ORIGINAL ARTICLE

# Heat Shock Protein Gene Family of the *Porphyra seriata* and Enhancement of Heat Stress Tolerance by *PsHSP70* in *Chlamydomonas*

**Hong-Sil Park · Won-Joong Jeong · EuiCheol Kim ·
Youngja Jung · Jong Min Lim · Mi Sook Hwang ·
Eun-Jeong Park · Dong-Soo Ha · Dong-Woog Choi**

**Abstract** Heat shock proteins and molecular chaperones
are key components contributing to survival in the abiotic
stress response. *Porphyra seriata* grows on intertidal rocks
exposed to dynamic environmental changes associated with
the turning tides, including desiccation and heat stress.
Analysis of the ESTs of *P. seriata* allows us to identify the
nine *HSP* cDNAs, which are predicted to be *PsHSP90*,
three *PsHSP70*, *PsHSP40* and *PsHSP20*, and three 5′-
truncated *HSP* cDNAs. RT–PCR results show that most of
the *PsHSP* transcripts were detected under normal cell
growth conditions as well as heat stress, with the exception
of two cDNAs. In particular, *PsHSP70b* and *PsHSP20*
transcripts were upregulated by heat stress. When the putative
mitochondrial *PsHSP70b* was introduced and overexpressed
in *Chlamydomonas*, transformed *Chlamydomonas* evidenced
higher rates of survival and growth than those of the wild
type under heat stress conditions. Constitutive overexpres-
sion of the *PsHSP70b* gene increases the transcription of the
*HSF1* as well as the *CrHSP20* and *CrHSP70* gene. These
results indicate that *PsHSP70b* is involved in tolerance to
heat stress and the effects on transcription of the *CrHSP20*
and *CrHSP70* genes.

**Keywords** *Porphyra seriata* · HSP · Heat tolerance ·
*Chlamydomonas*

Hong-Sil Park and Won-Joong Jeong equally contributed to this paper.

H.-S. Park · E. Kim · Y. Jung · D.-W. Choi (✉)
Department of Biology Education and Kumho Life Science
Laboratory, Chonnam National University,
Kwangju 500-757, South Korea
e-mail: dwchoi63@chonnam.ac.kr

W.-J. Jeong · J. M. Lim
Plant Systems Engineering Research Center,
Korea Research Institute of Bioscience and Biotechnology,
Daejeon 305-806, South Korea

M. S. Hwang · E.-J. Park · D.-S. Ha
Marine Algae Research Institute,
National Fisheries Research and Development Institute,
Mokpo, South Korea

## Introduction

*Porphyra* is a red algal genus of laver and includes more
than 130 species. Several *Porphyra* species, including
*Porphyra yezoensis* and *Porphyra seriata*, have been
employed as edible seaweeds and have been cultivated in
aquaculture industries in East Asia (Miura 1988; Hwang et
al. 2005). Under natural conditions, most *Porphyra* grow in
the intertidal zone and are frequently exposed to a variety
of potentially stressful environmental conditions with the
turning tides (Sahoo et al. 2002). These changes include
changes in humidity, from drying to partial to total
immersion; changes in temperature, from heat stress to
freezing during hibernal low tides; osmotic shocks; and
light intensities (Hwang et al. 1997). It has been demon-
strated that plants carry out the appropriate physiological
and biochemical changes in order to deal with environ-
mental stresses (Bray et al. 2000). In the acclimation
process, the expression of genes involved in abiotic stress
tolerance including ion transporters, osmoprotectants, free-
radical scavengers, stress proteins such as heat shock
protein (HSP), and factors involved in signaling cascades
and transcriptional control are induced or upregulated (Bray
et al. 2000; Wang et al. 2004).

HSPs play an essential role in protecting cells, and the
folding and translocation of nascent proteins, the refolding

of denatured proteins, the disassembly of already formed protein aggregates, and so on, under both stress and non-stress conditions (Nelson et al. 1992; Hartl 1996; Feder and Hofmann 1999; Bray et al. 2000; Schroda and Vallon 2009). The presence of HSPs and their induction by environmental stresses have been shown to be a central tolerance mechanism in many organisms (Feder and Hofmann 1999; Ireland et al. 2004). Therefore, it is worthwhile to evaluate the role of HSPs in the adverse stress resistance mechanisms of seaweeds.

HSPs are grouped into five distinct classes named for their approximate molecular masses: HSP100, HSP90, HSP70, HSP60, and small HSP (sHSP) (Bray et al. 2000; Wang et al. 2004; Schroda and Vallon 2009). HSPs are located within both the cytoplasm and organelles, including the nucleus, mitochondria, chloroplasts, and ER (Boston et al. 1996). The HSP100 family chaperones are members of the large ATPase superfamily with a broad spectrum of diverse functional properties (Wang et al. 2004). HSP90 is distinct from other molecular chaperones in that most of its known substrates to date are signal transduction proteins such as steroid hormone receptors and signaling kinases (Wang et al. 2004; Schroda and Vallon 2009). Among the HSP families, HSP70s are a highly conserved and ubiquitous protein family (Karlin and Brocchieri 1998; Feder and Hofmann 1999; Renner and Waters 2007). They perform essential roles in the transport of nascent proteins across membranes into organelles, the folding of newly translated proteins, the repair of misfolded proteins, and helping to target damaged proteins for degradation (Nelson et al. 1992; Hartl 1996; Fink 1999). Under stress conditions, HSP70s are upregulated, participate in the refolding of denatured proteins, maintain cell homeostasis, and protect organisms against damage (Jolly and Morimoto 1999; Hartl and Hayer-Hartl 2002; Mayer and Bukau 2005; Tanaka et al. 2007). HSP70s are a kind of potential biomarker of environmental stresses and can be applied to the monitoring of environmental conditions (Ireland et al. 2004). The HSP60 family is a class of molecular chaperones that is evolutionarily homologous with *Escherichia coli* GroEL and found in prokaryotes (Hemmingsen et al. 1988; Hartl 1996). The small HSPs (sHSPs) are low molecular mass HSPs (12–40 kDa). In plants, sHSPs form a more diverse family than other HSPs/chaperones with regard to sequence similarity, cellular location, and functions (Vierling 1991). sHSPs function in responses to heat and other stresses, and some sHSPs are expressed during certain developmental stages (Boston et al. 1996).

The *Porphyra* are an important marine crop and have been proposed as a model plant to study the mechanisms underlying abiotic stress tolerance (Blouin et al. 2011). The recent sequencing of the complete genomes or transcriptome of algae allows us to identify the genes for abiotic stress tolerance in the genome. Herein, we describe the isolation and characterization of the cDNAs encoding for heat shock protein (HSP) from *P. seriata*. We isolated a total of nine cDNAs encoding for HSP from the *P. seriata* ESTs and named them *PsHSP90*, *PsHSP70*, *PsHSP40*, and *PsHSP20* according to their molecular weight and amino acid sequence homology. We analyze the physiological function of the putative mitochondrial *HSP*, *PsHSP70b* gene by using heterologous expression in *Chlamydomonas*.

## Material and Methods

### Organisms and Growth Condition

*P. seriata* was collected and maintained via in vitro cultivation under controlled conditions. Leafy gametophytes of *P. seriata* were cultured in modified Grund medium (McLachlan 1973) at 10°C with irradiation of 80 μmol photon $m^{-2}$ $s^{-1}$ provided by cool-white fluorescent lamps with a photoperiod of 14:10 (light/dark) in a growth room. For heat treatment, growth bottles containing *P. seriata* were transferred to a 25°C growth chamber with the same light intensity and photoperiod.

*Chlamydomonas reinhardtii* strain cc-125 (mt+) was grown in Tris–acetate–phosphate (TAP) medium (Harris 1989). *Chlamydomonas* cells were cultured at 25°C in TAP liquid medium with shaking at 200 rpm under continuous cool fluorescent light (50 μmol photon $m^{-2}$ $s^{-1}$).

### Isolation and Sequence Analysis of PsHSP cDNA

In a previous study, we generated 3,979 ESTs from two *P. seriata* cDNA libraries constructed from the gametophyte thalli at 10°C (control) and under high-temperature (25°C) conditions, respectively (Kim et al. 2011). The ESTs were analyzed for *Porphyra* heat shock protein gene resources. The individual ESTs were searched against the GenBank nr database, using a BLASTX algorithm. The putative HSP cDNAs were identified via keyword searches with BLASTX results.

