POLISH POLAR RESEARCH
vol. 40, no. 3, pp. 273–292, 2019 doi: 10.24425/ppr.2019.129674

Expression patterns of molecular chaperone genes in Antarctic psychrophilic yeast, *Glaciozyma antarctica* PI12 in response to heat stress

Nur Athirah YUSOF[1], Clemente Michael Vui Ling WONG[1*], Abdul Munir Abdul MURAD[2], Farah Diba ABU BAKAR[2], Nor Muhammad MAHADI[3], Ahmad Yamin Abdul RAHMAN[4], Nursyafiqi ZAINUDDIN and Mohd Nazalan Mohd NAJIMUDIN[4]

[1] *Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*

[2] *School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor, Malaysia*

[3] *Malaysia Genome Institute, Jalan Bangi, 43000 Kajang, Selangor Darul Ehsan, Malaysia*

[4] *School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*

[*] *coresponding author <michaelw@ums.edu.my>*

Abstract: Microbes living in the polar regions have some common and unique strategies to respond to thermal stress. Nevertheless, the amount of information available, especially at the molecular level is lacking for some organisms such as Antarctic psychrophilic yeast. For instance, it is not known whether molecular chaperones in Antarctic yeasts play similar roles to those from mesophilic yeasts when they are exposed to heat stress. Therefore, this project aimed to determine the gene expression patterns and roles of molecular chaperones in Antarctic psychrophilic *Glaciozyma antarctica* PI12 that was exposed to heat stress. *G. antarctica* PI12 was grown at its optimal growth temperature of 12ºC and later exposed to heat stresses at 16ºC and 20ºC for 6 hours. Transcriptomes of those cells were extracted, sequenced and analyzed. Thirty-three molecular chaperone genes demonstrated differential expression of which 23 were up-regulated while 10 were down-regulated. Functions of up-regulated molecular chaperone genes were related to protein binding, response to a stimulus, chaperone binding, cellular response to stress, oxidation, and reduction, ATP binding, DNA-damage response and regulation for cellular protein metabolic process. On the other hand, functions of down-regulated molecular chaperone genes were related to chaperone-mediated protein complex assembly, transcription, cellular macromolecule metabolic process, regulation of cell growth and ribosome biogenesis. The findings provided information on how molecular chaperones work

together in a complex network to protect the cells under heat stress. It also highlights the evolutionary conserved protective role of molecular chaperones in psychrophilic yeast, *G. antarctica*, and mesophilic yeast, *Saccharomyces cerevisiae*.

Key words: Antarctic, psychrophile, molecular chaperone, heat-stress, transcriptome.

Introduction

The temperature rise in the polar region due to the continuous unabated emission of greenhouse gas has been reported to be the primary cause of sea level rising (DeConto and Pollard 2016). In facing the threat of global warming, we need to increase our knowledge on how the residents of polar regions, especially the eukaryotic organisms, response toward thermal stress. Various studies on adaptations of cold-adapted psychrophiles to extreme conditions such as extremely low temperature, low energy environment, limited water, and nutrient availability, high hydrostatic pressure, oxidative stress, and high solar irradiation, have been reported (D'Amico *et al*. 2003; Boo *et al*. 2013; Firdaus-Raih *et al*. 2018; Wong *et al*. 2019). Nevertheless, studies on how psychrophiles response to heat or elevated temperatures are limited.

Heat or heat-shock is significant stress to many microorganisms, and it represents a significant barrier to their optimal growth and survival. There are numerous deleterious effects to the internal cell organization due to heat-shock such as cytoskeletal defects, aggregation of filaments, disruption of actin and tubulin network, loss of mitochondria and breakdown of dynamic processes such as translocation and transportation (Toivola *et al*. 2010; Szalay *et al*. 2007). Heat-shock also hits cellular regulations causing aggregation to the ribosomal proteins, the formation of stress granules in the cytosol, increase in membrane fluidity, global decrease in translation initiation components with other proteins affecting mRNA function and many more (Piper et al. 2003; Vigh et al. 2007; Buchan and Parker 2009). Cells can grow best within their optimal range of temperatures and become thermotolerant with moderate temperature increments. When cells are exposed to thermal stress, induction of a set string of genes encoding heat-shock proteins is expressed to protect cells from death (Verghese *et al*. 2012).

Many heat-shock proteins (HSPs) function as molecular chaperones to protect proteins in the cells from thermal-induced damage. Various molecular chaperones are referred to as HSPs because they are induced when cells are exposed to heat (Joplin *et al*. 1990). However, they are also produced when exposed to other forms of stresses, such as oxygen starvation and exposure to heavy metals, alcohol and low temperatures. Molecular chaperones can be classified into three subclasses based on their actions of functions. The first group is called the 'folding' chaperones that rely on ATP-driven conformational changes to assist

the folding or unfolding of macromolecular structures that, in turn, affects their assembly and disassembly (Ellis 2007). The second subclass of the chaperone is the 'holding' chaperones that maintain partially folded proteins on their surface to await the availability of folding chaperones upon stress abatement. The third subclass of the chaperone is the disaggregating chaperone that promotes the solubilization of proteins that have become aggregated as a result of stress (Baneyx and Mujacic 2004). Therefore, besides assisting a large variety of folding activities, molecular chaperones also reduce polypeptide aggregation, assist refolding of non-native proteins and solubilize aggregates for refolding (Ben-Zvi and Goloubinol 2001; Tomoyasu *et al*. 2001; Mogk *et al*. 2003). Some chaperones are reported to mediate degradation of proteins that failed to fold correctly to minimize accumulation of aggregates in cells (Huang *et al*. 2001). Moreover, extensive studies have shown that molecular chaperones are vital not only in proper protein folding but also act as the primary defense against physiologically stressful conditions (Tomoyasu *et al*. 2001). However, there is limited information on the expression patterns of molecular chaperone genes and their roles in Antarctic psychrophilic yeast under heat stress.

A psychrophilic yeast, *Glaciozyma antarctica* PI12 was isolated from sea ice collected in the vicinity of Casey Station (66°21'25"S; 110°37'09"E), Antarctica (Boo *et al*. 2013). The genome of this psychrophilic yeast has 7857 putative genes, out of which 89 are putative molecular chaperones. Among them are those coding for sHSPs, TRiC/Cpn60 subunits, HSP90s, HSP70s, AAA proteins, HSP40s/J Domain proteins, HSFs, cold shock proteins, ubiquitin proteins and CS-domain proteins (Yusof *et al*. 2015; Firdaus-Raih *et al*. 2018). Parallel to these findings, the *Saccharomyces cerevisiae* genome contains at least 60 known chaperones, namely sHSPs, CCT/ TRiC, and prefoldin complexes, Hsp90s, Hsp70s, Hsp60, AAA+ ATPases (Hsp104), and Hsp40s (Gong *et al*. 2009). Much of what we have known regarding the molecular chaperones in eukaryotic cells has been revealed in the yeast model, *S. cerevisiae*. However, it is not clear how similar are the responses of *G. antarctica* PI12 and *S. cerevisiae* towards heat stress. Hence, the objectives of this project were to determine the gene expression patterns and the roles of molecular chaperones in *G. antarctica* PI12 in response to heat stress.

