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HEAT SHOCK PROTEINS AND THERMAL RESISTANCE IN YEAST

Lee McAlister and David B. Finkelstein

Department of Biochemistry, The University of Texas Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235

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SUMMARY

A rapid shift in cultivation temperature of Saccharomyces cerevisiae from 23° to 36° results in protection from death due to extreme heat treatment (52°). The level of this acquired thermal resistance shows an excellent correlation with the cellular level of the heat shock proteins which are transiently induced by such a temperature shift.

INTRODUCTION

All organisms thus far examined exhibit elevated synthesis of a small set of proteins (heat shock proteins) upon rapid elevation of growth temperature beyond some fixed level. While this heat shock response has been most widely studied in Drosophila (1), equivalent responses have been observed in mammalian cell cultures (2), bacteria (3,4) and also in the yeast Saccharomyces cerevisiae (5,6). The fact that the patterns of protein synthesis are dramatically altered upon a marginal shift in cultivation temperature implies that these newly synthesized heat shock proteins must fulfill some essential cellular function. Since elevated temperature induces the heat shock responses we reasoned that the heat shock proteins might afford the cells with some protection from thermal injury. In this paper we demonstrate that shifting an actively growing culture of S. cerevisiae from 23° to 36° (both temperatures within the normal growth range for the organism, 7), results in a transient protection of the culture from death due to exposure to 52°. This thermal resistance correlates well with the cellular level of heat shock proteins.

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MATERIALS AND METHODS

Strains and Culture Conditions

Saccharomyces cerevisiae strain A364A (a, gal 1, adel, ade2, ural, his7, lys2, tyr1) and the temperature sensitive strain ts136 derived from A364A carrying the allele rna1 (8) were obtained from the Yeast Genetic Stock Center, Berkeley, California. Cultures were grown to mid log phase (2 x 10^7 cells/ml) at 23° or 36° in buffered low sulfate medium supplemented to meet the requirements of these strains (5). Temperature shifts were performed by pipetting cells into flasks preequilibrated in a 23° or 36° water bath.

Determination of Thermal Resistance

One milliliter aliquots of the yeast cultures were transferred to 16 x 125 mm test tubes and placed in a 52° water bath for 5 min. At the end of the heating period the cultures were rapidly cooled by placing in an ice bath. The heated culture (as well as an unheated control) were diluted into YPD (1% Bacto yeast extract, 2% peptone and 2% glucose) and spread onto YPD agar plates (YPD solidified with 2% Bacto agar) in order to determine percentage survival.

Quantitation of 100,000 Molecular Weight Heat Shock Polypeptide

Strain A364A was uniformly labelled by growth in buffered low sulfate medium containing $H_2{}^{35}SO_4$ (200 μli/ml) and subsequently maintained in logarithmic growth by frequent dilutions with fresh prewarmed medium containing isotope at the same specific activity. Samples of the culture were taken, SDS-soluble proteins were fractionated by electrophoresis on SDS-8% polyacrylamide slab gels, the gels autoradiographed and subsequently quantitated by densitometry as previously described (5).

RESULTS AND DISCUSSION

It has been previously shown that in yeast survival to thermal killing can vary with the stage of growth (9,10) or position in the cell cycle (10) as well as cultivation temperature (11). We have attempted to minimize variations between cultures in these studies by using only actively growing logarithmic cultures. When such actively growing yeast are challenged with a 5 min exposure to 52° it may be seen that the degree of viability is highly dependent upon the pretreatment regime (Table 1). In particular, while 52° treatment of a culture pregrown at either 23° or 36° results in almost complete killing, shifting the cultures from 23° to 36° for 90 min prior to imposition of the thermal stress results in a large enhancement of survival.

In order to test the hypothesis that the increased protection from thermal injury afforded by a 23° to 36° culture shift may be correlated with the cellular level of heat shock proteins the following experiment was per-

Table 1. Killing of Yeast Cells at Elevated Temperature

Cultivation temperature (°)	Percent survival*
23	0.055 ± 0.028 (6)
36	1.76 ± 0.54 (3)
23 → 36+	41.8 ± 5.0 (4)

Logarithmic phase Saccharomyces cerevisiae strain A364A were exposed to 52° for 5 min as outlined in Materials and Methods.

*Percentage survival is reported ± standard error of the mean. The number of independent determinations for each experiment is enclosed by parentheses.

+The culture was grown at 23° and shifted to 36° for 90 min prior to the heat kill.

formed. A culture of yeast growing actively at 23° was transferred to 36° and with time the ability to withstand thermal killing at 52° was examined. At the same time, the cellular level of one of the major inducible heat shock proteins (a polypeptide with an apparent MW of 100,000 as determined by SDS-polyacrylamide gel electrophoresis, 5) was quantitated. The results of this experiment (Fig. 1) demonstrate the excellent correlation between the ability to survive thermal injury and the cellular level of heat shock protein. Moreover, as may also be seen in Fig. 1, the addition of the protein synthesis inhibitor cycloheximide to the yeast culture prior to the shift from 23° to 36°, a treatment which prevents the appearance of the heat shock proteins (5), eliminates the acquisition of thermal resistance.

