694 pilA1	83.9 ± 21.6	24.8 ± 5.9	45.9 ± 5.3	44.8 ± 13.1
slr0272	77.8 ± 12.1	72.2 ± 29.1	45.9 ± 3.4	39.1 ± 10.3

Values are averages ± SD of three independent and each duplicate experiment (n = 6 for HL 15 min and HL 1 hr)
or averages ± SD of two independent and each duplicate experiment (n = 4 for HL 6 hr and HL 15 hr).
Orange boxes indicate values minus SD greater than 200. Yellow boxes indicate values greater than 200.
Deep blue boxes indicate values plus SD less than 50.   Pale blue boxes indicate values less than 50.
[a] Extent of induction or repression at each time point after the shift to HL is shown as a percentage of the level in LL.

clearly upregulated by HL. The *dnaJ* (*sll0897*) also was induced, but in this case the standard deviation was high. Interestingly, most of the transcripts for heat shock proteins were only transiently induced within 15 min (type 1), with the exception of *groES*, *groEL*, and *groEL-2*, which stayed at high transcript levels even 15 hr after the shift to HL and thus must be included in the type 2 category.

Of the four genes for fatty acid desaturase, *desA*, *desB*, and *desD* were induced within 15 min of exposure to HL (type 1, but the standard deviation of *desD* was relatively

high), whereas *desC* was constitutive. Note that the same three *des* genes were induced by low temperature in *Synechocystis* sp PCC 6803 (Los et al., 1997).

Genes that encode scavenging enzymes for ROS were expected to be upregulated under HL conditions in which production of ROS may be accelerated (Asada, 1994). However, only *sodB* and *slr1992*, which encodes glutathione peroxidase, were induced (type 1). Transcription of *katG*, *slr1171*, which encodes another glutathione peroxidase, and *sll0755*, which encodes thioredoxin peroxidase, was not affected significantly.

There are four *ftsH* homologs that encode the AAA-type protease in *Synechocystis* sp PCC 6803. Among them, *slr0228* and *slr1604* were induced upon the shift to HL. It has been observed that disruption of *slr0228* caused a 60% reduction in the amount of functional PSI (Mann et al., 2000). This *ftsH* gene may play a chaperone-like role in the regulation of PSI content under HL conditions.

Some hydrogenase-related genes, *hypA*, *ssl3044* (type 1), and *hypF* (type 2), were upregulated within 15 min, although *hox* genes, which encode the main structural components of hydrogenase (Appel and Schulz, 1996), were unaffected by the shift to HL.

Other ORFs belonging to the transiently induced group (type 1) might include *ssr2595* and *ssl2542*, which encode HL-inducible proteins (Dolganov et al., 1995), and two *nblA* genes (*ssl0452* and *ssl0453*) involved in the degradation of phycobilisome (Collier and Grossman, 1994). However, because in these cases the standard deviations were relatively high, conclusions regarding the effect of the HL treatment on their transcription must await confirmation.

Genes that encode components of the carboxysome (*ccm*; Price et al., 1998) and *rbc* genes, which encode ribulose bisphosphate (RuBP) carboxylase, were induced within 15 min, and their transcripts levels remained high even at 15 hr after the shift to HL (type 2).

Many genes involved in DNA replication, transcription, and translation were not induced within 15 min but were induced after approximately 1 hr (type 3). For example, three ribosomal operons (*sll1096* to *sll1101*, *sll1740* to *sll1747*, and *sll1799* to *sll1822*) seemed to be actively transcribed at this time point. This observation is not unexpected given that cells divided faster under HL.

Among genes that were transiently downregulated within 15 min, some were related to the biosynthesis of photosynthetic pigments (type 4). These include *hem* genes involved in tetrapyrrole biosynthesis, a heme oxygenase gene for phycobilin biosynthesis, and various genes for chlorophyll biosynthesis. Because the cellular contents of chlorophyll and phycocyanin decreased rapidly in HL, it is not surprising that these genes were downregulated upon transfer to HL.

