# Mitochondrial and cytoplasmic protein syntheses are not required for heat shock acquisition of ethanol and thermotolerance in yeast

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Heat shock acquisition of ethanol- and thermotolerance in *Saccharomyces cerevisiae* was not inhibited in cells incubated in the presence of cycloheximide or chloramphenicol. Respiratory-deficient ($\varrho^-$) mutants also characteristically exhibited the heat shock response. It was concluded that mitochondrial and cytoplasmic protein syntheses are not required for heat shock acquisition of ethanol and thermotolerance in yeast.

*Heat shock* *Protein synthesis* *Thermotolerance* *Ethanol-tolerance* *Yeast*

## 1. INTRODUCTION

It is now well established that heat shock treatment of a range of organisms, from bacteria to man, leads to the synthesis of a specific set of proteins, commonly termed heat shock proteins [1–3]. The synthesis of a similar, if not identical, set of proteins is also observed on exposure of cells to environmental stresses other than heat. These include cold [4,5], heavy metals [6,7], arsenite [1,8] and ethanol [9–11]. The functional significance of these stress proteins is unknown.

A clue as to a possible role for heat shock proteins comes from reports that agents, such as arsenite and ethanol, which are known to induce or enhance the synthesis of heat shock proteins, induce thermotolerance and, conversely, agents which induce thermotolerance also induce synthesis of heat shock proteins [9–11]. These observations have led to the general conclusion of a causal relationship between heat shock acquisition of thermotolerance and the synthesis of heat shock proteins.

We have previously reported the heat shock acquisition of ethanol tolerance in yeasts [12]. Here we present evidence that neither mitochondrial nor cytoplasmic protein synthesis is required for heat shock acquisition of thermal and ethanol tolerance in *Saccharomyces cerevisiae*.

## 2. MATERIALS AND METHODS

### 2.1. *Cultures*

*S. cerevisiae* CBS1171, CBS1237 and CBS1242 were obtained from the Centraalbureau voor Schimmelculturen (Delft, The Netherlands). *S. cerevisiae* ATCC26422, a saké yeast, was from the American Type Culture Collection (Rockville, MD). Cells were grown at 23°C to mid-log phase in media containing, per litre: 100 g glucose, 10 g Oxoid yeast extract, 5 g Oxoid bacteriological peptone, 3 g $KH_2PO_4$, 3 g $(NH_4)_2SO_4$, 25 mg $CaCl_2$ and 25 mg $MgSO_4 \cdot 7H_2O$.

### 2.2. *Conditions of heat shock*

Cultures grown at 23°C to mid-log phase were primary heat-shocked by rapidly raising the temperature to 37°C by placing flasks (20 ml media) in a hot water-bath, followed by incubation at 37°C for 30 min in an orbital shaker incubator

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operating at 180 rpm. Following primary heat shock (37°C/30 min), cultures were cooled to 23°C and ethanol was added. The concentration of ethanol in the media varied from 10.5% (w/v) to 16.5% (w/v) as indicated in the text. Secondary heat shock was attained by heating flasks in a hot water-bath to the required temperature (45–60°C) and incubating for 5 min.

### 2.3. *Protein synthesis inhibitors*

Cycloheximide (100 μg/ml) and chloramphenicol (4 mg/ml) were added to cultures 15 min prior to primary heat shock. Cultures were subsequently heat shocked, cooled to 23°C and incubation continued at 23°C in the presence of antibiotic and/or ethanol.

### 2.4. *Viability measurement*

Cell viability was determined using, as staining solution, 0.03% (w/v) methylene blue in Ringer solution as outlined in [13]. Percentage viability was calculated assuming clear cells as alive and stained cells as dead; 100–300 cells were scored for cell viability. Ability of viable cells to grow and reproduce was determined by washing cells with $H_2O$ and subculturing (1%, v/v, inoculum) in fresh media, lacking ethanol and the appropriate antibiotic. A growth curve, relating number of cells to time of growth at 23°C, was constructed.

