

Loss of heat-shock acquisition of thermotolerance in yeast is not correlated with loss of heat-shock proteins

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Yeast cells when subjected to a primary heat shock, defined as a temperature shift from 23 to 37°C for 30 min, acquired tolerance to heat stress (52°C/5 min). Primary heat shocked cells incubated at 23°C for up to 3 h, progressively lost thermotolerance but retained high levels of the major heat-shock proteins as observed on polyacrylamide gels. On the other hand, a temperature shift back up to 37°C for 30 min fully restored thermotolerance. The major high-molecular-mass heat-shock proteins (hsp) identified were of approximate molecular mass 100 kDa (hsp 100), 80 kDa (hsp 80) and 70 kDa (hsp 70). The results indicate that loss of heat-shock acquisition of thermotolerance is not correlated with loss of heat-shock proteins.

Yeast Thermotolerance Heat-shock protein

1. INTRODUCTION

All organisms so far tested respond to changes in their local environment by the rapid, preferential synthesis and accumulation of a set of proteins termed heat-shock or stress proteins [1–3]. First noted in *Drosophila* species in response to a heat shock [4,5], the synthesis of the so-called heat-shock proteins has been shown to be induced by agents other than heat [2,5,6].

Although the precise function of the various stress proteins remains obscure, their accumulation in the cell appears to afford protection against subsequent stress situations. Cells pretreated by exposure to a non-lethal heat shock exhibit enhanced rates of survival upon subsequent exposure to lethal temperatures. This phenomenon is referred to as acquired thermotolerance [7–9]. In yeast, a primary heat shock induces not only thermotolerance [9,10] but ethanol [11] and radiation resistance [12].

It is important to recognize, however, that some

heat-shock proteins are present at high constitutive levels in cells at normal physiological temperatures and may be subject to developmental regulation [13–17]. It is conceivable, therefore, that heat-shock proteins may play a more general role in cellular regulation and are not exclusive to stress conditions.

The present studies, which focus on the transient nature of the heat-shock response, show that the presence of heat-shock proteins in yeast does not necessarily correlate with cell thermotolerance.

2. MATERIALS AND METHODS

2.1. Growth of organism

Saccharomyces cerevisiae (ATCC 26422) was grown in media containing, per litre: 10 g yeast extract, 5 g bacteriological peptone, 3 g KH_2PO_4 , 3 g $(\text{NH}_4)_2\text{SO}_4$, 25 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 25 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 20 g glucose (GYP). Starter cultures were grown in 20 ml GYP medium on an orbital shaker operating at 180 rpm and 23°C. Test cultures (100 ml) were inoculated with 0.2 ml starter culture and grown to cell densities of 1×10^7 – 1×10^8 cells/ml (12–20 h).

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2.2. Primary heat shock and heat stress

Cells grown at 23°C were heat shocked by rapidly raising the temperature to 37°C and incubating for 30 min in an orbital shaker at 37°C. Cells thus treated were termed primary heat shocked cells. Heat stressed cells were defined as cells (23°C) heated directly and rapidly to 52°C/5 min or primary heat shocked cells similarly heated to 52°C/5 min. Following heat treatment, cells were rapidly cooled in an ice bath and incubated on an orbital shaker at 23°C. Cell viabilities were determined by dilution plate growth (GYP plus 2% agar) after 2–4 days at 30°C.

2.3. Polyacrylamide gel electrophoresis

Proteins were extracted from cells by centrifuging 1.5 ml culture in an Eppendorf microcentrifuge for 10 s and the pelleted cells broken in the presence of glass beads (0.45–0.50 mm diameter) in 200 µl of buffer consisting of 1 mM phenylmethylsulphonyl fluoride, 2 mM EDTA, 2% SDS, 1% mercaptoethanol, 2% Ficoll 400, 0.0025% bromophenol blue and 25 mM Tris-HCl, pH 7.0. Cells were broken by vortexing at 6 × 20 s intervals with cooling in between. The homogenate was centrifuged in an Eppendorf microcentrifuge for 10 min. The supernatant was removed and heated for 10 min at 70°C. Protein samples (7–15 µl) and molecular mass standards (LKB, Stockholm) were applied by microsyringe to loading wells of a 4.5–10% gradient acrylamide gel (1.5 mm thick × 160 mm wide × 160 mm length) and samples run at 5 mA until the tracking dye reached the base of the gel. Gels were fixed and stained using an AgNO₃-staining procedure [18].