Colonies containing putative HSP cDNA were selected and cultured, and then plasmid DNAs were purified using a Qiaquick Plasmid Extraction Kit (Qiagen, Hilden, Germany) and sequenced. Sequence editing and amino acid sequence prediction from the selected ESTs were conducted using the Sequencher program (Gene Code Corporation, Ann Arbor, MI, USA). The putative molecular weights and p*I* values of the deduced polypeptides were predicted with the DNASIS Max program (MiraiBio Inc., San Francisco, CA, USA). The alignments of the deduced amino acid sequences were conducted using the CLUSTAL W program (http://www.ebi.ac.uk/clustalw). The

phylogenetic tree was constructed using the program. Conserved motifs or domains are predicted by the Internet program Prosite at http://expasy.org/prosite.

## Gene-Specific RT–PCR

Total RNAs were prepared from leafy gametophyte thalli tissues, using a Plant RNeasy kit (Qiagen). The first-strand cDNAs were constructed from 2 μg of total RNA via reverse transcription in 20-μl reaction volumes, using oligo $(dT)_{17}$ primer and Superscript III reverse transcriptase, in accordance with the manufacturer's instructions (BRL Life Technologies, Carlsbad, CA, USA). The reactions were conducted for 60 min at 42°C, followed by 5 min of heating at 70°C. The first-strand cDNA reaction was diluted by a factor of 5, and then 2 μl of diluted cDNA were applied to a 50-μl PCR-amplification reaction, containing 5 μl of 10× PCR buffer [200 mM Tris–HCl (pH 8.4), 500 mM KCl], 1 μl of 10 mM dNTPs, 1 μl of each gene-specific primer (10 μM), and 2.5 U of ExTaq DNA polymerase (Takara, Shiga, Japan). PCR reactions were carried out for 35 cycles, each consisting of 30 s at 95°C, 30 s of 58 to 64°C, 90 s of 72°C, and 5 min of termination at 72°C. The annealing temperature and PCR cycle for each of the gene-specific primers were adjusted for optimal PCR reaction. The PCR products were separated on 1% agarose gels and stained with ethidium bromide for photography.

## Real-Time Quantitative RT–PCR

Reverse transcription (RT) was carried out using 2 μg of total RNA and 200 U of M-MLV reverse transcriptase (Promega, Madison, WI, USA), 50 μM oligo(dT), 500 μM of each dNTP, and 20 U of ribonuclease inhibitor. For real-time RT–PCR, EvaGreen Real-Time PCR kits (SolGent, Daejeon, Korea) were utilized according to the manufacturer's instructions with a light cycler, Opticon2 (MJ Research, Ramsey, MN, USA). Amplification was conducted using 40 cycles of 92°C for 20 s, 53°C to 60°C for 20 s, and 72°C for 20 s.

## Vector Construction for the PsHSP70b Expression in Chlamydomonas

The pCr102 vector, which harbors regulatory sequences with the psaD promoter and the terminator driven by the *Chlamydomonas* genome for transgene expression, as well as a hygromycin resistance gene (*aph7''*) under the control of the *tubB2* promoter and the *rbcS2* terminator were used for the selection of transformants. The open reading frame of the HSP70 gene was amplified via PCR and subcloned into a pCr102 vector using the NcoI and EcoRV sites, and named pCr102-PsHSP70b.

For the transformation of *Chlamydomonas*, the pCr102-PsHSP70b plasmid was linearized with XbaI and introduced into *Chlamydomonas* strain cc-125 via the glass bead method (Kindle 1990). Transformed colonies were selected on TAP agar medium containing 10 μg/ml of hygromycin after 7–14 days of growth.

## DNA and RNA Blot Analysis

*Chlamydomonas* genomic DNA was purified from 100-ml liquid cultures. For Southern blot analysis, 10 μg of genomic DNA was digested with NcoI, separated via 0.8% agarose gel electrophoresis, and then blotted onto Hybond-N nylon membrane (Amersham Biosciences, Uppsala, Sweden). To assess the expression of the introduced PsHSP70b gene, total RNAs were isolated from control and transformed *Chlamydomonas* cells grown at 25°C and separated on 1.2% agarose gel and subsequently transferred onto nylon membranes. The probe (a 0.9-kb fragment) corresponding to the PsHSP70 cDNA was prepared from XbaI/EcoRV digested transformation vector. For all blot analyses, hybridization with $^{32}$P-labeled probes and washing were conducted in accordance with the manufacturer's instructions. The membrane hybridized with $^{32}$P-labeled probe was exposed to a phosphor imaging plate (IP) for 2 days, and signals were detected using a Bio-Imaging Analyzer BAS-1800II (Fuji, Tokyo, Japan).

## Heterologous Expression and Analysis of the PsHSP70b Gene in Chlamydomonas

*Chlamydomonas* cells were grown in TAP medium containing hygromycin at 25°C under a 14-h light/10-h dark cycle. To evaluate high-temperature tolerance, the cells were grown at a concentration of approximately $2–4×$ $10^6$ cells/ml and concentrated to $10^7$ cells/ml and diluted to $10^2–10^4$ in fresh medium. Five microliters of diluted cells was inoculated onto agar plates. For heat stress treatment, the cells were incubated at 37°C for 5 days and subsequently transferred to a 25°C growth chamber under a 14-h light/10-h dark photocycle. *C. reinhardtii* strain cc-125 and transformed cells with the pCr102 vector, Hyg5414, were used as controls.

## Results and Discussion

### Identification of the *P. seriata HSPs* cDNAs

In a previous work, we generated 3,989 ESTs from two cDNA libraries constructed from *P. seriata* thalli under normal growth conditions and heat stress (Kim et al. 2011). EST analysis via keyword allowed us to identify the

cDNAs encoding for putative heat shock protein (HSP). The clustering of the *HSP* cDNA candidates generated five contigs and four unassembled ESTs, and represented nine unique *HSP* cDNAs (Table 1). The longest sister cDNA clone in the contig was selected as a representative EST for each HSP contig and was used in the determination of the full sequences.

Homology searching using the BLASTX program showed that the nine putative *HSP* cDNAs encoded for different HSPs. The characteristics of the deduced polypeptides from the nine Ps*HSP* cDNAs are summarized in Table 1. Based on the amino acid sequence homology and molecular weight of the deduced polypeptide, we named the genes *PsHSP* (*Porphyra seriata* heat shock protein) 90, *PsHSP70*, *PsHSP40*, and *PsHSP20*. The other three cDNAs, *PsHSP_O22*, *PsHSP_D07*, and *PsHSP_G17*, have 5′-truncated cDNA and sequence homology with putative HSP90, ER HSP70, and DnaJ-like protein, respectively. Figure 1 represents the amino acid sequence of the deduced polypeptide from the nine Ps*HSP* cDNAs. Five major families of *HSPs*—*HSP100*, *HSP90*, *HSP70*, *HSP60*, and small *HSP* (*sHSP*)—are conservatively recognized in plant and algae genomes (Wang et al. 2004; Schroda and Vallon 2009).

### Characterization of the *PsHSP*

Among the identified *PsHSPs*, the *PsHSP90* encodes for a 95.6-kDa polypeptide with 872 amino acids (p*I* 4.79). Prediction of the signal peptide in the PsHSP90 by the PrediSi program (Prediction of signal peptides, www.pridisi.de) indicated that 25 amino acid residues at the N terminus may be employed as a signal peptide for secretion. PsHSP90 evidences the highest amino acid sequence homology to ER HSP90 from *Ostreococcus lucimarinus*. HSP90 family members are highly conserved and harbor

the N-terminal ATPase domain. PsHSP90 harbors an ATPase domain in the N-terminal region (Fig. 1a). *HSP90* family includes seven members in the *Arabidopsis* genome. *Chlamydomonas* harbors three genes encoding for the *HSP90* family, which is located in the cytoplasm, plastid, and endoplasmic reticulum, respectively (Wang et al. 2004; Schroda and Vallon 2009). The C terminus of each subgroup of HSP contains a highly conserved motif, which is EEVD or EEID for cytosolic, and HDEL for the endoplasmic reticulum (ER) group (Guy and Li 1998; Sung et al. 2001a). PsHSP90 harbors the RDEL sequence in the C terminus, which is similar to the HDEL motif found in the ER HSP. These results indicate that PsHSP90 may be located within the ER. PsHSP_O22 evidences the highest amino acid sequence homology to HSP90 and the EDVD motif in the C-terminal end region. These results indicate that PsHSP_O22 may encode for a cytosolic HSP90. It has been reported that some HSP90 members are upregulated by abiotic stress and perform a function in stress adaptation in plants (Krishna and Gloor 2001), although cytosolic HSP90 has a role in the maturation of signal transduction proteins such as hormone receptors and signaling kinases (Richter and Buchner 2001) in animals.

