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Effects of high light on transcripts of stress-associated genes for the cyanobacteria *Synechocystis* sp. PCC 6803 and *Prochlorococcus* MED4 and MIT9313

Isabelle Mary,[1] Chao-Jung Tu,[2] Arthur Grossman[2] and Daniel Vaulot[1]

[1]Station Biologique, UMR 7127, CNRS et Université Pierre et Marie Curie, BP 74, F-29682 Roscoff cedex, France

[2]Carnegie Institution of Washington, Department of Plant Biology, 260 Panama Street, Stanford, CA 94305, USA

Correspondence
Isabelle Mary
mary@sb-roscoff.fr

Cyanobacteria constitute an ancient, diverse and ecologically important bacterial group. The responses of these organisms to light and nutrient conditions are finely controlled, enabling the cells to survive a range of environmental conditions. In particular, it is important to understand how cyanobacteria acclimate to the absorption of excess excitation energy and how stress-associated transcripts accumulate following transfer of cells from low- to high-intensity light. In this study, quantitative RT-PCR was used to monitor changes in levels of transcripts encoding chaperones and stress-associated proteases in three cyanobacterial strains that inhabit different ecological niches: the freshwater strain *Synechocystis* sp. PCC 6803, the marine high-light-adapted strain *Prochlorococcus* MED4 and the marine low-light-adapted strain *Prochlorococcus* MIT9313. Levels of transcripts encoding stress-associated proteins were very sensitive to changes in light intensity in all of these organisms, although there were significant differences in the degree and kinetics of transcript accumulation. A specific set of genes that seemed to be associated with high-light adaptation (*groEL*/*groES*, *dnaK2*, *dnaJ3*, *clpB1* and *clpP1*) could be targeted for more detailed studies in the future. Furthermore, the strongest responses were observed in *Prochlorococcus* MED4, a strain characteristic of the open ocean surface layer, where *hsp* genes could play a critical role in cell survival.

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INTRODUCTION

All living organisms must acclimate to environmental stresses that can potentially damage cellular processes (Asada, 1994). Under extreme environmental conditions many polypeptides will denature (Glover & Lindquist, 1998; Gottesman *et al.*, 1998); they lose their native, functional configuration and tend to aggregate. This process can be reversed to some extent, but when conditions become severe, aggregation can lead to irreversible damage of cellular functions and result in cell death (Herman & D'Ari, 1998). Bacteria respond to adverse environmental conditions by synthesizing a set of 'stress-associated' proteins that limit cellular damage by preventing the intracellular accumulation of unfolded and misfolded polypeptides (Lindquist & Craig, 1988). Most stress-associated polypeptides, also called heat-shock proteins (Hsp) because they were first observed in cells exposed to elevated temperatures, are either molecular chaperones or energy-dependent proteases. The chaperones play crucial roles in promoting folding, stabilization, solubilization, renaturation and degradation of polypeptides, and also facilitate the transport of polypeptides into specific cellular compartments and the assembly of multiprotein complexes (Bukau & Horwich, 1998; Gottesman *et al.*, 1997). The energy-dependent proteases perform targeted polypeptide degradation, modulating the availability of regulatory elements and removing non-functional but potentially harmful polypeptides that arise when polypeptides misfold, denature and/or aggregate (Gottesman, 1996). Many of these proteases function in conjunction with chaperones since substrates targeted for degradation may need to be unfolded prior to proteolysis.

Cyanobacteria are an ancient (over 3 billion years old), diverse and ecologically important group of eubacteria that evolved to compete in a wide range of habitats. As the only prokaryotes that perform oxygenic photosynthesis, they are major contributors to biomass accumulation on Earth. Since their apparatus for photosynthesis resembles that of plants, they have been used as model organisms by many scientists to investigate the structure and function of plant-type photosynthesis. *Synechocystis* sp. PCC 6803 is a

Abbreviations: HL, high light; LL, low light.

freshwater, unicellular, non-nitrogen-fixing cyanobacterium for which the complete nucleotide sequence of the genome has been determined (Kaneko *et al.*, 1996). This naturally transformable strain is one of the most popular organisms for genetic and physiological studies of photosynthesis and environmental gene regulation because of its ability to grow both photoautotrophically and photoheterotrophically. The cyanobacterium *Prochlorococcus*, the smallest known photosynthetic prokaryote, is a dominant primary producer throughout the oligotrophic oceans (Partensky *et al.*, 1999). It is ubiquitous within the 40°S to 40°N latitudinal band and is found both in surface waters, experiencing light intensities as high as 1500 μmol photons m^{-2} s^{-1}, and at 150 m depth, where light intensities fall below 1 μmol photons m^{-2} s^{-1}. The wide distribution of *Prochlorococcus* in the marine environment probably reflects the existence of several physiologically and genetically distinct ecotypes (Moore *et al.*, 1998). High-light-adapted genotypes occupy the upper, well-illuminated but nutrient-poor part of the water column whereas low-light-adapted genotypes are preferentially found at the bottom of the illuminated layer but in a nutrient-rich environment. These ecotypes differ in their optimal light intensity for growth (Moore *et al.*, 1998), pigment content (Moore *et al.*, 1995), light harvesting efficiency, sensitivity to trace metals (Mann, 2002) and nitrogen utilization ability (Moore *et al.*, 2002). Full genome information is available for *Prochlorococcus* MED4, which experiences growth in high light (HL) waters of the open oceans, and *Prochlorococcus* MIT9313, which typically grows in low light (LL) environments and is found deeper down in the water column (Rocap *et al.*, 2003).

Cyanobacteria possess stress-associated genes, encoding chaperones such as DnaK, DnaJ, GroEL or ClpB, and proteases such as ClpP (Glatz *et al.*, 1999). A number of these genes may be induced when cells are shifted to high temperatures (Eriksson *et al.*, 2001) or from LL to HL. In *Synechocystis* sp. PCC 6803, *clpB*, *htpG*, *dnaK*, *groES*, *groEL*, *groEL2* and *hsp17* transcripts all appeared to accumulate when cells were transferred from 20 to 300 μmol photons m^{-2} s^{-1} (Hihara *et al.*, 2001). These results are consistent with experiments performed under similar conditions by Huang *et al.* (2002). Stress-associated multigene family members appear to be differentially expressed under a variety of physiological conditions (Lindquist & Craig, 1988). As an example, cyanobacteria possess four *clpP* genes (Porankiewicz *et al.*, 1999; Schelin *et al.*, 2002). *clpP1* is monocistronic, while *clpP2* and *clpP3*, similar to the situation in *Escherichia coli*, appear to be arranged in an operon with *clpX* (Schelin *et al.*, 2002; Clarke *et al.*, 1998) and *clpP4* (also called *clpR*, encoding a protein that lacks the three active-site amino acids representative of ClpP proteases), respectively (Schelin *et al.*, 2002). Unlike most bacterial Clp proteins, and especially ClpP1 (Clarke *et al.*, 1998), the ClpP2, ClpP3, ClpP4 and ClpX polypeptides did not accumulate to high levels upon cold shock, oxidative stress or HL exposure of *Synechococcus* sp. PCC 7942 (Schelin *et al.*, 2002). Similarly, while the DnaK2 protein of *Synechococcus* sp. PCC 7942 exhibited a typical increased accumulation following heat shock, the levels of DnaK1 and DnaK3 did not change following the same treatment (Nimura *et al.*, 2001).

