J. Microbiol. Biotechnol. (2010), **20**(2), 403–409
doi: 10.4014/jmb.0907.07035
First published online 7 December 2009

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Saccharomyces cerevisiae Hsp30 is Necessary for Homeostasis of a Set of Thermal Stress Response Functions

Thakur, Suresh[1]* and Amitabha Chakrabarti[2]

[1]*Division of R&D Triesta Sciences HCG Tower, #8, P. Kalinga Rao Road, Sampangi Ram Nagar, Bangalore-560 027, India*
[2]*Division of Molecular Biology, Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi-110054, India*

Received: July 29, 2009 / Revised: September 8, 2009 / Accepted: September 21, 2009

***Saccharomyces cerevisiae* Hsp30 is a plasma membrane heat shock protein that is induced by various environmental stress conditions. However, the functional role of Hsp30 during diverse environmental stressors is not presently known. To gain insight into its function during thermal stress, we have constructed and characterized a Δ*hsp30* strain during heat stress. BY4741Δ*hsp30* cells were found to be more sensitive compared with BY4741 cells, when exposed to a lethal heat stress at 50°C. When budding yeast is exposed to either heat shock or weak organic acid, it inhibits Pma1p activity. In this study, we measured the levels of Pma1p in mutant and Wt cells both during optimal temperature and heat shock temperature. We observed that BY4741Δ*hsp30* cells showed constitutive reduction of Pma1p. To gain further insights into the role of Hsp30 during heat stress, we compared the total protein profile by 2D gel electrophoresis followed by identification of differentially expressed spots by LC–MS. We observed that contrary to that expected from thermal-stress-induced changes in gene expression, the Δ*hsp30* mutant maintained elevated levels of Pdc1p, Trx1p, and Nbp35p and reduced levels of Atp2p and Sod1p during heat shock. In conclusion, Hsp30 is necessary during lethal heat stress, for the maintenance of Pma1p and a set of thermal stress response functions.**

Keywords: Hsp30, heat stress, Pma1, stress response, thermotolerance

S. cerevisiae cells respond to stress by the induction of specific stress response functions. Exposure to thermal stress leads to the Hsf1-mediated transcriptional induction of genes encoding heat shock proteins (Hsps). Hsps are chaperones that promote refolding of misfolded proteins and prevent aggregation of denatured proteins generated by heat treatment [9, 16]. Recent evidence suggest that in addition to the well-characterized heat shock response, the hyper osmotic glycerol (Hog1)–mitogen activated protein kinase (MAPK) pathway [28] and the cell wall integrity (CWI) pathway are also activated [12] by thermal stress. In addition to the above, heat shocked cells accumulate trehalose and sphingoid bases [10] that are required for protection against thermal stress and for the activation of heat shock response, respectively.

Saccharomyces cerevisiae Hsp30 is a plasma membrane heat shock protein. It has seven predicted transmembrane domains, and may belong to Family A of the G protein coupled receptor superfamily of proteins (http://www.yeastgenome.org). Hsp30 expression is induced during exposure to a variety of stress conditions including heat shock, exposure to weak organic acids, hyperosmotic stress, oxidative stress, glucose limitation, exposure to alcohol, entry into stationary phase [15, 23], and low-shear microgravity environment [25]. The mechanism of Hsp30 induction and its role in providing resistance against multiple environmental stress conditions is however not well characterized. It inhibits Pma1H^+-ATPase activity when cells are exposed to either heat shock or weak organic acids [18], leading to the conservation of cellular ATP reserves during stress exposure.

HSP30 expression is independent of Hsf1 and partially dependent on Msn2 and Msn4 [23]. Two other activators of *HSP30* expression, namely Pci8 [24] and Sfl1p [4], have also been identified. Hsp30 is a part of the Pci8-activated stress regulon [24] that includes genes induced by various environmental stress conditions.

Since the only known function of Hsp30 is unable to account for its induction in response to a multitude of stressors, we have carried out a characterization of a Δ*hsp30* mutant during heat stress.

*Corresponding author
Phone: +91 80 40206105; Fax: +91 80 40206106;
E-mail: suresh.triesta@gmail.com

Materials and Methods

Strains, Chemicals, Growth Media, and Growth Conditions

The S. cerevisiae strains used in this study are listed in Table 1. Media components were purchased from Becton, Dikinson and Co., and all reagents used were of molecular biology grade. Unless stated otherwise, yeast cells were grown at 30°C in YPD (Bacto-yeast extract 1%, Bacto-peptone 2%, and dextrose 2%) media. Media containing glycerol (yeast extract, peptone, and glycerol; YPG) and acetate (yeast extract, peptone, and acetate; YPA) were prepared by adding glycerol and sodium acetate to a final concentration of 2% (v/v) and 2% (w/v), respectively, instead of dextrose in YPD. For solid media, 2% Bacto-agar was used [1].

