Translational thermotolerance in *Saccharomyces cerevisiae*

Elizabeth M. Hallberg, Richard L. Hallberg

Department of Biology, Syracuse University, Syracuse, NY 13244, USA.

Abstract While protein synthesis is rapidly inactivated in *Saccharomyces cerevisiae*, cells shifted from log growth at 30°C to 43°C, a 1-h 37°C treatment given to cells just prior to the shift to 43°C partially blocks this inactivation. By contrast, such a pre-heat shock treatment has no protective effect on translational inactivation at 45°C or higher. Cells allowed to approach stationary phase not only develop an enhanced thermotolerance relative to log cells but also exhibit a pronounced resistance to inactivation of protein synthesis at 43°C as well as at 45°C. We have found that this 'translational thermotolerance' can also be induced in *S. cerevisiae* by briefly treating log phase cells at 30°C with cycloheximide. Using such a procedure to induce stabilization of protein synthesis at 43°C, we have been able to show that heat shock-induced proteins are not responsible for the establishment of this protective effect. This work shows that enhanced thermotolerance can be induced in log cells even after a shift to 43°C, as long as a prior translational thermotolerance has been established. Furthermore, we show that the capacity of plateau cells to maintain translation at 43°C contributes significantly to their state of enhanced thermotolerance.

INTRODUCTION

Enhanced thermotolerance is an inducible state manifested by most organisms whereby a physiological stress increases the capacity of cells to survive longer at lethal temperatures (reviewed in Parsell et al 1994). The 'normal' stress most often used experimentally to induce this state is a brief exposure to an elevated, but non-lethal, temperature administered just before the shift to the lethal temperature. This prior heat treatment elicits the heat shock response and it has now been shown for some organisms that the accumulation of specific heat shock proteins (Hsps) are absolutely required for the manifestation of enhanced thermotolerance. In the case of *Saccharomyces cerevisiae*, an essential protein is Hsp104 (Sanchez and Lindquist 1990), while in *Drosophila melanogaster* and mammalian cells, Hsp70 and small Hsps appear to be crucial (Li and Laszlo 1985; Landry et al 1989; Li et al 1991; Solomon et al 1991; Mehlen et al 1993).

While the majority of studies have indicated that the pre-accumulation of at least some Hsp is necessary to

establish enhanced thermotolerance (summarized in Parsell et al 1994), a few have indicated otherwise (Hall 1983; Hallberg 1986; Laszlo 1988; Lee and Dewey 1988; Smith and Yaffee 1991). In the case of the ciliate, *Tetrahymena thermophila*, it was shown that a pre-accumulation of Hsps was not necessary for enhanced thermotolerance if cells had previously acquired the ability to maintain protein synthesis at what would normally have been a heat-inactivating temperature (Hallberg 1986; Kraus et al 1986). That is, under the appropriate conditions, Hsps could apparently be accumulated *after* a shift to a lethal temperature such that a state of enhanced thermotolerance could be established equivalent to that induced by a normal pre-heat shock treatment (Kraus et al 1986). Additional studies showed that ribosome modifications were involved in some way in the thermostabilization of protein synthesis (Hallberg et al 1981; Hallberg and Hallberg 1983; Kraus et al 1986). More recently, a small, RNA polymerase III transcribed, heat-induced RNA was shown to be required for translational stabilization to be established (Fung et al 1995). However, what step(s) in translation this RNA affects have not been identified, nor has it been shown whether modifications of other components of the protein synthetic machinery are involved.

It has been reported in a number of different organisms

Received 28 November 1995; Revised 13 December 1995;
Accepted 14 December 1995

Correspondence to: R. L. Hallberg, Tel: +1 315 443 1104;
Fax: +1 315 443 2156; E-mail: hallberg@mailbox.syr.edu

that the stabilization of protein synthesis at normally heat-inactivating temperatures can occur in cells that have experienced a prior stress (Altshuler and Mascarenhas 1982; Lin et al 1984; Lee and Dewey 1988; Beck and De Maio 1994). These stresses elicited the production of Hsps and it was suggested that they are in some way responsible for this so-called translational thermotolerance. In no cases, however, was it shown that the presence of the Hsps played any direct role in stabilizing protein synthesis at the elevated temperature and this remains an open question.