In this study we examined the responses of the *groES*, *groEL*, *dnaK* and *clp* transcripts in the freshwater cyanobacterium *Synechocystis* sp. PCC 6803, the marine HL-adapted strain *Prochlorococcus* MED4 and the marine LL-adapted strain *Prochlorococcus* MIT9313 following a shift from LL to HL. Using real-time, quantitative RT-PCR, the levels of transcripts were quantified over both the short term (30 min, 1 h) and relatively longer term (3, 6 and 12 h) following the shift to HL, allowing the determination of those heat-shock genes with elevated levels of expression following exposure to HL stress.

METHODS

Culture conditions. *Synechocystis* sp. PCC 6803 was cultivated in BG-11 medium (Rippka *et al.*, 1979) buffered with 10 mM Tris/HCl, pH 8·2, at 30 °C. Cultures were bubbled with 3 % CO_2 in air and illuminated with 30 μmol photons m^{-2} s^{-1} from incandescent bulbs. For HL treatments, cells in the mid-exponential growth phase (OD_{730} ~0·8) were diluted with fresh medium to an OD_{730} of ~0·2. The cells (50 ml cultures) were then placed in a temperature-controlled chamber maintained at 30 °C and exposed to 600 μmol photons m^{-2} s^{-1} white light for various lengths of time, as indicated in the text, prior to RNA isolation. *Prochlorococcus* MED4 and MIT9313 were cultivated in PCR-S11 medium (Partensky *et al.*, 1999) at 20 °C at 6 or 20 μmol photons m^{-2} s^{-1}. For HL treatments, cells in the mid-exponential growth phase (10^8 cells ml^{-1}, as measured by flow cytometry) were exposed to 40 or 200 μmol photons m^{-2} s^{-1} for various lengths of time prior to RNA isolation. The exact light conditions during the experiments are indicated in Results. Separate experiments were performed to assess the cell response to HL as measured by flow cytometry or gene expression (RT-PCR).

Flow-cytometric analysis. A 1 ml aliquot of the *Prochlorococcus* culture was sampled at each RNA sampling time following HL exposure for the analysis of flow-cytometric cell parameters (i.e. cell number, chlorophyll fluorescence) as described by Jacquet *et al.* (2001). Aliquots were fixed for 10 min with glutaraldehyde (0·25 % final concentration), frozen in liquid nitrogen and stored at −80 °C for later analysis. Samples were analysed with a FACSort flow cytometer (Becton Dickinson) with 488 nm excitation. Cell chlorophyll fluorescence was normalized to that of 0·95 μm fluorescent beads (Polysciences).

RNA isolation. RNA was isolated from pelleted cells that had been frozen at −80 °C, using a modification of the method of de Saizieu *et al.* (1998), as described by Bhaya *et al.* (2000). Briefly, 500 μl acidified phenol and 500 μl NAES (50 mM sodium acetate pH 5·1, 10 mM EDTA, 1 % SDS) were added to pelleted cells from 150 ml cultures; 100 mg glass beads (0·1 μm mean diameter, Bio-Rad) was placed in the suspension, which was then vortexed three times for 20 s each. This was followed by two phenol/chloroform (1 : 1, v/v) and one chloroform extraction. Nucleic acids were precipitated with 2 vols ethanol, resuspended in sterile, double-distilled H_2O and then treated for 1 h at room temperature with DNase I (20 U FPLC purified, Amersham), according to the protocol recommended by the manufacturer. DNase-treated samples were extracted with

phenol/chloroform (1 : 1), then with chloroform, and the RNA was precipitated from the aqueous phase upon addition of 2 vols ethanol. The final RNA pellet was dissolved in 50 µl 10 mM Tris/HCl pH 8·0, 1 mM EDTA and stored at −80 °C.

Real-time quantitative RT-PCR analysis of gene expression. For the reverse transcriptase (RT) reaction, 200 ng RNA was incubated with a mixture of PCR reverse primers (4 pmol per 20 µl final vol) (Table 1) for 10 min at 70 °C prior to adding 100 U Superscript II RT (Gibco-BRL). The RT reaction was performed at 42 °C for 1 h and stopped by placing the reaction at 72 °C for 10 min. SYBR Green PCR Master mix (Applied Biosystems) or LightCycler DNA Master SYBR Green I (Roche Applied Science) were used as recommended by the manufacturers; each reaction of 25 µl contained 1 µl of a specific, diluted cDNA (diluted 1/10 relative to the cDNA reaction mixture) and 0·4 µmol l^{-1} of the appropriate primers.

Sequences of whole genomes of *Synechocystis* sp. PCC 6803 (GenBank NC_000911), *Prochlorococcus* MED4 (=CCMP1378) (GenBank BX548174) and *Prochlorococcus* MIT9313 (GenBank BX548175) were retrieved from public databases.

Transcript levels of the *Synechocystis* sp. PCC 6803 *hsp* genes were analysed by real-time RT-PCR using the LightCycler System (Roche Applied Science). The amplification programme consisted of 1 cycle of 95 °C for 60 s followed by 40 cycles of 95 °C for 15 s, 55 °C for 20 s, 72 °C for 40 s. The relative levels of transcripts of *hsp* genes of the *Prochlorococcus* strains were analysed by real-time RT-PCR using the ABI Prism 5700 sequence detection system (Applied Biosystems). The amplification programme consisted of 1 cycle of 95 °C with a 60 s hold followed by 40 cycles of 95 °C with a 15 s hold and 60 °C with a 1 min hold. The *rps* (*slr1984*) and *rnpB* transcripts were used as external standards, for *Synechocystis* and *Prochlorococcus* respectively, since levels of these transcripts were not altered by HL conditions (Mary & Vaulot, 2003) (Fig. 1).

Each quantitative RT-PCR experiment was performed on two distinct biological samples (using separate cultures grown under identical conditions) with three replicates for the first, and two replicates for the second experiment. Quantification of the relative fold change in mRNA levels was calculated using the $\Delta\Delta C_T$ method normalized against the level of *rnpB* transcript as described in the Applied Biosystems user bulletin #2 (http://dna-9.int-med.uiowa.edu/RealtimePCRdocs/Compar_Anal_Bulletin2.pdf), using the equation $2^{-\Delta\Delta C_T} = 2^{-[(C_{T\,hsp,\,HL} - C_{T\,rnpB,\,HL}) - (C_{T\,hsp,\,LL} - C_{T\,rnpB,\,LL})]}$, where C_T is the threshold cycle for amplification of *hsp* or *rnpB* from LL or HL-stressed cultures.