We identified three cDNAs encoding for an approximate 70-kDa polypeptide and named them *PsHSP70a*, *PsHSP70b*, and *PsHSP70c* (Table 1). PsHSP70a is a 664-amino acid residue polypeptide (71.8 kDa, p*I* 4.94). PsHSP70b and PsHSP70c are polypeptides comprising 736 amino acids (76.9 kDa, p*I* 5.28) and 663 amino acids (72.4 kDa, p*I* 6.29). The PsHSP70 polypeptides harbor three motifs—IDLGTTNS, VYDLGGGTFDVSIL, and VLLVGGMTRVPKVVE—found in the HSP70 family, and also harbor the ATPase domain and substrate binding domain (Fig. 1b). The amino acid sequence alignment of three PsHSP70s showed that there were sequence variations, including deletions and substitutions, in the N

**Table 1** Heat shock proteins isolated *P. seriata*

| Gene | EST ID | AA no. | MW (kDa) | p*I* | *e* value[a] | Description |
|---|---|---|---|---|---|---|
| *PsHSP90* | Ps0251aK23 | 872 | 95.6 | 4.79 | 0.0 | Heat shock protein 90, endoplasmic reticulum (*Ostreococcus lucimarinus*) |
| *PsHSP70a* | Ps0251aA09 | 664 | 71.8 | 4.94 | 0.0 | Putative heat shock protein 70 (*Porphyra yezoensis*) |
| *PsHSP70b* | PS0174aL18 | 736 | 76.9 | 5.28 | 0.0 | Heat shock protein 70 (*Ostreococcus tauri*) |
| *PsHSP70c* | PS0222aH14 | 663 | 72.4 | 6.29 | 0.0 | Mitochondrial HSP70 (*Trypanosoma congolense*) |
| *PsHSP40* | PS0234aJ04 | 429 | 46.1 | 6.61 | 2e−95 | DnaJ (Hsp40) homolog (*Xenopus laevis*) |
| *PsHSP20* | Ps0221aO20 | 248 | 25.8 | 6.45 | 2e−14 | Heat shock protein Hsp20 (*Desulfatibacillum alkenivorans*) |
| *PsHSP_O22* | PS0172aO22[b] | na | na | na | 1e−132 | Putative heat shock protein 90 (*Porphyra yezoensis*) |
| *PsHSP_D07* | Ps0243aD07[b] | na | na | na | 1e−88 | ER luminal binding protein, Bip/heat shock protein (*Scherffelia dubia*) |
| *PsHSP_G17* | Ps0231aG17[b] | na | na | na | 8e−50 | DnaJ-like protein (*Griffithsia japonica*) |

*na* not available

[a] BLASTP results with deduced amino acid sequence

[b] 5′-truncated cDNA

**a**

PsHSP90
MGRHRAMRGLLAAAVVLGVASLSVAGDAGVDTAAAGEVKLDAPSSGGAAAAGEPAAAAPAGETFSFQAEVSRVMDIIIHSLYSNR
NIFLRELISNASDALDKIRFLALSDGGQLGSGDAAALEIRVRADKEARTLTLRDRGVGMTRAELIAKSGTRAFIDKLSS
AKGDSSNLIGQFGVGFYSAYLVADRVTVRSKHNAESAQYVWESGADQTFTIREDVGGPPLVRGTELTLHLKEDAGEYLETSKLKE
LIQKYSQFINFPIYLEVAEEIEVPIESPSDETADDDKPAEDKEGDDAEAGAAKATEAADDMKVEDGDDDAKPSDAKEEKPKTKKE
TVYKWLDVNEHKPIWTRDASEVTAEEYDSFFNTIAKLPGKPLAKSHFKAEGDVDFRSILYIPDKPPVDLYNNAESEKDAIKMYVR
RVLVTDKFEFGLLPRYLGFLIGIVDSSDDLPINVSREMLQQSKTLSVIKRKLVRKALDMIRGLSEKAKADAKPADEEVDMDAAKES
ADASADADDADKAAKASPADDDEGDKKPDPYVGFWKAYGKSIKLGIVEDSANRERLAKLLRFQTSKTNASDESDWASLDDYVDRM
KEDQEYIYYSSGESVEAIRRSPFLERLLEKDYEVLYLNEPIDEHMIMSLPDYQGSKVMSAEKDNFKFGDKDQKEAKERLDGVKAE
FRPLTKFLKTSLGDKVSKVRLSSRLSSTPCVLSTEQWGYSARMEIIMKAQAFSDPDSYKYMMPKAKVMEIHPGHPLIRKLRDLVV
ADDQSKQAAELAHLVYDTALVSSGFLMQDNSAFAERMYKMLRQVSGVDESATVEEIELPSSEPEKSPETKAAEADGSADSDAKED
APAASGAAKESPAAGSEDRDEL

PsHSP40/DnaJ
MFGGFPGMGGMPGGMPMGGMPGMRRGPAADTTKLYKTLGVSKDADPGQLKKAYRKLSLKYHPDKPGGDEEKFKEITHAFEVLSD
DEKRNIYDEYGEEGLSQHQSGGGGMDPTDVFAAMFGGGGGRSRGPRKGEDVVHRLNVSLNDLYNGRTSKLAIVRNRVCSGCNGCG
AKDPKLVTTCRSCNGEGVKIHHMQIAPGMVQRVQAECNVCGGVGSAIAPLDKCTKCGGDKVVKDRKVLEVHIAPGMQSGQKITFT
GEANDNPGLVPGDVVVILEQTEHPSFVRKGSNLIMVKEISLVDALCGVSFTVQQLDGRFLHIQSAPGATIKPDSIKSVPNEGMPT
WKRPYDKGYLFVRFKVNFPTNVSARQAHALVSVLGPRTPPDAPPDGFEVEECPLLDFSEEHARQTQNGGEAYDEDDGEEGRPRVQ
CAQS

PsHSP20
MAFVCAAAAASLGLRAPAATAAALGVAARRVRLAPPAAARVRMTSSATPHDAKDDNTDIKSDAQASARGHAPVHRRRGGGRGWRG
VPSLWGLSALRPDLLAGELAGAFDSPFFRAGGGLAASAWPSMAGAVADWAPRADLTVSADGKSYEWAVELPGMAKEDVKLSIDGD
VITVRGEKRSEREVLGVGTERLWGSFTRSVVAPADADLDKSGVKAVTKDGVLTVSFPKTSPPEEHVDKEDNTIPIMEE

PsHSP_O22
HEASLKEYVSRMKDGQKNIYYITGASKKAVENAPFLEKLKKKGFEVIYMTEPIDEYCVQQLKEYDGKKLVCTTKENMQLEESEEE
KAAREAEAKACETLCEVMKENLGEKVEKVVVSDRLADSPCILVTGEYGWSANMERIMSSQALRDNSLSTYMSSRKTLEINPTNSI
IRELRRRVEADKTDKTVKDLVSLLFDTAMLTSGFSLEEPHVFATRIQRMLMLGLQIDDDEEAGGEEKKQDEVFDELPPLEGGAAA
GGMEDVD

PsHSP_D07
GTRRIPKVQELLRGYFDGKELNKGVNPDEAVAFGAAVQGGILSNEGGSAGVVLLDVAPLSLGIETVGGVMSKIIERNTVIPTRKT
QTFTTVQDKQTTVSIQVYEGERAMTKDNRQLGKFDLNGIPAAARGTPQIEVTFEVDSNGILAVSAEDKASSAKEAITITNDKGRL
SKEEIERMVKEAEEYADADAEVKRKVEGKNSLETAIFSAKAAVEEGGSLVDKASDEDKESLKAAVKEAQDWLDESGEDATADDLE
GKLKDLQEIIQPIMAKGGAAGGGSGAAQGGEEDDTKEEL

PsHSP_D17
ARGSDCESRCTICGGSGFTVTTRRIGPGFIQQMRSPCSACGGTGKIVTECSTCPHGQFEQAEQVVLIEIVRGAFDGQRVAFEEMA
DELPGHATGALEFELDAAPHPTFRREGDDLHVRMPLSLAEALVGVDRTVTQLDGRSVPVQLSGVTQSLQTHRVAGEGMPRADSDG
AGDMVVTFWVEFPQWLTDAQRKVVLETFGSPPAASEASTASETAASIDGDADASKEDL