3. RESULTS

Control cells (23°C), when exposed to a heat stress (52°C/5 min), showed a marked drop in viability from about 8×10^7 cells/ml (fig.1a) down to $<1 \times 10^4$ cells/ml (fig.1b). By contrast, control cells which had been primary heat shocked (37°C/30 min) immediately prior to heat stress showed a high viability, around 2×10^7 cells/ml (fig.1c). Incubation of primary heat shocked cells at 23°C led to a progressive loss in thermotolerance as indicated by decreased cell viabilities of around 8×10^5 cells/ml and 2×10^4 cells/ml after 1 and 3 h, respectively, at 23°C

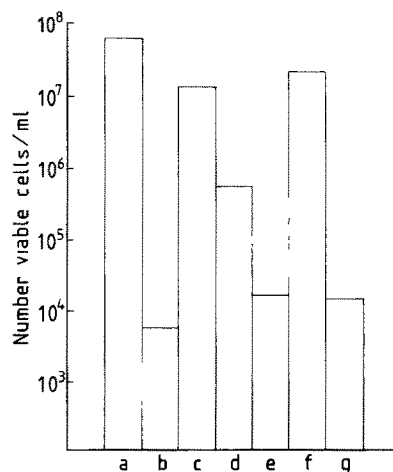


Fig. 1. Loss of thermotolerance following a primary heat shock (37°C/30 min). Heat stress was at 52°C/5 min. (a) Control 23°C (20 h) non-stressed cells; (b) control cells heat stressed directly to 52°C/5 min; (c) control cells, primary heat shocked followed by heat stress; (d) control cells, primary heat shocked, incubated at 23°C for 1 h followed by heat stress; (e) control cells, primary heat shocked, incubated at 23°C for 3 h followed by heat stress; (f) control cells, primary heat shocked, incubated at 23°C for 3 h, primary heat shocked again followed by heat stress; (g) control cells incubated for a further 3 h at 23°C followed by heat stress. Cell numbers determined by dilution plate count.

(fig.1d,e). On the other hand, a re-heat shock (37°C/30 min) after 1 or 3 h at 23°C fully restored heat-shock acquisition of thermotolerance (fig.1f).

Gel electrophoretic analyses of the protein pattern of control (23°C) and cells heat shocked (23–37°C) for 30 min, 1, 3 and 6 h are presented in fig.2. A newly synthesized protein designated hsp 100, corresponding to a molecular mass of approx. 100 kDa, was clearly defined on AgNO₃-stained gels following a heat shock. Two other heat-shock proteins, designated hsp 80 (approximate molecular mass 80 kDa) and hps 70 (approximate molecular mass 70 kDa) were clearly visible. These latter two proteins were also present in 23°C control cells and therefore represented heat-shock proteins whose syntheses have markedly increased as a result of a primary heat shock. It was noteworthy that the protein pattern of cells following prolonged incubation and growth (6 h) at 37°C corresponded closely to that of primary heat shocked cells. The protein pattern of cells

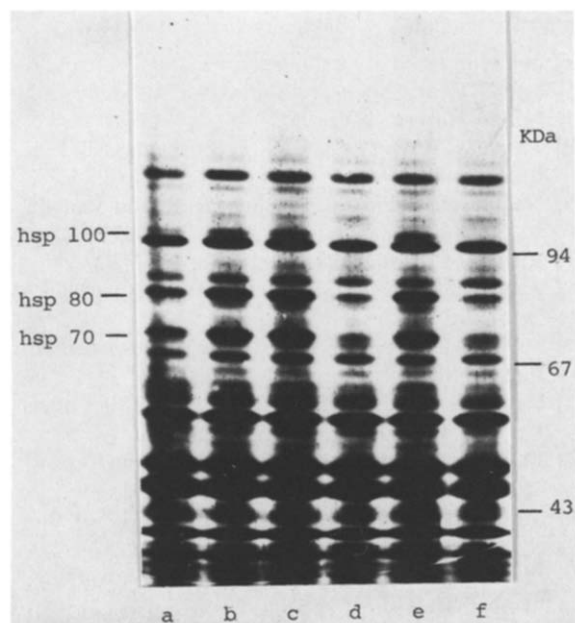


Fig.2. Polyacrylamide gel electrophoretic analysis of cells heat shocked for various times. Protein pattern for control 23°C cells primary heat shocked for (a) 30 min, (b) 1 h, (c) 3 h and (e) 6 h. Protein pattern for (d) control 23°C cells at the beginning of the experiment and (f) control 23°C cells, 6 h (incubation at 23°C on an orbital shaker) after the beginning of the experiment. Heat-shock proteins are designated as hsp 100, hsp 80 and hsp 70.

which had been primary heat shocked and subsequently incubated at 23°C for 1, 3 and 6 h is shown in fig.3. The major heat-shock proteins, hsp 100, hsp 80 and hsp 70 were clearly retained even after 6 h incubation at 23°C.