HSP30 Gene Disruption

S. cerevisiae BY4741Δ*hsp30* was constructed by PCR-mediated gene disruption [2, 14] using primers 1 and 2 (Table 2). Gene replacement was confirmed by the presence and absence of *LEU2* (Primers 5 and 6, Table 2) and *HSP30* coding regions (Primers 3 and 4, Table 2), respectively, by PCR amplification using genomic DNA from Δ*hsp30* cells as template.

Cell Survival Assay at 50°C

For measurement of cell survival following exposure at 50°C, 3-ml log phase cultures of BY4741 or BY4741Δ*hsp30* in minimal media containing appropriate supplements were subjected to thermal stress at either 50°C for different intervals of time or kept at 30°C (untreated control). Cells were then either spotted in 10-fold serial dilutions or plated at 500 cells/plate on appropriate plates followed by incubation at 30°C for 3 days. All experiments were performed in triplicates.

Isolation of Crude Membrane Fraction

Total membrane fraction was isolated from 25-ml log phase cultures of BY4741 and BY4741Δ*hsp30* [17, 26] that were either exposed to 40°C for 30 min or left untreated at 30°C. Heat shocked and untreated cells were precipitated and suspended in 100 µl of Buffer S (30 mM Tris-HCl, pH 8.5, 5 mM EDTA, 1 mM PMSF, 1 µM pepstatin, and 6 µM leupeptin) followed by cell lysis by vortexing in the presence of glass beads for 6×1 min with 1-min intervals on ice. The glass beads were washed twice with 500 µl of Buffer W (10 mM Tris-HCl, pH 7.5, 0.2 mM EDTA, 0.2 mM DTT, and 20% glycerol) and the pooled wash (1 ml) was centrifuged at 700 ×*g* for 10 min. The supernatant obtained was further centrifuged at 20,000 ×*g* for 30 min, and the precipitated membrane-enriched fraction was suspended in 100 µl of Buffer W and stored at −70°C. At this stage of purity, the membrane-enriched fraction had considerable cytoplasmic carryover (cytoplasmic Hsps and actin were detectable); the supernatant was however devoid of membrane proteins (Hsp30 and Pma1).

Table 1. Microbial strains and plasmids used in this study.

Strains	Genotype
BJ5416	MATα ura3-52 lys2-801 Δleu2Δ his3-Gal$^+$
A364A	MATa ura1 lys2 ade1 ade2 his7 tyr1gal1 SUC mal cup BIO
BY4741	MATa Δhis3Δ leu2 Δmet15 Δura3
BY4741Δ*hsp30*	MATa Δhis3 Δleu2Δmet15 Δ ura3 Δhsp30::LEU2

Immunoblotting

Yeast total protein was isolated from log phase cells [12] that were either untreated or subjected to different stress conditions for indicated periods of time. Protein was quantified by the Bio-Rad Bradford reagent with BSA as standard and electroblotted onto a PVDF membrane in a Bio-Rad semi-dry electrophoresis apparatus. The blot was probed with anti-Pma1 (yN-20) antibody (Santa Cruz Biotechnology, Inc.) and alkaline phosphatase conjugated secondary antibody (Santa Cruz Biotechnology, Inc.), and then developed with the alkaline phosphatase substrate 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium chloride (NBT). Anti-β-actin antibody (Abcam) was used in dilutions recommended by the manufacturer as an internal control.

Two-Dimensional Gel Electrophoresis and Peptide Mass Fingerprinting by LC–MS

One hundred µg of total protein isolated from log phase BY4741 and BY4741Δ*hsp30* cells following an exposure at 40°C for 30 min using the Bio-Rad protein isolation kit was resolved by two-dimensional gel electrophoresis; first by isoelectric focussing in a Bio-Rad 7-cm IPG strip (pH range 4–7) followed by SDS–PAGE and silver staining.

Table 2. Oligonucleotide primers used in this study.