**Fig. 1** Deduced amino acid sequence of the PsHSPs (**a**) and sequence alignment of PsHSP70 (**b**) from *P. seriata*. **a** Deduced amino acid sequences of the PsHSPs, PsHSP90, PsHSP40, PsHSP20, PsHSP_O22, PsHSP_D07, and PsHSP_D17. Amino acid residues are marked with *single letters*. The putative signal peptide is *underlined* and the ATPase domain is *double-underlined* in PsHSP90. The DnaJ domain and zinc finger DNA binding domain are *double-underlined* in PsHSP40. A putative α-crystallin domain was *double-underlined* in PsHSP20. The conserved amino acids motif, HDEL for the endoplasmic reticulum (ER) group and EEVD or EEID for the cytosolic group, are marked as *bold* in PsHSP90, PsHSP_O22, and PsHSP_D07. **b** Amino acid sequence alignment of the three HSP70s from *P. seriata*. Amino acid residues are marked with *single letters*. The putative signal peptide is *underlined* in PsHSP70b. The three amino acid motifs found in HSP70 are *boxed*. The conserved amino acids motif, EDID for the cytosolic group, is marked as *bold* in PsHSP70a. *Asterisk* (*) and *colon* (:) indicate identical and similar amino acid residues. Alignments of the deduced amino acid sequences were conducted with the Clustal X program

**b**

PsHSP70a  MADKKDVP--------------------------------------------------SGPSIG
PsHSP70b  MASAVSARTALARVGSCRPGLLGLAAGGGGGLAALRPAGHPAPSVVAAAAGAALRRWKSSDAGIAGDVIG
PsHSP70c  MMRRFSSRTAVSSVPFVR------ACSGG--------------------------K-----VQGDVIG
          *:                                                       *  **

PsHSP70a  IDLGTTYSCVGIWQNDRVEIIANDQGNRTTPSFVAFTDS-ERLIGDAAKNQVAINATNTVFDAKRMIGRK
PsHSP70b  IDLGTTNSCVAVMQGSEPRVIENAEGMRTTPSVVAFSADGEKLVGVPARRQAVTNPENTLFATKRLIGRR
PsHSP70c  IDLGTTYSCVAIMEGDKPRVIENAEGFRTTPSVVAFKGE-EKLVGHSAKRQAVTNPSSTFYAVKRFIGRR
          ****** ***.: :...  .:* * * *****.***.  .*.**.*:.*. *. *. .*.: :.**:***

PsHSP70a  FSDPSVQQDMKHFSFKVVQKEGDKPVVEVLYKGETKQFSPEEISAMVLSKMREVAESYLGKEVKTAVVTV
PsHSP70b  YDDSKTQEDIRQMPFKIVKGPNGDAWVS----AGGDNYSPSQVGAFVLQKMRETAEAFLGRKVSSAVVTV
PsHSP70c  FDDPATQKDIKAMPFKIVRHSNGDAWVEN---ANGKQFSPSQVGAFVLEKMKTAEAHLHKKVKNAVVTV
          :.*. .*:*:: .*.*:** .  .*.        * .:*.**:.*:**.**..*: .* :*:*..*****

PsHSP70a  PAYFNDSQRLATKDAGTIAGLHVSRIINEPTAAAIAYGLDRKTAGDTALERTVLIFDLGGGTFDVSLLSI
PsHSP70b  PAYFNDSQRQATKDAGKIAGLNVARIINEPTAASLAYGMDKADG------KLIAVYDLGGGTFDVSILEI
PsHSP70c  PAYFNDAQRQATKDAGQISGLTVHRIINEPTAAALAYGLEKTQD------RTIAVYDLGGGTDISILEI
          ******:** ****** *:** *.*********:.***::::      :* *:*******: *:**

PsHSP70a  EIDSLFEGVD-----FYSSLSRAKFEELCMDLFRGTLEPVEKVLRDAKAAKGDVDDIVLVGGSTRIPKVQS
PsHSP70b  NLPFITADAGGPKHMNMKLTRAKFESLVDPLVSRTLAPMKSCLKDAGVSSKEISDVLLVGGMTRVPKVVE
PsHSP70c  NLPFISANKDGPQHIQMMLSRSKFEGLVDNLVQRSVAPCKQCMKDAAVENKDIHEIVMVGGMTRMPKVVE
          ::    ::. *    :. * .**:***. * :. :.:  : *:  *..:..::*:**** **:***:*

PsHSP70a  LLVDFFNGKELSKSINPDEAVAYGAAVQAAILAGDTSERTQDLLLLDVAPLSLGIETAGGVMTPLIKRNT
PsHSP70b  TVKDFF-GKEPSKGVNPDEVVAMGAAIQGGVLRGDVKD-----ILLLDVTPLSLGLETLGGVFTRLINRNT
PsHSP70c  TVKNFF-GKEPFRGVNPDEAVASGAAIQGGVLRGDVSG-----ILLLDVTPLSLGIETLGGVFTRMIPKNT
          :.*.*** *:.: .*****.** ***:*.*:* **:      *****.*****.** ***.*:*:*

PsHSP70a  TIPTKKSQIFSTYADNQTGVLIQVYEGERTRTKDCGLLGKFELTGIPPAPRGQPQLEVVFDVDANGILNV
PsHSP70b  TIPTKKSQVFSTAADGQTQVGIKVGQGERDMFQDNSVLGSFDLVGIPPAPRGVPQVEVTFDIDANGILHV
PsHSP70c  TIPTKKSQTYSTAADNQTQVGIKVFQGEREMAADNQMLGQFDLVGIPPAPRGVPQIEVTFDIDANGICHV
          ********:: ** **.**.*  * .*. .. .  :** *:*.***********.**.** *:****** *

PsHSP70a  SATDKKSTGKQNKITITNDKGRLSKEQIEAMVQEAEKYKAEDEAAQAKIEARNGLENYCYNLKNTTVGDAT
PsHSP70b  SARDKATNKKQSIQIQSSGG-LSDAEVSEMVKRAEAYAQEDKQRREGIEAKNDADSLLYSTEKTLS---E
PsHSP70c  LAKDKATGKEQAITIQSNGG-LSKTEIERMIREGEQHADNDRKKREIVETKNNAETQLNTAEKQLG---E
          * **:*.*:: * *.. *.  **.:*:* *::.  :   .* ::*  :*.**.  : :  *: :  :

PsHSP70a  VKEKLSEDDAKAIETIVADTLAWLESNDNAEKEEYDAKLKEVEAVAHP--IMTKMYVAAGGGAPG-GMPDM
PsHSP70b  HREKMPADDVAAVESAMTALRAEVEKEDPSTKELKDLTDALQKASMKVGEAMYKASQAANASPSDGSADA
PsHSP70c  WKYVSDAEK-ANVRELLAALKKEMENPNSSKDDLAGATDKLQKAVMECGRTEYQQAAAQNQS-S-GAQQG
          :         :  .   :**  * * :   .* * :.  :   * . * :  * .*.  * . *

PsHSP70a  AGG----MGGAGAAPMDTGGADNPDITVEDID----------------------
PsHSP70b  ADAGAAGESGSKAENTKAGGAKADGAQEAEFRDADADSDGAKDAKDKKASGGGSA
PsHSP70c  GDQ---QQQQQQQQQSGEQKPEGEQK-----------------------------
          ..        .  :.:*

**Fig. 1** (continued)

To predict the subfamily of each PsHSP70, different types of HSPs from *Arabidopsis*, *Chlamydomonas*, and a few algae were employed to construct a phylogenetic tree using the Clustal X program. In the phylogenetic tree of HSP70s, PsHSP70b and PsHSP70c clustered together with mitochondrial HSP70s, and PsHSP70a and PsHSP_D07 were grouped with the cytosolic and ER HSP family, respectively (Fig. 2). The results of the phylogenetic tree of HSP70s and the motifs or putative signal peptides found in PsHSPs indicate that PsHSP70a is a cytosolic HSP70, and PsHSP70b may be transported to subcellular organelles, specifically the mitochondria. However, the conserved amino acid motif, PEAEYEEAKK, in the mitochondrion (MT) group was not detected in PsHSP70b and PsHSP70c. The signal peptide sequence for the subcellular localization was also not detected in PsHSP70c (Fig. 1b). The localization of PsHSP70 could be confirmed using a reporter gene system such as GFP.

