Microbiology (2014), **160**, 647–658 **DOI** 10.1099/mic.0.073478-0

Review

Cyanobacterial heat-shock response: role and regulation of molecular chaperones

Hema Rajaram,[1] Akhilesh Kumar Chaurasia[1,2] and Shree Kumar Apte[1]

Correspondence
Hema Rajaram
hemaraj@barc.gov.in

[1]Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai, 400085 India

[2]Samsung Biomedical Research Institute, School of Medicine, SKKU, Suwon, 440-746 Republic of Korea

Cyanobacteria constitute a morphologically diverse group of oxygenic photoautotrophic microbes which range from unicellular to multicellular, and non-nitrogen-fixing to nitrogen-fixing types. Sustained long-term exposure to changing environmental conditions, during their three billion years of evolution, has presumably led to their adaptation to diverse ecological niches. The ability to maintain protein conformational homeostasis (folding–misfolding–refolding or aggregation–degradation) by molecular chaperones holds the key to the stress adaptability of cyanobacteria. Although cyanobacteria possess several genes encoding DnaK and DnaJ family proteins, these are not the most abundant heat-shock proteins (Hsps), as is the case in other bacteria. Instead, the Hsp60 family of proteins, comprising two phylogenetically conserved proteins, and small Hsps are more abundant during heat stress. The contribution of the Hsp100 (ClpB) family of proteins and of small Hsps in the unicellular cyanobacteria (*Synechocystis* and *Synechococcus*) as well as that of Hsp60 proteins in the filamentous cyanobacteria (*Anabaena*) to thermotolerance has been elucidated. The regulation of chaperone genes by several *cis*-elements and *trans*-acting factors has also been well documented. Recent studies have demonstrated novel transcriptional and translational (mRNA secondary structure) regulatory mechanisms in unicellular cyanobacteria. This article provides an insight into the heat-shock response: its organization, and ecophysiological regulation and role of molecular chaperones, in unicellular and filamentous nitrogen-fixing cyanobacterial strains.

Received 24 September 2013
Accepted 31 January 2014

Introduction

Cyanobacteria or blue green algae originated as a group of photoautotrophs nearly 3.5 billion years ago (Brock, 1973) and were largely responsible for the initial oxygenation of Earth's atmosphere (Schopf, 1975). Today, as a group, they are ubiquitous in distribution and are often found in extreme environmental conditions such as hot springs (*Synechococcus* sp., >70 °C; *Oscillatoria terebriformis*, ~54 °C), frozen lakes of Antarctica (*Calothrixparetina*, *Nostoc* sp., *Synechococcus* sp., *Phormidium frigidum*), freshwater bodies (*Nostoc*, *Anabaena*, *Microcystis aerugenosa*, *Oscillatoria* sp., *Nodularia spunigena*) or brackish waters (*Anabaena* sp., *Aphanizomenon* sp., *Arthrospira* sp., *Microcystis* sp.), salt ponds (*Gleothece* sp., *Plectonema* sp., etc.), oceans (*Synechococcus elongatus*) and deserts (*Gloeocapsa* sp.). They also exhibit the ability to survive in extremes of temperatures from −60 to 74 °C (*Synechococcus lividus*), or salinity stress (*Aphanothece halophytica*) (Whitton & Potts, 2000). Filamentous nitrogen-fixing freshwater strains of *Nostoc* and *Anabaena* significantly contribute to the carbon and nitrogen economy of tropical paddy fields (Singh, 1950; Venkataraman, 1979). Recently, successful strain improvement of such microbes using sustainable and eco-friendly genetic engineering approaches has been reported (Chaurasia & Apte, 2009, 2011; Chaurasia *et al.*, 2013). Given their natural abundance in all ecological niches, studies on the stress responses of cyanobacteria are vital and relevant to their biotechnological exploitation, ranging from production of useful biomolecules (Lem & Glick, 1985) to nitrogen biofertilizers for rice cultivation under stressful environments (Singh, 1950).

The nitrogen fixation capability of most heterocystous cyanobacteria depends entirely on photosynthesis, as the electrons, ATP and carbon skeletons required for nitrogen fixation in heterocysts come as photosynthates from vegetative cells (Stewart, 1980; Wolk, 1968). Both photosynthesis and nitrogen fixation are adversely affected by heat and other stresses. Inactivation of the photosystems upon temperature upshift has been shown both in the unicellular cyanobacteria *Synechocystis* PCC6803 (Glatz *et al.*, 1999; Mamedov *et al.*, 1993) and *Synechococcus* PCC7942 (Eriksson & Clarke, 1996) and in the filamentous *Anabaena* sp. (Chaurasia & Apte, 2009; Rajaram & Apte,

Abbreviations: Hsp, heat-shock protein; HSR, heat-shock response; PSII, photosystem II; sHsp, small Hsp.

2008). Nitrogen fixation has been similarly found to be sensitive to temperatures above 42 °C in the heterocystous cyanobacteria *Anabaena cylindrica*, *Mastigocladus laminosus* (Pederson *et al.*, 1986), *Anabaena* sp. strain L-31 (Rajaram & Apte, 2003) and *Anabaena* PCC7120 (Chaurasia & Apte, 2009). Such adverse effects on vital metabolic processes emphasize the importance of studying the heat-shock response (HSR) in cyanobacteria. This article reviews currently available information on the cyanobacterial HSR in terms of its genomic organization, physiological role and regulation in the unicellular and filamentous cyanobacteria, with special emphasis on the Hsp60 proteins.

The HSR in cyanobacteria

Upon temperature upshift, cyanobacteria induce a set of proteins called the heat-shock proteins or Hsps, by transcriptional activation (Bhagwat & Apte, 1989; Borbély *et al.*, 1985; Rajaram & Apte, 2010; Rajaram *et al.*, 2001; Webb *et al.*, 1990). The growth temperature and the extent of temperature upshift determine the magnitude of induction (Lehel *et al.*, 1993a). GroEL, small Hsps and GroES are the most prominent Hsps that accumulate in cyanobacterial cells (Bhagwat & Apte, 1989; Blondin *et al.*, 1993; Rajaram & Apte, 2003; Roy *et al.*, 1999). The typical HSR in *Synechocystis* PCC6803 comprises about 90 proteins upregulated after 1 h of heat stress, with the major proteins being HspA, GroEL1, GroEL2, GroES, HtpG, DnaK2 and ClpB (Slabas *et al.*, 2006). Microarray data indicate transcriptional induction of the corresponding genes during heat stress (Suzuki *et al.*, 2006).

In *Synechocystis* PCC6803, change in growth temperature results in changes in the proportion of polyunsaturated fatty acids in membranes via the desaturase enzyme cascade, *desA*/*desB* (Murata *et al.*, 1992). Comparison of wild-type and *desA*/*desB* mutants of *Synechocystis* PCC6803, which contain only monounsaturated fatty acids, indicates that changes in protein dynamics during heat stress are solely dependent on protein stability rather than their interaction with neighbouring lipids (Laczkó-Dobos & Szalontai, 2009). Large-scale temperature-induced changes in ester bonds occur during the gel to liquid crystalline phase transition in the cytoplasmic membrane, but in thylakoid membranes protein structural changes occur only at high temperatures (Laczkó-Dobos & Szalontai, 2009). Different perturbations observed in the thylakoid and cytoplasmic membranes are due to the differences in membrane-associated protein complexes and the ratio of lipids to proteins, which is higher for cytoplasmic membranes in *Synechocystis* PCC6803 (Laczkó-Dobos & Szalontai, 2009). Studies with a mutant defective in the synthesis of Fab1 and using inhibitors of Fab1 synthesis (triclosan) and protein synthesis (chloramphenicol) have demonstrated that *de novo* fatty acid synthesis precedes high-temperature acclimatization of photosynthesis in *Synechocystis* PCC6803 (Nanjo *et al.*, 2010). Changes in the physical and structural properties of membranes are thought to play a key role in initiating the HSR (Horvárth *et al.*, 2012). Not only is the expression of membrane-associated Hsps controlled by changes in the composition and physical state of the lipid phase of the membrane, but so too is the association of pre-synthesized Hsps with the membrane during heat stress (Horvárth *et al.*, 2012). In higher photoautotrophs such as mosses, a lipid-based signalling cascade is activated, and there are changes in the transport and availability of Ca^{2+} during heat stress (Horvárth *et al.*, 2012). This underlines the importance of membranes during heat stress and acclimatization to change in growth temperature.

Induction of Hsp synthesis in *Anabaena* L-31was observed upon temperature upshift from 25 ± 2 °C to 39–45 °C, beyond which photo-bleaching occurred. The major Hsps synthesized upon exposure to 42 °C include the 10 kDa GroES, 16 kDa Hsp, 59 kDa GroEL, 61 kDa Cpn60, 70 kDa DnaK, 96, 98 and 100 kDa Hsps (Apte *et al.*, 1998; Bhagwat & Apte, 1989; Rajaram & Apte, 2003). A time-dependent induction of Hsps was seen, and proteins were classified as early or late Hsps, while those synthesized throughout the heat stress were termed long-term Hsps, such as GroEL, Cpn60 and GroES (Rajaram & Apte, 2003).

Anabaena strains display a distinct overlap in the synthesis of Hsps and that of other stress proteins, such as those induced by salinity and osmotic stress (Apte, 2001; Apte & Bhagwat, 1989; Apte *et al.*, 1998; Bhagwat & Apte, 1989). The GroEL proteins are commonly induced by almost all stresses, including heat stress (Apte *et al.*, 1998). Proteomic analyses of *Synechocystis* PCC6803 exposed to heat, salt or metal stresses similarly indicate the presence of several common proteins, including chaperones and sigma (Sig) factors (Castielli *et al.*, 2009). Protein denaturation occurs in response to almost all abiotic stresses, resulting in accumulation of denatured proteins in the cytosol. This, in turn, is known to evoke the HSR (Kanemori *et al.*, 1994) and explains why Hsps are induced in response to many different stresses (Bhagwat & Apte, 1989). Pre-exposure to sublethal temperature and early synthesis of elevated levels of Hsps alleviated UV-B toxicity in the cyanobacterium *Anabaena doliolum* and conferred cross tolerance to a variety of other abiotic stresses in *Anabaena* (Mishra *et al.*, 2009). However, pre-treatment with mild heat stress did not protect *Synechocystis* PCC6803 against subsequent exposure to salt stress, despite substantial overlap in the stress-induced proteins (Nikkinen *et al.*, 2012).