Materials and methods

Strain and culture conditions. — *Glaciozyma antarctica* PI12 was isolated from a sea ice sample collected in the vicinity of Davis station, Antarctica. It was identified previously by researchers at the Universiti Sains Malaysia (USM) based on its 18S rDNA sequence (NCBI GenBank accession no. DQ525623.1). *G. antarctica* PI12 was routinely cultivated in yeast peptone dextrose (YPD) broth (Difco) medium at its optimal growth temperature of 12°C (Boo *et al*. 2013).

Exposure of yeast culture to heat stress. — *G. antarctica* PI12 was grown in YPD broth medium in conical flasks at 12°C until mid-log phase. Subsequently, these cultures (three replicates each) were rapidly exposed to heat shocks at 16°C and 20°C for 6 hours. After the exposure period, the cultures were snap-frozen in liquid nitrogen and stored at -80°C prior to RNA extraction.

RNA extraction and cDNA preparation. — Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instruction. Extracted RNA was treated with RNase-free DNase (Qiagen) to remove traces of genomic DNA. Subsequently, the sample was purified using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. The oligo (dT) magnetic bead and magnet separator system in the Dynabeads mRNA purification kit (Invitrogen) was used to purify the mRNA. Purified mRNA was eluted with Tris-HCl buffer and subsequently, the mRNA fragmented under a heating condition. cDNA synthesis was carried out using the SuperScriptTM II reverse transcription kit (Invitrogen) according to the manufacturer's instruction. The quantity and quality of the cDNA library were determined using the Agilent 2100 Bioanalyzer and ABI StepOnePlus Real-Time PCR systems to ensure suitability for sequencing. Paired-ends high-throughput DNA sequencing was performed at the University of Hawaii using the Solexa Illumina DNA Sequencing platform.

Reads mapping and transcript assembly. — The transcript was assembled using the Trinity RNA-Seq *de novo* transcriptome assembly method (Haas *et al*. 2013). It was further cluster-compressed to reduce multi-mapping low hits on putative isoforms using CD-HIT-EST (Li and Godzik 2006). TransDecoder was used to get probable CDS while differential expression test was done via the negative binomial model via the DESEQ2 R package (Love *et al*. 2014).

Differential gene expression analysis. — CLC Genomics Workbench software was used to identify differentially expressed genes. Genes with significant differential expression were identified using the direct indication of q-value with the alpha significance of 0.05.

Analysis of Gene ontology enrichment. — The transcripts from *G. antarctica* PI12 were retrieved from *G. antarctica* genome data (http://www.mgi-nibm.my/glaciozyma_antarctica/) maintained by the Malaysia Genome Institute. The genes were imported into BLAST2GO for Gene Ontology analysis allowing gene functions to be determined. Protein identification and comparative analysis were done using BLASTP *Saccharomyces cerevisiae* public database (http://www.yeastgenome.org/). InterPro analysis was done to classify annotated genes into related families based on domains and important sites (https://www.ebi.ac.uk/interpro/).

Gene alignment and the construction of the phylogenetic was performed using the ClustalW software.

Results and discussion

Transcriptome analysis in response to heat stress in *G. antarctica*. — A total of 33 annotated molecular chaperones fulfilled the alpha significance at alpha 0.05 on the false discovery rate. They were identified to show significant level expression in response to heat stress (Table 1). Several stress genes induced upon heat stress in *G. antarctica* PI12 were also found in *S. cerevisiae*, such as the HSP70, HSP90, HSF, and AAA ATPase protein genes. These indicated that *G. antarctica* PI12 heat stresses transcriptional response was likely to be qualitatively and quantitatively comparable to other yeasts. Several families of molecular chaperones designated according to their molecular mass were found in both mesophilic and psychrophilic yeasts upon heat stress response which was HSP90, HSP70, and HSP60 that made up the chaperonin complex, the TCP1-Ring-Complex (TRiC). Figure 1 displays the comparison of gene expression using $\log_2$ fold change value between exposure at 16ºC/control and 20ºC/control after 6 hours exposure. Several genes were differentially expressed which reflected their importance in cellular regulation after the heat-shock treatment such as the TRiC chaperonin genes. Besides that, several other genes were upregulated by 2 to 4-fold upon exposure of the yeast cells to heat stress. HSP70 and HSP90 genes showed a strong response as early as 6 hours when cells were exposed to 16ºC and 20ºC. The shift from the optimal temperature of 12ºC to 16ºC and 20ºC (lethal temperature) resulted in major alterations in the pattern of the production of HSP70 and HSP90 proteins. These responses might play a major role to protect the cells, from thermal injury and death. The slight increase of heat-shock factors (HSF) presence after exposure to thermal stress did not provide a strong and convincing outcome to conclude that the increase was due to heat stress tolerance attainment.

The other interesting finding was seen in a group of molecular chaperones known as the J-domain protein or HSP40 chaperone. A total of 13 J-domain protein genes were identified to be expressed differentially when *G. antarctica* cells were exposed to heat stress. However, there was no distinct pattern in gene expression. Some of the J-domain protein genes showed an increase in expression, while some have reduced expression and a few were expressed constitutively. The difference in the gene expression levels showed the different role of each of the J-domain protein where the pattern of gene expression signified a distinct pathway in cells either for heat stress tolerance or cell regulation. These observations suggest that the presence of J-domain protein is crucial for heat stress tolerance. Another interesting finding was the AAA ATPases related genes. Their genes were found to be upregulated when cells were exposed to thermal stress. Their gene products were probably to protect the cells from injury caused by heat.

Table 1

Genes log2 fold change expressed in *G. antarctica* cells exposed to thermal assaults of 16ºC and 20º for 6 hours exposure.

Gene name	Log2 Fold Change		Gene description	Localization	Function
	16°C	20°C			
Transcript-1	1.312770777	1.6775427	HSP70	ribosome	response to stress (GO:0006950)
Transcript-2	2.637111994	2.8093974	HSP70	mitochondria	unfolded protein binding (GO:0051082)
Transcript-3	3.021100309	3.1832709	HSP70	cytosol	response to stress (GO:0006950)
Transcript-4	3.10434755	3.0391468	HSP70	mitochondria	unfolded protein binding (GO:0051082)
Transcript-5	1.070761399	1.7666742	HSP70	membrane	unfolded protein binding (GO:0051082)
Transcript-6	4.448943261	4.3459918	HSP90	cytosol	unfolded protein binding (GO:0051082)
Transcript-7	1.270396504	1.3422306	HSF	nucleus	protein folding chaperone (GO:0005515)
Transcript-8	1.726763738	1.6441914	HSF	unknown	unknown
Transcript-9	1.103812587	1.1378671	HSF	unknown	unknown
Transcript-10	-0.82586028	-1.024400	HSF	unknown	unknown
Transcript-11	-1.61609986	-1.395891	HSF	cytosol	transcription coactivator activity (GO:0003712)
Transcript-12	-1.02205340	-0.898101	HSF	unknown	transcription coactivator activity (GO:0003712)
Transcript-13	1.573067408	1.6129076	J domain-containing protein	nucleus	unfolded protein binding (GO:0051082)
Transcript-14	1.208911258	0.9130602	J domain-containing protein	cell tip	DNA damage response, detection of DNA damage (GO:0042769)
Transcript-15	-1.13267542	-1.287980	J domain-containing protein	cytosol	unfolded protein binding (GO:0051082)
Transcript-16	0.793057075	0.8902983	J domain-containing protein	cytosol	unfolded protein binding (GO:0051082)
Transcript-17	0.935539546	0.7410631	J domain-containing protein	cytosol	ligand binding (GO:0005488)