RESULTS

Synechocystis PCC 6803 was shifted from 30 to 600 µmol photons m^{-2} s^{-1} white light as studied before by Bhaya *et al.* (2000) and used as a reference. The HL-adapted *Prochlorococcus* MED4 was shifted from 20 µmol photons m^{-2} s^{-1} to 200 µmol photons m^{-2} s^{-1} conditions and the LL-adapted *Prochlorococcus* MIT9313 was shifted either from 20 µmol photons m^{-2} s^{-1} to 200 µmol photons m^{-2} s^{-1} or from 6 µmol photons m^{-2} s^{-1} to 40 µmol photons m^{-2} s^{-1}. The photoacclimation dynamics of *Prochlorococcus* growth were determined in both the MED4 and MIT9313 strains by monitoring variations in cell number and chlorophyll fluorescence by flow cytometry (Table 2). No significant change in cell number was observed for MED4 following a transfer from LL to HL, in agreement with Jacquet *et al.* (2001), or for MIT9313 shifted from 6 to 40 µmol photons m^{-2} s^{-1}. In contrast, a decrease in cell number was observed for MIT9313 shifted from 20 to 200 µmol photons m^{-2} s^{-1}. For both MED4 shifted from 20 to 200 µmol photons m^{-2} s^{-1} and MIT9313 shifted from 6 to 40 µmol photons m^{-2} s^{-1}, chlorophyll fluorescence did not change for the first 6 h and then decreased after 12 h. In contrast, MIT9313 shifted from 20 to 200 µmol photons m^{-2} s^{-1} exhibited a rapid decrease in cell fluorescence 3 h after the shift.

Changes in the level of each *hsp* mRNA after shifting *Prochlorococcus* MED4, *Prochlorococcus* MIT9313 and *Synechocystis* sp. PCC 6803 from LL to HL were analysed by real-time quantitative RT-PCR. Quantitative RT-PCR data (Tables 3–5) were reproducible, although in some cases, standard deviations were relatively high ($0{\cdot}08 > \mathrm{SD/mean} \approx 0{\cdot}37 > 0{\cdot}77$).

Overall, the increase in transcript levels in the *Prochlorococcus* strains was higher and more sustained over the period examined than that of *Synechocystis* sp. PCC 6803, although the light shift was more drastic in the latter case. In general, mRNA accumulated later in *Prochlorococcus* MED4 (6 h in HL) than in *Prochlorococcus* MIT9313 and *Synechocystis* sp. PCC 6803 (1 h in HL). In *Prochlorococcus* MIT9313, the increase in transcript levels was less for the transition from 6 to 40 µmol photons m^{-2} s^{-1} than for the transition from 20 to 200 µmol photons m^{-2} s^{-1}. In the remainder of the manuscript we will only consider the 20 to 200 µmol photons m^{-2} s^{-1} transition for the MIT9313 strain, except when explicitly mentioned.

Two genes encoding GroEL have been identified in the genome of the *Prochlorococcus* strains and *Synechocystis* sp. PCC 6803. The *groEL2* gene is monocistronic and the *groEL1* gene is part of a bicistronic operon with *groES*. In all of these strains, *groEL1* and *groES* showed similar patterns of expression in terms of intensity and timing (Tables 3–5). The strongest response was observed in *Prochlorococcus* MED4, while both *Synechococystis* PCC 6803 and *Prochlorococcus* MIT9313 exhibited moderate increases. Furthermore, the response occurred earlier (after 1 h) in *Prochlorococcus* MIT9313, in agreement with the cell response data. The *groEL2* response was not as pronounced as that of *groES*/*groEL1* and occurred later with respect to the transition time.

The second major chaperone system, the DnaK system, is encoded by multiple *dnaK*, *dnaJ* and *grpE* genes. In contrast to *Synechocystis* sp. PCC 6803, which has four *dnaK* genes (sll0086, sll0170, sll1932 and sll0058), the *Prochlorococcus* strains have only three. The polypeptides encoded by these genes in both *Synechocystis* sp. PCC 6803 and *Prochlorococcus* strains are very similar to those of analogous genes identified in *Synechococcus* sp. PCC 7942 (Nimura *et al.*, 2001). The *dnaK2* and *dnaK3* genes are well conserved among the three strains. In contrast, the *Prochlorococcus dnaK1* displayed low similarities to the analogous gene in *Synechocystis* sp. PCC 6803. Unlike

Table 1. Primers (5′–3′) specific for chaperone, protease and control genes used for quantitative RT-PCR reactions for *Synechocystis* sp. PCC 6803, *Prochlorococcus* MED4 and *Prochlorococcus* MIT9313