Heat-shock protein families and their contribution to cyanobacterial stress tolerance

Thermotolerance of cyanobacterial species, both unicellular and filamentous, is enhanced upon pre-treatment at sublethal temperatures, suggesting involvement of heat-shock genes/proteins in thermotolerance (Blondin *et al.*, 1993). A brief description of the functions of Hsps in cyanobacteria is provided in Table 1.

Table 1. Heat-shock family proteins of cyanobacteria

Family	Gene	Cyanobacteria	Function	Reference(s)
Hsp100	*clpBI*	*Synechococcus* PCC7942	Acquired thermotolerance	Eriksson & Clarke (1996)
	clpBII		Cold tolerance	Porankiewicz & Clarke (1997)
Hsp90	*htpG*	*Synechococcus* PCC7942	Innate and acquired thermotolerance, protection of photosynthetic apparatus	Tanaka & Nakamoto (1999), Sato *et al.* (2010)
Hsp70 Hsp40 GrpE	*dnaK*1/2/3, seven *dnaJ grpE*	*Synechocystis* PCC6803, *Synechococcus* PCC7942	DnaJ2: thermotolerance; DnaK2: RNA chaperone thermosensor	Varvasovszki *et al.* (2003), Watanabe *et al.* (2007b), Barthel *et al.* (2011)
Hsp60 Hsp10	*groEL*-1 (*groEL*), *groEL*-2 (*cpn60*)	*Synechocystis* PCC6803, *Anabaena* L-31	Thermotolerance, GroEL: nitrogen-fixing conditions; Cpn60: nitrogen-replete conditions	Chaurasia & Apte (2009), Rajaram & Apte (2008)
sHsp	*hsp16.6*/*hsp17*/*hspA*	*Synechocystis* PCC6803, *Synechococcus* PCC7942	Protects membrane fluidity, thermotolerance	Nakamoto *et al.* (2000), Horváth *et al.* (1998), Lee *et al.* (2000)

Hsp100 family. *Synechococcus* PCC7942 has two *clpB* (caseinolytic peptidase) genes (Eriksson & Clarke, 1996; Eriksson *et al.*, 2001). ClpBI is translated as a full-length 93 kDa protein or as a truncated version of about 79 kDa similar to other bacterial ClpB proteins. ClpBI levels are enhanced by heat stress (Clarke & Eriksson, 2000) and moderate cold stress (Porankiewicz & Clarke, 1997) and contribute to the acquired thermotolerance (Eriksson & Clarke, 1996) as well as cold tolerance (Porankiewicz & Clarke, 1997), both of which are severely affected upon deletion of the *clpBI* gene. The truncated ClpB-79 also confers thermotolerance and contributes about 30 % of the thermotolerance developed in *Synechococcus* PCC7942 (Clarke & Eriksson, 2000). The ClpBII protein, by contrast, is constitutively expressed as a full-length protein (Eriksson *et al.*, 2001). It does not contribute to acquired thermotolerance, as shown by its inability to complement a *clpBI* mutant of *Synechococcus* PCC7942 (Eriksson *et al.*, 2001). Thus, ClpBI has the greater physiological role in *Synechococcus*, especially upon exposure to stress.

Hsp90 family. The HtpG protein of the Hsp90 family of *Synechococcus* PCC7942 plays a role in several abiotic stresses, suggesting that it is more of a general stress protein (Hossain & Nakamoto, 2002, 2003; Tanaka & Nakamoto, 1999). Deletion of the *htpG* gene severely decreases both innate and acquired thermotolerance (Tanaka & Nakamoto, 1999), inhibits growth and photosynthetic activity in low temperature and high light conditions as well as during methyl viologen-induced oxidative stress (Hossain & Nakamoto, 2002, 2003). It is speculated that HtpG plays a role as a molecular chaperone during oxidative stress (Hossain & Nakamoto, 2003). Protection of the photosynthetic apparatus by HtpG possibly involves its interaction with phycobiliproteins to prevent their aggregation (Sato *et al.*, 2010). It also interacts with uroporphyrinogen decarboxylase, HemE (Saito *et al.*, 2008), indirectly regulating levels of coproporphyrin (Watanabe *et al.*, 2007a), and thereby those of phycobilins. HtpG thus primarily protects the photosynthetic machinery from heat and other stresses in cyanobacteria.

Hsp70/Hsp40/Hsp25 family. In bacteria, this family of proteins includes the 70 kDa DnaK along with its cohorts, i.e. the 40 kDa DnaJ and the 25 kDa GrpE proteins. Cyanobacteria possess multiple *dnaK* and *dnaJ* genes. *Synechocystis* PCC6803 and *Synechococcus* PCC7942 have three *dnaK* genes and seven *dnaJ* genes, while *Anabaena* PCC7120 has five *dnaK* and eight *dnaJ* genes (http://genome.microbedb.jp/cyanobase). Multiple *dnaK* and *dnaJ* genes have also been reported for *Escherichia coli* (Genevaux *et al.*, 2007). Of the three *dnaK* genes in the unicellular cyanobacteria, only *dnaK2* is induced under heat and other abiotic stresses (Rupprecht *et al.*, 2010; Sato *et al.*, 2007) and contributes to thermotolerance (Varvasovszki *et al.*, 2003). Among the seven DnaJ proteins, the one encoded by *sll0897* (DnaJ2) has been suggested to be the canonical Hsp (Düppre *et al.*, 2011). DnaJ2, along with DnaK2, acts as an RNA chaperone protecting the *psbAII* transcript from RNaseE-mediated degradation (Watanabe *et al.*, 2007b), and prevents inhibition of photosynthesis during stress. DnaK1 and DnaK2 proteins are localized in the cytoplasm. DnaK3 is targeted to the thylakoid membranes and may be involved in protein folding in thylakoids (Nimura *et al.*, 1996; Rupprecht *et al.*, 2007), or during the translation process on the surface of the thylakoid membrane (Katano *et al.*, 2006). The long C-terminal tail of DnaK3 is characteristic of all cyanobacterial DnaK3 proteins and has a well-conserved amino acid motif that is essential for the *in vivo* function of this protein in *Synechocystis* PCC6803 (Rupprecht *et al.*, 2010). The 25 kDa GrpE protein of *Synechocystis* PCC6803 and *Thermosynechococcus elongatus* BP-1 exists as a dimer and acts as a thermosensor, either through its N-terminal helix pair in *Synechocystis* PCC6803 or through its C-terminal four-helix bundle in *Thermosynechococcus elongatus* BP-1 (Barthel *et al.*, 2011).

Hsp60/Hsp10 family. The Hsp60/Hsp10 family, also referred to as the GroE chaperone machinery, comprises