Transcript-18	-2.59407129	-2.930641	J domain-containing protein	unknown	unknown
Transcript-19	-1.46236837	-1.715032	J domain-containing protein	nucleolus	cellular macromolecule metabolic process (GO:0044260)
Transcript-20	-0.78157824	-0.730511	J domain-containing protein	integral to membrane	response to heat (GO:0009408)
Transcript-21	-2.25636593	-2.423607	J domain-containing protein	unknown	transcription factor complex (GO:0005667)
Transcript-22	0.890986244	0.9163676	J domain-containing protein	unknown	cellular protein modification (GO:0044267)
Transcript-23	-0.97927338	-1.255140	J domain-containing protein	transport vesicle	unfolded protein binding (GO:0051082)
Transcript-24	1.913958074	1.9803085	J domain-containing protein	unknown	low-density lipoprotein receptor (GO:0050750)
Transcript-25	1.326521557	1.1974405	J domain-containing protein	unknown	metal ion binding (GO:0046872)
Transcript-26	1.475469091	1.347539	TRiC	cytosol	unfolded protein binding (GO:0051082)
Transcript-27	1.506360611	1.5624396	TRiC	ribosome	cellular response to stress (GO:0033554)
Transcript-28	1.088996167	1.2893943	TRiC	centriole	unfolded protein binding (GO:0051082)
Transcript-29	1.734516981	2.1207879	TRiC	nucleus	unfolded protein binding (GO:0051082)
Transcript-30	-1.14134298	-0.900197	AAA ATPase	mitochondria	protein binding (GO:0005488)
Transcript-31	0.830731866	1.3222925	AAA ATPase	mitochondria	isoform-specific homophilic binding (GO:0042802)
Transcript-32	2.713533264	1.6524344	AAA ATPase	integral to membrane	nucleotide binding (GO:0000166)
Transcript-33	1.197937163	2.2633874	AAA ATPase	membrane	response to stimulus (GO:0050896)

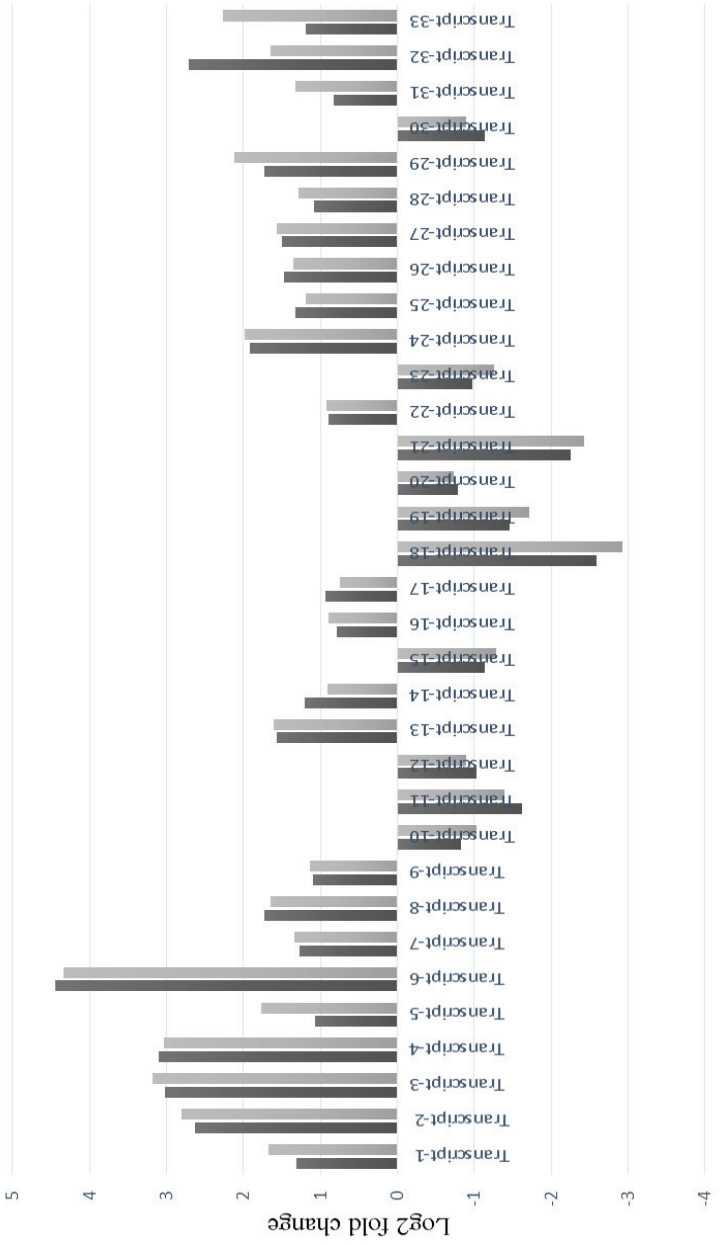

Fig. 1. Expression level of molecular chaperone genes $\log_2$ fold change when exposed to heat shock at 16ºC and 20ºC for 6 hours. Transcripts 1–5 = HSP70; Transcript 6 = HSP90; Transcripts 7–12 = HSF; Transcripts 13–25 = J domain-containing protein; Transcripts 26–29 = TRiC; and Transcripts 30–33 = AAA ATPase.

Gene ontology analysis of *G. antarctica* heat response. — The biological significance of the types of genes triggered by heat stress in *G. antarctica* has not been identified previously. Using Gene Ontology (GO) description, the details on the molecular function, biological process and cellular component were well-described. Among the heat stress-induced proteins were GO termed proteins that were associated with protein binding, response to a stimulus, chaperone binding, cellular response to stress, oxidation and reduction, ATP binding, DNA damage response and regulation for cellular protein metabolic.

HSP70 is one of the ubiquitous class of proteins in the chaperone system and the workhorse chaperone of eukaryotic cells compared to bacterial cells that greatly depend on GroEL/ES chaperonin for cell regulation (Frydman 2001). In eukaryotic cells, HSP70 involves in a large variety of cellular processes such as *de novo* protein folding, assembly of newly synthesized proteins, refolding of stress-denatured proteins, degradation of unstable proteins, protein transport and membrane translocation (Bukau *et al*. 2000; Hartl and Hayer-Hartl 2002; Young *et al*. 2003). HSP70s were found in the mitochondria, cytosol, ribosome, and membrane in *G. antarctica* PI12 (Table 1). By using $\log_2$ fold change value, it was obvious that the expression of all HSP70 genes increased between 1 to 3-fold when cells were exposed to heat stress at 16ºC and 20ºC for 6 hours (Table 1 and Figure 1). The upregulation of HSP70 genes was probably to protect the cells from thermal-caused injury, while the exposure to a much higher temperature of 20ºC triggered cells to further upregulate the expression of HSP70 genes to protect cells from death.