Of interest, some genes related to cell motility were repressed by HL. Not only the *pilT* (*sll1533*) and *pilC* (*slr0162*) listed in Table 1 (type 4) but also another *pilT* (*slr0161*) and *pilC* (*slr0163*) were downregulated within 15 min (but standard deviations were relatively high). Moreover, the *pilA1* gene, which encodes a major subunit of filamentous appendages, type IV pili (Bhaya et al., 2000; Yoshihara et al., 2001), also was repressed by HL (type 6).

DISCUSSION

Relationship between the Regulation of Transcript Levels and Acclimation Responses to HL

Photosynthetic organisms show various acclimation responses to changing light intensity in their environment. Using DNA microarray technology, we revealed the dynamics and regulation of transcript abundance of known and unknown genes during acclimation from LL to HL. Although changes in the transcript levels are not necessarily reflected by changes in the amount of corresponding proteins, our results may help to elucidate the mechanisms whereby *Synechocystis* sp PCC 6803 acclimate to the rise in light intensity. One may distinguish the responses of genes involved in the photosynthetic machinery from those engaged in "housekeeping" processes (see Table 1), leading to, for example, shortening of the generation time from 20 under LL to 6 hr at HL.

Generally, short-term acclimation responses that took place upon the shift to HL mainly included changes in the efficiency of energy transfer to the reaction center (see Introduction). For example, Bissati et al. (2000) reported that nonphotochemical quenching developed within 5 min after the shift to HL in *Synechocystis* sp PCC 6803. It was proposed that *rpaA* (*slr0115*), *rpaB* (*slr0947*), and *rpaC* (*sll1926*) are involved in energy transfer from the phycobilisomes to PSII in *Synechocystis* sp PCC 6803 (Ashby and Mullineaux, 1999; Emlyn-Jones et al., 1999). However, our analysis indicated that *rpaA* and *rpaB* were not affected by HL, whereas *rpaC* was downregulated throughout the exposure to HL, although the standard deviation was high. Therefore, possible involvement of *rpa* in short-term acclimation to HL has not been established. Similarly, our analysis identified some genes (listed in Table 1), the transcription of which altered strongly after the transfer to HL, but their possible involvement in processes of short-term acclimation must await further experimental verification.