Gene	*Synechocystis* sp. PCC 6803		*Prochlororcoccus* MED4		*Prochlororcoccus* MIT9313	
	Forward primer sequence	Reverse primer sequence	Forward primer sequence	Reverse primer sequence	Forward primer sequence	Reverse primer sequence
clpB1	AAACGTCCTGGTTTAGGAGC	GGGATTGTAATACCTGTTTCT	TTAGGGCCAACAGGAGTAGG	TTGCCCATCGGTAATAATCC	TGGCTCGAGGTGATGAGTTGT	GGAACGGTCGGCTTCTTCA
clpB2	AACTGATTTACAGCGGCAAG	CCAAACGGGTAGCCAAAGAC	CCAATCCTCATTGGTCATCC	AGGCGTTGTTGTTCCTATGC	TCGGTAAAACGGAGCTATCCA	GCCGCCTCGCTGTCAA
clpC	CGGCCTCGGACATAACTTCG	CATCCTCGAAGAAAAACGGG	ACTGCTGAGGTTGGTTCAGG	ATTTTGTTCCTGCGACAAGG	TCATGCTGGCCCAGGAA	CGGTGCCCACGAAATTATG
clpX	ATGCCCAAATACGACTCCCA	CTAAGCAGATTCCGGTTTAG	CGCCAGAAAGAGTGAAAACC	TTTGCTTTCATCAGGGTTGG	CCTGCTCATCGGTCCAACA	GGCCAGGGTCTGGGCTAATA
clpP1	ATGGAAATAACTGCTTTTCA	CTAAATCACTGCCATTGGTT	AGCTGGTATACCGGAGATGC	CCTCTTCTGCCCTTATTTGG	CAGGAACCAAAGGCCACAGA	GTTGGTGCAGCACGATTGAG
clpP2	ATGCCTATAGGTGTTCCCAG	TTAGTTATTGCCCATGGTGG	ATGATGGAATTGCGAACAGC	AGCCTGACGTTGTGAAGTCC	CGATGGGCGCCTTCCT	GGGCAGGGCGAGTCTTTT
clpP3	ATGATTCCAACCGTCATT	TTAAATAGGGTCACAGGCGG	GGAGGTTCGGTTTATGATGG	TCACTGGCTTGACCTCTTGC	CAAGTAGCAGATGCCCTCGTT	TCGGGATCTTCGGCTTCA
clpP4	AACTCCCGTTCTCCCTACCG	GGTGACGGGGGTAGTGGGGT	CGGTGGCTCTGTTACTGCTG	ATTTCAACTGCTTGGCCTTG	TGCGTGAACGGATGACATCT	TCGATTTGCCGAATCACTTG
dnaJ1	ATGGAACAAGTGCGGAA	TTAAAAATCAAAGAACTT	GAGTTTCTGGAGAGGGAAACG	ACCTGATGATTGCCTCTTGC	AGTTGCGCGTTCAAACAGAA	CGGGATAAAGCACACGAAGTC
dnaJ2	ATGGCATCAACAGATTTC	CTATGCCAACAAATTGG	–	–	GCGAAGTTCAAGGAGGTGAG	CGGCCAAACTGCTCATATTT
dnaJ3	GTGTCTTCTAATCCCCATTC	TTAGTTGATGAACCATGGTA	AAAGCTTTTCGAAGGGAAGC	ACAGGTGGTGGTTCTTCTGG	CCGCATAAGAACGCATGGT	CGAACGCCACGCTGATG
dnaJ4	GGGCGATTTTGCCGACATTT	TCATTTATGGAATAATCCCC	–	–	–	–
dnaK1	AACGGCAATTTTTTGAACGATT	TTGGGCCGTTTGCTGAAC	AACTTCGGTACAGAGAAAGAAGG	TTCTTCTTCGTTCCAAGTCTCC	TCCTCGATCTCCCCACCAATC	GCCGGCCCAGATCAAACT
dnaK2	ACAGTCAGTTAGAGGGTTCC	CTAATCGATCGCTTCATAGT	TGGTGGCTCAACTAGAATGC	CCTGCAAAACATGAATCTCG	TGTTGGCATTGACCTTGGAA	GCTTGCCACCCTCCATTACA
dnaK3	AGGTCACCGGAGCTGTGATTA	TTGCCGTTGGGAATCGTT	GGGATTGATTTGGGTACTGC	AATTCCCCTTCCTTCATTGC	TGCCGGCAGGCTCTTG	CAGCAGTGGGCTCGTTGAG
dnaK4	GCAAAGATCCCAACCAAGGA	CTATTTCTCGGCTCAGAGA	–	–	–	–
groEL1	ATGGCTAAATCCATCATTTA	GGTTTCTTCGATCTGGCGAC	CGCTGAGGGTAATGAAAAGG	AAAGCTTTATCAGCCCATTCC	TCGCAACCTTGGTGGTCAA	CTTCACGGCAGCCACATTC
groEL2	GGAAAGAATATGCTGCTTCT	TTACATCATGCCCATGCCTCC	GACTGCTGGTGATGGAACAA	GCTCCCGCAGCTATATTTTT	GCCCTGCTGTTGCTCACA	TGGGCACAAGATCGGAAAC
groES	ATGACTTCTATGGCCGCTAT	TAGGCAACGGAGGCCAAAA	TCACTTACAGTCTCTACAGTCAAACC	TCTGAAAGTAAGACGTACTCATCTCC	CCAGGCCCCCGAAGTG	CCATACCTGCGTACTTGCTGTAA
grpE	GGGAACCCACCGCTGAA	CCCGCACCAACTCCTCAA	CAACTAAAACCCGAAAGTGAGG	GGCTCTTGTGAATTTTGTTGG	GCTTGTTGATCGGTGCTCAA	GCCCAACAACCCGCATT
hsp17	GGATACCCATAGCACCGAAA	AGGTACAGGAATAACCCGGC	–	–	–	–
htpG	GCATTGCTGATTACATTGCC	TTACTGTTTGGTCAAACGGG	AAGAAGAATGGCCTCAATGG	AGGTGAGCCATCACATGACC	AACCGAATCCTTGGCACAAG	CCGAGGCGAGCCATGTC
rps	ATGCCTTCTTCCCTCCAA	AACTTTTTTCCAGTTGCTC	–	–	–	–
rnpB	–	–	CCCATATGGTCAGGCTTGC	GGCAATCATCTATCTAGGACTGG	TGCAAGCAAATCCCCAACTG	GCATGCCGTGGAGTTGTG

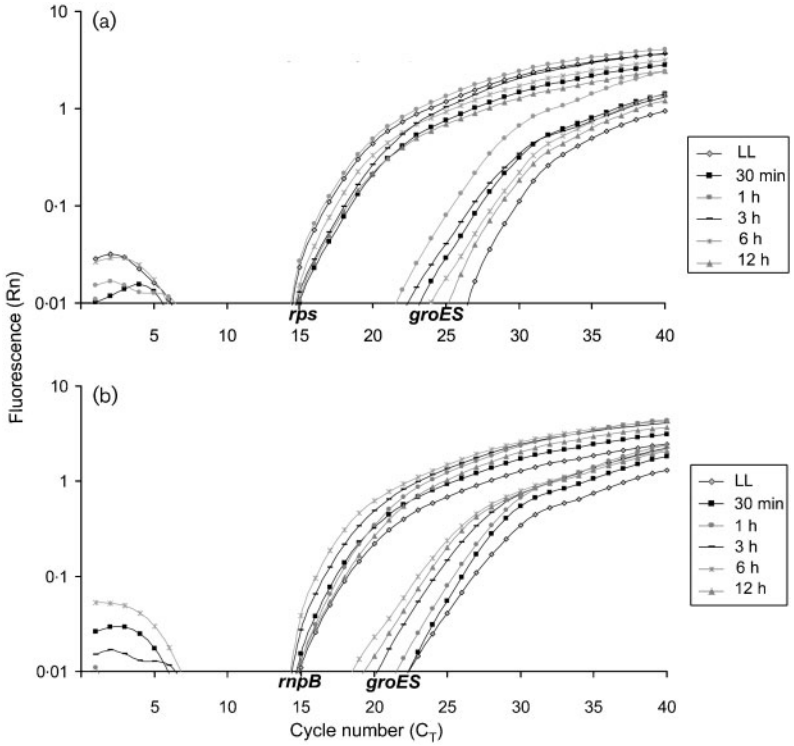

Fig. 1. Analysis of *groES* and control genes by real-time quantitative RT-PCR. Fluorescence vs amplification cycle during HL stress was determined using a 1/10 cDNA dilution. Increases in fluorescence, which were due to the cleavage of the reporter dye as the PCR proceeded, relative to the starting values of delta-normalized reporter fluorescence (Rn), were determined and plotted by the instrument against cycle number. (a) *groES* and *rps* (control) for *Synechocystis* sp. PCC 6803. (b) *groES* and *rnpB* (control) for *Prochlorococcus* MED4.