Oligonucleotide primer	Sequence (5'–3')
HSP30 (DP) F	GTTTGAGACTTTAATATCTTTTGATTACTAAAAACAACAAATTTCAAATGAAAGGAAAGGTGAGAGCGCCGGAA
HSP30 (DP) R	TTTGAAATGTGTTAAGCAAAGAATGATTAAGACAATCTCAAGCTGCTCTATTAAGCAAGGATTTTCTTAACTTC
HSP30F	GAATTCGGATCCATGAACGATACGCTATCAAGCTTT
HSP30R	CTCGAGCCCGGGCTAAGCAGTATCTTCGACAGCTTG
LEU2F	AAAGGAAAGGTGAGAGCGCCGGAA
LEU2R	TTAAGCAAGGATTTTCTTAACTT
TRX1 F	ATGGTTACTCAATTCAAAACTGCC
TRX1 R	TTAAGCATTAGCAGCAATGGCTTG
PGK1 F	ATGTCTTTATCTTCAAAGTTGTCT
PGK1 R	TTATTTCTTTTCGGATAAGAAAGC
YAL037C-A F	ATGTCAATCTCGTTTCCAAAAATG
YAL037C-A R	GTATAAAAAGAGTATTATGTTATT
PDC1 F	ATGTCTGAAATTACTTTGGGTAAA
PDC1 R	GACTTCCTTTTCGGATTCAGCATC
SOD1F	ATGGTTCAAGCAGTCGCAGTGTTA
SOD1 R	TTAGTTGGTTAGACCAATGACACC
ATP2 F	ATGGTTTTGCCAAGACTATATACT
ATP2 R	AACTGGGACGGAGATAGGGCCACC
NBP35 F	ATGACTGAGATACTACCACATGTA
NBP35 R	CTATACATCCCCCACAGCATCTCG

The procedure was carried out as described in the Bio-Rad instruction manual. Differentially expressed protein spots were cut out manually from the gel and subjected to peptide mass fingerprinting by liquid chromatography mass spectrometry (LC–MS), followed by sequence similarity analysis at The Centre For Genomic Application (an Institute of Genomic and Integrative Biology Collaboration), New Delhi, India.

RNA Isolation and RT–PCR

Total RNA was isolated by the LETS buffer method [1] from log phase cells that were exposed to 40°C for 30 min. Total RNA was subjected to DNase treatment, followed by purification, and was then converted to cDNA using the Qiagen one-step RT–PCR kit, according to the instructions of the manufacturer. RT–PCR for *PGK1*, *YAL037C-A*, *PDC1*, *SOD1*, *ATP2*, and *NBP35* were carried out using 250 ng of cDNA and respective primers (Table 2). RT–PCR products were analyzed by agarose gel electrophoresis and ethidium bromide staining followed by documentation with a Fluor Chem FC2 instrument from Alpha Innotech.

RESULTS AND DISCUSSION

Initial experiments performed in the laboratory indicated *S. cerevisiae* BJ5416 to be more resistant to thermal stress compared with A364A. A comparison of proteins expressed in BJ5416 *vis-à-vis* A364A cells following exposure at 40°C for 30 min showed expression of Hsp42 and Hsp30 exclusively in BJ5416 cells (data not shown). To investigate the individual roles of Hsp42 and Hsp30 in providing thermotolerance, we constructed and characterized appropriate deletion strains.

Cell Survival and Pma1p Levels in Δ*hsp30* cells During Thermal Stress

To determine if Hsp30 is required for cell viability during exposure to thermal stress, we exposed BY4741 and BY4741Δ*hsp30* cells to 50°C for different intervals of time and estimated cell survival following the exposure. The *HSP30* deletion strain was more sensitive to an exposure at 50°C compared with BY4741 cells (Fig. 1A). This sensitivity was however observed only under exposure to thermal stress and when subsequent growth was carried out in minimal media. Viable cell count was also determined for both strains upon exposure at 50°C (data not shown); the results obtained were consistent with the data in Fig. 1A.

Hsp30 inhibits Pma1H$^+$-ATPase activity during heat shock treatment and thereby conserves cellular ATP reserves during stress exposure [3]. To further characterize the above, we next determined Pma1 protein levels and activity in BY4741 *vis-à-vis* BY4741Δ*hsp30* cells at 30°C and during exposure at 40°C.

In agreement with previous reports [18], we observed a decline in Pma1p levels following heat shock in BY4741 cells (Fig. 1B, panel 1). Membrane preparations from the Δ*hsp30* mutant however showed significantly lowered

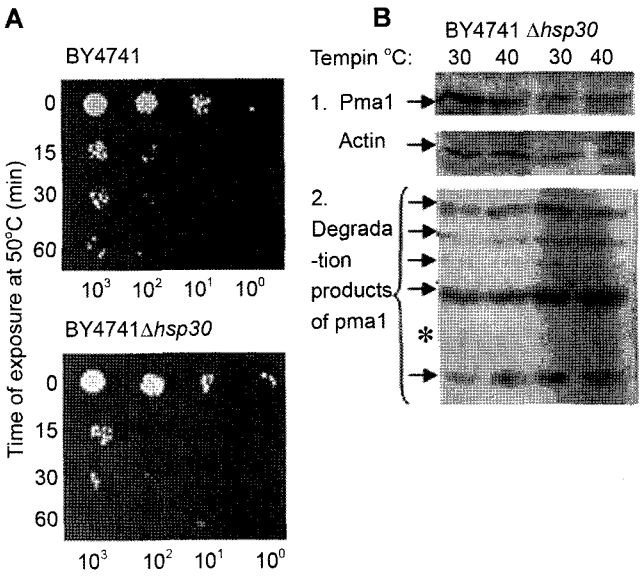

Fig. 1. Role of Hsp30 in thermotolerance.