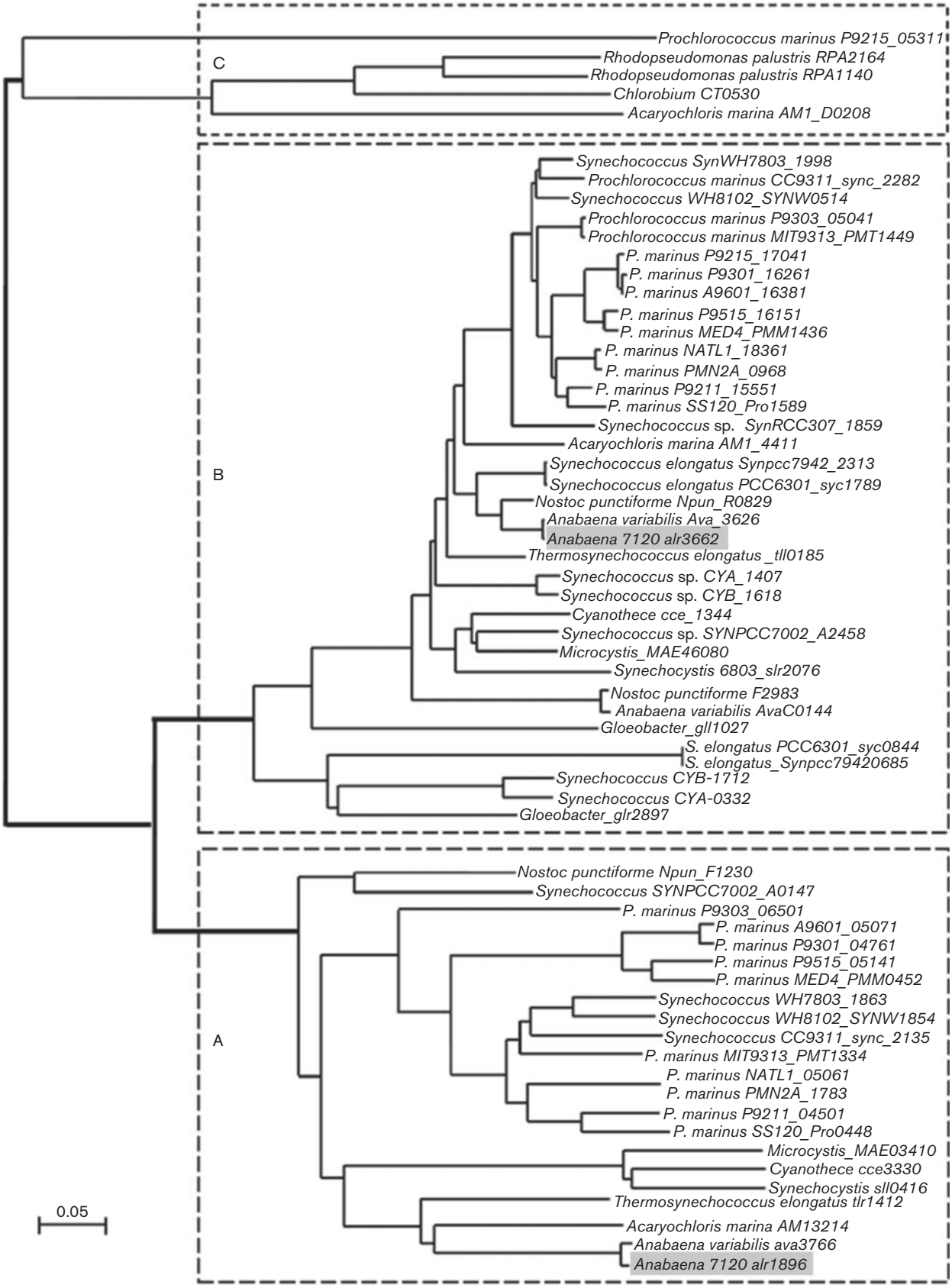
C
Prochlorococcus marinus P9215_05311
Rhodopseudomonas palustris RPA2164
Rhodopseudomonas palustris RPA1140
Chlorobium CT0530
Acaryochloris marina AM1_D0208
B
Synechococcus SynWH7803_1998
Prochlorococcus marinus CC9311_sync_2282
Synechococcus WH8102_SYNW0514
Prochlorococcus marinus P9303_05041
Prochlorococcus marinus MIT9313_PMT1449
P. marinus P9215_17041
P. marinus P9301_16261
P. marinus A9601_16381
P. marinus P9515_16151
P. marinus MED4_PMM1436
P. marinus NATL1_18361
P. marinus PMN2A_0968
P. marinus P9211_15551
P. marinus SS120_Pro1589
Synechococcus sp. SynRCC307_1859
Acaryochloris marina AM1_4411
Synechococcus elongatus Synpcc7942_2313
Synechococcus elongatus PCC6301_syc1789
Nostoc punctiforme Npun_R0829
Anabaena variabilis Ava_3626
Anabaena 7120 alr3662
Thermosynechococcus elongatus _tll0185
Synechococcus sp. CYA_1407
Synechococcus sp. CYB_1618
Cyanothece cce_1344
Synechococcus sp. SYNPCC7002_A2458
Microcystis_MAE46080
Synechocystis 6803_slr2076
Nostoc punctiforme F2983
Anabaena variabilis AvaC0144
Gloeobacter_gll1027
S. elongatus PCC6301_syc0844
S. elongatus_Synpcc79420685
Synechococcus CYB-1712
Synechococcus CYA-0332
Gloeobacter_glr2897
A
Nostoc punctiforme Npun_F1230
Synechococcus SYNPCC7002_A0147
P. marinus P9303_06501
P. marinus A9601_05071
P. marinus P9301_04761
P. marinus P9515_05141
P. marinus MED4_PMM0452
Synechococcus WH7803_1863
Synechococcus WH8102_SYNW1854
Synechococcus CC9311_sync_2135
P. marinus MIT9313_PMT1334
P. marinus NATL1_05061
P. marinus PMN2A_1783
P. marinus P9211_04501
P. marinus SS120_Pro0448
Microcystis_MAE03410
Cyanothece cce3330
Synechocystis sll0416
Thermosynechococcus elongatus tlr1412
Acaryochloris marina AM13214
Anabaena variabilis ava3766
Anabaena 7120 alr1896
0.05

Fig. 1. Neighbour-joining tree of GroEL proteins among cyanobacterial genera. The phylogenetic relationship of GroEL among cyanobacterial genera clustered into three groups boxed as clusters A, B and C. Cluster A represents the monocistronic GroEL2 (Cpn60) while cluster B is GroEL1, which is encoded in a bicistronic operon with GroES. Cluster C represents the ancient cyanobacterium *Prochlorococcus* sp., which shares high similarity with the chlorophyll *d*-containing cyanobacterium *Acaryochloris marina*, purple non-sulfur bacterium *Rhodopseudomonas* sp. and green sulfur bacterium *Chlorobium* sp. The text following the taxon represents the species name and the annotated number of either of the two *hsp60* genes of the species. The *hsp60* genes of *Anabaena* PCC7120 have been highlighted. The scale bar of 0.05 indicates the amount of genetic change. The protein sequences obtained from http://genome.microbedb.jp/cyanobase/ were subjected to CLUSTAL W analyses using the BioEdit (Hall, 1999) program, to generate the phylogenetic tree.

the 59 kDa GroEL and 10 kDa GroES proteins encoded by the bicistronic *groESL* operon (Tilly *et al.*, 1983). Although most bacteria possess a single *groE* operon, a significant proportion of bacteria, including cyanobacteria, have multiple *groE* operons or *groEL* genes (Lund, 2009). The cyanobacterial Hsp60/Hsp10 family is characterized by the presence of a 10 kDa GroES and two 60 kDa Hsps, i.e. GroEL-1 (GroEL), encoded by the bicistronic *groESL* operon, and GroEL-2 (Cpn60), encoded by the monocistronic *groEL-2*/*cpn60* gene (Chitnis & Nelson, 1991; Furuki *et al.*, 1996; Kaneko *et al.*, 2001; Lehel *et al.*, 1993b; Rajaram *et al.*, 2001; Tanaka *et al.*, 1997; Webb *et al.*, 1990). The monocistronic *groEL-2*/*cpn60* genes of cyanobacteria are phylogenetically in Group A, while the bicistronic *groESL* operons fall in Group B (Fig. 1). *Prochlorococcus marinus* P9215, containing chlorophyll *b* and a small number of genes (~2000) (Chisholm *et al.*, 1988), constitutes a separate cluster (Group C) which shares high similarity with chlorophyll *d*-containing cyanobacterium *Acaryochloris marina*, the purple non-sulfur *Rhodopseudomonas* sp. and the green sulfur *Chlorobium* sp. (Fig. 1).

Both the Hsp60 proteins, the 59 kDa GroEL (GroEL-1) and 61 kDa Cpn60 (GroEL-2), possess the signature sequence 'GPKGRN' and exhibit an overall sequence similarity of 60 %. However, a 'GGM' tail comprising six 'GGM' repeats is present at the C terminus of Cpn60, but is absent in GroEL. This pattern has been observed across almost all bacterial species that have two *groEL* genes, one as part of a bicistronic operon and the other as a monocistronic gene. GroEL-1 exhibits higher chaperone activity as well as ATPase activity than GroEL-2 (Cpn60), both in *Synechococcus elongatus* PCC7942 (Huq *et al.*, 2010) and in *Anabaena* (A. A. Potnis *et al.*, unpublished results). The GroES protein does not significantly assist the chaperonin activity of GroEL (Huq *et al.*, 2010). This is unlike the situation in *E. coli*, where interaction with GroES is essential for optimal chaperonin activity of GroEL (Horwich *et al.*, 2006; Paul *et al.*, 2007), but not for the unfolding activity, i.e. release of misfolded peptides from the GroEL cavity (Priya *et al.*, 2013). The identified mobile loop region in *E. coli* GroES, which is essential for interaction with GroEL (Landry *et al.*, 1993), is absent in *Anabaena* GroES. The corresponding interacting region in *E. coli* GroEL (Zeilstra-Ryalls *et al.*, 1993), as well as the phosphorylation site, Y-477, in the conserved 'GYNAAT' motif in the C-terminal region of *E. coli* GroEL (Martin *et al.*, 1993), is present in Cpn60, but not in GroEL of *Anabaena*. The interaction of GroES with Cpn60 needs to be verified biochemically. The GroES protein of *Synechocystis* PCC6803 undergoes phosphorylation, possibly by the protein kinase Spk, which is essential for its *in vivo* activity (Zorina *et al.*, 2011).

Relative expression of the two Hsp60 proteins is dependent on N-status in *Anabaena*. Enhanced synthesis of the 59 kDa GroEL protein is observed in response to heat stress irrespective of N-status during growth, although it is relatively higher under nitrogen-fixing conditions (Rajaram & Apte, 2003, 2008). However, the 61 kDa Cpn60 shows enhanced expression during heat stress only under nitrogen-fixing conditions. Under N-supplemented growth, its levels are high at ambient temperature, but completely repressed during heat stress (Rajaram & Apte, 2008). The decreased level of Cpn60 protein during heat shock in N-replete conditions is due to (i) inhibition of transcription as shown by rifampicin-based experiments, and (ii) probable proteolytic degradation of the Cpn60 protein accumulated under control growth conditions (Rajaram & Apte, 2008). This difference in expression of the two Hsp60 proteins affects the thermotolerance of *Anabaena* in an N-status-dependent manner.

Exposure to heat stress has a bacteriostatic effect on *Anabaena*, which exhibits no growth at 42 °C, possibly due to decreased photosynthetic and nitrogenase activity in the nitrogen-fixing conditions (Rajaram & Apte, 2003) and inhibition of photosynthetic and nitrate reductase activity under N-replete conditions (Rajaram & Apte, 2008). However, these bacteria exhibit remarkable recovery from heat stress, possibly due to the continuous synthesis of the two Hsp60 proteins right through the heat stress and their high stability even after return to normal growth conditions (Rajaram & Apte, 2003), unlike the transient synthesis of Hsps observed in other bacteria. The association of Hsp60 proteins with carboxysomes of *Anabaena* PCC7120 (Jäger & Bergman, 1990) and repression of the synthesis of *cpn60* in the dark or in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) in *Synechocystis* PCC6803 (Glatz *et al.*, 1997) suggests a possible role for Hsp60 proteins in the assembly of multimeric photosynthetic complexes in cyanobacteria. Although the association of cyanobacterial Hsp60 proteins with nitrogenase has not been shown, a requirement of GroEL for assembly and activity of the nitrogenase proteins has been demonstrated in *Klebsiella*

(Govezensky *et al.*, 1991) and *Bradyrhizobium* (Fischer *et al.*, 1993).