HSP70s have highly specialized functions such as the regulation of the heat stress response and membrane translocation of proteins (Kang et al. 1990; Tomoyasu et

terminus and C terminus (Fig. 1b). PsHSP70a harbors an EDID motif at the C-terminal end, which is similar to the EEID motif found in cytosolic HSP. The prediction of signal peptides using the PrediSi program (www.pridisi.de) demonstrates that PsHSP70b harbors a putative signal peptide in its N-terminal region. These results indicate that PsHSP70a may be located in the cytosol and PsHSP70b may be transported into subcellular organelles. PsHSP_D07 evidences the highest amino acid sequence homology to the ER luminal binding protein, Bip, and EEDD motif in the C-terminal region (Table 1, Fig. 1a). These results indicate that PsHSP_D07 may encode for an ER HSP70.

**Fig. 2** Phylogenetic tree of the PsHSP70s with HSPs from *Arabidopsis* and *Chlamydomonas*. The phylogenetic tree of the HSP70 family member was constructed via the unrooted neighbor-joining method using the ClustalX program. Accession numbers of the HSPs from *Chlamydomonas* and *Arabidopsis* followed the name of each HSP
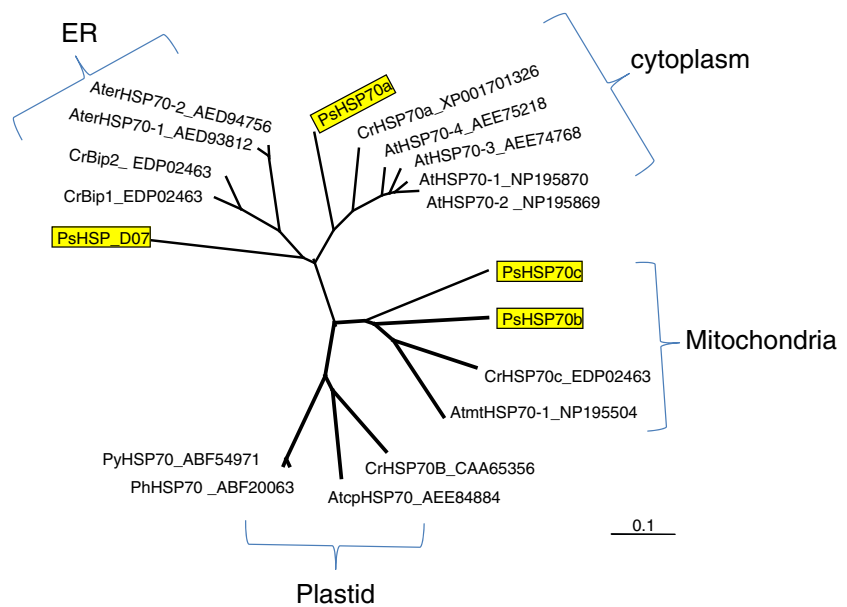


al. 1998). In higher plants, HSP70 genes are generally encoded by multigene families. Recently, the completion of genome sequencing in some model plants and unicellular algae demonstrates that *Arabidopsis thaliana* harbors 14 HSP70 genes consisting of five cytoplasmic, three ER, two mitochondrial, and two chloroplast members, and *C. reinhardtii* have nine *HSP70* genes of two cytoplasmic and four mitochondrial or chloroplast members, and three ER members (Schroda and Vallon 2009), and *C. merolae* have four *HSP70* genes with one each for cytoplasmic, ER, mitochondrial, or chloroplast members, based on cDNA or genomic DNA annotation (Renner and Waters 2007).

The *PsHSP40* encodes for 429 amino acids (46.1 kDa, p*I* 6.61), which are similar to the HSP70 cochaperones, DnaJ(HSP40). PsHSP40 harbors a DnaJ domain at residues 34 to 96 and a zinc finger DNA binding domain at residues 150 to 235 (Fig. 1a). HSP70 cochaperones are usually involved in the regulation of the ATPase activity of their HSP70 partner; they supply it with substrates, and/or connect it with other factors involved in protein folding or degradation (Hennessy et al. 2005). These HSP70 cochaperones were classified into several classes with DnaJ-domain protein regulating ATPase, GrpE-type nucleotide exchange factor, CHIP1 homolog mediate ubiquitinylation of trapped subtract and the HOP-like protein, which mediate the interaction of HSP70 and HSP90. PsHSP_G17 also evidences the highest level of amino acid sequence homology to the DnaJ-like protein and harbors a DnaJ domain (Table 1, Fig. 1a). These results indicate that PsHSP_G17 may encode for a DnaJ/HSP40. The DnaJ/HSP40 interacts, via the DnaJ domain, with its HSP70 partner and stimulates its ATPase activity and supplies it with specific substrates (Liberek et al. 1991; Han and

Christen 2003). The DnaJ-domain proteins are multigene family in *Chlamydomonas* and land plants. Among these, few J-domain proteins harbor the zinc finger domain. PsHSP40 may be one of the DnaJ proteins harboring the zinc finger DNA biding domain in the *Porphyra* genome.

*PsHSP20* encodes for a 25.8-kDa polypeptide with 248 amino acids (p*I* 6.45) and is similar to the HSP20 family (Table 1). The PsHSP20 polypeptides harbor an α-crystallin domain at residues 139 to 228, which are 90 conserved amino acids at the C-terminal region of the sHSPs (Fig. 1a). The sHSPs are low molecular HSPs with 12–40 kDa and synthesized responses to heat and other stresses. Plants contain a large number of different *sHSP* genes. In *Arabidopsis*, 13 different sHSPs are grouped into six classes based on their intracellular localization and sequence relatedness (Scharf et al. 2001). A total of eight *sHSP* genes are identified from the genome of *Chlamydomonas* (Schroda and Vallon 2009). The sHSP are not themselves able to refold non-native proteins. They have a high capacity to bind to non-native proteins, probably via hydrophobic interaction, and to stabilize and prevent non-native aggregation. Plant sHSPs respond to a broad range of environmental stresses, including heat, cold, drought, salinity, and oxidative stress (Wang et al. 2004).

Expression of *PsHSP* Genes

In an effort to evaluate the expression of each *PsHSP* gene under heat stress conditions, total RNAs were isolated and subsequently subjected to RT–PCR with *PsHSP* gene-specific primer (Table 2). Figure 3 shows the expression of each *PsHSP* gene under normal growth condition and heat stress. RT–PCR results show that most of the *PsHSP* genes are expressed under normal growth conditions, as

**Table 2** Lists of primers for the *PsHSP* and *CrHSP* genes used in PCR

| Gene | Primer | |
| --- | --- | --- |
| *PsHSP990* | GTGATCAAACGCAAGCTCGT | ACGCGAAGGACGACAACACAGATA |
| *PsHSP70a* | CTTACCTCGGCAAGGAGGTC | GACACGTCAAAAGTGCCACC |
| *PsHSP70b* | TTACGATGACAGCAAGACGCAGGA | TACACTGCAATCAGCTTGCCATCC |
| *PsHSP70c* | CATGCAAACCGAGATCAACC | CGATCTCGTGGATGTCCTTG |
| *PsHSP40* | TGAAGAAGGCGTACCGAAAGCTGA | TCCGCCCATTGTACAGGTCATTCA |
| *PsHSP20* | ACGCGAAGGACGACAACACAGATA | TCCCTCAGCCTCTACTCTTCCATGAT |
| *PsHSP_D07* | TTGAGGTGACGTTTGAGGTGGACT | AACAACATACTTACAAGCGCCGCC |
| *PsHSP_G17* | GTGCAGCTGTCGGGTGTAAC | TTCGACCCAAAAAGTCACCA |
| *PsHSP_O22* | AAGGTGGAGAAGGTTGTGGTGTCA | GTGTCAAACAGCAGGCTCACCAAA |
| *CrHSP90* | ATTATTGCCGGCATCAAGAC | CTTACGCGGACTTCTTCCAG |
| *CrHSP70* | AGCACTGATGCTGTGTTTCG | GGTGCCATCTCGGTTTCTTA |
| *CrHSP70b* | GTAGCCACGTCTGCTGTTGA | TTTGCATGTCCATTCCTTCA |
| *CrHSP33* | CTGTGCCAGCAGACCTACAA | TGACTACCTGGGTCACGACA |
| *CrHSP22* | GATTGAGGCGTGAGGATGAC | ATGCAGGCCTAAGAATGTGC |
| *TubA1* | CTCGCTTCGCTTTGACGGTG | CGTGGTACGCCTTCTCGGC |

well as heat stress. However, the transcripts of two *PsHSP* genes, *PsHSP70c* and *PsHSP_G17*, were not observed under the conditions tested. These results indicate that *PsHSP70c* and *PsHSP_G17* are expressed at relatively low levels or at different life stages, either in a tissue-specific manner or under other stimuli. According to the RT–PCR results, it may prove difficult to evaluate the relative expression patterns of the *HSP* genes, which are expressed abundantly in the cell. However, Fig. 3 shows that the transcript levels of *PsHSP70b* and *PsHSP20* were increased by heat stress. Many plant genes are induced or upregulated in response to heat stress. The genes expressed under heat stress conditions might also be involved in cellular thermotolerance. Among the *PsHSP* genes, *PsHSP70b* and *PsHSP20* are upregulated by heat stress, and may be involved in the thermotolerance of *Porphyra*.