Synechocystis sp. PCC 6803, which has four *dnaJ* genes (sll1666, sll1933, sll0093 and slr0897), *Prochlorococcus* MIT9313 and MED4 possess three and two *dnaJ* homologues, respectively. *dnaJ3* shares strong identity (about 60 %) among the three strains, as does *dnaJ2*, which has 51 % identity between *Synechocystis* sp. PCC 6803 and *Prochlorococcus* MIT9313. In contrast, *Prochlorococcus dnaJ1* is not well conserved (about 34 % identity) but may still be the paralogous gene to *dnaJ1* of *Synechocystis* sp. PCC 6803 because it is located close to *dnaK3* in both strains. Furthermore, *grpE* is in the same operon as *dnaJ3* in the *Prochlorococcus* strains, whereas it is in an operon with a *dnaK* gene (sll0058) in *Synechocystis* sp. PCC 6803. Among the *dnaK* transcripts, that of *dnaK2* increased strongly in all

Table 2. Cell number and chlorophyll fluorescence of *Prochlorococcus* strains MED4 and MIT9313 as measured by flow cytometry during HL exposure

Strain	HL incubation (h)	Cell no. (cells ml^{-1})	Chlorophyll fluorescence
Prochlorococcus MED4	0	$1{\cdot}4\times10^{8}$	1·2
(20–200 μmol photons m^{-2} s^{-1})	0·5	$1{\cdot}2\times10^{8}$	1·4
	1	$1{\cdot}7\times10^{8}$	1·3
	3	$1{\cdot}1\times10^{8}$	1·2
	6	$9{\cdot}6\times10^{7}$	1·3
	12	$1{\cdot}4\times10^{8}$	0·7
Prochlorococcus MIT9313	0	$8{\cdot}0\times10^{7}$	2·7
(20–200 μmol photons m^{-2} s^{-1})	1	$7{\cdot}8\times10^{7}$	2·6
	3	$5{\cdot}9\times10^{7}$	1·5
	6	$7{\cdot}3\times10^{7}$	0·7
	12	$4{\cdot}1\times10^{7}$	0·9
Prochlorococcus MIT9313	0	$9{\cdot}9\times10^{6}$	6·0
(6–40 μmol photons m^{-2} s^{-1})	0·5	$1{\cdot}2\times10^{7}$	6·0
	1	$1{\cdot}3\times10^{7}$	6·1
	3	$1{\cdot}4\times10^{7}$	5·9
	6	$1{\cdot}7\times10^{7}$	5·8
	12	$1{\cdot}6\times10^{7}$	5·1

Table 3. Relative change in *hsp* transcript levels of *Synechocystis* PCC 6803 following a shift from LL (30 μmol photons m^{-2} s^{-1}) to HL (600 μmol photons m^{-2} s^{-1}), as monitored by real-time quantitative RT-PCR

Values >5 are in bold. Relative values at $t=0$ are calculated relative to the level of *rps*.

Gene	ID	Relative level $\times 10^{-1}$ at $t=0$	HL incubation (induction ratio with respect to LL)				
			0·5 h	1 h	3 h	6 h	12 h
clpB1	slr1641	0·12	1·31±0·40	**5·53±1·12**	4·02±1·50	0·60±0·32	0·74±0·20
clpB2	slr0156	0·78	0·90±0·41	0·65±0·24	0·91±0·12	0·70±0·23	0·61±0·12
clpC	sll0020	0·32	1·02±0·23	0·84±0·19	0·53±0·40	0·41±0·12	0·49±0·29
clpX	sll0535	0·14	0·59±0·32	0·86±0·23	0·68±0·29	0·75±0·43	0·31±0·12
clpP1	slr0542	1·33	4·02±1·00	**6·12±3·02**	**7·31±4·03**	**5·41±2·51**	2·02±0·48
clpP2	sll0534	0·31	1·10±0·32	1·27±0·61	0·70±0·21	0·85±0·50	0·95±0·29
clpP3	slr0165	0·76	1·10±0·38	2·00±0·35	1·81±1·05	1·50±0·62	2·05±1·25
clpP4	slr0164	0·90	1·03±0·24	3·01±0·90	1·89±0·48	1·75±0·25	1·19±0·65
dnaJ1	sll1933	0·29	0·62±0·23	1·46±0·36	1·01±0·46	1·52±1·03	1·12±0·20
dnaJ2	slr0093	0·91	1·02±0·34	0·59±0·23	0·42±0·25	0·37±0·19	0·56±0·22
dnaJ3	sll1666	0·95	0·98±0·15	2·12±0·24	4·20±1·86	2·12±1·02	1·53±0·54
dnaJ4	sll0897	0·70	1·53±0·61	1·42±0·22	0·71±0·54	0·50±0·23	0·71±0·33
dnaK1	slr0086	1·90	0·98±0·31	1·61±0·22	3·51±0·52	1·12±1·01	0·86±0·40
dnaK2	sll0170	1·69	1·12±0·27	**5·41±1·02**	1·66±0·55	0·82±0·32	0·83±0·40
dnaK3	sll1932	0·78	1·01±0·30	1·40±0·57	1·57±0·61	0·54±0·17	0·51±0·32
dnaK4	sll0058	0·23	1·38±0·11	1·13±0·15	2·43±0·67	3·19±0·69	0·96±0·42
groEL1	slr2076	3·70	4·03±0·42	**6·52±2·68**	**5·99±1·20**	4·01±2·23	2·52±1·03
groEL2	sll0416	1·88	1·23±0·51	2·20±0·91	4·07±2·09	3·22±1·90	4·12±1·36
groES	slr2075	2·52	**6·52±2·31**	**7·80±1·97**	**7·53±2·90**	**5·23±2·64**	3·99±1·23
grpE	sll0057	4·04	1·26±0·96	1·75±0·54	1·52±0·50	1·14±0·63	0·62±0·21
hsp17	sll1514	1·03	**5·34±1·06**	1·86±0·24	1·09±0·23	0·89±0·17	0·79±0·11
htpG	sll0430	1·25	1·41±0·61	1·89±0·95	2·94±0·83	2·25±1·14	1·17±1·01

Table 4. Relative change in *hsp* transcript levels of *Prochlorococcus* MED4 following a shift from LL (20 μmol photons m^{-2} s^{-1}) to HL (200 μmol photons m^{-2} s^{-1}), as monitored by real-time quantitative RT-PCR

Values >5 are in bold and values >10 are in bold italic. Relative values at $t=0$ are calculated relative to the level of *rnpB*.

Gene	GenBank no.	Relative level $\times 10^{-1}$ at $t=0$	HL incubation (induction ratio with respect to LL)				
			0·5 h	1 h	3 h	6 h	12 h
clpB1	NP_892698	1·80	2·15±0·65	2·01±0·60	3·25±1·24	**7·34±3·05**	2·09±1·14
clpB2	NP_892328	0·32	0·97±0·10	1·09±0·19	1·22±0·43	0·74±0·15	0·66±0·22
clpC	NP_893205	0·08	4·12±2·10	**5·03±1·87**	***12·54±4·15***	***13·08±5·44***	2·67±1·36
clpX	NP_893774	0·11	0·54±0·21	0·56±0·22	0·39±0·20	0·64±0·36	0·55±0·12
clpP1	NP_892860	1·99	3·50±1·54	**6·50±2·19**	**5·14±2·32**	***12·15±3·28***	3·04±1·12
clpP2	NP_893773	0·74	3·17±1·76	3·23±0·59	4·32±1·64	**6·24±2·56**	1·76±0·50
clpP3	NP_893431	1·90	1·09±0·44	2·13±0·44	1·77±0·81	***11·34±2·13***	3·45±1·17
clpP4	NP_893430	0·31	1·03±0·72	2·11±0·45	2·36±0·90	3·65±2·01	1·75±1·01
dnaJ1	NP_893014	0·67	0·98±0·22	0·77±0·19	0·75±0·39	2·13±1·08	1·45±1·01
dnaJ3	NP_893591	1·87	***11·45±3·56***	***10·99±4·12***	***13·67±4·99***	2·87±1·31	2·56±1·03
dnaK1	NP_893549	0·27	**5·05±2·31**	**8·54±3·12**	1·74±0·39	1·17±0·24	1·68±0·52
dnaK2	NP_893821	0·85	0·85±0·38	3·96±1·23	***22·80±4·23***	***25·03±5·43***	***12·82±5·02***
dnaK3	NP_893015	0·13	1·10±0·66	1·22±0·50	1·50±0·78	**8·56±5·76**	1·58±1·00
groEL1	NP_893553	1·20	2·16±0·56	2·81±1·05	3·10±1·19	***10·11±5·01***	4·96±1·12
groEL2	NP_892570	4·02	1·35±0·51	2·54±1·13	3·41±1·20	**6·76±3·04**	***15·01±5·02***
groES	NP_893554	4·50	1·28±0·95	**7·72±2·86**	***11·22±3·54***	***17·05±4·15***	***16·08±5·53***
grpE	NP_892137	2·10	1·23±0·44	1·91±0·34	1·86±0·45	0·68±0·23	0·750±0·19
htpG	NP_893019	0·20	4·55±2·13	4·98±2·21	2·57±1·12	2·11±0·65	2·09±1·09