Generally, there is closer agreement between long-term acclimation responses of photosynthesis and upregulation and downregulation of genes detected in this study. Figure 4 shows the time-dependent changes in transcript levels that are involved in HL acclimation. In some cases, the induction or repression of genes involved in long-term acclimation could be completed within 15 min, whereas changes in protein levels were observed for hours or even days. Transcripts of housekeeping genes for DNA replication, transcription, and translation accumulated approximately 1 hr after the shift to HL. Acclimation of photosynthetic machinery to cope with the sudden shift to HL seems to come first, followed by changes in the housekeeping processes to support the faster cell division under HL.
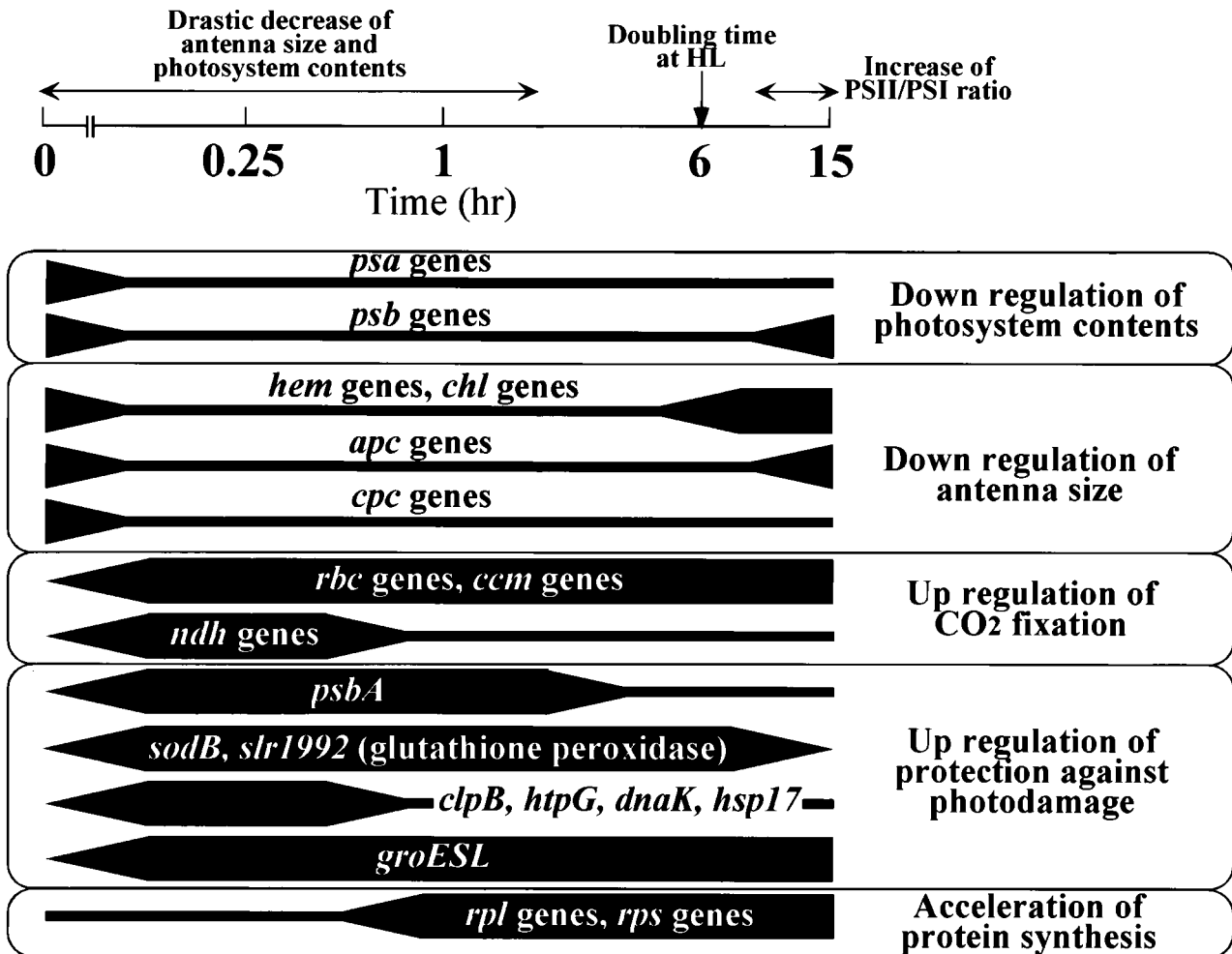
Figure 4. A Scheme Representing the Relationship between Changes of Transcript Levels and Acclimation to HL.

The physiological changes of cells during acclimation to HL (300 μmol photons m^{-2} sec^{-1}) are shown above the time scale expressed logarithmically. Changes of transcript levels shown by DNA microarray analyses are indicated below the time scale. The thickness of lines roughly represents the amount of transcripts.

In cyanobacteria, the PSII/PSI ratio generally increases upon the shift to HL (Kawamura et al., 1979; Murakami and Fujita, 1991; Hihara et al., 1998). Declining PSI content would be expected to lower the susceptibility of the cells to HL damage particularly under prolonged exposure (Hihara and Ikeuchi, 1998; Hihara et al., 1998). Data presented here provide evidence that, in *Synechocystis* sp PCC 6803, alteration of the transcript levels of genes that encode various subunits of the photosystems may be involved in the determination of photosystem stoichiometry under changing light conditions. Repression of *psa* genes after the shift to HL was far more pronounced and for longer duration than that of *psb* genes. After 15 hr of HL, the level of *psa* transcripts was 50% lower than for LL, whereas that of *psb* was close to the LL level.