In land plants, the *sHSPs* family of *PsHSP20* are the most abundant heat stress-induced proteins, and their transcription levels are increased by various stresses such as cold, drought, and oxidants, in addition to heat stress (Sabehat et al. 1998; Soto et al. 1999; Schroda and Vallon 2009).

*HSP70* genes are generally encoded for by multigene families; some members of the *HSP70* family are constitutively expressed and other members are expressed in response to heat and other abiotic stresses (Fu et al. 2009; Tominaga et al. 2010). The constitutively expressed *HSP70* are frequently involved in assisting in the folding of de novo synthesized polypeptides and the import or translocation of precursor proteins (Frydman 2001; Wang et al. 2004). Other family members that respond to environmental fluctuation may be involved in facilitating refolding and proteolytic degradation of non-native proteins (Hartl 1996; Miernyk 1997). The *PsHSP70a* that is constitutively expressed may be located in the cytosol and involved in the folding or translocation of newly synthesized polypeptides. The *PsHSP70b* may be involved in abiotic stress tolerance in *Porphyra*.
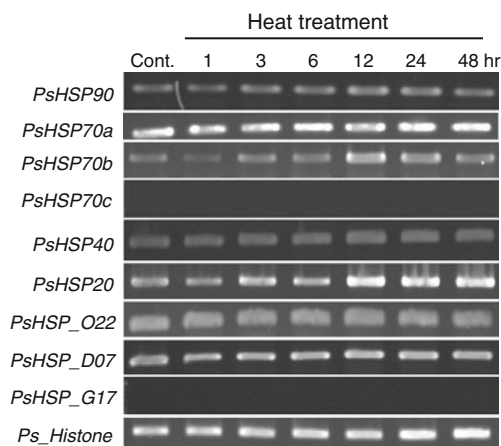


**Fig. 3** Expression pattern of the *PsHSP* gene under heat stress conditions. Total RNAs were purified from gametophyte thalli and employed for RT–PCR. For heat treatment, gametophyte thalli were transferred into an incubator at 25°C, and then the thallus tissues were harvested at 1-, 3-, 6-, 12-, 24-, and 48-h intervals. The annealing temperatures and PCR cycles were adjusted for some of the primer sets in order to optimize the PCR reactions. The PCR products were separated on 1% agarose gels and stained with ethidium bromide for photography

### Increase of Thermotolerance by Expression of the *PsHSP70b* Gene in *Chlamydomonas*

The *PsHSP70b* gene was increased at the transcriptional level under heat stress condition. In order to determine the physiological functions of the *PsHSP70b* gene, we introduced

the complete coding sequence of the *PsHSP70b* into *C. reinhardtii* strain cc-125 under the control of the psaD promoter (Fig. 4a). The vector alone was also introduced into *C. reinhardtii* and named Hyg5414. Five *PsHSP70b*-transformed *Chlamydomonas* lines were selected on a medium containing hygromycin, and the introduction and expression of the *PsHSP70b* gene were confirmed via DNA blot and RNA blot analysis (Fig. 4b). These results indicate that the *PsHSP70b* gene was introduced into the *Chlamydomonas* genome and expressed. The RNA blot data demonstrate that the transcript level of the *PsHSP70b* in *Chlamydomonas*
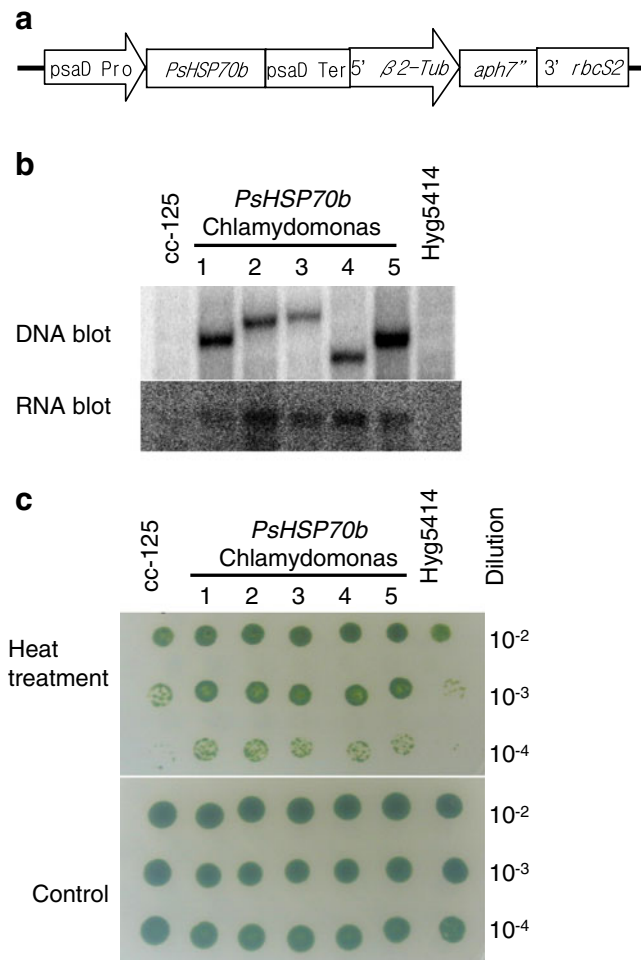


**Fig. 4** Effect of the *PsHSP70b* on the growth of *Chlamydomonas* under heat stress conditions. **a** Vector map for the expression of *PsHSP70b* in *Chlamydomonas*. **b** Verification of introduction and expression of the *PsHSP70b* gene in transformed *Chlamydomonas*. To assess the introduction of *PsHSP70b*, genomic DNA was digested with NcoI and hybridized with the *PsHSP70b* gene probe. The expression of the *PsHSP70b* was evaluated via RNA blot analysis. Total RNA were purified from *Chlamydomonas* at normal growth condition (25°C). cc-125; wild type, Hyg5414; vector control. **c** Survival and growth pattern of the wild-type and transformed *Chlamydomonas* under heat stress conditions. *Chlamydomonas* were heat treated at 37°C for 5 days and transferred into a 25°C growth chamber, then cultured for 7 days. Then $10^7$ cells/ml were diluted to $10^2$–$10^4$ in fresh medium and 5 μl of diluted cells was inoculated onto agar plates

varied in each transformant line (Fig. 4b). The *PsHSP70b* gene was expressed at much higher levels in transformant line 2 and line 4 than in other lines.

In order to assess the thermotolerance of the *Chlamydomonas* harboring the *PsHSP70b* gene, cc-125 and Hyg5414, and *PsHSP70b Chlamydomonas* lines were subjected to 37°C (heat treatment) for 5 days or not (control), and then transferred into an incubator at 25°C. One week later, *Chlamydomonas* cells grew at similar levels on the control culture plates (Fig. 4c). However, when heat stress was applied to the cells, the *PsHSP70b Chlamydomonas* grew at much higher levels than cc-125 and Hyg5414 (Fig. 4c). These results indicate that the *Porphyra PsHSP70b* gene contributes to high-temperature tolerance in *Chlamydomonas*.