While the phenomenon of translational stabilization in *T. thermophila* is well documented, and the ability to manipulate these organisms for molecular genetic studies has dramatically improved recently (Kahn et al 1993; Gaertig et al 1995), we wanted to see if a more genetically tractable system, viz. *S. cerevisiae*, displayed similar properties. If so, this would potentially provide another way of mechanistically evaluating translational thermotolerance. The studies reported herein show that a number of stimuli that elicit translational stabilization in *T. thermophila* have similar effects in *S. cerevisiae*. Furthermore, we have found that protein synthesis can be stabilized in yeast without the prior accumulation of Hsps and that it is possible for *S. cerevisiae* to establish enhanced thermotolerance by accumulating Hsps (presumably Hsp104) even *after* being shifted to a normally lethal temperature.

MATERIALS AND METHODS

Yeast strains and growth media

The *S. cerevisiae* strain W303 was used in all the experiments described herein. The growth medium used in these studies (YPD), contained 1% yeast extract, 2% dextrose, and 2% peptone. Cell viabilities (see below) were determined by plating cells on YPD-containing agar. To radioactively label proteins, either for fluorography or scintillation counting, [³⁵S]-methionine (Tran³⁵S-label, ICN) was added to the YPD medium to give a final concentration of 30 µCi/ml.

Cell viability measurements

Aliquots of cells exposed for various periods of time to either 43 or 45°C were vortexed, diluted several times, and then plated out and incubated at 30°C. Colonies were counted 2–3 days later. Viabilities were expressed as the fraction of the number of colonies formed by cells exposed for a particular length of time at 43°C relative to zero-time controls. Triplicate counts were made in all cases.

Protein extraction, electrophoresis and Western analysis

Proteins were isolated from concentrated cell suspensions by the addition of one-part cell suspension to one-part 1.8 M NaOH, 7.4% β-mercaptoethanol. After incubation on ice for 10–15 min, an equal volume of acetone and a 0.5 volume of 100% trichloroacetic acid were added and the mixture left on ice for an additional 15 min. The precipitated proteins were collected by centrifugation, washed twice in acetone, dried and then redissolved in standard SDS containing electrophoresis sample buffer. For amino acid incorporation measurements, triplicate aliquots of the re-dissolved proteins were re-precipitated with 10% TCA, caught on filters, dried and counted in a liquid scintillation counter. SDS gel electrophoresis was carried out on 10% acrylamide gels and the proteins transferred to nitrocellulose filters as described previously (Hallberg et al 1993). Western analyses were carried out as previously described (Hallberg et al 1993) using polyclonal antisera generated against *S. cerevisiae* Hsp60 and Hsp104 (Sanchez and Lindquist 1990).

RESULTS

To determine whether translation in *S. cerevisiae* can be made less susceptible to thermal inactivation, we exposed early log phase cells to a 1-h 37°C treatment and then transferred them directly to 43°C. Immediately thereafter, and at successive intervals, cells were pulse-labeled with [³⁵S]-methionine. As a negative control, we also labeled early log cells transferred directly from 30 to 43°C. Total cell proteins were separated by 10% SDS-PAGE and fluorographed. From the pattern of labeling (Fig. 1A) it was clear that, while amino acid incorporation declined at 43°C in both cases, the loss of detectable protein synthesis was faster in the non-treated cells (Fig. 1A, lanes 1–3) when compared with the pre-heat shocked cells (Fig. 1A, lanes 4–6). Similar experiments were carried out another five times and the averages of the overall amino acid incorporations quantitated and plotted (Fig. 1B). The loss of amino acid incorporation was approximately 2.5 times more rapid in the naive cells than in the pre-heat shocked cells. Qualitatively similar results were obtained when we examined cells exposed to 41°C or 42°C with and without pre-heat shocks (data not shown). By contrast, cells transferred to 45°C with or without a 37°C pre-treatment showed identical rates of loss of protein synthesis capacity (Fig. 1C). Thus, a prior heat shock appeared to increase the ability of the translational apparatus to withstand the inactivating effects of heat at 43°C but not 45°C. While it could be argued that the differences in incorporation that we see are not a true indication of protein synthesis differences, but are due to differences in amino acid