A. Cell survival at 50°C. Log phase BY4741 and BY4741Δ*hsp*30 cells in 5 ml of minimal media containing appropriate supplements were exposed to either 50°C for different intervals of time or left untreated at 30°C and spotted in 10-fold serial dilutions such that the respective spots contained 10^3, 10^2, 10^1, and 10^0 cells, as indicated in the figure, and incubated at 30°C for 3 days. Experiments were performed in triplicate and representative data are shown. **B.** Pma1 protein levels in BY4741 and BY4741Δ*hsp*30 cells. Panel 1: 30-μg membrane fractions, isolated from BY4741 and BY4741Δ*hsp*30 strains that were either heat shocked at 40°C for 30 min or left at 30°C, were electroblotted onto PVDF membranes and the upper half on the blot was probed with anti-Pma1 Ab (1:10^3); blots were developed with alkaline phosphatase conjugated secondary Ab and alkaline phosphatase substrate BCIP and NBT. The lower half of the blot used to detect Pma1 was probed with anti-β-actin Ab and developed as above. Panel 2: Proteolytic degradation of Pma1 in BY4741 and BY4741Δ*hsp*30 cells. Same as panel 1, except that a 1:200 dilution of the anti-Pma1 Ab was used in the experiment. The region of the blot below the Pma1 band is shown.

Pma1p at 30°C, and no further reduction in Pma1p was observed when the deletion strain was subjected to heat shock (Fig. 1B, panel 1) at 40°C for 30 min. When total membrane proteins were analyzed as above with lower dilutions of anti-Pma1 Ab (Fig. 1B, panel 2), we detected elevated levels of polypeptides lower in molecular weight than Pma1p in BY4741 cells exposed to 40°C and in Δ*hsp30* cells under both optimal growth conditions and following heat shock at 40°C for 30 min. The data indicated proteolytic degradation of Pma1p under optimal growth conditions in Δ*hsp30* cells and during heat shock treatment in both BY4741 and Δ*hsp30* cells. The characteristic 50 kDa band obtained by proteolytic degradation of Pma1p [5] is indicated by an asterisk (Fig. 1B). Hsp30 may hence be required for stabilization of Pma1p during optimal growth conditions.

The effect of *HSP30* deletion on Pma1-ATPase activity and extracellular pH levels was consistent with Hsp30-mediated inhibition of Pma1 activity at 40°C in BY4741

cells and lack of such inhibition in the deletion strain (data not shown; [18]).

Results demonstrated a requirement for Hsp30 in cell survival during exposure to lethal heat stress and also in the stabilization of cellular Pma1p levels under optimal growth conditions. The negative regulation of Pma1 expression plays a crucial role in cellular stress response by restricting ATP utilization during exposure to stress. Its expression [5, 21] and protein levels [15] are downregulated during stress exposure. The reduction in Pma1 protein level in Δ*hsp30* cells under optimal growth condition is similar to that observed when cells are exposed to thermal stress; it is hence possible that *HSP30* deletion leads to inappropriate induction of certain stress response functions that lead to the observed reduction in Pma1p levels. The two major functions required for cell survival during exposure to thermal stress, namely the induction of heat shock proteins and the degradation of accumulated insoluble proteins following prolonged exposure to elevated temperatures, were however unaffected by *HSP30* deletion (data not shown).

Comparative Analysis of Proteins Expressed in BY4741 Cells and BY4741Δ*hsp30* Cells

Proteins expressed in BY4741 and BY4741Δ*hsp30* cells under optimal growth conditions and following heat shock at 40°C for 30 min were compared by two-dimensional gel electrophoresis followed by silver staining. The differentially expressed proteins (indicated by arrows in Fig. 2) were identified by peptide mass spectrometry followed by sequence similarity analysis (Table 3). Significant differences were observed in the above comparison following exposure of BY4741 and BY4741Δ*hsp30* cells at 40°C for 30 min. Spots indicated by arrows but not numbered could not be