Comparative analysis between psychrophilic yeast, *G. antarctica*, and mesophilic yeast, *S. cerevisiae* revealed some interesting findings. In *S. cerevisiae*, the SSA HSP70s encode four genes; SSA1, SSA2, SS3 and SS4 where all of these genes play different, significant roles in *S. cerevisiae* response towards heat stress (Table 2). SSA1 and SSA2 are well known to be important for a wide range of protein homeostatic functions in cells such as protein folding, translocation, and degradation where these can be seen from their expression level which are constitutively expressed and induced under stress conditions (Sharma *et al*. 2009). In contrast, SSA3 and SSA4 are expressed only under stress conditions, induced upon heat-shock, high express in strains deleted for SSA1/2 and are transcriptionally derepressed in the absence of the constitutive HSP70s (Nelson *et al*. 1992). Functions of HSP70 in *G. antarctica* were parallel to HSP70 in *S. cerevisiae* whereby their gene expression levels towards thermal assault reflected their possible functions as molecular chaperones. In *G. antarctica*, several HSP70 protein-coding genes with high homology to those from *S. cerevisiae* were found to be significantly upregulated after exposure to thermal stress. In *S. cerevisiae*, SSC1 has been found to be one of the distinct HSP70s within the mitochondria serving two primary functions, to assist protein translocation and protein folding (Baumann *et al*. 2000). Extensive studies on

Table 2

Homology of transcript-1 to 5 from *G. antarctica* to proteins in *S. cerevisiae*.

Gene ID	Protein hits with *S. cerevisiae*	Score (bits)	E-value	Description
Transcript-1	SSA4	401.1	4.00E-216	highly induced HSPs upon stress plays a role in SRP-dependent cotranslational protein-membrane targeting and translocation
Transcript-2	SSC1	781.6	1.10E-228	involved in protein translocation and folding
Transcript-3	SSE1	419	1.50E-119	component of the heat shock protein *Hsp90* chaperone complex; binds unfolded proteins
Transcript-4	SSZ1	210.6	8.00E-57	binds ATP, interacts with Zuo1p (a *DnaJ* homolog) to form a ribosome--associated complex, also involved in pleiotropic drug resistance
Transcript-5	KAR2	755.2	9.30E-221	mediate protein folding in the ER and may play a role in ER export of soluble proteins; regulates the unfolded protein response

HSP70 have indicated the importance of SSC1 in promoting proper protein folding. A study using wild type yeast cells showed an enzyme dihydrofolate reductase folded properly into its mature form and therefore is resistant to proteolysis. In contrast, the dihydrofolate reductase in SSC1-mutant cell was almost completely sensitive to proteinase K (Kang *et al*. 1990). In addition, studies on protein folding showed that denatured luciferase refolded to its functional state in the presence of SSC1, confirming its role as a molecular chaperone which assists proper protein folding (Liu *et al*. 2001). SSC1 is vital to stabilize and maintain unfolded or partially folded proteins in a soluble state until the non-native proteins are properly folded in a correct manner (Wagner *et al*. 1994). In *G. antarctica*, the expression level of a gene homolog to yeast SSC1 (Transcript-2) was upregulated 2.6-fold when cells were exposed to heat stress at 16ºC and increased to 2.8-fold when cells were exposed to a higher temperature (Tables 1 and 2). This observation suggests that the presence of SSC1 for non-native proteins binding is critical for recovery during thermal stress. The SSE1 is a member of HSP110, a divergent of HSP70 chaperone class where both HSP110 and HSP70 shared similar domain architecture of an amino-terminal ATPase domain and a substrate binding domain (Yasuda *et al*. 1995; Ishihara *et al*. 1999; Oh *et al*. 1999). In yeast, the SSE1 acquire

"holdase" activity where it has the capability to hold the non-native proteins and stabilize them for proper protein folding preventing the accumulation of aggregates (Brodsky *et al*. 1999; Oh *et al*. 1999). SSE1 was reported to be weakly associated with the HSP90 where studies reported the absence of SSE1 caused a decline of HSP90 activity (Liu *et al*. 1999). In *G. antarctica*, a gene homolog (Transcript-3) of SSE1 was upregulated 3-fold and increase fairly to 3.1-fold when cells were exposed to 16ºC and 20ºC. This finding supports the function of SSE1 plausibly as one of the vital components in cells in maintaining cell homeostasis preventing the formation of aggregates or non-native proteins that could cause toxicity and cell lethality during thermal stress. In *S. cerevisiae*, the SSZ1 is the atypical cytosolic HSP70 superfamily that has been shown to activate transcription factors associated with induction of genes related to the stress response, lipid metabolism, the ER-associated degradation pathway (ERAD) and dismissal of cytotoxic compounds (Bosis *et al*. 2009). Recently, a proteomic study on *S. cerevisiae* reported that SSZ1 gene is differentially expressed in response to heat-shock with a median fold change of 3.3 (Mackenzie *et al*. 2016). From our *G. antarctica* transcriptome data, the expression of SSZ1 gene homolog (Transcript-4) was upregulated 3.1-fold when cells were exposed to 16ºC and remained relatively constant around 3.0-fold change at 20ºC (Tables 1 and 2). The exact biological function of SSZ1 towards thermal stress is vague, however, its importance in cells is indispensable. Mutagenesis studies have shown that KAR2 carries a primary function in preventing aggregation of partially folded proteins where the absence of it would cause an accumulation of aggregates in the endoplasmic reticulum (Simon *et al*. 1995). In *G. antarctica*, the expression of KAR2 gene homolog (Transcript-5) was upregulated 1 to 1.7-fold when cells were exposed to temperatures higher than growth optimal temperature, 12ºC. It is likely that KAR2 homolog from *G. antarctica* interacts with partially folded proteins upon proteotoxic stress and possibly many other substrates as part of cell regulation. Hence, the upregulation of all HSP70 genes in *G. antarctica* correlated well with the functions of HSP70 genes in *S. cerevisiae* in response to thermal stress.

HSP90. — HSP90 is a dimeric molecular chaperone which is evolutionarily conserved and highly abundant in cells that is important in the regulation of many fundamental cellular processes including cell cycle control, cell survival, hormone signaling and response to cellular stress (Zhao *et al*. 2005; Wandinger *et al*. 2008; Richter *et al*. 2010). HSP90 is found in the eukaryotic cell cytosol, nucleus and organelles and some studies reported that HSP90 could also be found on the cell surface of various cell types, suggesting a distinct extracellular chaperoning activity (Eustace *et al*. 2004; Sidera *et al*. 2008; Trepel *et al*. 2010). The importance of HSP90 has been reported in yeast whereby two of its genes, HSC82 and HSP82 that encoded HSP90 were expressed constitutively

and inducible upon exposure to heat-shock (Morano *et al*. 1999). It was reported that HSP90 regulates the formation of the correct conformation and activation of hundreds of proteins referred to as HSP90 client proteins (Zhao *et al*. 2005; McClellan *et al*. 2007). Unlike the HSP70 which recognize non-native proteins indiscriminately including unfolded and misfolded proteins, HSP90 plays a primary role in the final step of protein maturation by collaborating with HSP70, a myriad of co-chaperones and client proteins by forming complex macromolecular structures (Pratt and Toft 2003). In contrast to HSP70 which binds to the nascent polypeptide chain, the association with HSP90 occurs at a later stage of the client folding process (Li *et al*. 2012). In *G. antarctica*, the HSP90 gene (Transcript-6) was differentially expressed with the highest fold-of-change of more than four compared to other genes. Exposure to heat stress indeed triggered the HSP90 gene expression level for specific purposes presumably for thermal stress response. Interestingly, the gene expression level remained high when cells were exposed to a higher temperature which was lethal to cells. This finding supports the idea that HSP90 plays significant roles not only for cell regulation and other fundamental cellular processes but it may be one of the vital components of the cells as a shield to protect from thermal injury and possibly cell death.