We have previously demonstrated that synthesis of heat shock proteins does not require continuous exposure of a culture to 36°. Shifting yeast from 23° to 36° for a brief period results in the induction of the ability to synthesize heat shock proteins even after the culture is returned to 23° (5). Similarly, as shown in Table 2, shifting a culture to 36° for a brief period results in the acquisition of the thermal resistant phenotype and this phenotype is maintained when the culture is returned to 23°C.

Further correlations between the cellular level of heat shock proteins and temperature resistance can be made by use of a yeast strain (ts136)

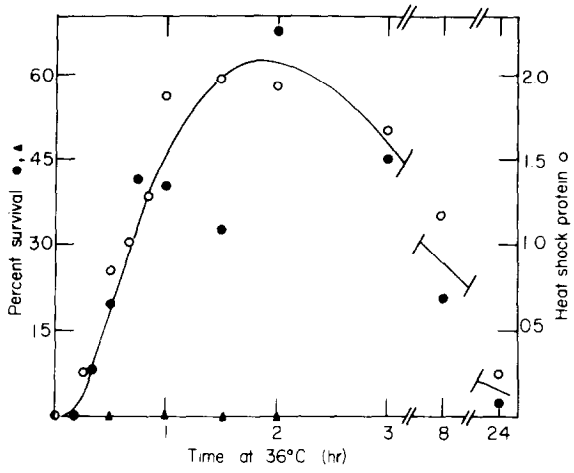

Figure 1. Induction of resistance to heat kill and 100,000 molecular weight heat shock polypeptide in A364A cells following a shift from 23° to 36°. A culture of A364A, grown to mid log phase in buffered low sulfate medium at 23°, was divided into two portions. One portion of the culture received 100 µg/ml of cycloheximide (Δ) and the other portion of the culture received no additions (O). Thirty sec. after the addition of cycloheximide both cultures were shifted to 36° and subsequently maintained in logarithmic growth by frequent dilutions with fresh prewarmed medium. With time, samples were removed and subjected to a 5 min. heat kill at 52°. Percentage survival was determined as outlined in Materials and Methods. The increase in level of the 100,000 molecular weight heat shock polypeptide as a percentage of total protein following a shift in culture temperature from 23° to 36° was determined as outlined in Materials and Methods (O).

harboring a mutant gene (rna 1) temperature sensitive at 36° for RNA transport from the nucleus to the cytoplasm (8). While strains of yeast carrying this allele fail to synthesize heat shock proteins when shifted from 23° to 36° they do continue to synthesize preshift proteins,

Table 2. Heat Kill of Yeast Following Transient Exposure to 36°

Cultivation temperature (°)	time,min	Percent Survival A364A	Percent Survival ts136
23	continuous	0.02	0.03
23 → 36	continuous, 20	28.5	0.8
23 → 36 → 23	continuous 20,60	9.0	10.1

Cultures of Saccharomyces cerevisiae strain A364A and strain ts136 were grown to mid log phase at 23° in buffered low sulfate medium. The cultures were shifted to 36° for 20 min and then returned to 23° for 60 min. Percentage of cells surviving a 52° heat kill was determined as outlined in Materials and Methods.

albeit with a decreasing rate, as the preexisting cytoplasmic mRNA decays (6). As seen in Table 2, shifting strain ts136 to 36° for 20 minutes results in no increase in thermal resistance. Thus, transport of RNA from nucleus to cytoplasm is required for both the synthesis of heat shock proteins and the acquisition of thermal resistance following a 23° to 36° shift. Furthermore, when a culture of strain ts136 which has been previously shifted from 23°C to 36°C is subsequently returned to 23°C, synthesis of heat shock proteins now begins (L.M. and D.B.F., submitted for publication); under these conditions thermal resistance is acquired (Table 2). This result is exactly as predicted if an increased level of heat shock proteins is the causitive agent of thermal resistance. We thus conclude that while continued exposure to 36° is not alone sufficient to elicit temperature resistance, conditions which allow cells to synthesize heat shock proteins are also permissive for the acquisition of thermal resistance.

If the correlation between thermal resistance and the cellular level of heat shock proteins is valid, it should be possible to select for mutants which overproduce heat shock proteins under non heat shock conditions by selecting for the expected phenotype; viz., enhanced survival following acute thermal stress. Such mutants have in fact been isolated by an enrichment procedure which selects repeatedly for survivors of a 52° heat exposure (D.B.F. and S. Strausberg, unpublished).

While it is clear that thermal stress of an organism is an extreme environmental insult which causes a wide range of injury and that protection could in fact be due to many mechanisms, the strong correlation between thermal resistance and the cellular level of heat shock proteins suggests that protection from heat kill may in some instances be provided by heat shock proteins. It is hoped that further examination of temperature resistant mutants which overproduce heat shock proteins will provide some insight into the exact function of the heat shock proteins and their regulation.

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