4. DISCUSSION

In the present studies, thermotolerance induced by a primary heat shock was rapidly lost when cells were stored at 23°C. After 1 h at 23°C thermotolerance declined appreciably and was essentially lost thereafter. Results essentially identical to the above were obtained by the author in [19]. After 30 min storage at 23°C acquired thermotolerance was virtually unaffected. However, viability decreased to approx. 40–50% at 1 h and 20% by 1.5 h. It was noted that the thermotolerant stage was lost rapidly at 23°C and could not be ac-

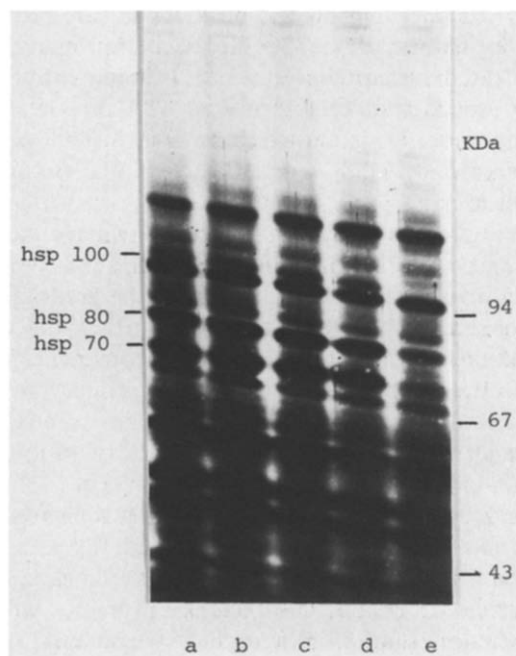


Fig.3. Protein pattern of primary heat shocked cells following incubation for various times at 23°C. (e) Control 23°C cells; (d) primary heat shocked cells and after incubation at 23°C for (c) 1 h; (b) 3 h and (a) 6 h. Heat-shock proteins are designated as hsp 100, hsp 80 and hsp 70.

counted for merely by dilution of heat-shock proteins, since the doubling time at 23°C was 3–4 h. Authors in [9] reported that when cells were returned to 23°C following a 20 min heat shock (36°C), the phenotype was maintained at a high level. It was noteworthy however, that after 1 h storage, thermotolerance decreased to 9% viable cells as compared to 28.5% viable cells observed immediately following primary heat shock.

It was highly significant that in the present studies loss of thermotolerance was not correlated with concomitant loss of heat-shock proteins. Cells which had been heat shocked from 23°C to 37°C/30 min followed by incubation at 23°C for up to 3 h progressively lost thermotolerance but retained high levels of the major heat-shock proteins as observed on gels. On the other hand, a temperature shift back up to 37°C fully restored thermotolerance. Thus a causal relationship be-

tween the elevated heat shock temperature per se rather than the presence of heat-shock proteins, is implicated in thermotolerance. It is noteworthy, in this respect, that cells grown at 37°C are intrinsically more resistant to heat stress than cells grown at 23°C ([20], Cavicchioli and Watson, unpublished).

The protein patterns for cells primary heat shocked to 37°C for 30 min, 1 and 3 h were qualitatively similar. In particular, the continued presence of hsp 70 and hsp 80 as well as the induced protein hsp 100, remained unequivocal. Indeed, the protein pattern of cells primary heat shocked 23°C to 37°C for 1–3 h was essentially analogous to cells grown continuously (6 h) at 37°C.

There is evidence from systems other than yeast, that heat-shock proteins are retained for a considerable time following a heat shock. In *Neurospora crassa*, heat-shock proteins were detectable within 15 min of heat treatment [21]. On reversion to normal growth temperature the heat-shock protein bands persisted for at least up to 6 h.

It is worth emphasising that most published data deal with the products or rate of heat-shock protein synthesis following a rise in temperature. Constitutive levels of heat-shock proteins have been generally ignored. It is well established in yeast that the rate of heat-shock protein synthesis peaks at 20–90 min following a heat shock and then declines with continued incubation at the primary heat shock temperature, generally 36–37°C [9,22–24], reverting to a protein pattern similar to that of 23°C grown cells [22]. Critical examination of the latter observation shows that the levels of a 100 kDa protein (hsp 100 taken as a measure of heat-shock protein synthesis) relative to total protein, peaked at 90 min and had only decreased to approx. 65% (~1.6% of total protein) of the peak level (~2.5% of total protein) after 8 h at 36°C.

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