Fig. 1 The effects of a 37°C heat shock on amino acid incorporation at 43°C and 45°C. W303 cells in early log growth in YPD at 30°C were transferred directly either to 43°C, 45°C, or 37°C for 1 h before being shifted to the higher temperature. At times, after the final temperature shift, aliquots of cells were pulsed labeled with [³⁵S]-methionine for 30 min. Total cell protein extracts were made and either TCA precipitated for scintillation counting or separated by 10% SDS-PAGE and fluorographed. (A) Fluorogram of labeled proteins from cells shifted to 43°C with (lanes 4–6) or without (lanes 1–3) an intervening 37°C heat shock, and labeled beginning at 0 h (lanes 1, 4), 1.25 h (lanes 2, 5), or 2.5 h (lanes 3, 6) after the shift to 43°C. Equal amounts of protein were run in each lane. (B) Relative incorporation of labeled methionine in cells treated as in (A). Triplicate samples of proteins from identical numbers of cells labeled, beginning at the times indicated, were collected on filters and counted, their averages calculated, and the radioactive incorporation relative to the zero-time labeling determined and plotted. The data shown were obtained in five separate experiments. (C) Relative incorporation of labeled methionine in cells treated as in (B) but shifted to 45°C instead.

uptake in the two cell populations, the results of experiments described below rule out that possibility.

Yeast cells at normal temperatures entering plateau stages of growth become more thermotolerant and accumulate a number of the same stress proteins that are induced by elevated temperatures (reviewed in Werner-Washburne et al 1993). To see whether the translational apparatus of such stationary cells also exhibit greater resistance to thermal inactivation at high temperatures, cells grown at 30°C for 3 days in YPD were shifted to 43°C and the stability of amino acid incorporation was determined (Fig. 2A). In these cells, even more so than in heat shocked log phase cells (Fig. 1A), incorporation of amino acids was essentially unaffected at the elevated temperature over a 5-h period (Fig. 2A; lanes 5–8), although by 22 h such cells showed no detectable amino acid incorporation (data not shown). Furthermore, unlike in log cells heat shocked at 37°C, protein synthesis at 45°C in plateau cells still exhibited an increased resistance to heat inactivation (Fig. 2B). Not until the temperature exceeded 47.5°C was protein synthesis inactivated with the same kinetics as naive or pre-heat shocked log phase cells (data not shown). Thus the depletion of nutrients, another stressful situation, elicited the accumulation of Hsps and brought about the increased resistance of the translational machinery to thermal inactivation.

Two questions were generated by the previous observations:

1. Is there a relationship between Hsp accumulation and the increased stabilization of translation?
2. Does the stabilization of translation contribute to thermotolerance?

Both of the treatments we had used to stabilize protein synthesis induced Hsp accumulation and enhanced thermotolerance. In order to begin answering either of the above questions, we needed a way to induce translational protection without simultaneously inducing Hsp accumulation (if, in fact, such a situation were possible). In 1985, Hallberg et al. found that protein synthesis inhibitors can induce enhanced thermotolerance and increased resistance to thermal inactivation of protein synthesis in *T. thermophila*. Therefore, we treated yeast cells growing at 30°C with cycloheximide at 10 µg/ml for 2 h, washed out the cycloheximide, and determined both the inactivation of amino acid incorporation (Fig. 3A) and cell viability (Fig. 3B) after a shift to 43°C. In both regards, the cycloheximide-treated cells were indistinguishable from cells that had received a 1-h 37°C treatment prior to being shifted to 43°C.