The overexpression of *HSP70s* in plants, which is correlated with enhanced stress tolerance, including thermotolerance, has been previously reported. The overexpression of an ER HSP70 homologue BiP gene in tobacco was shown previously to alleviate water stress and ER stress induced by tunicamycin treatment (Leborgne-Castel et al. 1999; Alvim
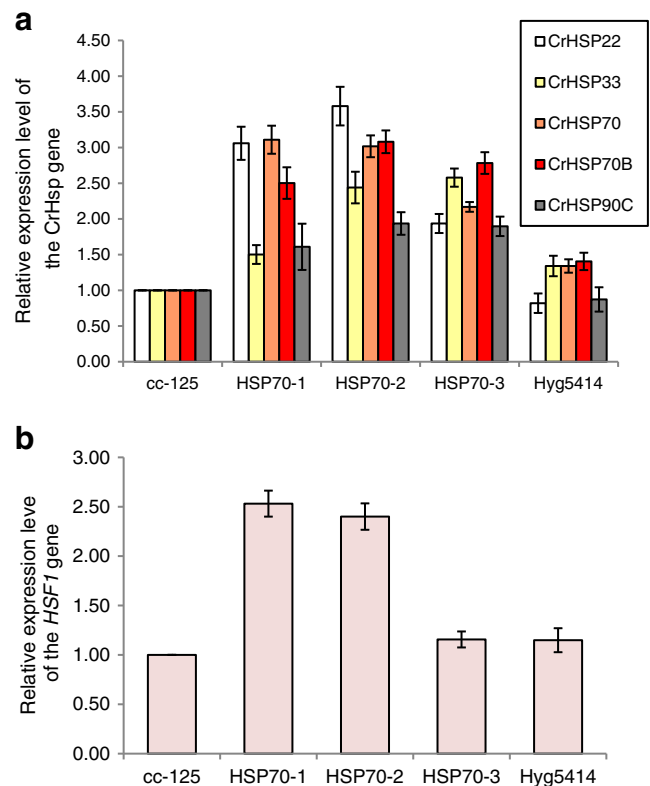


**Fig. 5** Expression of the *CrHSPs* (**a**) and *HSF1* gene (**b**) in transformed *Chlamydomonas* harboring *PsHSP70b*. Total RNAs were purified from wild-type *Chlamydomonas* (cc-125), transformed *Chlamydomonas* (HSP70-1, HSP70-2, HSP70-3) with *PsHSP70b* gene, and empty vector control (Hyg5414) under normal growth conditions, and the transcription level of the *Chlamydomonas HSP* gene (*CrHSPs*) and *HSF1* gene were analyzed via real-time RT–PCR. The *TubA1* gene was used as an internal control

et al. 2001). Overexpression of DnaK from cyanobacteria (*Aphanothece halophytica*) enhances heat and salt stress tolerance in transgenic tobacco plants (Sugino et al. 1999; Ono et al. 2001). The constitutive overexpression of cytosolic HSP made *Hsc70-1* plants more tolerant to heat shock, but *Hsc70-1* also appeared to be deleterious in terms of viability, growth, and development (Sung and Guy, 2003). Transformants overexpressing *Hsc70-1* induced reductions in root and shoot meristem activities, thus resulting in dwarfism in those plants (Sung and Guy 2003; Cazale et al. 2009). The elevated basal levels of *HSC70-1* in transgenic plants led to delayed heat shock response of several heat shock genes (Sung and Guy 2003). The different phenotypic changes observed in these studies suggest that HSP70s localized to different subcellular compartments participate in different cellular processes. The specific roles of individual HSP70 proteins in organisms are likely to be determined by their location in different subcellular compartments, by differential expression of *HSP70* genes in specific cells and at different stages of development or via interaction with specific sets of HSP70-associated proteins (Michaud et al. 1997; May and Soll 2000; Sung et al. 2001b).

### PsHSP70b Increases the Transcription of CrHSP Genes

Cellular thermotolerance involves many processes, including alterations of membrane permeability and gene transcription and enzyme function (Vierling 1991; Kampinga et al. 1995; Sangwan et al. 2002). HSP/molecular chaperones are key components contributing to cellular homeostasis in cells under heat stress conditions. Figure 4 suggests that *Chlamydomonas* cells harboring the *PsHSP70b* gene evidence much higher thermotolerance than normal cells. Therefore, we hypothesized that the heterologously expressed *PsHSP70b* may stimulate the expression of the *CrHSP* genes in the *Chlamydomonas* genome. In order to address this hypothesis, we assessed the transcription level of five *Chlamydomonas HSP* (*CsHSP*) genes including the *CrHSP22*, *CrHSP33*, *CrHSP70*, *CrHSP70b*, and *CrHSP90* genes (Table 2) in the *Chlamydomonas* harboring the *PsHSP70b* under normal growth conditions (Fig. 5a). Figure 5a shows that the *CrHSP22* and *CrHSP70* genes were increased by up to 3- to 5-fold in *PsHSP70b* transformants under normal growth conditions without heat stress. However, transcripts of the *CrHSP33* and *CrHSP90* genes were detected at levels similar to those in the control cells (Fig. 5a). It has been shown that the small *HSP* (*sHSP*) and *HSP70* responded to heat stress in higher plants. These results indicate that heterologously expressed *PsHSP70b* stimulates the transcription of endogenous *CrHSP* genes. How, then, is heterologous *PsHSP70b* involved in the activation of *CrHSP* gene transcription? It is known that the transcription of the *HSP* genes is mediated by a specific

class of transcription factors, termed heat shock factors (HSFs) (Morimoto 1998). *Chlamydomonas* HSF1 is weakly expressed under non-stress conditions and is induced rapidly by heat shock (Schulz-Raffelt et al. 2007).

In order to determine whether *HSF1* mediates the transcription of the *CrHSPs* gene in *PsHSP70b Chlamydomonas* under no heat stress, we assessed the transcription level of the *HSF1* gene. Figure 5b shows that transcripts of the *HSF1* gene were elevated in the transformed *Chlamydomonas*, line *PsHSP70b*-1 and *PsHSP70b*-2 under normal growth conditions, although transcripts of the *HSF1* gene were slightly upregulated in line *PsHSP70b*-3. These results indicate the possibility that heterologously expressed *PsHSP70b* may activate the transcription of the *HSF1* gene, which may increase the transcript level of the *CrHSP22* and *CrHSP70* genes. However, previous reports indicate that HSP70 acts as a negative regulator of the HSF in animals and higher plants. HSF undergoes stress-induced trimerization, and binding of the HSP70 to HSF inhibits the trimerization of the HSF (Rabindran et al. 1993; Lee et al. 1995). However, *Chlamydomonas* HSF1 appears to be constitutively trimeric as in yeast, and its interaction with HSP70 may be involved in the early steps of folding (Sorger and Nelson 1989; Schulz-Raffelt et al. 2007). Therefore, negative regulation of the HSF1 by HSP70 may not apply to *Chlamydomonas*. Heterologously expressed PsHSP70b may be perceived as a signal molecule and may be involved in activation of the signal pathway for heat tolerance. The substrates for the PsHSP70b involved in those responses remain to be identified.

Genetic approaches with a specific *HSP* mutant cannot readily detect mutant phenotypes, probably as the result of a high level of functional redundancy. Therefore, overexpression systems have been shown to be a useful tool for assigning the functions of an *HSP* gene. The constitutively expressed *PsHSP70b* gene in *Chlamydomonas* shows that this gene is involved in enhanced heat stress tolerance. This system might be employed for studies of the physiological and molecular functions of the *PsHSP70b*.

### References

Alvim FC, Carolino SMB, Cascardo JCM, Nunes CM, Martinez CA, Otoni WC, Fontes EPB (2001) Enhanced accumulation of BiP in transgenic plants confers tolerance to water stress. Plant Physiol 126:1042–1054

Blouin NA, Brodie JA, Grossman AC, Xu P, Brawley SH (2011) *Porphyra*: a marine crop shaped by stress. Trends Plant Sci 16:29–37

Boston RS, Viitanen PV, Vierling E (1996) Molecular chaperones and protein folding in plants. Plant Mol Biol 32:191–222

Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses. In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Biologists, Rockville, pp 1158–1203

Cazale A-C, Clement M, Chiarenza S, Roncato M-A, Pochon N, Creff A, Marin E, Leonhardt N, Noel L (2009) Altered expression of cytosolic/nuclear Hsc70-1 molecular chaperone affects development and abiotic stress tolerance in *Arabidopsis thaliana*. J Exp Bot 60:2653–2664

Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 61:243–282

Fink AL (1999) Chaperone-mediated protein folding. Physiol Rev 79:425–449

Frydman J (2001) Folding of newly translated proteins in vivo: the role of molecular chaperones. Annu Rev Biochem 70:603–647

Fu W, Yao J, Wang X, Liu F, Fu G, Duan D (2009) Molecular cloning and expression analysis of a cytosolic HSP70 gene from *Laminara japonica* (Laminariacease, Phaeophyta). Mar Biotechnol 11:738–747

Guy CL, Li QB (1998) The organization and evolution of the spinach stress 70 molecular chaperone gene family. Plant Cell 10:539–556

Han W, Christen P (2003) Mechanism of the targeting action if DnaJ in the DnaK molecular chaperone system. J Biol Chem 278:19038–19043