Heat-shock factor (HSF). — HSFs are transcriptional activators of heat-shock genes in eukaryotes. In the absence of cellular stress, HSF is repressed by the association with heat-shock proteins and therefore not active. Cellular stress such as temperature increase causes proteins in the cell to misfold. As a response, the heat-shock proteins will bind to the misfolded proteins and dissociate from HSF (Parsell and Lindquist 1993; Zou *et al*. 1998). *S. cerevisiae* possesses a single essential HSF gene known as HSF1, while distinct HSF isoforms have been identified in humans (Liu *et al*. 1997). The expression of HSF genes in *G. antarctica* was intriguing whereby some were seen upregulated while others were downregulated when cells were exposed to heat (Figure 1). Although half of the HSF genes were identified with unknown function (Table 1), the HSFs (Transcript-11 and 12) related to transcription coactivator were down-regulated. Under the permissive condition, the eukaryotic heat-shock transcription factor such as HSF1 is kept in a complex with HSP90, HSP70 and HSP40 proteins. The HSF1 is primed for degradation by these chaperones, thereby reducing its cellular level and keeping the heat-shock genes untranscribed (Zou *et al*. 1998; Rodriguez *et al*. 2008; Richter *et al*. 2010). In cells, the regulation of HSF1 is complex where it involves phosphorylation, posttranslational modifications, and oligomerization of complex proteins (Prahlad and Morimoto 2009; Akerfelt *et al*. 2010). The induction of HSF1 gene expression is due to the disturbance of protein homeostasis in cells under thermal stress whereby the HSFs are the critical components for HSPs regulation (Voellmy and Boellmann 2007). As

cells were subjected to thermal stress, the expression profile of HSF genes in *G. antarctica* splendidly explained the roles of HSFs where their gene expression was induced by the presence of non-native proteins for HSP genes regulation. The downregulation of HSF genes was probably due to the presence of other unemployed molecular chaperones where transcription of certain genes was repressed due to thermal stress to cells.

Interestingly in *G. antarctica*, genome annotation identified several genes that were related to the HSF family proteins. The importance of HSFs is undeniable where they mediate gene expression of HSP genes in response to heat-shock. It was possible that *G. antarctica* acquired more than one HSF and possible isoforms to cope with environmental-induced stress, promote rapid response at the transcription level and coordinate cell defense in this context, the thermal stress. Phylogenetic tree analysis showed that these genes were evolutionary related and possibly share similarities in functions and architecture (Figure 2). These genes that encoded the HSF family proteins contain highly conserved motifs which were i) the winged helix DNA-binding proteins consists of two wings, three alpha helices, and three beta-sheets and ii) heat-shock factor DNA binding transcriptional activator which binds specifically to heat-shock promoter elements (Liu *et al*. 1997).

TRiC — The chaperonin containing *t*-complex polypeptide-1 ring complex, which is also known as TRiC plays a central role in cellular homeostasis by assisting the folding of ~10% of newly synthesised proteins which includes

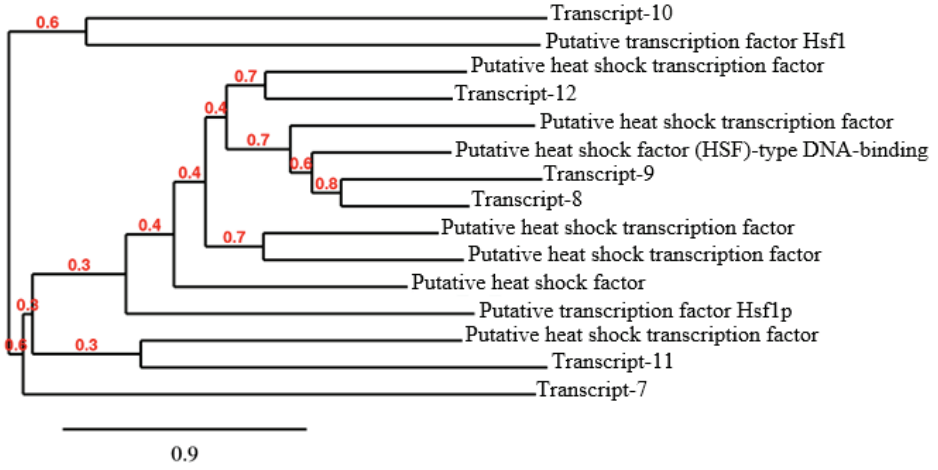

Fig. 2. Phylogenetic tree of all *G. antarctica* genes encoded for HSFs family proteins inferred by the neighbor-joining method based on the amino acid sequences. 100 bootstraps were used to infer the reliability of branching points. The scale bar indicated the number of amino acid substitution in every site. All HSFs gene found in the transcriptome data were labeled as transcript-7 to 12.

tubulins, actins, luciferin, Von Hippel-Lindau disease tumor suppressor (VHL), histone deacetylase 3 and other client proteins (Frydman *et al*. 1994; Feldman *et al*. 1999; Guenther *et al*. 2002; Valpuesta *et al*. 2002). TRiC complex has been known to be the gatekeeper of cellular protein homeostasis especially in assisting a large number of protein folding of multiple structural classes and complex topology (Thulasiraman *et al*. 1999; Dekker *et al*. 2008; Yam *et al*. 2008). Hence, the cells' metabolic systems could have gone haywire if the TRiC complex is structurally prone to be compromised by temperature fluctuation. Our data show that the *G. antarctica* TRiC subunits were easily identified by the high similarities of the amino acids with other mesophilic TRiC which were the *S. cerevisiae* (PDB ID: 4V81) and the mammalian TRiC of *Bos taurus* (PDB ID: 3IYG) with significant E-value of 0 for all TRiC proteins. All identified TRiC genes of *G. antarctica* were upregulated one to 2-fold when cells were exposed to thermal stress. The upregulation of *G. antarctica* TRiC genes in both temperatures 16ºC and 20ºC (lethal) shows that the chaperonin is important for cell regulations at elevated growth temperatures.

J-domain containing proteins. — The J-domain containing proteins or J-proteins are also known as the HSP40s (Kampinga and Craig 2010) due to their molecular mass in yeast and mammalian cells are the largest class of cofactor for HSP70s. Their primary functions in cells are to bind to non-native proteins, deliver them to the HSP70s, interact with the HSP70s ATPase domain and stimulate the ATP hydrolysis for protein folding. In *S. cerevisiae*, a total of 22 J-domain proteins have been identified which display highly conserved signature (Kampinga and Craig 2010). Besides, J-proteins are localized in most cellular locations such as mitochondria, ER lumen and ER membrane where this possibly increases the efficiency in HSP70 activities in promoting cellular processes. All of the J-proteins play significant roles where the major cytoplasmic J-proteins bind vast client proteins and accelerate ATP hydrolysis in HSP70, some bind to only specific clients while others not at all (Morano *et al*. 1999). Recent studies on *G. antarctica* genome revealed some 30 J-Domain where 13 genes were identified with significant expression in the transcriptome data. Some of the *G. antarctica* J-Domain protein genes were upregulated and some were downregulated when cells were exposed to heat stress (Table 1 and Figure 1). In both *S. cerevisiae* and *Caenorhabditis elegans*, the J-proteins were found to support disaggregation by efficiently extract substrate from aggregates and assist protein folding to native state (Goloubinoff *et al*. 1999). Several J-protein genes expression were upregulated when *G. antarctica* cells were exposed to thermal stress. These J-proteins most probably delivered the misfolded proteins to the HSP70 to stimulate ATP hydrolysis for protein folding. However, our data also suggested that some of the J-protein genes were down-regulated when cells were subjected to thermal stress. As J-proteins serve as sensors for protein folding linking the HSP70 systems for

stress response and normal cellular growth, it may be proposed that the down-regulation of certain J-protein genes can be due to their functions as growth regulator which was repressed during cell stress. This can be seen from the yeast ortholog, Mas5 where its expression as growth rate sensor regulating G/S entry was indeed repressed when cells were subjected to stress (Ferrezuelo *et al*. 2012). This mechanism is probably one of the adaptive approaches to provide efficient and highly sensitive adjustment in energy consumption means for cells to adapt to stress by temporary lowering or inhibiting cell growth.