While no Hsps could have been accumulated during the time the cells were in the presence of cycloheximide,

Fig. 2 Amino acid incorporation in plateau cells at 43°C and 45°C. W303 cells that had been growing in YPD at 30°C for 3 days were transferred to either 43°C or 45°C and their incorporation of [³⁵S]-methionine into proteins then determined by fluorography of SDS-PAGE separated proteins (A), or by scintillation counting of TCA-precipitated proteins (B). As a control, early log cells growing at 30°C were given a 1 h 37°C exposure to induce translational thermotolerance and they were treated in a similar fashion to the plateau cells. Cells were labeled as in Figure 1(A). Fluorogram of proteins labeled at 43°C. Lanes 1–4: pre-heat shocked log cells; lanes 5–8: plateau cells. The times labeling was initiated after the shift to 43°C: 0 h, lanes 1, 5; 1.5 h, lanes 2, 6; 3 h, lanes 3, 7; 4.5 h, lanes 4, 8. The same amounts of protein were run in each lane. The lower T_0 incorporation in plateau cells relative to the controls is in large part due to the overall decrease in rate of protein synthesis in these cells (Werner-Washburne et al 1993). (B) Relative incorporation of labeled methionine into protein in cells treated as in (A) but shifted to 45°C instead. The relative incorporation was measured as in Figure 1.

it was possible that, during the time it took to wash the cells out of the drug and transfer them to 43°C, a stress response might have occurred thereby resulting in the accumulation of Hsps. We tested that possibility by measuring levels of Hsp104, a strongly inducible heat shock protein, and the one protein already shown to be absolutely necessary for the establishment of enhanced thermotolerance in *S. cerevisiae* (Sanchez and Lindquist 1990; Sanchez et al 1992). We found that, at the time that cycloheximide-treated cells were transferred to 43°C, the detectable level of Hsp104 in these cells was indistinguishable from that found in the control cells growing at 30°C (Fig. 3C, lanes 1 and 3). Furthermore, in the cycloheximide pre-treated cells, Hsp104 accumulated at 43°C to a level that was comparable to that found in cells that had received a 1-h 37°C heat treatment (Fig. 3C, lanes 2, 4, 5).

The cycloheximide treatment itself was not inducing the pre-accumulation of Hsp104 mRNA. This was inferred from the observation that cycloheximide-treated, and subsequently washed, cells showed no detectable increase in Hsp104 over a 2-h period if maintained at 30°C rather than being shifted to 43°C (data not shown). Also, Hsp104 did accumulate at 43°C in the non-pretreated controls

but, reflecting the fact that protein synthetic capacity decreased more rapidly in these cells, this occurred to a much lesser degree (Fig. 3C, lanes 6–8). This difference in Hsp104 accumulation presumably accounts, at least in part, for the viability differences.

Our results thus far confirmed three things:

1. the increase in thermal stability of protein synthesis does not require a normal heat shock response
2. the *prior* accumulation of Hsps (particularly Hsp104) is not required for the establishment of enhanced thermotolerance at 43°C
3. the differences we see in amino acid incorporation at 43°C truly reflect differences in protein synthesis capacity and not differences in uptake.

However, we could not conclude whether, in cells treated with cycloheximide, the stabilization of protein synthesis is required in any way for the establishment of enhanced thermotolerance. Consequently, we carried out the following experiment. Log cells at 30°C were treated with cycloheximide as before but, after washing out the drug and just before a shift to 43°C, the culture was split and 0.5 of the cells were re-exposed to cycloheximide. The viabilities of these two different cell populations were

Fig. 3 The effect of protein synthesis inhibition on translational and enhanced thermotolerance. W303 cells in early log growth in YPD at 30°C were treated with cycloheximide (10 $\mu\text{g}/\text{ml}$) for 2 h after which they were washed twice, resuspended in fresh YPD and then shifted to 43°C. The effect of this treatment on the relative incorporation of $[^{35}\text{S}]$ -methionine at 43°C (A) and its effect on the viability of cells exposed to 43°C (B) was determined. Relative incorporation was determined as in Figure 1. Control cells (with or without a 37°C heat shock) received no cycloheximide treatment. Cell viability (colony-forming ability) was determined as described in Materials and Methods. Western analyses (C) measuring Hsp104 levels in cells treated in various ways were carried out as described in Materials and Methods. Lanes 1 and 2: Log cells growing at 30°C (lane 1) were shifted to 37°C for 1 h (lane 2). Lanes 3–5: Log cells growing at 30°C were treated with cycloheximide (10 $\mu\text{g}/\text{ml}$) for 2 h, the cycloheximide washed out, and the cells shifted to 43°C. Protein was isolated from cells at 0 h (lane 3), 1 h (lane 4) and 2 h (lane 5) after the shift to 43°C. Lanes 6–8: Log cells growing at 30°C were shifted to 43°C and protein was isolated from cells at 0 h (lane 6), 1 h (lane 7) and 2 h (lane 8) after the shift to 43°C. As an internal control to monitor the amounts of protein run in each lane, the levels of the mitochondrial chaperonin, Hsp60, were measured.

