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

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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 thermostolerance 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.

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 (Calothrix paretina, Nostoc sp., Synechococcus sp., Phormidium frigidum), freshwater bodies (Nostoc, Anabaena, Microcystis aeruginosa, Oscillatoria sp., Nodularia spumigena) or brackish waters (Anabaena sp., Aphanizomenon sp., Arthrospira sp., Microcystis sp.), salt ponds (Gleiothece 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 (Aphanathece 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,
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 (Horváth 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 (Horváth et al., 2012). In higher phototrophs such as mosses, a lipid-based signalling cascade is activated, and there are changes in the transport and availability of Ca²⁺ during heat stress (Horváth 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-31 was 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 doliolium* 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

<table>
<thead>
<tr>
<th>Family</th>
<th>Gene</th>
<th>Cyanobacteria</th>
<th>Function</th>
<th>Reference(s)</th>
</tr>
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<tbody>
<tr>
<td>Hsp100</td>
<td>clpB</td>
<td>Synechococcus PCC7942</td>
<td>Acquired thermotolerance</td>
<td>Eriksson &amp; Clarke (1996)</td>
</tr>
<tr>
<td></td>
<td>hspA</td>
<td>Synechococcus PCC7942</td>
<td>Innate and acquired thermotolerance, protection of photosynthetic apparatus</td>
<td>Porankiewicz &amp; Clarke (1997)</td>
</tr>
<tr>
<td>Hsp90</td>
<td>htpG</td>
<td>Synechococcus PCC7942</td>
<td>DnaJ2: thermostability; DnaK2: RNA chaperone thermosensor</td>
<td>Varvasovszki et al. (2003), Watanabe et al. (2007b), Barthel et al. (2011)</td>
</tr>
<tr>
<td>Hsp60 Hsp10</td>
<td>groEL-1 (groEL), groEL-2 (cpn60)</td>
<td>Synechocystis PCC6803, Anabaena L-31</td>
<td>Protects membrane fluidity, thermotolerance</td>
<td>Nakamoto et al. (2000), Horváth et al. (1998), Lee et al. (2000)</td>
</tr>
<tr>
<td>sHsp</td>
<td>hsp16.6/hsp17/hspA</td>
<td>Synechocystis PCC6803, Synechococcus PCC7942</td>
<td></td>
<td></td>
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**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 *clpB* gene. The truncated ClpB-79 also confers thermotolerance and contributes about 30% of the thermotolerance developed in *Synechococcus* PCC7942 (Clarke & Eriksson, 2000). The ClpBI 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 (Saito 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
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 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 groEL-2/cpn60 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 Hsp 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...
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 GroESL 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, 2008). 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 (Tórók 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 ClpB1, ClpC2, HspA, HtpG and Slr1674, while the acquired thermotolerance was affected by CpnC2, Hik34, HspA and HypA1 (Rowland et al., 2010).
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 σ32 and σ24 (Mecsas et al., 1993).

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 Δhik34 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 Δhik34 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 Δhik34 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 (Gunnellius 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 HrC (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


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