AAA ATPases. — The AAA ATPases proteins form diverse superfamily found in all organisms which extract energy from ATP hydrolysis for diverse cellular processes such as protein unfolding and degradation, peroxisome biogenesis, biosynthesis, DNA recombination, replication and repair (Snider *et al*. 2008). The AAA ATPase proteins are responsible for repairing and refolding damaged proteins that form non-functional aggregates. Destroying and synthesizing new proteins consume more energy compared to protein refolding and repairing hence, the AAA ATPase proteins play a major role as the protein proteolytic machinery as part of the stress response (Richter *et al*. 2010). These AAA ATPase proteins are remarkable as they exhibit either chaperone or protease function based on the cell surrounding. When cells are subjected to heat-shock, these proteins will either refold the non-native proteins or degrade the proteins if they were severely damaged (Spiess *et al*. 1999). In yeast, its proteolytic system is the most highly induced genes involving proteosomal degradations as part of heat-shock response (Richter *et al*. 2010). Additionally, the expression of AAA ATPase protein gene (Transcript-33) in *G. antarctica* was found to be upregulated one-fold higher in cells, which were exposed to 20ºC when compared to 16ºC. The induction of AAA ATPase related protein-coding genes in *G. antarctica* probably played an essential role in activating protein proteolytic activity or degradation depending on the stress that the cells endured. There was one gene related to AAA ATPase proteins that were down-regulated (Transcript-30), possibly because it was involved in cellular processes that were not feasible during thermal stress conditions and therefore was repressed as part of thermal adaptation strategy.

Conclusions

In general, the thermal heat response studies carried out on bacterial and eukaryotic systems provide an insight into the strategies they employ. They are found to use one or more similar strategies. However, the survival programs of each organism may trigger different regulation levels of heat-shock response, promoting unique stress management systems. Temperature is the major barrier to life for all organisms where each organism has its way of sensing heat functioning like a thermometer before

sending a message to cells for regulation of adaptation strategies. In yeast, a small heat-shock protein, HSP26 acts as the temperature-regulated molecular chaperone, where its dissociation induced by heat is essential for other chaperones activities (Haslbeck *et al*. 1999). Interestingly, we have discovered the homolog of HSP26 in *S*. *cerevisiae* that plays a vital role in promoting thermotolerance in cells and showed significant up-regulation due to thermal stress in *G*. *antarctica* PI12 (Yusof *et al*. 2016). Furthermore, the fluidity or rigidity of membranes seems to act as the monitor or "thermometer" for cells to orchestrate heat-shock and even cold shock responses (Los *et al*. 2013). Another temperature sensing mechanism is through the accumulation of degraded proteins or aggregates in cells due to heat-shock. When cells started to accumulate non-native disfunctional proteins, cells will send feedback to induce expression of related genes that play major roles in thermal adaptations before the heat-shock disrupt the cellular homeostasis causing cell death (Klinkert and Narberhaus 2009). The present study identified differentially expressed genes in *G*. *antarctica* under chronic heat stress. From the transcriptome data, we identified several common genes shared between *G*. *antarctica* and *S*. *cerevisiae* which suggest that these chaperones might play similar roles as a defense mechanism towards heat-shock. Interestingly, some of the findings reflect the diversity of adaptive mechanism acquire by this psychrophilic yeast such as the regulation levels of the identified molecular chaperones and the presence of certain genes such as the presence of HSFs that need further studies to elucidate their functions. Information on the expression of genes under heat-shock is valuable for us to understand the network of molecular chaperones and more importantly in the context of protein folding related to thermal adaptation in psychrophilic yeast.

Acknowledgments. — The funding supports from the Ministry of Science, Technology, and Innovation (MOSTI), Malaysia, under the Antarctica Flagship Programme (Sub-Project 1: FP1213E036), and Science Fund Project No: 08-05-MGI-GMB001 are gratefully acknowledged. We would like to thank Mr. Ahmad Faisal Mohamad and Ms. Nurohaida Ab Aziz for compiling the transcriptome data for analysis, those who are involved in the structural project of *Glaciozyma antarctica* in Malaysia Genome Institute and others who assisted for technical assistance and helpful discussions.

References

AKERFELT M., MORIMOTO R.I. and SISTONEN L. 2010. Heat-shock factors: integrators of cell stress, development and lifespan. *Nature Reviews Molecular Cell Biology* 11: 545–555.

BANEYX F. and MUJACIC M. 2004. Recombinant protein folding and misfolding in *Escherichia coli*. *Nature Biotechnology* 22: 1399–1408.

BAUMANN F., MILISAV I., NEUPERT W., and HERRMANN J.M. 2000. Ecm10, a novel hsp70 homolog in the mitochondrial matrix of the yeast *Saccharomyces cerevisiae*. *FEBS Letters* 487: 307–312.

BEN-ZVI A.P. and GOLOUBINOFF P. 2001. Mechanisms of disaggregation and refolding of stable protein aggregates by molecular chaperones. *Journal of Structural Biology* 135: 84–93.

Boo S., Wong C., Rodrigues K., Najimudin N., Murad A. and Mahadi N. 2013. Thermal stress responses in Antarctic yeast, *Glaciozyma antarctica* PI12, characterized by real-time quantitative PCR. *Polar Biology* 36: 381–389.

Bosis E., Salomon D., Ohayon O., Sivan G., Bar-Nun S. and Rabinovich E. 2009. Ssz1 restores endoplasmic reticulum-associated protein degradation in cells expressing defective Cdc48-Ufd1-Npl4 complex by upregulating Cdc48. *Genetics* 184: 695–706.

Brodsky J.L., Werner E.D., Dubas M.E., Goeckeler J.L., Kruse K.B. and McCracken A.A. 1999. The requirement for molecular chaperones during endoplasmic reticulum-associated protein degradation demonstrates that protein export and import are mechanistically distinct. *The Journal of Biological Chemistry* 274: 3453–3460.

Buchan J.R. and Parker R. 2009. Eukaryotic stress granules: the ins and outs of translation. *Molecular Cell* 36: 932–941.

Bukau B., Deuerling E., Pfund C. and Craig E.A. 2000. Getting newly synthesized proteins into shape. *Cell* 101: 119–122.

D'Amico S., Marx J.C., Gerday C. and Feller G. 2003 Activity–stability relationships in extremophilic enzymes. *The Journal of Biological Chemistry* 278: 7891–7896.

DeConto R.M. and Pollard D. 2016. Contribution of Antarctica to past and future sea-level rise. *Nature* 531: 591–597.