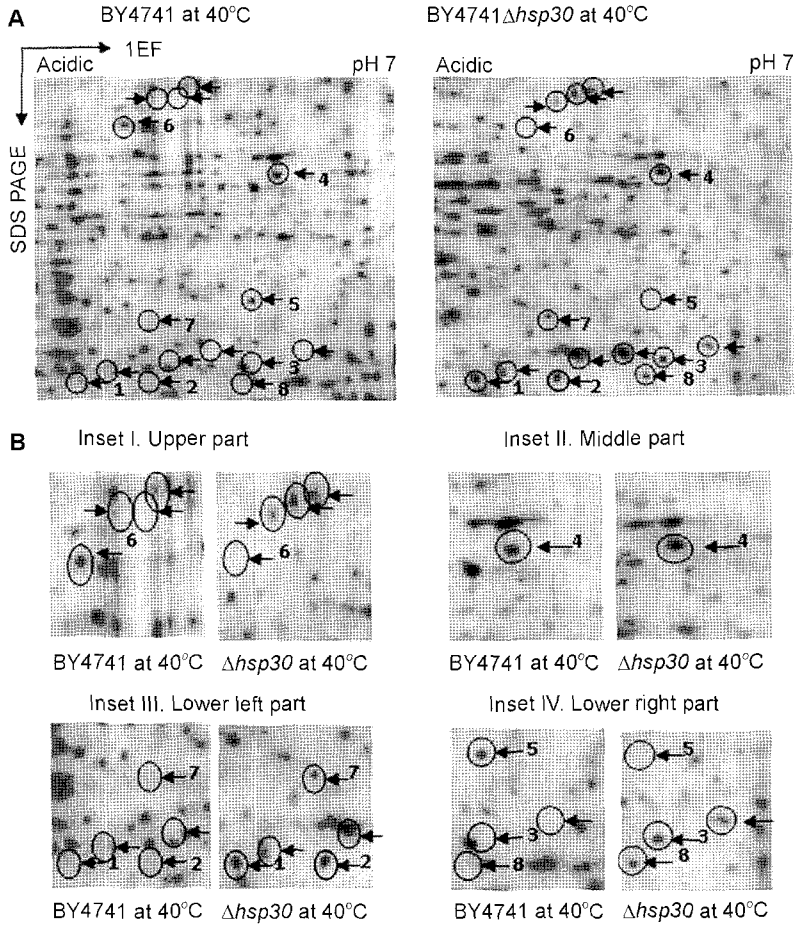

Fig. 2. Protein expression in BY4741 *vis-à-vis* BY4741Δ*hsp30* cells at 40°C.
A. One hundred μg of total cellular protein isolated from log phase BY4741 and BY4741Δ*hsp30* strains that were heat shocked at 40°C for 30 min was resolved by two-dimensional gel electrophoresis; first by isoelectric focusing in a Bio-Rad 7-cm, pH 4–7, IPG strip, followed by SDS–PAGE. The gel was stained with Bio-Rad silver reagent and the differentially expressed spots numbered 1 to 8 (indicated by arrows) were analyzed by LC–MS. No clear sequences were obtained from spots that are not numbered. For the sake of clarity, data from the right two-thirds (5.3 cm of 7 cm) of the gel is presented. **B.** Enlarged images of the upper (Inset I), middle (Inset II), lower left (Inset III), and lower right (Inset IV) parts of the two-dimensional gels containing proteins from BY4741 and Δ*hsp30* cells exposed to 40°C.

Table 3. Identification and regulation of proteins differentially expressed in BY4741 *vis-à-vis* BY4741Δ*hsp30* at 40°C by LC–MS.

Spot number	Yeast protein from SGD	Regulation in Δ*hsp30* cells (↑ Up ↓ Down)	Protein score	Matched peptide	Sequence coverage	Matched sequences (residue numbers)
1	Trx1	↑	30.1 bits (71)	KTASEFDSAIAQDKL	6–15	15
2	Pgk1	↑	21.6 bits (47)	KKYGVTDKI	379–387	9
3 and 8	Yal037c-a	↑	22.3 bits (49)	KVNNNIILFL	21–30	10
4	Pdc1	↑	26.9 bits (62)	KGSIDEQHPRY	249–259	11
5	Sod1	↓	20.9 bits (45)	MVQAVAVLKG	1–10	10
6	Atp2	↓	27.8 bits (58)	KAVLEGKY	448–455	8
7	Nbp35	↑	24.1 bits (54)	KEICESLPKG	47–56	10

identified either because of low amounts of protein or failure of LC–MS reaction. No discernable differences were observed in the proteome of the two strains during growth under optimal conditions (data not shown). Two-dimensional gel electrophoresis followed by identification of differentially expressed protein spots by mass spectrometry between BY4741Wt and Δ*hsp30* cells revealed the upregulation of Trx1, Pgk1, Yal037c-a, and Pdc1and downregulation of Sod1 and Atp2 in Δ*hsp30* cells (Table 3) during heat stress.