Mutation of either *groEL* or *cpn60* was lethal to *Anabaena* PCC7120, indicating that the two Hsp60 proteins are vital and non-redundant (Rajaram & Apte, 2008). By contrast, the recombinant *Anabaena* strains individually overexpressing the GroES/EL or Cpn60 proteins exhibited superior growth to wild-type *Anabaena* PCC7120 (Chaurasia & Apte, 2009; Rajaram & Apte, 2008), suggesting a possible limitation on the availability of GroEL under ambient conditions. Continuous overexpression of GroEL protected against heat and salt stress and inhibited protein aggregation under nitrogen-fixing conditions (Chaurasia & Apte, 2009). Overexpression of Cpn60, by contrast, helped sustain photosynthetic and nitrate reductase activity for up to 4 days of heat stress, compared with 24 h of activity in wild-type *Anabaena* PCC7120 (Rajaram & Apte, 2008). Possible physiological roles of Hsps in general, and those of GroEL and Cpn60 proteins, depending on nitrogen-status are schematically depicted in Fig. 2.

Small Hsps. In general, the bacterial small Hsps (sHsps) are characterized as 12–13 kDa proteins with an α-crystallin domain, capable of forming oligomers and exhibiting an ATP-independent chaperone activity (Caspers *et al.*, 1995). Cyanobacterial sHsp has been annotated as Hsp16.6, Hsp17 or HspA, all of which relate to the same protein referred to as HspA in Fig. 2. In *Synechocystis* PCC6803, HspA acts as a chaperone and interacts with about 42 different proteins at high temperatures. It may offer protection to proteins involved in diverse cellular activities (Basha *et al.*, 2004). The *hspA* gene mutation in *Synechocystis* PCC6803 causes a decrease in growth rate and photosynthetic activity during normal growth conditions, and reduces viability at higher temperatures (Horváth *et al.*, 1998; Lee *et al.*, 1998, 2000). In the unicellular cyanobacterium *Synechococcus* PCC7942, overexpression of HspA enhanced thermotolerance, possibly by protecting photosystem II and phycobilisomes (Nakamoto *et al.*, 2000). HspA of *Anabaena* PCC7120 forms large oligomers, and exhibits chaperone activity to protect citrate synthase from thermal aggregation at 43 °C *in vitro* (Liu *et al.*, 2005). HspA also stabilizes heat-stressed membranes, and targets proteins to chaperone-mediated folding (Török *et al.*, 2001). The cellular localization of HspA shifts from thylakoids to cytosol and then back to thylakoids during heat stress (Nitta *et al.*, 2005). This indicates multiple roles for HspA, ranging from folding of proteins to stabilization of thylakoid and periplasmic membranes.

The thermosensitive nature of photosynthesis is primarily due to the thermally most sensitive component of photosynthesis, photosystem II (PSII). Transcriptional studies and gene knockout analyses in *Synechocystis* PCC6803 showed that the basal thermotolerance of PSII was controlled by ClpBI, Cpnc2, HspA, HtpG and Slr1674, while the acquired thermotolerance was affected by Cpnc2, Hik34, HspA and HypA1 (Rowland *et al.*, 2010).

Regulation of cyanobacterial *hsp* genes

In bacteria, most *hsp* genes are regulated either positively by σ^{32} (RpoH) or negatively by HrcA (Yura & Nakahigashi, 1999). Of the two regulators, only the gene encoding HrcA is present in all cyanobacteria (Nakamoto *et al.*, 2003; Singh *et al.*, 2006; Rajaram & Apte, 2010). Constitutive expression of Hsp60 proteins in *hrcA* mutants of *Synechocystis* PCC6803 (Nakamoto *et al.*, 2003) and *Anabaena* PCC7120 (Rajaram & Apte, 2010) indicates that the negative regulation of these genes is indeed mediated through HrcA. In general, regulation occurs by binding of the HrcA dimer to a 9 bp inverted repeat element (TTAGCACTC-N_9-GAGTGCTAA) known as the CIRCE (controlling inverted repeat of chaperone expression) element (Zuber & Schumann, 1994) at ambient temperatures. The CIRCE element is present in the promoter region upstream of the *groESL* operon as well as the *cpn60* (*groEL-2*) gene of both *Synechocystis* (Kojima & Nakamoto, 2007) and *Anabaena* (Rajaram & Apte, 2010), partially overlapping with the −10 region in the *groESL* promoter and both −10 and −35 regions in the *cpn60* promoter. The HrcA repressor downregulates expression of the *groESL* operon and the *cpn60* gene by binding specifically to the CIRCE element in the dimeric form, but is unable to do so as a monomer (Rajaram & Apte, 2010). Denaturation and monomerization of HrcA during heat stress dissociates it from the CIRCE element and upregulates the downstream *hsp* genes (Rajaram & Apte, 2010). In addition to the upregulation of the *groESL* operon and the *groEL2* gene, in the Δ*hrcA* mutant of *Synechocystis* PCC6803, about 22 other genes, including *dnaK2*, are upregulated, while about 13 genes are downregulated (Singh *et al.*, 2006), indicating that the CIRCE/HrcA system may be a more generic regulator of heat-shock genes in cyanobacteria.

In cyanobacteria, the *hsp60* genes are additionally regulated by other *cis*-elements as well as *trans*-acting proteins, which include the K-box, N-box and H-box, as well as alternative sigma factors. In *Synechocystis* PCC6803, a *cis*-element, designated the K-box (GTTCGG-NNAN-CCNNAC), is located upstream of the promoter region and positively regulates expression of *groEL-1*, *groEL-2* and *dnaK* genes in response to both heat and light (Kojima & Nakamoto, 2007). The same element regulates expression of the *groESL* operon only in response to light in *Anabaena* (Rajaram & Apte, 2010). The modulation of expression in response to light intensity is also observed for the *htpG* and *hspA* genes in *Synechocystis* PCC6803 (Asadulghani *et al.*, 2003). The N-box is detected upstream of the *groEL-1* gene of *Synechocystis* PCC6803 and *groEL-2* (*cpn60*) of *Anabaena* PCC7120 and positively regulates expression of the downstream gene in response to light in the presence of a DNA-binding protein (Kojima & Nakamoto, 2007). An additional distinct heat-regulatory element (an 11 bp inverted repeat element), designated the H-box, is present upstream of the *groESL* operon of *Anabaena* and negatively regulates expression of the *groESL* promoter without the aid of a *trans*-acting protein. The possible melting of the stem–loop