Harris EH (1989) The *Chlamydomonas* sourcebook: a comprehensive guide to biology and laboratory use. Academic, San Diego

Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381:571–579

Hartl FU, Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295:1852–1858

Hemmingsen SM, Woolford C, van der Viles SM, Tily K, Dennis DT, Georgopoulos CP, Hendrix RW, Ellis RJ (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature 333:330–334

Hennessy F, Nicoll WS, Zimmermann R, Cheetham ME, Blatch GL (2005) Not all J domains are created equal: implications for the specificity of HSP40–HSP70 interaction. Protein Sci 14:1697–1709

Hwang MS, Chung IK, Oh YS (1997) Temperature responses of *Porphyra tenera* Kjellman and *P. yezoensis* Ueda (Bangiales, Rhodophyta) from Korea. Algae 12:207–213

Hwang MS, Kim SM, Ha DS, Baek JM, Kim HS, Choi HG (2005) DNA sequences and identification of *Porphyra* cultivated by natural seeding on the southwest coast of Korea. Algae 20:183–196

Ireland HE, Harding SJ, Bonwick GA, Jones M, Smith CJ, Williams JHH (2004) Evaluation of heat shock protein 70 as a biomarker of environmental stress in *Fucus serratus* and *Lemna minor*. Biomarkers 9:139–155

Jolly C, Morimoto RI (1999) Stress and the cell nucleus: dynamics of gene expression and structural reorganization. Gene Expr 7:261–270

Kampinga HH, Brunsting JF, Stege GJ, Burgman PW, Konings AW (1995) Thermal protein denaturation and protein aggregation in cells made thermotolerant by various chemicals: role of heat shock proteins. Exp Cell Res 219:536–546

Kang PJ, Ostermann J, Shilling J, Neupert W, Craig EA, Pfanner N (1990) Requirement for HSP70 in the mitochondria matrix for translocation and folding of precursor protein. Nature 348:137–143

Karlin S, Brocchieri L (1998) Heat shock protein 70 family: multiple sequence comparisons, function, and evolution. J Mol Evol 47:565–577

Kim E, Park HS, Jung YJ, Jeong WJ, Park HS, Hwang MS, Park EJ, Gong YG, Choi DW (2011) Identification of the high-temperature response genes from *Porphyra seriata* (Rhodophyta) ESTs and enhancement of heat tolerance of *Chlamydomonas*

(Chlorophyta) by expression of the *Porphyra HTR2* gene. J Phycol 47:821–828

Kindle KL (1990) High-frequency nuclear transformation of *Chlamydomonas reinhardtii*. Proc Natl Acad Sci USA 87:1228–1232

Krishna P, Gloor G (2001) The HSP90 family of proteins in *Arabidopsis thaliana*. Cell Stress Chaperones 6:238–246

Leborgne-Castel N, Jelitto-Van Dooren DPWM, Crofts AJ, Denecke J (1999) Overexpression of BiP in tobacco alleviates endoplasmic reticulum stress. Plant Cell 11:459–470

Lee JH, Hubel A, Schoffl F (1995) Depression of the activity of genetically engineered heat shock factor causes constitutive synthesis of heat shock proteins and increased thermotolerance in transgenic *Arabidopsis*. Plant J 8:603–612

Liberek K, Marszalek J, Ang D, Georgopoulos C, Zylicz M (1991) *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc Natl Acad Sci USA 88:2874–2878

May T, Soll J (2000) 14-3-3 proteins form a guidance complex with chloroplast precursor proteins in plants. Plant Cell 12:53–64

Mayer MP, Bukau B (2005) HSP70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 62:670–684

McLachlan J (1973) Growth media—marine. In: Stein JR (ed) Handbook of Phycological methods. Cambridge University Press, New York

Michaud S, Marin R, Westwood JT, Tanguay RM (1997) Cell-specific expression and heat-shock induction of HSPs during spermatogenesis in *Drosophila melanogaster*. J Cell Sci 110:1989–1997

Miernyk JA (1997) The 70 kDa stress-related proteins as molecular chaperones. Trends Plant Sci 2:180–187

Miura A (1988) Taxonomic studies of *Porphyra* species cultivated in Japan, referring to their transition to the cultivated variety. J Tokyo Univ Fish 75:311–325

Morimoto RI (1998) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors molecular chaperones, and negative regulators. Genes Dev 12:3788–3796

Nelson RJ, Ziegelhoffer T, Nicolet C, Werner-Washburne M, Craig EA (1992) The translation machinery and 70 kD heat shock protein cooperate in protein synthesis. Cell 71:97–105

Ono K, Hibino T, Kohinata T, Suzuki S, TanakaY NT, Takabe T, Takabe T (2001) Overexpression of DnaK from a halotolerant cyanobacterium *Aphanothece halophytica* enhances the high-temperature tolerance of tobacco during germination and early growth. Plant Sci 160:455–461

Rabindran SK, Haroun RI, Clos J, Wisniewski J, Wu C (1993) Regulation of heat shock factor trimer formation: role of a conserved leucine zipper. Science 259:230–234

Renner T, Waters ER (2007) Comparative genomic analysis of the HSP70s from five diverse photosynthetic eukaryotes. Cell Stress Chaperones 12:172–185

Richter K, Buchner J (2001) HSP90: chaperoning signal transduction. J Cell Physiol 188:281–290

Sabehat S, Lurie D, Weiss D (1998) Expression of small heat-shock proteins at low temperatures—a possible role in protecting against chilling injuries. Plant Physiol 117:651–658

Sahoo D, Tang X, Yarish C (2002) *Porphyra*—the economic seaweed as a new experimental system. Curr Sci 83:1313–1316

Sangwan V, Orvar BL, Beyerly J, Hirt H, Dhindsa RS (2002) Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways. Plant J 31:629–638

Scharf KD, Siddique M, Vierling E (2001) The expanding family of *Arabidopsis thaliana* small heat stress protein and a new family of proteins containing α-crystallin domains (Acd proteins). Cell Stress Chaperones 6:225–237

Schroda M, Vallon O (2009) Chaperones and proteases. In: Stern DB (ed) *Chlamydomonas* source book 2nd edition, vol volume 2. Elsevier, San Diego

Schulz-Raffelt M, Lodha M, Schroda M (2007) Heat shock factor 1 is a key regulator of the stress response in *Chlamydomonas*. Plant J 52:286–295

Sorger PK, Nelson HC (1989) Trimerization of a yeast transcriptional activator via a coiled-coil motif. Cell 59:807–813

Soto A, Allona I, Collada C, Guevara MA, Casado R, Rodriguez-Cerezo E, Aragoncillo C, Gomez L (1999) Heterologous expression of a plant small heat-shock protein enhances *Escherichia coli* viability under heat and cold stress. Plant Physiol 120:521–528

Sugino M, Hibino T, Tanaka Y, Nii N, Takane T, Takabe T (1999) Overexpression of DnaK from a halotolerant cyanobacterium *Aphanothece halophytice* acquires resistance to salt stress in transgenic tobacco plants. Plant Sci 146:81–88

Sung DY, Guy CL (2003) Physiological and molecular assessment of altered expression of Hsc70-1 in *Arabidopsis*. Evidence for pleiotropic consequences. Plant Physiol 132:979–987

Sung DY, Kaplan F, Guy CL (2001a) Plant HSP70 molecular chaperones: protein structure, gene family, expression and function. Physiol Plant 113:443–451

Sung DY, Vierling E, Guy CL (2001b) Comprehensive expression profile analysis of the *Arabidopsis* HSP70 gene family. Plant Physiol 126:789–800

Tanaka KI, Namba T, Arai Y, Fujimoto M, Adachi H, Sobue G, Takeuchi K, Nakai A, Mizushima T (2007) Genetic evidence for a protective role for heat shock factor 1 and heat shock protein 70 against colitis. J Biol Chem 282:23240–23252

Tominaga H, Coury DA, Amno H, Kakinuma M (2010) Isolation and characterization of a cDNA encoding a heat shock protein 70 from a sterile mutant of *Ulva pertusa* (Ulvales, Chlorophyta). Ecotoxicology 19:577–588

Tomoyasu T, Ogura T, Tatsuta T, Bukau B (1998) Level of DnaK and DnaJ provide tight control of heat shock gene expression and protein repair in *Escherichia coli*. Mol Microbiol 20:567–581

Vierling E (1991) The roles of heat shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 42:579–620

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperons in the abiotic stress response. Trends Plant Sci 9:244–252