Expression levels of the genes encoding Trx1, Pgk1, Yal037c-a, Pdc1, Sod1, and Atp2 were further confirmed by RT–PCR between BY4741 wild type and Δ*hsp30* cells during heat stress (Fig. 3) using appropriate primers (Table 2). Results demonstrated that genes encoding Trx1, Pgk1, Yal037c-a, Pdc1, and Nbp35 were upregulated in Δ*hsp30*, whereas Sod1 and Atp2 were downregulated in these cells during thermal stress (Fig. 3). These results are in corroboration with the differential expression of these protein in Δ*hsp30* cells during thermal stress (Fig. 2).

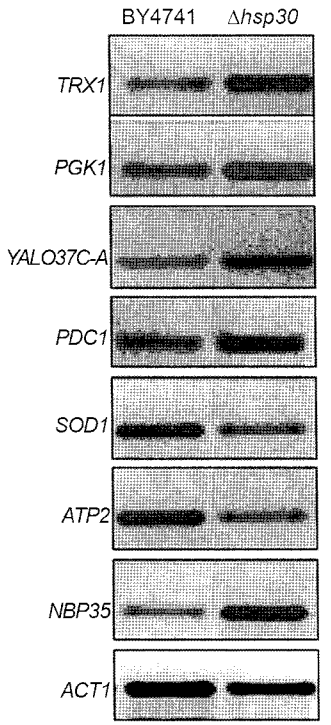

Fig. 3. Confirmation of differential expression of the genes encoding Trx1, Pgk1, Yal037c-a, Pdc1, Sod1, and Atp2 by RT–PCR.

About 250 ng of total cellular RNA isolated from BY4741 and BY4741Δ*hsp30* strains following exposure to heat stress at 40°C for 30 min was converted to cDNA. RT–PCR was performed using appropriate primers (Table 2). *ACT1* mRNA was used as an internal control.

Significant among the above alterations include reduced levels of Atp2p and Sod1p and elevated levels of Pdc1p and a Pgk1p fragment in Δ*hsp30* cells. This observation is suggestive of a shift from aerobic to fermentative growth during exposure of Δ*hsp30* cells to heat shock conditions. Pdc1p is the major of three pyruvate decarboxylase isozymes and is a key enzyme in alcohol fermentation [11, 22]. Atp2 is the β-subunit of F_1F_0 ATPase and is essential for oxidative ATP synthesis [19, 20, 27]. An increased level of Pdc1p and a reduced level of Atp2p in Δ*hsp30* cells suggest fermentative utilization of the available carbon source and lowering of oxidative generation of ATP, respectively. The cytoplasmic thioredoxin Trx1p is believed to supply reducing equivalents to the enzymes ribonucleotide reductase (Rnr1p, Rnr2p, Rnr3p, Rnr4p) and 3'-phosphoadenosine 5'-phosphosulfate (PAPS) reductase (Met16p) [7, 8] in addition to its role in protection against oxidative and reductive stresses. Increased levels of Trx1p might be an adaptive mechanism in the deletion strain required to support growth of Δ*hsp30*cells under thermal stress. The lower Sod1p content of Δ*hsp30* cells might be a consequence of lower levels of Atp2p and consequently due to lowered oxidative metabolism in the deletion strain.

It was of considerable interest to note that genes encoding proteins that are expressed only in the deletion strain (Pdc1p, Trx1p, and Nbp35p) are normally downregulated under heat shock conditions [5], whereas those encoding the two proteins that were detectable only in wild-type cells following exposure at 40°C (Atp2p and Sod1p) are upregulated during heat shock [5]. The above data suggest a shift from oxidative to fermentative growth and deviation from established stress-induced gene expression in Δ*hsp30* cells.

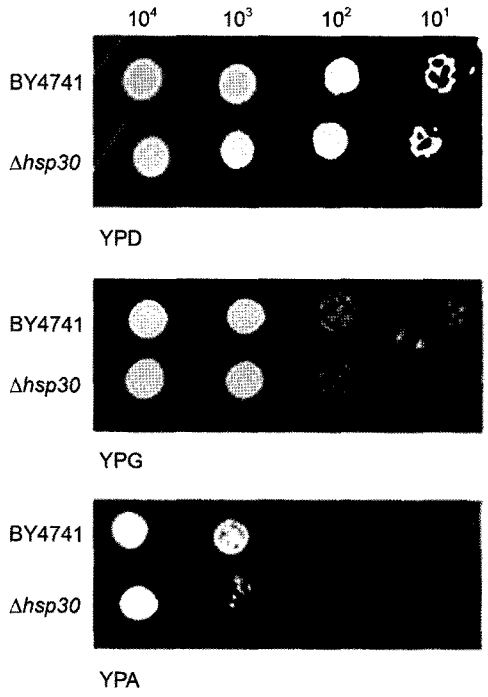

Fig. 4. Effect of fermentable and non-fermentable carbon sources on the growth of Δ*hsp30* cells under optimal growth conditions. Log phase cultures of the above strains in YPD, YPG, and YPA were spotted in 10-fold serial dilutions in respective media, as indicated in the figure, and incubated at 30°C for 3 days.