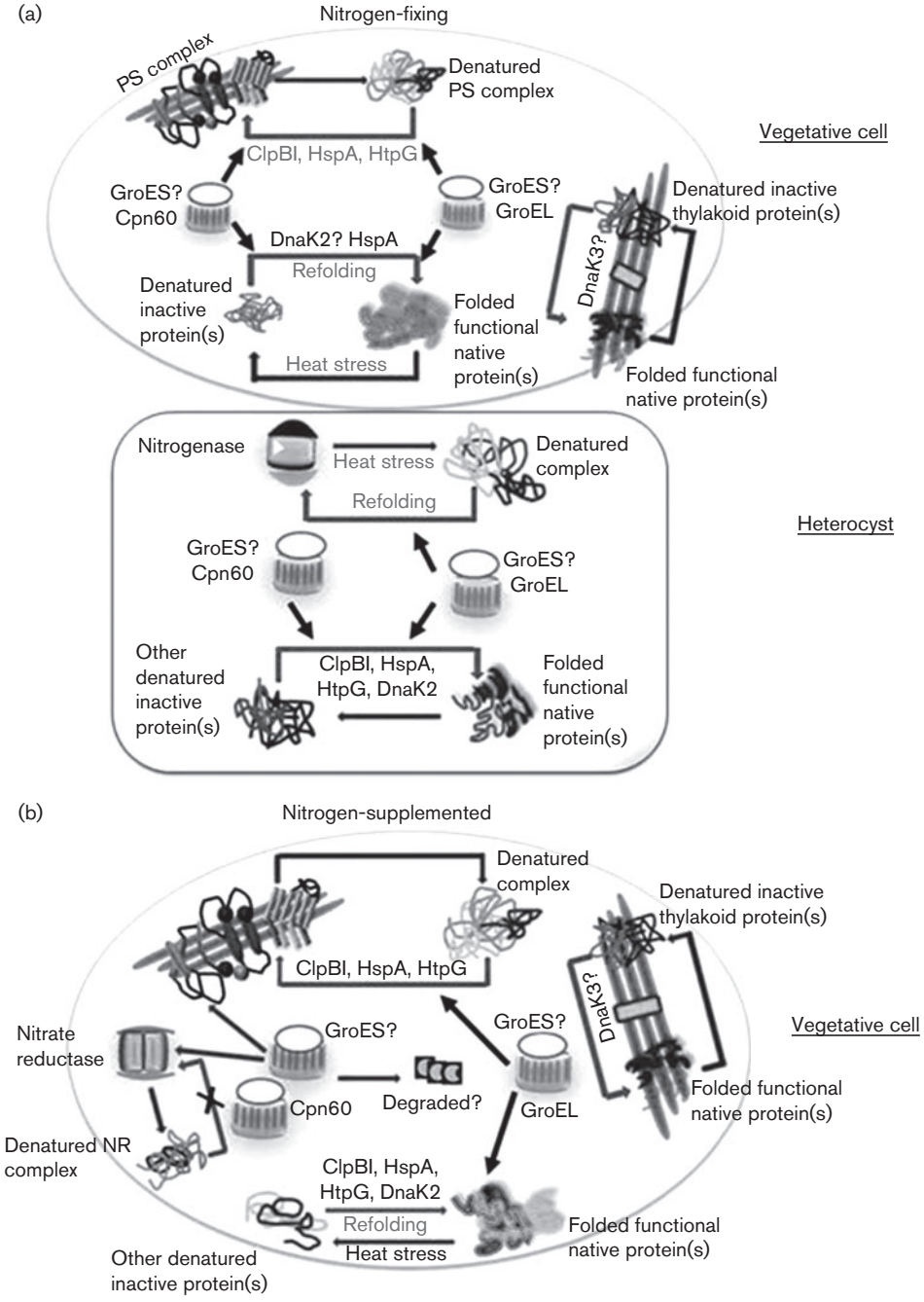

Fig. 2. Schematic representation of probable *in vivo* roles of Hsps in different cell types of cyanobacteria. (a) Under nitrogen-fixing conditions, showing both vegetative cells and heterocysts for *Anabaena* (filamentous cyanobacteria). (b) Under nitrogen-supplemented conditions, exhibiting a single cell type (vegetative cell). The arrow pointing from native protein to denatured protein indicates heat denaturation, while the arrow in the reverse direction signifies refolding. In addition to the general cytosolic and thylakoid/membrane-associated proteins, three specific complexes, namely the photosynthetic complex (PS complex) in (a) and (b), nitrogenase complex in (a) and nitrate reductase (NR) in (b) are indicated. Refolding of the PS and NR complexes by Cpn60 under control growth conditions in N-supplemented medium is shown by violet arrows in (b). The PS complex and other thylakoid proteins are shown on different thylakoids only for clarity of representation; this does not indicate that they are present on different thylakoids. Of the different Hsps, GroES is indicated by an oval shape, while GroEL and Cpn60 proteins by striped cylindrical shapes and DnaK by a thin rectangular shape. The question mark beside GroES indicates that it is not known whether GroES associates with GroEL or Cpn60. DnaK3 protein associated with thylakoids is indicated by an orange cylinder. The results obtained regarding the role of individual Hsps in *Synechocystis*, *Synechococcus* and *Anabaena* have been extrapolated to represent the possible scenario in cyanobacteria.

structure of the H-box element during heat stress results in increased transcription of the downstream *groESL* operon, and accounts for a further increase in GroEL levels during heat stress in the *hrcA* mutant of *Anabaena* (Rajaram & Apte, 2010). The regulation of cyanobacterial *hsp60* genes by natural ecophysiological factors, such as heat, light stress and combined nitrogen availability, is summarized in Fig. 3. The mechanism of upregulation of Cpn60 under nitrogen-supplemented conditions is not known, but overrides the negative regulation by HrcA under ambient temperatures in the presence of combined nitrogen.

The unicellular cyanobacterium *Synechocystis* PCC6803 has several sigma factors involved in transcriptional regulation (Imamura *et al.*, 2003). Comparative transcriptome analyses of individual *sigB*, *sigD* and *hrcA* deletion mutants of *Synechocystis* PCC6803 have revealed a complex regulation of expression of the chaperone genes in *Synechocystis* PCC6803 (Singh *et al.*, 2006). Of the *hsp* genes positively regulated by SigB and SigE, only the *hsp60* genes are negatively regulated by HrcA, while others such as *hspA* and *htpG* are not (Singh *et al.*, 2006). SigE seems to play a greater role in regulating the expression of *hsp* genes in the absence of SigB, suggesting interplay between SigB and SigE (Singh *et al.*, 2006), similar to that observed in *E. coli* between σ^{32} and σ^{24} (Mecsas *et al.*, 1993).

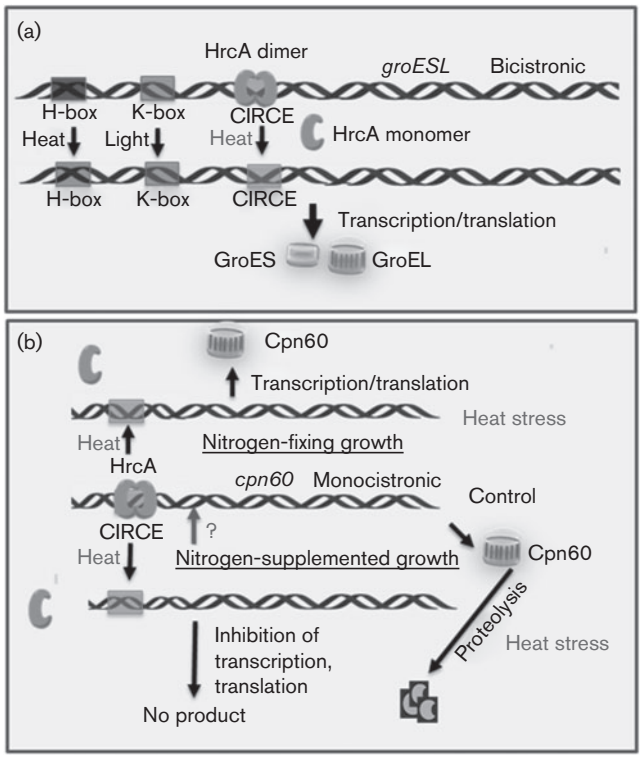

Fig. 3. Schematic representation of regulation of *hsp60* genes in response to heat, light and nitrogen stress in cyanobacteria. (a) Regulation of the *groESL* operon by heat and light stress in *Anabaena*. The H-box, K-box and CIRCE elements are shown by rectangular boxes. Melting of the H-box is shown by a transparent rectangular box. (b) Regulation of the *cpn60* gene in response to heat stress and N-status in *Anabaena*. The positive regulation by combined nitrogen availability is shown by an arrow, by an as yet undeciphered mechanism. The HrcA dimer, binding to the CIRCE element shown as a rectangular box, is represented as two peanut shaped objects, which upon exposure to heat is monomerized.

Among the several histidine kinases (Hik) identified in *Synechocystis* PCC6803, Hik34 has been found to be involved in negative regulation of heat-shock genes (Slabas *et al.*, 2006; Suzuki *et al.*, 2006). Deletion of *hik34* results in increased transcript levels of a few *hsp* genes, i.e. *groESL*, *htpG* and *hspA*, in addition to at least nine other genes and decreased expression of about 11 genes, including *sigE* (Suzuki *et al.*, 2006). Although the transcription of *groEL-2* and *dnaK2* was not significantly affected in the Δ*hik34* mutant of *Synechocystis*, their expression levels decreased upon overexpression of the Hik34 protein in *Synechocystis* PCC6803 (Suzuki *et al.*, 2006). The change in transcript levels of different genes correlated well with the changes in the corresponding protein levels in most cases in the Δ*hik34* mutant (Suzuki *et al.*, 2006). In addition to the enhanced levels of the heat-shock proteins, the levels of proteins involved in the protein biosynthesis machinery also showed an increase, both under control conditions and at elevated temperature in the Δ*hik34* mutant (Slabas *et al.*, 2006).

Another recently identified HSR in *Synechocystis* PCC6803 is Sll1130, which as a tetramer binds an inverted repeat element, regulating expression of several heat-shock genes, such as *htpG*, *hspA*, *isiA* and *isiB*, and a few hypothetical protein genes (Krishna *et al.*, 2013). The derepression of these *hsp* genes occurs due to a sudden decrease in both the transcript and the protein levels of *sll1130* during heat shock (Krishna *et al.*, 2013).

In addition to the *trans*-acting repressors/inducers or sigma factors, RNA-based regulation of heat-shock genes has also been reported in the unicellular cyanobacterium *Synechococcus* PCC7942. The *hsp17* (*hspA*) gene of *Synechococcus* PCC7942 has a short 5′ untranslated region characterized by a hairpin having an asymmetrical loop. Reporter gene assays and point mutations in the untranslated region which affected the stability of the hairpin–loop structure confirmed involvement of this region in the regulation of translation of the downstream gene (Kortmann *et al.*, 2011). This opens the possibility of the use of RNA as a thermosensor, enabling regulation of expression of the downstream heat-shock genes (Kortmann *et al.*, 2011). Regulation of cyanobacterial *hsp* genes by different *cis*-acting elements and *trans*-proteins is schematically described in Fig. 4. However, at present, it is not known if these regulators interact with each other and/or can override each other.

Modulation of the levels of the heat-shock regulatory proteins affects thermotolerance in cyanobacteria. Mutation of the *hrcA* gene, the primary negative regulator of the *hsp60* genes, enhances thermotolerance in both *Synechocystis* (Nakamoto *et al.*, 2003) and *Anabaena* (H.

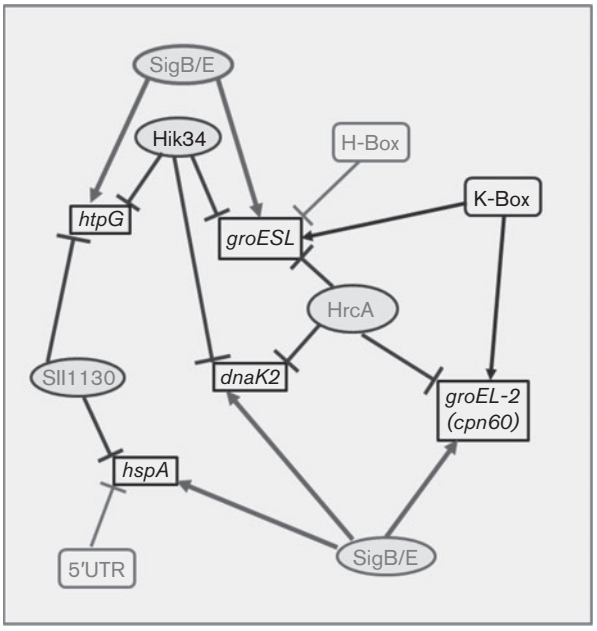

Fig. 4. Schematic representation of the regulation of major *hsp* genes in response to heat stress in cyanobacteria. The different *hsp* genes are italicized and shown in rectangular boxes, the proteins in oval shaped boxes and the *cis*-elements in rectangular boxes with curved edges. Positive regulation of the different *hsp* genes is indicated by arrows and negative regulation by 'T's. UTR, untranslated region.