Chlorophyll and phycocyanin content per cell declined drastically within 3 hr of HL. Simultaneously, phycobilisome size and photosystem content probably were reduced to avoid absorption of excess light energy. These changes may originate from the downregulation of genes that encode enzymes for the biosynthesis of photosynthetic pigments (*hem* and *chl* genes), structural components of phycobilisome (*apc* and *cpc* genes), and subunits of photosystems (*psa* and *psb* genes). Although many *psb* genes were downregulated or did not change after the HL, *psbA* genes, which encode D1, were strongly upregulated. It is likely that the elevated level of *psbA* transcripts (Mohamed and Jansson, 1989) makes the increasing turnover rate of the D1 protein under HL conditions possible.

In contrast with photochemical reactions downregulated by HL, CO_2 fixation is upregulated. Because the capacity for

CO_2 fixation by RuBP carboxylase is rate limiting under saturating light intensity, it is not surprising that *rbc* genes that encode large and small subunits of RuBP carboxylase and *ccm* genes encoding components of the CO_2-concentrating mechanism were induced. Upregulation of *ndh* genes, especially the *ndhF3* operon involved in high affinity CO_2 uptake (Ohkawa et al., 1998, 2000) may help to increase the availability of CO_2 under HL.

Despite the increasing energy consumption during HL, the cells could suffer damage from ROS at HL. Genes encoding ROS scavenging enzymes and genes that have chaperonin-like roles such as heat shock proteins were upregulated.

Existence of Common Signal Transduction Pathway(s) for Different Environmental Changes

The HL treatment led to the induction of several genes that, apparently, are not directly involved with the photosynthetic activity such as heat and cold shock–related genes (Table 1). This is in agreement with the report (Glatz et al., 1997) that induction of *groEL-2* by heat shock was largely suppressed by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or dark conditions. Similarly, the cold shock–inducible *des* genes were upregulated by a shift from dark to light but not in the presence of DCMU (Kis et al., 1998). These observations may indicate that different environmental changes may lead to the induction of similar sets of genes via common signaling pathway(s). In fact, it may enable one to distinguish between a specific response to a certain environmental stimuli and general responses to a number of stressors (Schwarz and Grossman, 1998), possibly mediated by the redox state of component(s) on the photosynthetic electron transport chain. Further application of DNA microarray methodology on RNA isolated after exposure to various environmental conditions may help to clarify stress-sensing mechanisms and networks of gene expression for acclimation responses.

Advantages and Limitations of the DNA Microarray Method

Substantial amount of information on the modulation of transcript levels during acclimation from LL to HL was obtained using the DNA microarray, providing an entire profile of gene expression in *Synechocystis* sp PCC 6803. However, we find it necessary to discuss some of the difficulties involved with the application of this technique and possible artifacts. First, we could not detect genes induced or repressed by HL in ~1000 weak spots. Some of these genes such as those encoding for regulatory components (e.g., signal transduction and transcription factors) are of great interest but are present in low abundance. They may have responded to the changing light regimen, but we could not detect them due to inevitable false hybridization with probes derived from abundant rRNA. The extent of masking may vary depending on the fortuitous sequence similarity of each gene with rRNA. To increase the sensitivity of the DNA microarray, it might be necessary to remove rRNA before the reverse transcription reaction by, for example, subtraction with biotin-labeled antisense rRNA (Su and Sordillo, 1998). Second, we could not distinguish between highly homologous genes with the current version of CyanoCHIP. For example, although we obtained independent data for *psbA2* and *psbA3* spots, they seem to be identical (Figure 3) probably because only the coding regions of ORFs were fixed on the CyanoCHIP, and *psbA2* and *psbA3* are 99% identical. Highly homologous genes may result in cross-hybridization and wrong interpretation of the results. Third, although both synthesis and degradation determine the abundance of a specific transcript, we could not distinguish between them with the DNA microarray analysis. It might be necessary to perform microarray analyses in the presence or absence of inhibitors of RNA synthesis.