Table 5. Relative changes in *hsp* transcript levels for *Prochlorococcus* MIT9313 as monitored by real-time quantitative RT-PCR following a shift from LL (4 or 20 μmol photons m^{-2} s^{-1}) to HL (40 or 200 μmol photons m^{-2} s^{-1})

Values >5 are in bold and values >10 are in bold italic. Relative values at $t=0$ are calculated relative to the level of *rnpB*.

Gene	GenBank no.	Relative level $\times 10^{-1}$ at $t=0$	HL incubation (induction ratio with respect to LL)				Relative level $\times 10^{-1}$ at $t=0$	HL incubation (induction ratio with respect to LL)				
			Prochlorococcus MIT9313 (20–200 μmol photons m^{-2} s^{-1})					*Prochlorococcus* MIT9313 (6–40 μmol photons m^{-2} s^{-1})				
			1 h	3 h	6 h	12 h		0·5 h	1 h	3 h	6 h	12 h
clpB1	NP_894282	1·01	**6·54±2·34**	**8·66±3·09**	**6·86±2·55**	4·56±1·77	1·20	2·09±1·07	1·49±0·59	2·86±0·56	1·38±0·42	1·09±0·23
clpB2	NP_895909	0·99	2·97±1·09	2·13±1·02	1·45±1·11	0·54±0·13	1·54	0·78±0·33	0·76±0·17	0·80±0·09	0·67±0·27	0·55±0·20
clpC	NP_894892	0·23	0·46±0·23	0·45±0·09	0·39±0·08	0·49±0·18	0·66	0·79±0·15	0·84±0·16	1·01±0·52	0·99±0·29	1·65±0·78
clpX	NP_893894	0·54	0·67±0·22	0·33±0·15	1·54±0·12	1·63±1·02	0·12	0·39±0·13	0·54±0·14	0·72±0·17	0·45±0·24	0·80±0·21
clpP1	NP_894508	1·82	1·06±0·57	2·13±0·61	1·95±0·35	1·38±0·11	1·45	0·78±0·39	0·91±0·26	0·59±0·37	3·43±1·19	2·34±0·90
clpP2	NP_893895	1·02	0·64±0·23	0·52±0·11	0·56±0·20	0·35±0·04	0·19	0·81±0·51	0·74±0·28	0·55±0·17	0·49±0·08	0·34±0·19
clpP3	NP_894147	0·50	0·34±0·14	0·36±0·15	0·45±0·13	0·25±0·07	0·30	0·60±0·23	0·85±0·13	1·02±0·14	1·56±1·10	0·98±0·27
clpP4	NP_894148	0·07	0·64±0·32	0·60±0·24	0·57±0·19	0·39±0·15	0·17	0·60±0·29	0·51±0·26	0·47±0·21	0·56±0·10	0·39±0·17
dnaJ1	NP_895802	0·18	1·39±1·03	2·16±1·12	2·34±0·72	3·42±1·23	0·13	0·46±0·14	0·49±0·23	0·37±0·21	0·49±0·22	0·58±0·18
dnaJ2	NP_895375	0·74	**5·47±2·25**	**5·52±1·14**	3·52±1·73	2·24±1·03	0·90	0·72±0·32	0·52±0·21	0·68±0·33	0·54±0·15	1·57±0·18
dnaJ3	NP_895322	0·83	2·90±1·17	**7·54±1·98**	***17·23±2·99***	***24·33±7·43***	0·66	**8·18±1·65**	**9·25±3·12**	**8·44±3·05**	**5·25±2·03**	3·22±1·49
dnaK1	NP_895271	0·14	0·56±0·08	0·43±0·28	0·69±0·41	0·39±0·20	0·84	1·21±0·32	1·72±1·02	1·83±0·72	**5·01±1·79**	1·13±0·25
dnaK2	NP_894535	0·12	***12·13±2·43***	**9·07±4·10**	2·35±0·36	1·06±0·53	0·68	**6·24±2·85**	4·39±2·02	**5·52±3·05**	**7·21±2·23**	3·89±1·76
dnaK3	NP_896079	0·13	2·32±1·09	3·45±1·14	3·04±0·78	2·12±1·07	0·25	1·42±0·98	3·69±2·04	2·56±1·43	1·45±0·79	1·34±1·02
groEL1	NP_895276	4·01	***11·12±5·45***	**5·46±1·04**	**8·43±3·25**	4·67±1·07	6·11	3·33±1·17	1·98±0·49	2·24±1·01	3·67±1·50	0·99±0·25
groEL2	NP_895161	1·20	4·78±1·52	3·52±1·03	3·24±1·23	2·56±0·58	2·50	1·53±1·03	0·95±0·56	1·24±0·20	1·52±0·58	2·17±1·04
groES	NP_895277	3·17	**6·21±2·15**	**5·03±2·12**	2·01±1·06	1·99±0·44	5·86	3·05±0·76	3·84±1·41	1·88±0·42	1·93±1·01	1·29±0·43
grpE	NP_893854	2·32	**6·74±2·02**	**6·10±1·22**	3·88±1·54	2·17±1·02	3·21	0·78±0·21	0·99±0·31	1·29±0·18	1·54±0·43	0·69±0·31
htpG	NP_894528	0·55	***10·12±3·54***	***11·34±5·22***	**7·09±3·16**	**5·32±2·56**	0·76	1·55±1·02	1·46±0·49	1·79±0·56	2·37±0·67	1·76±0·55

strains (Tables 3–5). The *dnaK1* and *dnaK3* transcript levels increased moderately in *Prochlorococcus* MED4 and *Synechocystis* sp. PCC 6803. Some of these transcripts, including *dnaK2* in *Prochlorococcus* MED4 and *dnaJ3* in *Prochlorococcus* MIT9313, remained high even after 12 h of HL exposure. The *dnaJ3* mRNA level increased dramatically in all strains, whereas *dnaJ2* mRNA accumulated to a lesser extent in *Prochlorococcus* MIT9313. Furthermore, *grpE* mRNA accumulated only in *Prochlorococcus* MIT9313 (Tables 3–5).