then determined as a function of time at 43°C. We found that, as before, enhanced thermotolerance was established by the cycloheximide pre-treatment (Fig. 4A), but was only manifested if cells were allowed to synthesize proteins at 43°C. That is, cells induced with cycloheximide at 30°C, but prevented from synthesizing proteins at 43°C, showed a loss of viability identical to that of cells shifted directly from 30°C to 43°C. That the presence of cycloheximide at 43°C was not intrinsically cytotoxic, was shown by the fact that cells pretreated at 37°C for 1 h, and then exposed to cycloheximide at the time of the shift to 43°C, exhibited a normal enhanced thermotolerance over a 4-h period (Fig. 4A).

We interpret the results of all these experiments as indicating that, if cells have already accumulated Hsps prior to their being shifted to 43°C, further protein synthesis at 43°C does not increase their degree of induced thermotolerance, at least as seen over a 4-h period. However, should Hsps not be present at the time of the shift to 43°C, enhanced thermotolerance can still be established if, and only if, the translational machinery has been induced to become more resistant to thermal inactivation. This then allows the rapid accumulation at 43°C of sufficient Hsp levels to increase cell viability to that found in cells that had experienced a prior heat shock. These results also reinforce the conclusion that

Fig. 4 The effects of protein synthesis inhibition at 43°C on the induction and maintenance of enhanced thermotolerance. The viabilities of log phase (A) and plateau (B) cells treated with cycloheximide while at 43°C were determined (see Materials and Methods for details). The various treatment regimens for log cells (A) are indicated in the legend box. The cycloheximide pre-treatment at 30°C was as described in Figure 3. The 37°C pre-treatment was for 1 h. The concentration of cycloheximide present in cell cultures at 43°C was 10 µg/ml. (B) A culture of cells growing at 30°C in YPD for 3 days was divided in half. To one-half, cycloheximide was added to 10 µg/ml. Both halves were then transferred to 43°C. Samples were taken at the indicated times for viability determinations.

the accumulation of heat shock protein (presumably Hsp104) is required for enhanced thermotolerance whether that temperature is 43°C, 45°C or higher.

Having seen that the accumulation of Hsps at 43°C can have an affect on thermotolerance, we wanted to know if Hsps synthesized in plateau cells at 43°C contributed in any way to their enhanced thermotolerant state. To test that idea we took cells that had been in culture for 3 days, split the culture, adding cycloheximide to one and nothing to the other, and then shifted the cells to 43°C. At intervals over a 24-h period we measured the viabilities of the two cell populations. As seen in Figure 4B, while viabilities of drug-treated and control cells were similar for the first 10 h, after that cells that had been prevented from continued synthesis of Hsps at 43°C showed a rapid decline in their state of thermotolerance. Again, as a control on the possible cytotoxicity of cycloheximide at elevated temperatures, we compared the thermotolerances of drug-treated and control plateau cells shifted to 48°C, a temperature at which protein synthesis can not be stabilized. We found no differences (data not shown). In light of our previous results we interpret these data as indicating that plateau cells have an increased thermotolerance at 43°C as a result of their prior accumulation of Hsps at 30°C. However, as they can also continue synthesizing Hsps at 43°C, they are either able to replace Hsps that may become non-functional or are degraded, or they may further increase the overall levels of Hsps at 43°C;

this then allows them to maintain a reasonably high viability level over a 24-h period. Cycloheximide-treated cells at 43°C would be prevented from augmenting their Hsp104 amounts and would, therefore, lose thermotolerance more rapidly.