Rajaram & S. K. Apte, unpublished results), due to the constitutive and elevated expression of the Hsp60 and GroES proteins (Nakamoto *et al.*, 2003; Rajaram & Apte, 2010). Among the sigma factors that positively regulate the expression of several *hsp* genes, SigB by itself regulates the short-term HSRs and acquired thermotolerance, and, along with SigD, has a role in general high-temperature responses (Tuominen *et al.*, 2006). A decrease in thermotolerance of *Synechocystis* PCC6803 occurs upon deletion of *sigC*, although SigC is not a known regulator of any heat-shock genes. This was found to be an indirect effect of a change in expression of genes related to carbon concentration mechanisms (Gunnelius *et al.*, 2010; Tuominen *et al.*, 2008). Inactivation of *hik34* or *sll1130* genes, both of which negatively regulate a number of heat-shock genes, allows the *Synechocystis* cells to recover from heat stress faster, thereby enhancing thermotolerance (Krishna *et al.*, 2013; Slabas *et al.*, 2006).

Conclusions

Molecular chaperones are essential components of many cellular functions. Their action involves (i) folding of nascent proteins during or after translation in the cytosol (Young *et al.*, 2004), (ii) transport of unfolded proteins and their subsequent folding in the Sec-dependent secretion pathway (Kim & Kendall, 2000), (iii) protein conformational homeostasis (Beissinger & Buchner, 1998) and (iv) protection of the photosynthetic apparatus from stress-induced damage (Katano *et al.*, 2006; Nimura *et al.*, 1996; Rupprecht *et al.*, 2007; Sato *et al.*, 2010).

Gene duplication and synonymous-redundancy may not be a general rule in Hsps of cyanobacteria, as has been demonstrated by the individual importance, organization, regulation and physiological division of labour in various vital processes for ClpB (Clarke & Eriksson, 2000; Eriksson *et al.*, 2001), DnaK (Rupprecht *et al.*, 2007, 2010; Varvasovszki *et al.*, 2003) and GroEL (Chaurasia & Apte, 2009; Rajaram & Apte, 2008, 2010). Notwithstanding their requirement for optimal function of several cellular processes, the Hsps in cyanobacteria are generally present in low abundance under normal growth conditions, but are upregulated at multiple levels in a need-based manner.

Cyanobacterial *hsp* genes are repressed by *cis*-elements, such as H-box and CIRCE in *groESL* of *Anabaena* (Rajaram & Apte, 2010) or 5′ untranslated regions of *hsp17* (Kortmann *et al.*, 2011), or by *trans*-acting proteins, such as HrcA (Kojima & Nakamoto, 2007; Nakamoto *et al.*, 2003; Rajaram & Apte, 2010), Hik34 (Slabas *et al.*, 2006) and Sll1130 (Krishna *et al.*, 2013), or by a combination of various sigma factors (Singh *et al.*, 2006; Tuominen *et al.*, 2006, 2008). Recent studies have revealed a novel role for GroEL in accumulation and stabilization of mutations for novel protein function (Tokuriki & Tawfik, 2009) or in codon usage (Warnecke & Hurst, 2010). A cumulative effect of GroES/EL overexpression has been observed on genome evolution (Bogumil & Dagan, 2012). This may offer a plausible reason why GroES/EL is not made in excess despite being beneficial, and is tightly regulated according to cellular needs. Maintenance of chaperone protein levels in appropriate stoichiometry to the unfolded proteins, through a complex web of regulation, probably ensures their availability on demand and circumvents the possible complications that may arise due to their accumulation in the cells.

The multiple roles of Hsps in cyanobacterial physiology suggest that their constitutive expression may help enhance stress tolerance, as has been observed for several Hsps in cyanobacteria. This possibly could be put to biotechnological use, such as in the production of biofertilizer for stressful environments (Chaurasia & Apte, 2009) or for production of biofuel, as has been demonstrated for *Clostridium acetobutylicum* ATCC 824 (Tomas *et al.*, 2003) and *E. coli* (Zingaro & Papoutsakis, 2013).

References

Apte, S. K. (2001). Coping with salinity/water stress: cyanobacteria show the way. *Proc Indian Natl Acad Sci* **B67**, 285–310.

Apte, S. K. & Bhagwat, A. A. (1989). Salinity-stress-induced proteins in two nitrogen-fixing *Anabaena* strains differentially tolerant to salt. *J Bacteriol* **171**, 909–915.

Apte, S. K., Fernandes, T., Badran, H. & Ballal, A. (1998). Expression and possible role of stress-responsive proteins in *Anabaena*. *J Biosci* **23**, 399–406.

Asadulghani, S., Suzuki, Y. & Nakamoto, H. (2003). Light plays a key role in the modulation of heat shock response in the cyanobacterium *Synechocystis* sp. PCC 6803. *Biochem Biophys Res Commun* **306**, 872–879.

Barthel, S., Rupprecht, E. & Schneider, D. (2011). Thermostability of two cyanobacterial GrpE thermosensors. *Plant Cell Physiol* **52**, 1776–1785.

Basha, E., Lee, G. J., Breci, L. A., Hausrath, A. C., Buan, N. R., Giese, K. C. & Vierling, E. (2004). The identity of proteins associated with a small heat shock protein during heat stress *in vivo* indicates that these chaperones protect a wide range of cellular functions. *J Biol Chem* **279**, 7566–7575.

Beissinger, M. & Buchner, J. (1998). How chaperones fold proteins. *Biol Chem* **379**, 245–259.

Bhagwat, A. A. & Apte, S. K. (1989). Comparative analysis of proteins induced by heat shock, salinity, and osmotic stress in the nitrogen-fixing cyanobacterium *Anabaena* sp. strain L-31. *J Bacteriol* **171**, 5187–5189.

Blondin, P. A., Kirby, R. J. & Barnum, S. R. (1993). The heat shock response and acquired thermotolerance in three strains of cyanobacteria. *Curr Microbiol* **26**, 79–84.

Bogumil, D. & Dagan, T. (2012). Cumulative impact of chaperone-mediated folding on genome evolution. *Biochemistry* **51**, 9941–9953.

Borbély, G., Surányi, G., Korcz, A. & Pálfi, Z. (1985). Effect of heat shock on protein synthesis in the cyanobacterium *Synechococcus* sp. strain PCC 6301. *J Bacteriol* **161**, 1125–1130.

Brock, T. D. (1973). Evolutionary and ecological aspects of cyanophytes. In *The Biology of Blue-Green Algae*, pp. 487–500. Edited by N. G. Carr & B. A. Whitton. Oxford: Blackwell.

Caspers, G. J., Leunissen, J. A. & de Jong, W. W. (1995). The expanding small heat-shock protein family, and structure predictions of the conserved ''α-crystallin domain''. *J Mol Evol* **40**, 238–248.

Castielli, O., De la Cerda, B., Navarro, J. A., Hervás, M. & De la Rosa, M. A. (2009). Proteomic analyses of the response of cyanobacteria to different stress conditions. *FEBS Lett* **583**, 1753–1758.

Chaurasia, A. K. & Apte, S. K. (2009). Overexpression of the *groESL* operon enhances the heat and salinity stress tolerance of the nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC7120. *Appl Environ Microbiol* **75**, 6008–6012.

Chaurasia, A. K. & Apte, S. K. (2011). Improved eco-friendly recombinant *Anabaena* sp. strain PCC7120 with enhanced nitrogen biofertilizer potential. *Appl Environ Microbiol* **77**, 395–399.

Chaurasia, A. K., Adhya, T. K. & Apte, S. K. (2013). Engineering bacteria for bioremediation of persistent organochlorine pesticide lindane (γ-hexachlorocyclohexane). *Bioresour Technol* **149**, 439–445.

Chisholm, S. W., Olson, R. J., Zettler, E. R., Goericke, R., Waterbury, J. B. & Welschmeyer, N. A. (1988). A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* **334**, 340–343.

Chitnis, P. R. & Nelson, N. (1991). Molecular cloning of the genes encoding two chaperone proteins of the cyanobacterium *Synechocystis* sp. PCC 6803. *J Biol Chem* **266**, 58–65.

Clarke, A. K. & Eriksson, M. J. (2000). The truncated form of the bacterial heat shock protein ClpB/HSP100 contributes to development of thermotolerance in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J Bacteriol* **182**, 7092–7096.

Düppre, E., Rupprecht, E. & Schneider, D. (2011). Specific and promiscuous functions of multiple DnaJ proteins in *Synechocystis* sp. PCC 6803. *Microbiology* **157**, 1269–1278.

Eriksson, M. J. & Clarke, A. K. (1996). The heat shock protein ClpB mediates the development of thermotolerance in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J Bacteriol* **178**, 4839–4846.

Eriksson, M. J., Schelin, J., Miskiewicz, E. & Clarke, A. K. (2001). Novel form of ClpB/HSP100 protein in the cyanobacterium *Synechococcus*. *J Bacteriol* **183**, 7392–7396.

Fischer, H. M., Babst, M., Kaspar, T., Acuña, G., Arigoni, F. & Hennecke, H. (1993). One member of a *gro-ESL*-like chaperonin multigene family in *Bradyrhizobium japonicum* is co-regulated with symbiotic nitrogen fixation genes. *EMBO J* **12**, 2901–2912.

Furuki, M., Tanaka, N., Hiyama, T. & Nakamoto, H. (1996). Cloning, characterization and functional analysis of *groEL*-like gene from thermophilic cyanobacterium *Synechococcus vulcanus*, which does not form an operon with *groES*. *Biochim Biophys Acta* **1294**, 106–110.

Genevaux, P., Georgopoulos, C. & Kelley, W. L. (2007). The Hsp70 chaperone machines of *Escherichia coli*: a paradigm for the repartition of chaperone functions. *Mol Microbiol* **66**, 840–857.

Glatz, A., Horváth, I., Varvasovszki, V., Kovács, E., Török, Z. & Vigh, L. (1997). Chaperonin genes of the *Synechocystis* PCC 6803 are differentially regulated under light–dark transition during heat stress. *Biochem Biophys Res Commun* **239**, 291–297.

Glatz, A., Vass, I., Los, D. A. & Vigh, L. (1999). The *Synechocystis* model of stress: from molecular chaperones to membranes. *Plant Physiol Biochem* **37**, 1–12.