Growth of Δ*hsp30* Cells in Non-Fermentable Carbon Sources

Given the above, we specifically wanted to determine possible phenotypic effects of increased Pdc1p and decreased Atp2p levels in Δ*hsp30* cells at 40°C. For this purpose, we either exposed log phase BY4741 and BY4741Δ*hsp30* cells in YPD, YPG, and YPA to heat shock conditions at 40°C for 30 min or left them untreated, followed by spotting 10-fold serial dilutions on solid media containing either a fermentable or non-fermentable carbon source. Results demonstrated that untreated Δ*hsp30* cells grew slower than BY4741 cells in non-fermentable carbon sources (Fig. 4). Exposure at 40°C in either fermentable or non-fermentable media followed by plating in YPD, YPG, and YPA plates did not show any further growth defect in Δ*hsp30* cells over the unexposed control (data not shown). Although Δ*hsp30* cells elaborated higher levels of Pdc1p and lower levels of Atp2p as compared with BY4741 cells, cell survival of the deletion strain at 40°C for 30 min was not absolutely dependent on the presence of fermentable carbon source in the medium. Results however suggest a preference for fermentative growth in the deletion strain.

It is evident from this study that Hsp30 is required during thermal stress and is also necessary for a set of thermal stress response functions; however, the exact mechanism of how a membrane protein (Hsp30) is involved in such function is not exactly known. Further experiments are necessary to establish the mechanism of Hsp30.

Acknowledgments

The help, support, and encouragements of Dr. G. Ilavazhagan Director, DIPAS and Dr. Soma Sarkar DIPAS, DRDO Delhi, India, are gratefully acknowledged. We are also grateful to Prof. Rajendra Prasad and Dr. S. Biswas of Jawaharlal Nehru University, New Delhi, for providing us with *S. cerevisiae* A364A, and BJ5416, respectively; and to Dr. A. Bachhawat from IMTECH, CSIR, Chandigarh, for the generous gift of *S. cerevisiae* BY4741. This work was supported by a financial fund of the Defence Research and Development Organization (DRDO), Ministry of Defence, Government of India.

References

1. Adams, A., D. E. Gottschling, C. A. Kaiser, and T. Stearns. 1997. *Methods in Yeast Genetics*. Cold Spring Harbour Laboratory Press.
2. Baudin, A., O. Kalogeropoulos, A. Denouel, F. Lacroute, and C. Cullin. 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acid Res.* **21:** 3329–3330.
3. Braley, R. and P. W. Piper. 1997. The C-terminus of yeast plasma membrane H^+-ATPase is essential for the regulation of this enzyme by heat shock protein Hsp30, but not for stress activation. *FEBS Lett.* **418:** 123–126.
4. Galeote, V. A., H. Alexandre, B. Bach, P. Delobel, S. Dequin, and B. Blondin. 2007. Sfl1p as an activator of *HSP30* gene in *Saccharomyces cerevisiae*. *Curr. Genet* **52:** 55–63.
5. Gasch, A. P., P. T. Spellman, C. M. Kao, O. C. Harel, M. B. Eisen, G. Storz, D. Botstein, and P. O. Brown. 2000. Genomic expression programs in the response of yeast cells to environmental change. *Mol. Biol. Cell* **11:** 4241–4257.
6. Gong, X. and A. Chang. 2001. A mutant plasma membrane ATPase Pma1-10 is defective in stability at the yeast cell surface. *Proc. Natl. Acad. Sci. U.S.A.* **98:** 9104–9109.
7. Gonzalez-Porque, P., A. Bladesten, and P. Reichard. 1970. Purification of a thioredoxin system from yeast. *J. Biol. Chem.* **245:** 2363–2370.
8. Gonzalez-Porque, P., A. Bladesten, and P. Reichard. 1970. The involvement of the thioredoxin system in the reduction of methionine sulfoxide and sulfate. *J. Biol. Chem.* **245:** 2371–2374.
9. Hendrick, J. P. and F. U. Hartl. 1993. Molecular chaperone functions of heat-shock proteins. *Annu. Rev. Biochem.* **62:** 349–384.
10. Jenkins, G. M., A. Richards, T. Wahl, C. Mao, L. Obeid, and Y. Hannun. 1997. Involvement of yeast sphingolipids in the heat stress response of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **272:** 32566–32572.