DISCUSSION

While our results show that translation in *S. cerevisiae* can be made more resistant to thermal inactivation, we do not know what component(s) of the protein synthetic apparatus is affected by the pre-treatments that bring about these changes. It is, however, fair to conclude that, at least in one instance, no newly synthesized protein components are absolutely required, since inhibiting protein synthesis itself induces enhanced thermostability. This must mean that physiologically-induced modifications of pre-existing components, and/or the accumulation of new RNA species, is involved. There are a number of reported modifications to components of the translational apparatus induced by heat, starvation, or other physiologically stressful situations (reviewed in Panniers 1994). These include phosphorylation or dephosphorylation of initiation factors (Duncan and Hershey 1989; Duncan et al 1995), modifications of ribosomal proteins (Hallberg et al 1981; McMullin and Hallberg 1986) and changes in ribosome conformation (Hallberg et al 1981; Kraus et al 1986). Whether any of these situations, or

some as yet unrecognized ones, are mechanistically responsible for establishing translational thermotolerance remains to be determined.

Our studies indicate that the preaccumulation of stress-induced proteins is not required for translational thermotolerance, but they do not indicate whether or not constitutively expressed molecular chaperones may be required. The yeast proteins Ssb1 and Ssb2, members of the Hsp70 family, have been shown to be associated with nascent polypeptides and required for translation to occur normally (Nelson et al 1992). Neither of the genes encoding these proteins is stress inducible; rather their transcriptions actually decrease at higher temperatures (Craig et al 1993). Nevertheless, the functions of Ssb1 and Ssb2 are presumably still required for translational efficiency at these elevated temperatures. It is unknown if other constitutive molecular chaperones may be required.

With regard to the possibility of new RNA species being involved, we have recently shown that in *T. thermophila* a small, cytoplasmic, heat-induced RNA (G8 RNA) is required for the full manifestation of translational stabilization at normally lethal temperatures (Fung et al 1995). Although this RNA has been shown to be quantitatively ribosome-associated (Kraus et al 1987; Hallberg and Hallberg 1989), its function is unknown at present. We have searched for a homologous species of RNA in *S. cerevisiae*, but so far have been unsuccessful in identifying one. Whether the gene for such an RNA does or does not exist in yeast, or in any other organism, remains to be rigorously determined. Nevertheless, the similarities between *S. cerevisiae* and *T. thermophila* with regard to protein synthesis stabilization argue that some similarities in mechanisms exist as well.

While translation is thermostabilized in plateau cells, heat shocked cells, and in cells treated with cycloheximide, the mechanisms whereby this stabilization is achieved need not be identical. The fact that the thermal stabilities of translation in heat shocked log phase cells and in plateau cells are so different argues that qualitative differences exist. One way of addressing this question, as well as identifying the particular components whose modifications are required to achieve greater thermal resistance of protein synthesis inhibition, is to identify mutants unable to respond to one or more of the stimuli that elicit this response. We are currently pursuing such a search.

It is important to note that, whatever the stress-induced responses are that allow protein synthesis to continue at lethal temperatures, they are functionally distinguishable from the apparent activities of the protein chaperones characterized thus far. That is, these protein chaperones do not appear to *prevent* the loss of functions at elevated temperatures, but rather promote and accelerate the repair of damaged proteins and restoration of functions

after cells are returned to a permissive, non-lethal temperature (Parsell et al 1994). However, There may be unidentified functions of protein chaperones that promote the continued operation of processes at higher temperatures. If such functions do exist, they would, most likely, be manifested at temperatures within a few degrees of the normally minimum lethal temperature in a manner similar to what we find for protein synthesis stabilization.

Our studies provide no alternative explanations for the reports (Hall 1983; Smith and Yaffe 1991) that indicate that Hsp accumulation need not be required for establishing enhanced thermotolerance in *S. cerevisiae*. In these studies, thermotolerance was assayed at 50°C and above, temperatures at which protein synthesis is inactivated rapidly whether or not cells have been pre-heat shocked. Rather, our data are consistent with all previous reports indicating that establishing enhanced thermotolerance in yeast requires the accumulation of Hsps, but they also show that this accumulation can occur after cells have been exposed to a temperature just above the minimum lethal temperature, if the translational apparatus has been appropriately modified. Whatever these modifications are, they presumably reflect another way in which cells have adopted alternative strategies for increasing the likelihood of survival under physiologically stressful situations.