Naturally, we could not mention all the ORFs and many hypothetical genes are indeed transcribed. Some of them responded strongly to the HL treatment, suggesting that they may have a significant role, yet to be unraveled, in further studies.

METHODS

Strains and Culture Conditions

A glucose-tolerant wild-type strain of *Synechocystis* sp PCC 6803 was grown at 32°C in BG-11 medium (Stanier et al., 1971) with 20 mM Hepes-NaOH, pH 7.0, under continuous illumination provided by fluorescent lamps. Cells were grown in volumes of 50 mL in test tubes (3 cm in diameter) and bubbled by air containing 1.0% (v/v) CO_2. Cell density was estimated as A_{730} with a spectrophotometer (model UV-160A; Shimadzu, Kyoto, Japan).

For the low light (LL) samples, cells were grown at 20 μmol photons m^{-2} sec^{-1} for at least 1 day and harvested at a cell density of $A_{730} = 0.6$ to 1.0. For the exposure to high light (HL) for 15 min and 1 hr, cells grown at 20 μmol photons m^{-2} sec^{-1} to a cell density of $A_{730} = 0.1$ to 0.2 were transferred to HL (300 μmol photons m^{-2} sec^{-1}) without dilution. For the exposure to HL for 6 and 15 hr, cells grown in LL were diluted to adjust the cell density to 0.1 (for 6-hr samples) or 0.05 (for 15-hr samples) and transferred to HL. After incubation in HL for 6 or 15 hr, the cell density was $A_{730} \approx 0.2$; thus, self-shading of cells was minimized.

Isolation of Total RNA

Total RNA was isolated using the RNeasy Midi kit (Qiagen, Hilden, Germany). The standard protocol for breakage of cells was modified as follows. In brief, approximately 100 mL of LL-grown cells ($A_{730} = 0.6$ to 1.0) or 200 mL of HL-grown cells ($A_{730} \approx 0.2$) was broken with

a Mini-Bead Beater (Biospec, Bartlesville, OK) with zircon beads (100 μm in diameter; Biospec) for three pulses of 50 sec at 4°C. After removal of the beads by centrifugation, the volume of cell lysate was adjusted to 3.8 mL with the buffer provided with the kit. After a brief centrifugation, 2.8 mL of 100% ethanol was added to the supernatant. Total RNA then was isolated using a spin column (provided as part of the kit) according to the manufacturer's instructions (Qiagen) and used for the labeling reaction.

Preparation of Labeled cDNA

Fluorescently labeled cDNA probes were prepared from the total RNA pool by direct incorporation of fluorescent nucleotide analogs during the first-strand reverse transcriptase reaction. Each 40 μL of labeling solution consisted of 20 μg of total RNA, 2 ng of λRNA as an internal control, 300 pmol of random hexamer, 0.5 mM each of dATP, dCTP, and dGTP, 0.2 mM dTTP, 100 units of RNase inhibitor (TaKaRa, Kyoto, Japan), 8 μL of 5 $\times$ reaction buffer provided with reverse transcriptase (TaKaRa), and 3 nmol of either Cy3-dUTP or Cy5-dUTP (Amersham Pharmacia, Uppsala, Sweden). The solution was incubated at 65°C for 5 min and then at 25°C for 2 min, and then 38 units of reverse transcriptase (AMV Reverse Transcriptase XL; TaKaRa) was added, and the reverse transcription was performed at 42°C for 2 hr. Another 38 units of reverse transcriptase was added 1 hr after the beginning of reverse transcription. Unincorporated fluorescent nucleotides were removed using Centri-Sep spin columns (Princeton Separations, Adelphia, NJ). After ethanol precipitation, pellets were dissolved in 8 and 7 μL of water for probes labeled with Cy3 and Cy5, respectively.