11. Kellermann, E., P. G. Seeboth, and C. P. Hollenberg. 1986. Analysis of the primary structure and promoter function of a pyruvate decarboxylase gene (*PDC1*) from *Saccharomyces cerevisiae*. *Nucleic Acid Res.* **14:** 8963–8977.
12. Lee, S. G., M. F. Schwartz, J. K. Duong, and D. F. Stern. 2003. Rad53 phosphorylation site clusters are important for rad53 regulation and signaling. *Mol. Cell Biol.* **23:** 6300–6314.
13. Levin, D. E. 2005. Cell wall integrity signaling in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **69:** 262–291.
14. Lorenz, M. C., R. S. Muir, E. Lim, J. McElver, S. C. Weber, and J. Heitman. 1995. Gene disruption with PCR products in *Saccharomyces cerevisiae*. *Gene* **158:** 113–117.
15. Panaretou, B. and P. Piper. 1992. The plasma membrane of yeast acquires a novel heat-shock protein (Hsp30) and displays a decline in proton pumping ATPase levels in response to both heat shock and entry to stationary phase. *Eur. J. Biochem.* **206:** 635–640.
16. Parsell, D. A. and S. Lindquist. 1993. The function of heat-shock proteins in stress tolerance: Degradation and reactivation of damaged proteins. *Annu. Rev. Genet.* **27:** 437–496.
17. Perzov, N., H. Nelson, and N. Nelson. 2000. Altered distribution of yeast plasma membrane ATPase as a feature of vacuolar H+-ATPase null mutants. *J. Biol. Chem.* **275:** 40088–40095.
18. Piper, P. W., C. Ortiz-Calderon, C. Holyoak, P. Coote, and M. Cole. 1997. Hsp30, the integral plasma membrane heat shock protein of *Saccharomyces cerevisiae*, is a stress-inducible regulator of plasma membrane H^+-ATPase. *Cell Stress Chaperones* **2:** 12–24.
19. Reinders, J., K. Wanger, R. P. Zahedi, D. Stojanovski, B. Eyrich, M. Van der Laan, *et al.* 2007. Profiling phosphoproteins of yeast mitochondria reveals a role of phosphorylation in assembly of the ATP synthase. *Mol. Cell Proteomics* **6:** 1896–1906.
20. Saltzgaber-Muller, J., S. P. Kunapulli, and M. G. Douglas. 1983. Nuclear gene coding the yeast mitochondrial adenosine triphosphatase complex, isolation of ATP2 coding the F1-ATPase beta subunit. *J. Biol. Chem.* **258:** 11465–11470.
21. Schmitt, M., P. Schwanewilm, J. Ludwig, and F. Lichtenberg. 2006. Use of PMA1 as a housekeeping biomarker for assessment of toxicant-induced stress in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **72:** 1515–1522.
22. Seeboth, P. G., K. Bohnsack, and C. P. Hollenberg. 1990. pdc1(0) mutants of *Saccharomyces cerevisiae* give evidence for an additional structural PDC gene: Cloning of PDC5, a gene homologous to PDC1. *J. Bacteriol.* **172:** 678–685.
23. Seymour, I. J. and P. W. Piper. 1999. Stress induction of HSP30, the plasma membrane heat shock protein gene of *Saccharomyces cerevisiae*, appears not to use known stress-regulated transcription factors. *Microbiology* **145:** 231–239.
24. Shalev, A., L. Valásek, C. A. Pise-Masison, M. Radonovich, L. Phan, J. Clayton, *et al.* 2001. *Saccharomyces cerevisiae* protein Pci8p and human protein eIF3/Int-6 interact with eIF3 core complex by binding to eIF3 core complex subunits. *J. Biol. Chem.* **276:** 34948–34957.
25. Sheehan, K. B., K. McInneenry, B. P. Gage, S. D. Altenburg, and L. E.Hyman. 2007. Yeast genomic expression patterns in response to low-shear modeled microgravity. *BMC Genomics* **8:** 3.
26. Sorin, A., G. Rosas, and R. Rao. 1997. PMR1, a Ca^{2+}-ATPase in yeast golgi, has properties distinct from sarco/endoplasmic Reticulum and plasma membrane calcium pumps. *J. Biol. Chem.* **272:** 9895–9901.
27. Takeda, M., A. Vassarotti, and M. G. Douglas. 1985. Nuclear genes coding the yeast mitochondrial adenosine triphosphatase complex: Primary sequence analysis of ATP2 encoding the F1-ATPase beta-subunit precursor. *J. Biol. Chem.* **260:** 15458–15465.
28. Winkler, A., C. Arkind, C. P. Mattison, A. Burkholder, K. Knoche, and I. Ota. 2002. Heat stress activates the yeast high-osmolarity glycerol mitogen-activated protein kinase pathway, and protein tyrosine phosphatases are essential under heat stress. *Eukaryot. Cell* **1:** 163–173.