The *clp* family of genes in the three strains consists of single *clpC* and *clpX* genes and multiple *clpB* and *clpP* genes. The ClpP ATP-dependent protease is encoded by four distinct genes. The *clpP1* gene appears to be monocistronic while *clpP2* and *clpP3* are each part of bicistronic operons with *clpX* and *clpP4*, respectively. Of the two *clpB* genes, only *clpB1* mRNA levels rose following HL exposure of the three strains. As in *Synechocystis* sp. PCC 6803, the *clpP1* transcript level markedly increased in *Prochlorococcus* MED4 ($>$10-fold), whereas *clpP2*, *clpP3* and *clpC* mRNA accumulated to high levels in *Prochlorococcus* MED4 only. In contrast, many transcripts encoding Clp protease components, with the exception of *clpB1* and *clpB2*, did not increase following HL exposure of *Prochlorococcus* MIT9313 (Tables 3 and 4).

Finally, the *htpG* transcript accumulated in the three strains but to a lesser extent in *Prochlorococcus* MED4 and *Synechocystis* PCC 6803 (Tables 3–5).

DISCUSSION

Light is one of the most important environmental factors that governs cyanobacterial growth. While phenotypic features such as chlorophyll content respond quite slowly to changes in light levels (Table 2), the expression of certain classes of genes may be modulated on a relatively short time scale. This is the case, for example, for the two-component system genes (Mary & Vaulot, 2003) or for the *hsp* genes examined in the present paper. Cyanobacteria have several *hsp*-like genes that encode chaperones and specific proteases (Schelin *et al.*, 2002; Glatz *et al.*, 1999). While the number of genes in the compact genome of *Prochlorococcus* is reduced for some gene categories (e.g. there are only 12 genes encoding two-component systems in MED4, compared to the $\sim$80 genes in this category on the genome of *Synechocystis* sp. PCC 6803; Mary & Vaulot, 2003), these strains have almost the same number of *hsp* genes as *Synechocystis* sp. PCC 6803. The only exceptions are *dnaK4*, *dnaJ4* and the small *hsp17* gene (Table 1), which have no equivalent in either of the *Prochlorococcus* strains, and *dnaJ2*, which is absent in *Prochlorococcus* MED4 but present in MIT9313.

The transcripts for *dnaJ2* and *dnaJ4* do not increase strongly in *Synechocystis* sp. PCC 6803 during HL exposure, suggesting that they may not play a crucial role in this acclimation process. In contrast, the *dnaK4* transcript increased moderately and the *hsp17* transcript showed a strong increase in response to HL stress in *Synechocystis* sp. PCC 6803. Furthermore, the CIRCE (controlling inverted repeat of chaperone expression) binding repressor, HrcA, which is thought to regulate expression of some chaperone genes (Glatz *et al.*, 1997), is absent from the *Prochlorococcus* MED4 genome but present in the two other strains, which suggests that another mechanism may be involved in the regulation of *hsp* expression in this HL-adapted strain.

Besides these differences, the three cyanobacterial strains share many features of *hsp* gene organization and induction. In many cases, the *hsp* transcripts increase in abundance soon after exposure to HL and then decline during the later stages of acclimation. This probably reflects the capacity of the cells to reach a homeostatic condition in which the physiology of the cell has been modified with respect to the capture and utilization of excitation energy under HL conditions. Such a change is illustrated by the lower chlorophyll fluorescence observed after 12 h of HL exposure in *Prochlorococcus* (Table 2). Interestingly, when *Prochlorococcus* MIT9313 cells were shifted from 6 to 40 μmol photons m^{-2} s^{-1}, there was no significant increase in the level of most *hsp* transcripts, and little decrease in chlorophyll fluorescence, suggesting that low-amplitude changes in light levels do not induce drastic responses, and that the cells are able to quickly acclimate without major structural rearrangements.

Interestingly, the *hsp* genes that exhibit the strongest responses to HL exposure [i.e. *groEL* and *groES* (Tables 4–6)] also show the most sustained accumulation of transcripts (over the 12 h period following transfer to HL). This is especially apparent with *Prochlorococcus* MED4 (Table 3). The polypeptides encoded by such transcripts may be very important (either singly or in combinations) for maintenance of cellular function under specific stress conditions, such as HL. Among the three strains examined, *groEL1* and *groES* are arranged in an operon and encode the most-conserved Hsps. In contrast, the GroEL2 polypeptide is much less conserved. The GroEL/GroES system is a major chaperone system in all bacteria and the ways in which this system responds to stress conditions have been extensively studied in cyanobacteria (Hihara *et al.*, 2001; Clarke & Eriksson, 1996; Apte *et al.*, 1998). For example, the transcripts *groEL1* and *groEL2* have been shown to accumulate in response to elevated temperatures (Kovacs *et al.*, 2001). In all three strains *groES* and *groEL1* showed somewhat similar levels and kinetics of mRNA accumulation, while the level of the *groEL2* transcript increased later with respect to the HL exposure (Tables 3–5). This suggests that the two *groEL* genes may be regulated differently, as hypothesized previously for *Synechocystis* sp. PCC 6803 (Rajaram *et al.*, 2001).

While not all *dnaK* and *dnaJ* family members are present in the three cyanobacterial strains examined, the common DnaK2 and DnaJ3 polypeptides appear to be highly conserved. Furthermore, HL triggers a pronounced increase in

Table 6. Comparative induction of *hsp* transcripts in *Synechocystis* sp. PCC 6803, *Prochlorococcus* MED4 and *Prochlorococcus* MIT9313 following a shift of the cells from LL to HL

+, >5-fold induction; ++, >10-fold induction.

Gene	HL incubation (induction ratio with respect to LL)																			
	Synechocystis PCC 6803 (30–600 µmol photons m^{-2} s^{-1})					*Prochlorococcus* MED4 (20–200 µmol photons m^{-2} s^{-1})					*Prochlorococcus* MIT9313 (20–200 µmol photons m^{-2} s^{-1})				*Prochlorococcus* MIT9313 (6–40 µmol photons m^{-2} s^{-1})				
	0·5 h	1 h	3 h	6 h	12 h	0·5 h	1 h	3 h	6 h	12 h	1 h	3 h	6 h	12 h	0·5 h	1 h	3 h	6 h	12 h
clpB1		+							+		+	+	+	+					
clpB2																			
clpC							+	++	++										
clpX																			
clpP1		+	+	+			+	+	++										
clpP2									+										
clpP3									++										
clpP4																			
dnaJ2																			
dnaJ1											+	+							
dnaJ3						++	++	++				+	++	++	+	+	+	+	
dnaJ4																			
dnaK1						+	+											+	
dnaK2		+						++	++	++	++	+			+		+	+	
dnaK3									+										
groEL1		+	+						++		++	+	+						
groEL2			+						+	++									
groES	+	+	+	+			+	++	++	++	+	+							
grpE											+	+							
hsp17	+																		
htpG											++	++	+	+					

the *dnaK2* and *dnaJ3* transcripts in all three of the cyanobacteria following HL exposure (see Tables 3–5), suggesting an important role in HL acclimation.