Dekker C., Stirling P.C., McCormack E.A., Filmore H., Paul A., Brost R.L., Costanzo M., Boone C., Leroux M.R., and Willison K.R. 2008. The interaction network of the chaperonin CCT. *EMBO Journal* 27: 1827–1839.

Ellis J.R. 2007. Protein Misassembly: Macromolecular crowding and molecular chaperones. *Advances in Experimental Medicine and Biology* 594: 1–13.

Eustace B.K. and Jay D.G. 2004. Extracellular roles for the molecular chaperone, hsp90. *Cell Cycle* 3: 1098–1100.

Ferrezuelo F., Colomina N., Palmisano A., Garí E., Gallego C., Csikász-Nagy A. and Aldea M. 2012. The critical size is set at a single-cell level by growth rate to attain homeostasis and adaptation. *Nature Communications* 3: 1012.

Feldman D.E., Thulasiraman V., Ferreyra R.G. and Frydman J. 1999. Formation of the VHL–elongin BC tumor suppressor complex is mediated by the chaperonin TRiC. *Molecular Cell* 4: 1051–1061.

Firdaus-Raih M., Hashim N.H.F., Bharudin I., Abu Bakar M.F., Huang K.K., Alias H., Lee B.K.B., Mat Isa M.N., Mat-Sharani S., Sulaiman S., Tay L.J., Zolkefli R., Muhammad Noor Y., Law D.S.N., Abdul Rahman S.H., Md-Illias R., Abu Bakar F.D., Najimudin N., Abdul Murad A.M. and Mahadi N.M. 2018. The *Glaciozyma antarctica* genome reveals an array of systems that provide sustained responses towards temperature variations in a persistently cold habitat. *PLoS One* doi: 10.1371/journal.pone.0189947.

Frydman J. 2001. Folding of newly translated proteins in vivo: the role of molecular chaperones. *Annual Reviews of Biochemistry* 70: 603–647.

Frydman J., Nimmesgern E., Ohtsuka K. and Hartl F.U. 1994. Folding of nascent polypeptide chains in a high molecular mass assembly with molecular chaperones. *Nature* 370: 111.

Goloubinoff P., Mogk A., Zvi A.P.B., Tomoyasu T. and Bukau B. 1999. Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. *Proceedings of the National Academy of Sciences of the United States of America* 96: 13732–13737.

Gong Y., Kakihara Y., Krogan N., Greenblatt J., Emili A., Zhang Z. and Houry W.A. 2009. An atlas of chaperone-protein interactions in *Saccharomyces cerevisiae*: implications to protein folding pathways in the cell. *Molecular Systems Biology* 5:275.

GUENTHER M.G., YU J., KAO G.D., YEN T.J. and LAZAR M.A. 2002. Assembly of the SMRT–histone deacetylase 3 repression complex requires the TCP-1 ring complex. *Genes and Development* 16: 3130–3135.

HAAS B.J., PAPANICOLAOU A., YASSOUR M., GRABHERR M., BLOOD P.D., BOWDEN J., COUGER M.B., ECCLES D., LI B., LIEBER M., MACMANES M.D., OTT M., ORVIS J., POCHET N., STROZZI F., WEEKS N., WESTERMAN R., WILLIAM T., DEWEY C.N., HENSCHEL R., LEDUC R.D., FRIEDMAN N. and REGEV A. 2013. *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols* 8: 1494–1512.

HARTL F.U. and HAYER-HARTL M. 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295: 1852–1858.

HASLBECK M., WALKE S., STROMER T., EHRNSPERGER M., WHITE H.E., CHEN S., SAIBIL H.R. and BUCHNER J. 1999. Hsp26: a temperature-regulated chaperone. *The EMBO Journal* 18: 6744–51.

HUANG H.C., SHERMAN M.Y., KANDROR O. and GOLDBERG A.L. 2001. The molecular chaperone DnaJ is required for the degradation of a soluble abnormal protein in *Escherichia coli*. *The Journal of Biological Chemistry* 276: 3920–3928.

ISHIHARA K., YASUDA K. and HATAYAMA T. 1999. Molecular cloning, expression and localization of human 105 kDa heat-shock protein, hsp105. *Biochimia et Biophysica Acta* 1444: 138–142.

JOPLIN K.H., YOUCUM G.D. and DENLINGER D.L. 1990. Cold shock elicits expression of heat-shock proteins in the flesh fly, *Sarcophaga crassipalpis*. *Advances in Insect Physiology* 36: 825–834.

KAMPINGA H.H. and CRAIG E.A. 2010. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nature Reviews Molecular Cell Biology* 11: 579–592.

KANG P.J., OSTERMANN J., SHILLING J., NEUPERT W., CRAIG E.A. and PFANNER N. 1990. Requirement for hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins. *Nature* 348: 137–143.

KLINKERT B. and NARBERHAUS F. 2009. Microbial thermosensor. *Cell and Molecular Life Sciences* 66: 2661–76.

LI J., SOROKA J. and BUCHNER J. 2012. The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. *Biochimia et Biophysica Acta* 1823: 624–35.

LI W.Z. and GODZIK A. 2006. Cd-Hit: A fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22: 1658–1659.

LIU Q., KRZEWSKA J., LIBEREK K. and CRAIG E.A. 2001. Mitochondrial Hsp70 Ssc1: role in protein folding. *The Journal of Biological Chemistry* 276: 6112–6118.

LIU X.D., LIU P.C., SANTORO N. and THIELE D.J. 1997. Conservation of a stress response: human heat-shock transcription factors functionally substitute for yeast HSF. *EMBO Journal* 16: 6466–6477.

LIU X.D., MORANO K.A. and THIELE D.J. 1999. The yeast Hsp110 family member, Sse1, is an Hsp90 cochaperone. *Journal of Biological Chemistry* 274: 26654–26660.

LOS D.A., MIRONOV K.S. and ALLAKHVERDIEV S.I. 2013. Regulatory role of membrane fluidity in gene expression and physiological functions. *Photosynthesis Research* 116: 489–509.

LOVE M.I., HUBER S. and ANDERS S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15: 550.

MACKENZIE R.J., LAWLESS C., HOLMAN S.W., LANTHALER K., BEYNON R.J., GRANT C.M., HUBBARD S.J. and EYERS C.E. 2016. Absolute protein quantification of the yeast chaperone under conditions of heat-shock. *Proteomics* 16: 2128–2140.

MCCLELLAN A.J., XIA Y., DEUTSCHBAUER A.M., DAVIS R.W., GERSTEIN M. and FRYDMAN J. 2007. Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. *Cell* 131: 121–135.

MOGK A., DEUERLING E., VORDERWULBECKE S., VIERLING E. and BUKAU B. 2003. Small heat-shock proteins, ClpB and the DnaK system form a functional triade in reversing protein aggregation. *Molecular Microbiology* 50: 585–595.

MORANO K.A., SANTORO N., KOCH K.A. and THIELE D.J. 1999. A *trans*-Activation Domain in Yeast Heat-shock Transcription Factor Is Essential for Cell Cycle Progression during Stress. *Molecular and Cellular Biology* 19: 402–411.

NELSON R.J., HESCHL M.F. and CRAIG E.A. 1992. Isolation and characterization of extragenic suppressors of mutations in the SSA hsp70 genes of *Saccharomyces cerevisiae*. *Genetics* 131: 277–285.