Govezensky, D., Greener, T., Segal, G. & Zamir, A. (1991). Involvement of GroEL in *nif* gene regulation and nitrogenase assembly. *J Bacteriol* **173**, 6339–6346.

Gunnelius, L., Tuominen, I., Rantamäki, S., Pollari, M., Ruotsalainen, V., Tyystjärvi, E. & Tyystjärvi, T. (2010). SigC sigma factor is involved in acclimation to low inorganic carbon at high temperature in *Synechocystis* sp. PCC 6803. *Microbiology* **156**, 220–229.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Horváth, I., Glatz, A., Varvasovszki, V., Török, Z., Páli, T., Balogh, G., Kovács, E., Nádasdi, L., Benkö, S. & other authors (1998). Membrane physical state controls the signaling mechanism of the heat shock response in *Synechocystis* PCC 6803: identification of *hsp17* as a ''fluidity gene''. *Proc Natl Acad Sci U S A* **95**, 3513–3518.

Horváth, I., Glatz, A., Nakamoto, H., Mishkind, M. L., Munnik, T., Saidi, Y., Goloubinoff, P., Harwood, J. L. & Vigh, L. (2012). Heat shock response in photosynthetic organisms: membrane and lipid connections. *Prog Lipid Res* **51**, 208–220.

Horwich, A. L., Farr, G. W. & Fenton, W. A. (2006). GroEL-GroES-mediated protein folding. *Chem Rev* **106**, 1917–1930.

Hossain, M. M. & Nakamoto, H. (2002). HtpG plays a role in cold acclimation in cyanobacteria. *Curr Microbiol* **44**, 291–296.

Hossain, M. M. & Nakamoto, H. (2003). Role for the cyanobacterial HtpG in protection from oxidative stress. *Curr Microbiol* **46**, 70–76.

Huq, S., Sueoka, K., Narumi, S., Arisaka, F. & Nakamoto, H. (2010). Comparative biochemical characterization of two GroEL homologs from the cyanobacterium *Synechococcus elongatus* PCC 7942. *Biosci Biotechnol Biochem* **74**, 2273–2280.

Imamura, S., Yoshihara, S., Nakano, S., Shiozaki, N., Yamada, A., Tanaka, K., Takahashi, H., Asayama, M. & Shirai, M. (2003). Purification, characterization, and gene expression of all sigma factors of RNA polymerase in a cyanobacterium. *J Mol Biol* **325**, 857–872.

Jäger, K. M. & Bergman, B. (1991). Localization of a multifunctional chaperonin (GroEL protein) in nitrogen-fixing *Anabaena* PCC 7120. *Planta* **183**, 120–125.

Kaneko, T., Nakamura, Y., Wolk, C. P., Kuritz, T., Sasamoto, S., Watanabe, A., Iriguchi, M., Ishikawa, A., Kawashima, K. & other authors (2001). Complete genomic sequence of the filamentous

nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120. *DNA Res* **8**, 205–213.

Kanemori, M., Mori, H. & Yura, T. (1994). Induction of heat shock proteins by abnormal proteins results from stabilization and not increased synthesis of σ^{32} in *Escherichia coli*. *J Bacteriol* **176**, 5648–5653.

Katano, Y., Nimura-Matsune, K. & Yoshikawa, H. (2006). Involvement of DnaK3, one of the three DnaK proteins of cyanobacterium *Synechococcus* sp. PCC7942, in translational process on the surface of the thylakoid membrane. *Biosci Biotechnol Biochem* **70**, 1592–1598.

Kim, J. & Kendall, D. A. (2000). Sec-dependent protein export and the involvement of the molecular chaperone SecB. *Cell Stress Chaperones* **5**, 267–275.

Kojima, K. & Nakamoto, H. (2007). A novel light- and heat-responsive regulation of the *groE* transcription in the absence of HrcA or CIRCE in cyanobacteria. *FEBS Lett* **581**, 1871–1880.

Kortmann, J., Sczodrok, S., Rinnenthal, J., Schwalbe, H. & Narberhaus, F. (2011). Translation on demand by a simple RNA-based thermosensor. *Nucleic Acids Res* **39**, 2855–2868.

Krishna, P. S., Rani, B. R., Mohan, M. K., Suzuki, I., Shivaji, S. & Prakash, J. S. (2013). A novel transcriptional regulator, Sll1130, negatively regulates heat-responsive genes in *Synechocystis* sp. PCC6803. *Biochem J* **449**, 751–760.

Laczkó-Dobos, H. & Szalontai, B. (2009). Lipids, proteins, and their interplay in the dynamics of temperature-stressed membranes of a cyanobacterium, *Synechocystis* PCC 6803. *Biochemistry* **48**, 10120–10128.

Landry, S. J., Zeilstra-Ryalls, J., Fayet, O., Georgopoulos, C. & Gierasch, L. M. (1993). Characterization of a functionally important mobile domain of GroES. *Nature* **364**, 255–258.

Lee, S., Prochaska, D. J., Fang, F. & Barnum, S. R. (1998). A 16.6-kilodalton protein in the *cyanobacterium Synechocystis* sp. PCC 6803 plays a role in the heat shock response. *Curr Microbiol* **37**, 403–407.

Lee, S., Owen, H. A., Prochaska, D. J. & Barnum, S. R. (2000). HSP16.6 is involved in the development of thermotolerance and thylakoid stability in the unicellular cyanobacterium, *Synechocystis* sp. PCC 6803. *Curr Microbiol* **40**, 283–287.

Lehel, C., Gombos, Z., Torok, Z. & Vigh, L. (1993a). Growth temperature modulates thermotolerance and heat shock response of cyanobacterium *Synechocystis* PCC6803. *Plant Physiol Biochem* **31**, 81–88.

Lehel, C., Los, D., Wada, H., Györgyei, J., Horváth, I., Kovács, E., Murata, N. & Vigh, L. (1993b). A second *groEL*-like gene, organized in a *groESL* operon is present in the genome of *Synechocystis* sp. PCC 6803. *J Biol Chem* **268**, 1799–1804.

Lem, N. W. & Glick, B. R. (1985). Biotechnological uses of cyanobacteria. *Biotechnol Adv* **3**, 195–208.

Liu, X., Huang, W., Li, M. & Wu, Q. (2005). Purification and characterization of two small heat shock proteins from *Anabaena* sp. PCC 7120. *IUBMB Life* **57**, 449–454.

Lund, P. A. (2009). Multiple chaperonins in bacteria – why so many? *FEMS Microbiol Rev* **33**, 785–800.

Mamedov, M. D., Hayashi, H. & Murata, N. (1993). Effects of glycine betaine and unsaturation of membrane lipids on heat stability of photosynthetic electron transport and phosphorylation reactions in *Synechocystis* PCC6803. *Biochim Biophys Acta* **1142**, 1–5.

Martin, J., Geromanos, S., Tempest, P. & Hartl, F. U. (1993). Identification of nucleotide-binding regions in the chaperonin proteins GroEL and GroES. *Nature* **366**, 279–282.

Mecsas, J., Rouviere, P. E., Erickson, J. W., Donohue, T. J. & Gross, C. A. (1993). The activity of sigma E, an *Escherichia coli* heat-inducible sigma-factor, is modulated by expression of outer membrane proteins. *Genes Dev* **7**, 2618–2628.

Mishra, Y., Chaurasia, N. & Rai, L. C. (2009). Heat pretreatment alleviates UV-B toxicity in the cyanobacterium *Anabaena doliolum*: a proteomic analysis of cross tolerance. *Photochem Photobiol* **85**, 824–833.

Murata, N., Wada, H. & Gombos, Z. (1992). Modes of fatty-acid desaturation in cyanobacteria. *Plant Cell Physiol* **33**, 933–941.

Nakamoto, H., Suzuki, N. & Roy, S. K. (2000). Constitutive expression of a small heat-shock protein confers cellular thermotolerance and thermal protection to the photosynthetic apparatus in cyanobacteria. *FEBS Lett* **483**, 169–174.

Nakamoto, H., Suzuki, M. & Kojima, K. (2003). Targeted inactivation of the *hrcA* repressor gene in cyanobacteria. *FEBS Lett* **549**, 57–62.

Nanjo, Y., Mizusawa, N., Wada, H., Slabas, A. R., Hayashi, H. & Nishiyama, Y. (2010). Synthesis of fatty acids *de novo* is required for photosynthetic acclimation of *Synechocystis* sp. PCC 6803 to high temperature. *Biochim Biophys Acta* **1797**, 1483–1490.

Nikkinen, H. L., Hakkila, K., Gunnelius, L., Huokko, T., Pollari, M. & Tyystjärvi, T. (2012). The SigB σ factor regulates multiple salt acclimation responses of the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Physiol* **158**, 514–523.

Nimura, K., Yoshikawa, H. & Takahashi, H. (1996). DnaK3, one of the three DnaK proteins of cyanobacterium *Synechococcus* sp. PCC7942, is quantitatively detected in the thylakoid membrane. *Biochem Biophys Res Commun* **229**, 334–340.

Nitta, K., Suzuki, N., Honma, D., Kaneko, Y. & Nakamoto, H. (2005). Ultrastructural stability under high temperature or intensive light stress conferred by a small heat shock protein in cyanobacteria. *FEBS Lett* **579**, 1235–1242.

Paul, S., Singh, C., Mishra, S. & Chaudhuri, T. K. (2007). The 69 kDa *Escherichia coli* maltodextrin glucosidase does not get encapsulated underneath GroES and folds through *trans* mechanism during GroEL/GroES-assisted folding. *FASEB J* **21**, 2874–2885.

Pederson, D. M., Daday, A. & Smith, G. D. (1986). The use of nickel to probe the role of hydrogen metabolism in cyanobacterial nitrogen fixation. *Biochimie* **68**, 113–120.

Porankiewicz, J. & Clarke, A. K. (1997). Induction of the heat shock protein ClpB affects cold acclimation in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J Bacteriol* **179**, 5111–5117.