### 2.5. *Isolation and characterization of respiratory-deficient ($\varrho^-$) mutants*

The method adopted for the isolation of mutants was to add a high concentration (100 μg/ml) of ethidium bromide (Sigma) to an actively growing yeast culture and incubating for 24 h at 23°C. The tetrazolium overlay procedure [14] and respiratory measurements with an oxygen electrode (Rank) were used to confirm the respiratory-deficient nature of the mutants.

## 3. RESULTS

Heat shock acquisition of ethanol tolerance in *S. cerevisiae* is shown in fig.1. In the absence of cycloheximide, primary heat-shocked cells, defined as cells heat-shocked from 23–37°C for 30 min, were markedly more resistant to ethanol-induced cell death as compared to control cells (23°C). After 7 h incubation at 23°C, at which time control cells were essentially all dead, primary heat-shocked cells were still 80% viable. Cells previously incubated (15 min) and then primary heat-shocked (37°C/30 min) in the presence of cycloheximide also showed a marked enhancement of ethanol tolerance.

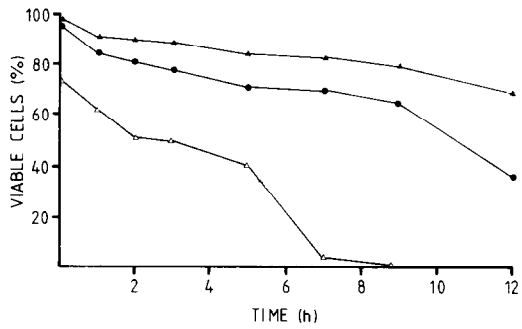


Fig.1. Effect of cycloheximide on heat shock acquisition of ethanol tolerance in strain ATCC26422. The ethanol concentration was 15% (w/v). (△) Control cells, 23°C, (▲) primary heat-shocked cells, 37°C/30 min, (●) control cells incubated 15 min in the presence of cycloheximide (100 μg/ml), and then primary heat-shocked, 37°C/30 min.

It was noteworthy that cycloheximide-treated cells were subjected to 3 environmental stresses, namely, cycloheximide, heat shock and high concentrations of ethanol. Nevertheless, these stressed cells retained viability and ability to reproduce after transfer to fresh media lacking ethanol. However, long term (>10 h) incubation in the presence of cycloheximide and ethanol led to a decrease in cell viability.

The effect of chloramphenicol, a known inhibitor of yeast mitochondrial protein synthesis [15], on heat shock acquisition of thermal tolerance is shown in fig.2. The presence of chloramphenicol had little effect on heat shock acquisition of thermal tolerance. Primary heat-shocked cells, when subjected to a secondary heat shock (55°C/5 min), were 80% viable immediately after the heat stress. Over a period of 3 h, cell viability fluctuated between 70–80% before decreasing to 70%, 4 h after the initial secondary heat shock. By contrast, the viability of control cells (23°C) after secondary heat shock was markedly lower and stabilized at 30%, 4 h after the initial heat stress. Similar results were obtained

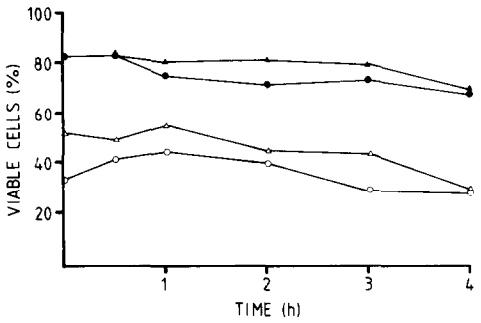


Fig.2. Effect of chloramphenicol on heat shock acquisition of thermotolerance in strain ATCC26422. (△) Control cells heat-stressed, 23–55°C/5 min, (○) control cells preincubated in the presence of chloramphenicol (4 mg/ml) and then heat-stressed 23–55°C/5 min, (▲) primary heat-shocked cells (37°C/30 min) followed by a secondary heat shock (55°C/5 min), (●) control cells preincubated 15 min in chloramphenicol (4 mg/ml) before primary heat shock (37°C/30 min) followed by a secondary heat shock (55°C/5 min).

on heat shock acquisition of ethanol tolerance (not shown).