OH J., EASTON D., MURAWSKI M., KANEKO Y. and SUBJECK J.R. 1999. The chaperoning activity of Hsp110. Identification of functional domains by the use of targeted deletions. *The Journal of Biological Chemistry* 274: 15712–15718.

PARSELL D.A. and LINDQUIST S. 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annual Review of Genetics* 27: 437–96.

PIPER P.W., MILLSON S.H., MOLLAPOUR M., PANARETOU B., SILIGARDI G., PEARL L.H. and PRODROMOU C. 2003. Sensitivity to Hsp90-targeting drugs can arise with mutation to the Hsp90 chaperone, cochaperones and plasma membrane ATP binding cassette transporters of yeast. *European Journal of Biochemistry* 270: 4689–4695.

PRAHLAD V. and MORIMOTO R.I. 2009. Integrating the stress response: lesson for neurodegenerative diseases from *C. elegans*. *Trends in Cell Biology* 19: 52–61.

PRATT W.B. and TOFT D.O. 2003. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Experimental Biology and Medicine* 228: 111–133.

RICHTER K., HASLBECK M. and BUCHNER J. 2010. The heat-shock response: life on the verge of death. *Molecular Cell* 40: 253–266.

RODRIGUEZ F., ARSÈNE-PLOETZE F., RIST W., RÜDIGER S., SCHNEIDER-MERGENER J., MAYER M.P. and BUKAU B. 2008. Molecular basis for regulation of the heat-shock transcription factor sigma32 by the DnaK and DnaJ chaperones. *Molecular Cell* 32: 347–58.

SHARMA D., MARTINEAU C.N., LE DALL M.T., REIDY M., MASISON D.C. and KABANI M. 2009. Function of *SSA* subfamily of Hsp70 within and across species varies widely in complementing *Saccharomyces cerevisiae* cell growth and prion propagation. *PLoS One* 4: e6644.

SIDERA K. and PATSAVOUDI E. 2008. Extracellular HSP90: conquering the cell surface. *Cell Cycle* 7: 1564–1568.

SIMONS J.F., FERRO-NOVICK S., ROSE M.D. and HELENIUS A. 1995. BiP/Kar2p serves as a molecular chaperone during carboxypeptidase Y folding in yeast. *The Journal of Cell Biology* 130: 41–49.

SNIDER J., THIBAULT G. and HOURY W.A. 2008. The AAA+ superfamily of functionally diverse proteins. *Genome Biology* 9: 216.

SPIESS C., BEIL A. and EHRMANN M. 1999. A temperature-dependent switch from chaperone to protease in a widely conserved heat-shock protein. *Cell* 97: 339–47.

SZALAY M.S., KOVÁCS I.A., KORCSMÁROS T., BÖDE C. and CSERMELY P. 2007. Stress-induced rearrangements of cellular networks: Consequences for protection and drug design. *FEBS Letters* 581: 3675–3680.

THULASIRAMAN V., YANG C.F., and FRYDMAN J. 1999. *In vivo* newly translated polypeptides are sequestered in a protected folding environment. *EMBO Journal* 18: 85–95.

TOIVOLA D.M., STRNAD P., HABTEZION A. and OMARY M.B. 2010. Intermediate filaments take the heat as stress proteins. *Trends in Cell Biology* 20: 79–91.

TOMOYASU T., MOGK A., LANGEN H., GOLOUBINO P. and BUKAU B. 2001. Genetic dissection of the roles of chaperones and proteases in protein folding and degradation in the *Escherichia coli* cytosol. *Molecular Microbiology* 40: 397–413.

TREPEL J., MOLLAPOUR M., GIACCONE G. and NECKERS L. 2010. Targeting the dynamic HSP90 complex in cancer. *Nature Reviews Cancer* 10: 537–549.

VALPUESTA J.M., MARTÍN-BENITO J., GÓMEZ-PUERTAS P., CARRASCOSA J.L. and WILLISON K.R. 2002. Structure and function of a protein folding machine: the eukaryotic cytosolic chaperonin CCT. *FEBS Letters* 529: 11–16.

VERGHESE J., ABRAMS J., WANG Y. and MORANO K.A. 2012. Biology of the heat-shock response and protein chaperones: budding yeast (*Saccharomyces cerevisiae*) as a model system. *Microbiology and Molecular Biology Reviews* 76: 115–58.

VIGH L., NAKAMOTO H., LANDRY J., GOMEZ-MUNOZ A., HARWOOD J.L. and HORVATH I. 2007. Membrane regulation of the stress response from prokaryotic models to mammalian cells. *Annals of the New York Academy of Sciences* 1113: 40–51.

VOELLMY R. and BOELLMANN F. 2007. Chaperone regulation of the heat-shock protein response. *Advances in Experimental Medicine and Biology* 594: 89–99.

WAGNER I., ARLT H., VAN DYCK L., LANGER T. and NEUPERT W. 1994. Molecular chaperones cooperate with PIM1 protease in the degradation of misfolded proteins in mitochondria. *EMBO Journal* 13: 5135–5145.

WANDINGER S.K., RICHTER K. and BUCHNER J. 2008. The Hsp90 chaperone machinery. *The Journal of Biological Chemistry* 283: 18473–18477.

WONG C.M.V.L., BOO S.Y., VOO C.L.Y., ZAINUDDIN N. and NAJIMUDIN N. 2019. A comparative transcriptomic analysis provides insights into the cold-adaptation mechanisms of a psychrophilic yeast, *Glaciozyma antarctica* PI12. *Polar Biology 42*: 541–553.

YAM A.Y., XIA Y., LIN H.T., BURLINGAME A., GERSTEIN M. and FRYDMAN J. 2008. Defining the TRiC/CCT interactome links chaperonin function to stabilization of newly made proteins with complex topologies. *Nature Structural and Molecular Biology* 15: 1255–1262.

YASUDA K., NAKAI A., HATAYAMA T. and NAGATA K. 1995. Cloning and expression of murine high molecular mass heat-shock proteins, hsp105. *The Journal of Biological Chemistry* 270: 29718–29723.

YOUNG J.C., BARRAL J.M. and ULRICH HARTL F. 2003. More than folding: localized functions of cytosolic chaperones. *Trends in Biochemical Sciences* 28: 541–547.

YUSOF N.A., ABU BAKAR F.D., ILLIAS R.M., MAHADI N.M. and MURAD A.M.A. 2015. In silico characterization of the *Glaciozyma antarctica* genome: mining the molecular chaperones. *Malaysian Applied Biology Journal* 44 : 161–165.

YUSOF N.A., HASHIM N.H., BEDDOE T., MAHADI N.M., ILLIAS R.M., BAKAR F.D. and MURAD A.M.A. 2016. Thermotolerance and molecular chaperone function of an SGT1-like protein from the psychrophilic yeast, *Glaciozyma antarctica*. *Cell Stress and Chaperones* 21: 707–15.

ZHAO R., DAVEY M., HSU Y.C., KAPLANEK P., TONG A., PARSONS A.B., KROGAN N., CAGNEY G., MAI D., GREENBLATT J., BOONE C., EMILI A. and HOURY W.A. 2005. Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the Hsp90 chaperone. *Cell* 120: 715–727.

ZOU J., GUO Y., GUETTOUCHE T., SMITH D.F. and VOELLMY R. 1998. Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell 94*: 471–480.

Received 22 May 2018
Accepted 20 May 2019