Priya, S., Sharma, S. K., Sood, V., Mattoo, R. U., Finka, A., Azem, A., De Los Rios, P. & Goloubinoff, P. (2013). GroEL and CCT are catalytic unfoldases mediating out-of-cage polypeptide refolding without ATP. *Proc Natl Acad Sci U S A* **110**, 7199–7204.

Rajaram, H. & Apte, S. K. (2003). Heat-shock response and its contribution to thermotolerance of the nitrogen-fixing cyanobacterium *Anabaena* sp. strain L-31. *Arch Microbiol* **179**, 423–429.

Rajaram, H. & Apte, S. K. (2008). Nitrogen status and heat-stress-dependent differential expression of the *cpn60* chaperonin gene influences thermotolerance in the cyanobacterium *Anabaena*. *Microbiology* **154**, 317–325.

Rajaram, H. & Apte, S. K. (2010). Differential regulation of *groESL* operon expression in response to heat and light in *Anabaena*. *Arch Microbiol* **192**, 729–738.

Rajaram, H., Ballal, A. D., Apte, S. K., Wiegert, T. & Schumann, W. (2001). Cloning and characterization of the major *groESL* operon from a nitrogen-fixing cyanobacterium *Anabaena* sp. strain L-31. *Biochim Biophys Acta* **1519**, 143–146.

Rowland, J. G., Pang, X., Suzuki, I., Murata, N., Simon, W. J. & Slabas, A. R. (2010). Identification of components associated with thermal acclimation of photosystem II in *Synechocystis* sp. PCC6803. *PLoS ONE* **5**, e10511.

Roy, S. K., Hiyama, T. & Nakamoto, H. (1999). Purification and characterization of the 16-kDa heat-shock-responsive protein from the thermophilic cyanobacterium *Synechococcus vulcanus*, which is an α-crystallin-related, small heat shock protein. *Eur J Biochem* **262**, 406–416.

Rupprecht, E., Gathmann, S., Fuhrmann, E. & Schneider, D. (2007). Three different DnaK proteins are functionally expressed in the cyanobacterium *Synechocystis* sp. PCC 6803. *Microbiology* **153**, 1828–1841.

Rupprecht, E., Düppre, E. & Schneider, D. (2010). Similarities and singularities of three DnaK proteins from the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol* **51**, 1210–1218.

Sato, M., Nimura-Matsune, K., Watanabe, S., Chibazakura, T. & Yoshikawa, H. (2007). Expression analysis of multiple *dnaK* genes in the cyanobacterium *Synechococcus elongatus* PCC 7942. *J Bacteriol* **189**, 3751–3758.

Sato, T., Minagawa, S., Kojima, E., Okamoto, N. & Nakamoto, H. (2010). HtpG, the prokaryotic homologue of Hsp90, stabilizes a phycobilisome protein in the cyanobacterium *Synechococcus elongatus* PCC 7942. *Mol Microbiol* **76**, 576–589.

Saito, M., Watanabe, S., Yoshikawa, H. & Nakamoto, H. (2008). Interaction of the molecular chaperone HtpG with uroporphyrinogen decarboxylase in the cyanobacterium *Synechococcus elongatus* PCC 7942. *Biosci Biotechnol Biochem* **72**, 1394–1397.

Schopf, J. W. (1975). Precambrian paleobiology: problems and perspectives. *Annu Rev Earth Planet Sci* **3**, 213–249.

Singh, R. N. (1950). Reclamation of 'Usar' lands in India through blue-green algae. *Nature* **165**, 325–326.

Singh, A. K., Summerfield, T. C., Li, H. & Sherman, L. A. (2006). The heat shock response in the cyanobacterium *Synechocystis* sp. strain PCC 6803 and regulation of gene expression by HrcA and SigB. *Arch Microbiol* **186**, 273–286.

Slabas, A. R., Suzuki, I., Murata, N., Simon, W. J. & Hall, J. J. (2006). Proteomic analysis of the heat shock response in *Synechocystis* PCC6803 and a thermally tolerant knockout strain lacking the histidine kinase 34 gene. *Proteomics* **6**, 845–864.

Stewart, W. D. P. (1980). Some aspects of structure and function in N_2-fixing cyanobacteria. *Annu Rev Microbiol* **34**, 497–536.

Suzuki, I., Simon, W. J. & Slabas, A. R. (2006). The heat shock response of *Synechocystis* sp. PCC 6803 analysed by transcriptomics and proteomics. *J Exp Bot* **57**, 1573–1578.

Tanaka, N. & Nakamoto, H. (1999). HtpG is essential for the thermal stress management in cyanobacteria. *FEBS Lett* **458**, 117–123.

Tanaka, N., Hiyama, T. & Nakamoto, H. (1997). Cloning, characterization and functional analysis of *groESL* operon from thermophilic cyanobacterium *Synechococcus vulcanus*. *Biochim Biophys Acta* **1343**, 335–348.

Tilly, K., VanBogelen, R. A., Georgopoulos, C. & Neidhardt, F. C. (1983). Identification of the heat-inducible protein C15.4 as the *groES* gene product in *Escherichia coli*. *J Bacteriol* **154**, 1505–1507.

Tokuriki, N. & Tawfik, D. S. (2009). Chaperonin overexpression promotes genetic variation and enzyme evolution. *Nature* **459**, 668–673.

Tomas, C. A., Welker, N. E. & Papoutsakis, E. T. (2003). Overexpression of *groESL* in *Clostridium acetobutylicum* results in increased solvent production and tolerance, prolonged metabolism, and changes in the cell's transcriptional program. *Appl Environ Microbiol* **69**, 4951–4965.

Török, Z., Goloubinoff, P., Horváth, I., Tsvetkova, N. M., Glatz, A., Balogh, G., Varvasovszki, V., Los, D. A., Vierling, E. & other authors (2001). *Synechocystis* HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone-mediated refolding. *Proc Natl Acad Sci U S A* **98**, 3098–3103.

Tuominen, I., Pollari, M., Tyystjärvi, E. & Tyystjärvi, T. (2006). The SigB σ factor mediates high-temperature responses in the cyanobacterium *Synechocystis* sp. PCC6803. *FEBS Lett* **580**, 319–323.

Tuominen, I., Pollari, M., von Wobeser, E. A., Tyystjärvi, E., Ibelings, B. W., Matthijs, H. C. & Tyystjärvi, T. (2008). Sigma factor SigC is required for heat acclimation of the cyanobacterium *Synechocystis* sp. strain PCC 6803. *FEBS Lett* **582**, 346–350.

Varvasovszki, V., Glatz, A., Shigapova, N., Jósvay, K., Vígh, L. & Horváth, I. (2003). Only one *dnaK* homolog, *dnaK2*, is active transcriptionally and is essential in *Synechocystis*. *Biochem Biophys Res Commun* **305**, 641–648.

Venkataraman, G. S. (1979). Algal inoculation in rice fields. In *Nitrogen and Rice*, pp. 311–321. Edited by N. C. Brady. La Banos, Phillipines: International Rice Research Institute.

Warnecke, T. & Hurst, L. D. (2010). GroEL dependency affects codon usage – support for a critical role of misfolding in gene evolution. *Mol Syst Biol* **6**, 340.

Watanabe, S., Kobayashi, T., Saito, M., Sato, M., Nimura-Matsune, K., Chibazakura, T., Taketani, S., Nakamoto, H. & Yoshikawa, H. (2007a). Studies on the role of HtpG in the tetrapyrrole biosynthesis pathway of the cyanobacterium *Synechococcus elongatus* PCC 7942. *Biochem Biophys Res Commun* **352**, 36–41.

Watanabe, S., Sato, M., Nimura-Matsune, K., Chibazakura, T. & Yoshikawa, H. (2007b). Protection of *psbAII* transcript from ribonuclease degradation *in vitro* by DnaK2 and DnaJ2 chaperones of the cyanobacterium *Synechococcus elongatus* PCC 7942. *Biosci Biotechnol Biochem* **71**, 279–282.

Webb, R., Reddy, K. J. & Sherman, L. A. (1990). Regulation and sequence of the *Synechococcus* sp. strain PCC 7942 *groESL* operon, encoding a cyanobacterial chaperonin. *J Bacteriol* **172**, 5079–5088.

Whitton, B. A. & Potts, M. (2000). *The Ecology of Cyanobacteria: Their Diversity in Time and Space*. Berlin: Springer.

Wolk, C. P. (1968). Movement of carbon from vegetative cells to heterocysts in *Anabaena cylindrica*. *J Bacteriol* **96**, 2138–2143.

Young, J. C., Agashe, V. R., Siegers, K. & Hartl, F. U. (2004). Pathways of chaperone-mediated protein folding in the cytosol. *Nat Rev Mol Cell Biol* **5**, 781–791.

Yura, T. & Nakahigashi, K. (1999). Regulation of the heat-shock response. *Curr Opin Microbiol* **2**, 153–158.

Zeilstra-Ryalls, J., Fayet, O., Baird, L. & Georgopoulos, C. (1993). Sequence analysis and phenotypic characterization of groEL mutations that block λ and T4 bacteriophage growth. *J Bacteriol* **175**, 1134–1143.

Zingaro, K. A. & Papoutsakis, E. T. (2013). GroESL overexpression imparts *Escherichia coli* tolerance to *i*-, *n*-, and 2-butanol, 1,2,4-butanetriol and ethanol with complex and unpredictable patterns. *Metab Eng* **15**, 196–205.

Zorina, A., Stepanchenko, N., Novikova, G. V., Sinetova, M., Panichkin, V. B., Moshkov, I. E., Zinchenko, V. V., Shestakov, S. V., Suzuki, I. & other authors (2011). Eukaryotic-like Ser/Thr protein kinases SpkC/F/K are involved in phosphorylation of GroES in the cyanobacterium *Synechocystis*. *DNA Res* **18**, 137–151.

Zuber, U. & Schumann, W. (1994). CIRCE, a novel heat shock element involved in regulation of heat shock operon *dnaK* of *Bacillus subtilis*. *J Bacteriol* **176**, 1359–1363.

Edited by: S. Spiro