Among the *dnaK* genes, the *dnaK2* transcripts accumulated to a greater extent than those of the other *dnaK* genes (Tables 3–5). This agrees with previous results showing that, upon shifting *Synechococcus* sp. PCC 7942 cells to elevated temperatures, the level of DnaK1 and DnaK3 polypeptides remained relatively constant while that of DnaK2 increased (Nimura *et al.*, 2001). Hihara *et al.* (2001) demonstrated that HL caused increased accumulation of *dnaK2* (*sll0170*) transcripts in *Synechocystis* sp. PCC 6803. This agrees with the observation that in most eukaryotes, Hsp70s (the DnaK homologues) are represented by a multigene family in which the different family members are differentially expressed (Lindquist & Craig, 1988; Queitsch *et al.*, 2000).

In parallel to what was observed for the *dnaK* genes, *dnaJ3* mRNA accumulated more than that of the other *dnaJ* transcripts following HL exposure in *Prochlorococcus* MED4 and MIT9313, and it was the only *dnaJ* that increased in HL in *Synechocystis* sp. PCC 6803. Another common feature of the three strains examined in this study is the contiguous genomic locations of *dnaJ1* and *dnaK3*, with the former immediately downstream of the latter. Interestingly, while these transcripts show some increase in HL in the three cyanobacterial strains (with the least change in *Synechocystis* sp. PCC 6803), these increases are neither marked nor as sustained as observed for *dnaK2* and *dnaJ3* (Tables 3–5). In *Synechococcus* sp. PCC 7942, the *dnaJ1* homologue was shown to be essential for growth and was detected in thylakoid membranes (Nimura *et al.*, 2001), suggesting that DnaK3 and DnaJ1 might function cooperatively with respect to photosynthesis.

While the ClpPs and ClpC proteases also appear conserved in *Synechocystis* sp. PCC 6803 and the *Prochlorococcus* strains, the responses of these genes to HL is much more variable (Tables 3–5). These proteases, one of the best-studied families in *E. coli*, are composed of regulatory, ATPase/chaperone polypeptides associated with protease subunits. The three cyanobacteria studied here have two different *clpB* genes, as in *Synechococcus* sp. PCC 7942 (Eriksson *et al.*, 2001). In the present study, *clpB1* transcripts transiently increased while *clpB2* mRNA levels did not change following the transfer to HL (Tables 3–5).

ClpB1 was shown to be essential for resistance to high- and low-temperature stress in *Synechococcus elongatus* (Eriksson & Clarke, 1996; Porankiewicz & Clarke, 1997). While ClpB2 has been suggested to be an essential constitutive protein with general chaperone activity (Eriksson *et al.*, 2001), our data indicate that ClpB1 might have a stress-related function. The *clpP* transcripts also accumulated in *Synechocystis* sp. PCC 6803 and *Prochlorococcus* MED4 after HL exposure, especially *clpP1*. Interestingly, all of the *clp* transcripts declined in *Prochlorococcus* MIT9313 following exposure to HL, except for *clpP1*. ClpP1 was previously shown to be induced strongly during acclimation of *Synechococcus* sp. PCC 7942 to either low temperature or UV-B, but was not induced by heat shock (Clarke *et al.*, 1998; Porankiewicz *et al.*, 1998). Similarly, neither ClpP2, ClpP3 nor ClpP4 (ClpR) was shown to increase upon heat or cold shock (Schelin *et al.*, 2002; Porankiewicz *et al.*, 1998), whereas all four Clp proteins were induced by HL intensities to different degrees, but again the most pronounced increase was for ClpP1 in *Synechococcus* sp. PCC 7942 (Clarke *et al.*, 1998). ClpX, ClpP3 and ClpR were found to be primarily constitutive proteins, critical for cell viability in this strain (Schelin *et al.*, 2002). These data suggest the complexity in the control and importance of the different *clp* genes in cyanobacteria in response to stress conditions. The arrangement of *clp* genes is conserved in the genomes of several cyanobacteria including *Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 7942, *Nostoc punctiforme* and *Prochlorococcus marinus* (Schelin *et al.*, 2002). For example, the *clpP2* gene is part of an operon with *clpX* and, therefore, ClpP2 may preferentially associate with ClpX and not with other Clp/Hsp100 proteins, such as ClpC (Porankiewicz *et al.*, 1999). In the present study, *clpX* mRNA did not accumulate in any of the three strains following HL exposure (Tables 3–5), raising the possibility that ClpX in cyanobacteria may function as an independent chaperone that is not functionally linked to ClpP (Levchenko *et al.*, 1995).

The *htpG* transcript accumulated in all strains following exposure to HL (Tables 3–5), which is consistent with recent microarray studies (Hihara *et al.*, 2001; Huang *et al.*, 2002). Moreover, it has been shown that HtpG is essential for survival during heat (Tanaka & Nakamoto, 1999) and cold stress (Hossain & Nakamoto, 2002), and is also involved in acclimation of *Synechococcus* sp. PCC 7942 to oxidative stress (Hossain & Nakamoto, 2003).

Overall, *hsp* genes were induced to higher levels for longer periods after the light shift for *Prochlorococcus* MED4 than for either *Prochlorococcus* MIT9313 or *Synechocystis* sp. PCC 6803. For example, while in *Synechocystis* sp. PCC 6803 only the *clpP1* transcript level rose substantially following exposure to HL and no *clpP* transcript accumulated in *Prochlorococcus* MIT9313, transcripts for all four of the *clpP*s strongly increased in *Prochlorococcus* MED4. Furthermore, while transcripts encoding ClpC (Kaneko *et al.*, 1996; Clarke & Eriksson, 1996) declined following HL exposure of *Synechocystis* sp. PCC 6803 (Eriksson *et al.*, 2001) and *Prochlorococcus* MIT9313, they showed a sustained increase in *Prochlorococcus* MED4. *Prochlorococcus* MED4 is found in ocean surface waters, where light intensities can reach up to 1500 μmol photons m^{-2} s^{-1} at midday, nutrient levels can be extremely low (Partensky *et al.*, 1999), and the cells probably experience oxidative stress. Our results for *Prochlorococcus* MED4 suggest that considerable protein misfolding and aggregation could take place in surface oceanic waters and that *hsp* genes could be critical for survival in such an extreme environment. Another example of adaptation of *Prochlorococcus* MED4 to a HL environment is the high number (22) of *hli* genes encoded by its genome relative to the genomes of the LL-adapted strains (Bhaya *et al.*, 2002); this gene family is critical for cyanobacterial survival during HL exposure (He *et al.*, 2001),

In conclusion, based on expression patterns, at least seven genes, *groEL1/ES*, *groEL2*, *dnaK2*, *dnaJ3*, *clpB1* and *clpP1*, are suspected to play a key role in the acclimation of cyanobacteria to HL (Table 6). Interestingly, these genes are among the most conserved in the three strains considered. They should be targeted for more detailed analyses including protein expression and genetic studies.

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