ACKNOWLEDGMENTS

We are grateful to Jeff Schatz and Susan Lindquist for the gifts of antibodies directed against yeast Hsp60 and Hsp104, respectively. This work was supported by NIH grant GM 46302.

REFERENCES

- Altshuler M and Mascarenhas JP (1982) Heat shock proteins and effects of heat shock in plants. *Plant Mol. Biol.* **1**: 103–115.
- Beck SC and De Maio A (1994) Stabilization of protein synthesis in thermotolerant cells during heat shock. Association of heat shock protein-72 with ribosomal subunits of polysomes. *J. Biol. Chem.* **269**: 21803–21811.
- Craig EA, Gambill BD and Nelson RJ (1993) Heat shock proteins: Molecular chaperones of protein synthesis. *Microbiol. Rev.* **57**: 402–414.
- Duncan RF, Cavener DR and Qu S (1995) Heat shock effects on phosphorylation of protein synthesis initiation factor proteins eIF4E and eIF-2 alpha in *Drosophila*. *Biochemistry* **34**: 2985–2997.
- Duncan RF and Hershey JW (1989) Protein synthesis and protein phosphorylation during heat stress, recovery, and adaptation. *J. Cell Biol.* **109**: 1467–1481.
- Fung PA, Gaertig J, Gorovsky MA and Hallberg RL (1995) Requirement of a small cytoplasmic RNA for the establishment of thermotolerance. *Science* **268**: 1036–1039.
- Gaertig J, Gu L, Hai B and Gorovsky MA (1995) Gene replacement and high-frequency vector-mediated electrotransformation in *Tetrahymena*. *Nucleic Acids Res.* **22**: 5391–5401.