Features of DNA Microarrays

DNA microarrays (CyanoCHIP version 0.8) were provided by TaKaRa. On this microarray, polymerase chain reaction (PCR) fragments of the C-terminal 1-kb coding regions of 3079 open reading frames (ORFs) were fixed, which covered all ORFs in the genome of *Synechocystis* sp PCC 6803 except some genes that encode transposase. In cases of genes smaller than 1 kb, the full length of the ORF was amplified and fixed. Approximately 90% of the PCR products fulfilled the requirement for concentration of 0.1 mg mL^{-1} and sufficient purity. In addition to the ORFs mentioned above, there were four spots each of 16S rRNA, 23S rRNA, λ phage DNA (as an internal control), and human transferrin receptor gene (as a negative control) and 44 spots of diluted solution of rRNAs used as positional markers. These spots (approximately 150 μm in diameter) were aligned in an area of 18.0 $\times$ 18.0 mm. Duplicate sets of DNA spots were fixed on the upper and lower parts of the DNA microarray to verify the reproducibility of experiments.

Hybridization and Washing Conditions of the DNA Microarray

Hybridization buffer (22 μL) containing 4 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl and 0.015 M sodium citrate), 0.2% SDS, 5 $\times$ Denhardt's solution (1 $\times$ Denhardt's solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% BSA), and 100 ng μL^{-1} salmon sperm DNA was spread onto the microarray surface, covered with a cover slip, and sealed with glue. Prehybridizations were performed at 65°C for 1 hr followed by washing of the microarrays with 0.2 $\times$ SSC and drying by brief centrifugation. Hybridization was performed with 22 μL of

hybridization solution containing 4 μL of Cy3 probe, 7 μL of Cy5 probe, and 11 μL of 2 $\times$ hybridization buffer at 65°C overnight. Washing was done at 65°C in 2 $\times$ SSC for 5 min, in 0.2 $\times$ SSC, and 0.1% SDS for 5 min, followed by three washes in 0.2 $\times$ SSC at room temperature, and then the microarrays immediately were spun dry.

Image Acquisition and Analysis

Microarrays were scanned with two wavelengths for Cy3 (570 nm) and Cy5 (660 nm) by using a laser fluorescent scanner (418 Array Scanner; Affymetrix, Santa Clara, CA) with three different photomultiplier gains. Data analysis was performed using Imagene version 3.0 software (BioDiscovery, Los Angeles, CA). The raw data obtained with the highest photomultiplier gain were routinely used for quantification. For spots in which signal intensity was saturated, the raw data of the lower photomultiplier gain were reanalyzed. The fluorescence intensity of each spot in both Cy3 and Cy5 images was quantified, and fluorescence levels of the local background were subtracted. Normalization of Cy3 and Cy5 images were performed by adjusting the total signal intensities of two images ("global normalization"). Because extraneously added controls such as λRNA could not correct errors in steps that proceeded probe labeling, inaccuracy in the quantification of RNA samples had a substantial effect on the results normalized by such methods. Thus, more reliable global normalization was used, although λDNA spots were provided on CyanoCHIP. After normalization, the expression of each gene under HL conditions was shown as a percentage of the LL levels. For example, the induction rate in HL was calculated from the ratio of Cy5 to Cy3 signals in the experiment represented in Figure 1. To obtain reliable data, we performed three independent experiments (cell culture, RNA isolation, labeling of probes, and hybridization all were performed independently) for the time points of 15 min and 1 hr and two experiments for the time points of 6 and 15 hr. Because the upper and lower parts on CyanoCHIP gave us duplicate results from single hybridizations, we had six, six, four, and four results for the time points of 15 min, 1 hr, 6 hr, and 15 hr, respectively. All induction rates described here are shown as averages and standard deviations of six or four results. The analyzed data are available at http://www.genome.ad.jp/kegg/expression.

RNA Gel Blot Analysis

Total RNA (15 μg) extracted as described above was fractionated on a 1.2% denaturing agarose gel, blotted, and probed with PCR-amplified DNAs. Labeling of probes and detection was performed using enhanced chemiluminescence direct nucleic acid labeling and detection systems (Amersham Pharmacia) according to the manufacturer's instructions.

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