Heat shock acquisition of thermal and ethanol tolerance was also exhibited by respiratory-deficient ($\varrho^-$) mutants of *S. cerevisiae*. Control cells (23°C) died within 24 h after exposure to high concentrations of ethanol (fig.3). Primary heat-shocked cells, on the other hand, were 80% viable after 24 h, decreasing to 30% after 48 h. A

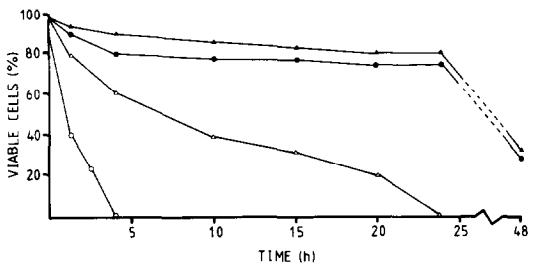


Fig.3. Heat shock acquisition of ethanol and thermotolerance in a respiratory-deficient ($\varrho^-$) mutant of strain ATCC26422. The ethanol concentration was 13.5% (w/v). (△) Control cells 23°C, (○) control cells heat-stressed, 23–52°C/5 min, (▲) primary heat-shocked cells (37°C/30 min), (●) primary heat-shocked cells (37°C/30 min) followed by a secondary heat shock (52°C/5 min).

primary heat shock protected cells against both ethanol and thermal (52°C/5 min) death. Control cells, heated directly to 52°C/5 min, were the most stress sensitive and showed little resistance to ethanol or thermal stress. Cell death occurred 4 h after the initial heat stress.

## 4. DISCUSSION

It has been established that exposure of yeast cells, grown at 23°C, to a primary heat shock at sublethal temperature (36–37°C) results in protection from temperatures (52–55°C) which would normally lead to rapid cell death [16,17]. Furthermore, authors in [17] have reported loss of heat shock acquisition of thermotolerance in cells pretreated with cycloheximide, to inhibit heat shock protein synthesis. We have confirmed, by sodiumdodecylsulphate-polyacrylamide gel electrophoresis, that cycloheximide added to yeast cells prior to heat shock at 37°C, inhibits the synthesis of heat shock proteins (unpublished). On the other hand, our present results unequivocally show that cycloheximide had little effect on the heat shock acquisition of thermal and ethanol tolerance in yeast. These results were obtained in over 30 separate experiments and using 4 different strains of *Saccharomyces*.

We conclude, therefore, that heat shock proteins are not obligatory for the expression of heat shock acquisition of thermal and ethanol tolerance in yeasts.

The question then arises as to the role of mitochondrial protein synthesis in the yeast heat shock response. We have approached this question by using chloramphenicol to inhibit mitochondrial protein synthesis and by using respiratory-deficient ($\varrho^-$) mutants, induced by ethidium bromide mutagenesis of respiratory-competent ($\varrho^+$) yeasts.

Cells preincubated (15 min) and then heat-shocked in the presence of chloramphenicol still acquired thermal (fig.2) and ethanol tolerance. Similarly, respiratory-deficient ($\varrho^-$) mutants exhibited heat shock acquisition of thermal and ethanol tolerance (fig.3). It is noteworthy that chloramphenicol-treated cells (unpublished) and respiratory-deficient mutants ([18], unpublished) characteristically synthesize heat shock proteins in response to a primary heat shock.

There is recent evidence in the literature to support the conclusion that heat shock proteins are not directly involved in thermotolerance. The author in [19] has independently reported that thermotolerance in yeast does not require cytoplasmic protein synthesis and authors in [9] have shown that heat shock protein synthesis is not a sufficient condition for thermotolerance in Morris hepatoma cells.

Further support comes from recent gene cloning experiments on yeast heat shock proteins. Authors in [20] have cloned a major heat shock-inducible protein ($M_r$ 90000) into yeast. Under conditions where this heat shock protein represented up to 1/5 of the total protein synthesized during a temperature upshift from 23 to 36°C, no effect on the synthesis of other major heat shock proteins and no alterations in the phenotypic response of heat shock acquisition of thermotolerance were noted.

## ACKNOWLEDGEMENTS

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