- Hall BG (1983) yeast thermotolerance does not require protein synthesis. *J. Bacteriol.* **156**: 1363–1365.
- Hallberg EM, Shu Y and Hallberg RL (1993) Loss of mitochondrial hsp60 function: non-equivalent effects on matrix-targeted and intermembrane-targeted proteins. *Mol. Cell Biol.* **13**: 3050–3057.
- Hallberg RL (1986) No heat shock protein synthesis is required for the induced thermostabilization of the translational machinery. *Mol. Cell Biol.* **6**: 2267–2270.
- Hallberg RL and Hallberg EM (1983) Characterization of a cycloheximide-resistant *Tetrahymena thermophila* mutant which also displays altered growth properties. *Mol. Cell Biol.* **3**: 503–510.
- Hallberg RL and Hallberg EM (1989) Heat shock in *Tetrahymena* induces the accumulation of a small RNA homologous to eukaryotic 7SL RNA and E. coli 4.5S RNA. In: *Stress-Induced Proteins, UCLA Symposia on Molecular and Cellular Biology*, Vol. 96, eds M Pardue, J Feramisco and S Lindquist, Alan R Liss, New York, 107–116.
- Hallberg RL, Wilson PG and Sutton CA (1981) Regulation of ribosome phosphorylation and antibiotic sensitivity in *Tetrahymena thermophila*: a correlation. *Cell* **25**: 47–56.
- Hallberg RL, Kraus KW and Hallberg EM (1985) Induction of acquired thermotolerance in *Tetrahymena thermophila*. Effects of protein synthesis inhibitors. *Mol. Cell Biol.* **5**: 2061–2069.
- Kahn RW, Anderson BH and Brunk CF (1993) Transformation of *Tetrahymena thermophila* by microinjection of a foreign gene. *Proc. Natl. Acad. Sci. USA* **90**: 9295–9299.
- Kraus KW, Hallberg EM and Hallberg RL (1986) Characterization of a mutant strain of *Tetrahymena thermophila* unable to develop normal thermotolerance. *Mol. Cell Biol.* **6**: 3854–3861.
- Kraus KW, Good P and Hallberg RL (1987) A heat shock induced, polymerase III-transcribed RNA selectively associates with polysomal ribosomes in *Tetrahymena thermophila*. *Proc. Natl. Acad. Sci. USA* **84**: 383–387.
- Landry J, Chretien P, Lambert H, Hickey E and Weber L (1989) Heat shock resistance conferred by expression of the human Hsp27 gene in rodent cells. *J. Cell Biol.* **109**: 7–15.
- Laszlo A (1988) Evidence for two states of thermotolerance in mammalian cells. *Int. J. Hyperthermia* **4**: 513–526.
- Lee YJ and Dewey WC (1988) Thermotolerance induced by heat, sodium arsenite, or puromycin: Its inhibition and difference between 43 degrees C and 45 degrees C. *J. Cell Physiol.* **135**: 397–406.
- Li GC and Laszlo A (1985) Thermotolerance in mammalian cells: A possible role for heat shock proteins. In: *Changes in Eukaryotic Gene Expression in Response to Environmental Stress*, eds BG Atkinson and DB Walton, Academic Press, Orlando, Florida, 227–254.
- Li GC, Li LG, Liu YK, Mak JY, Chen LL and Lee WM (1991) Thermal response of rat fibroblasts stably transformed with the 70-kDa heat shock protein-encoding gene. *Proc. Natl. Acad. Sci. USA* **88**: 1681–1685.
- Lin C-Y, Roberts JK and Key JL (1984) Acquisition of thermotolerance in soybean seedlings: Synthesis and accumulation of heat shock proteins and their cellular localization. *Plant Physiol.* **74**: 152–161.
- Lindquist S (1986) The heat shock response. *Ann. Rev. Biochem.* **55**: 1151–1191.
- McMullin TW and Hallberg RL (1986) An effect of heat shock on ribosome structure: the appearance of a new ribosome-associated protein. *Mol. Cell Biol.* **6**: 2527–2535.
- Mehlen P, Briolay J, Smith L, Diaz-Latoud L, Fabre N, Pauli D and Arrigo A-P (1993) Analysis of the resistance to heat and hydrogen peroxide stresses in COS cells transiently expressing wild type or deletion mutants of the *Drosophila* 27-kDa heat-shock protein. *Eur. J. Biochem.* **215**: 277–284.
- Nelson RJ, Ziegelhoffer T, Nicolet M, Werner-Washburne M and Craig EA (1992) The translational machinery and seventy kilodalton heat shock protein cooperate in protein synthesis. *Cell* **71**: 97–105.
- Panniers R (1994) Translational control during heat shock. *Biochimie* **76**: 737–747.
- Parsell DA, Taulien J and Lindquist S (1994) Heat shock proteins and stress thermotolerance. In: *The Biology of Heat Shock Proteins and Molecular Chaperones*, eds RI Morimoto, A Tissieres and C Georgopoulos, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 457–494.
- Peterson NS and Mitchell HK (1981) Recovery of protein synthesis after heat shock: prior heat treatment affects the ability of cells to translate mRNA. *Proc. Natl. Acad. Sci. USA* **78**: 1708–1711.
- Sanchez Y and Lindquist SL (1990) HSP104 required for induced thermotolerance. *Science* **249**: 1112–1115.
- Sanchez Y, Taulien J, Borkovich KA and Lindquist S (1992) Hsp104 is required for tolerance to many forms of stress. *EMBO J.* **11**: 2357–2364.
- Smith BJ and Yaffe MP (1991) Uncoupling thermotolerance from the induction of heat shock proteins. *Proc. Natl. Acad. Sci. USA* **88**: 11091–11094.
- Solomon JM, Rossi JM, Golik K, McGarry T and Lindquist S (1991) Changes in hsp70 alter thermotolerance and heat-shock regulation in *Drosophila*. *New Biologist*; **3**: 1106–1120.
- Werner-Washburne M, Braun E, Johnston GC and Singer RA (1993) Stationary phase in the yeast *Saccharomyces cerevisiae*. *Microbiol. Rev.* **57**: 383–401.
- Widelitz RB, Magun BE and Gerner EW (1986) Effects of cycloheximide on thermotolerance expression, heat-shock protein synthesis, and heat-shock protein mRNA accumulation in rat fibroblasts. *Mol. Cell Biol.* **6**: 1088–1094.