Thermotolerance and the Heat-shock Response in *Candida albicans*

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At elevated temperatures, yeast cells of *Candida albicans* synthesized nine heat-shock proteins (HSPs) with apparent molecular masses of 98, 85, 81, 76, 72, 54, 34, 26 and 18 kDa. The optimum temperature for the heat-shock response was 45 °C although HSPs were detected throughout the range 41–46 °C. Protein synthesis was not observed in cells kept at 48 °C. Yeast cells survived exposure to an otherwise lethal temperature of 55 °C when they had previously been exposed to 45 °C. The thermotolerance induced during incubation at 45 °C required protein synthesis, since protection was markedly reduced by trichodermin. Mercury ions induced a set of three stress proteins, one of which corresponded in size to an HSP, and cadmium ions evoked one stress protein seemingly unrelated to the HSPs observed after temperature shift.

INTRODUCTION

Prokaryotic and eukaryotic cells respond to a sudden change of temperature by the increased production of an array of proteins called heat-shock proteins (HSPs). These HSPs may be induced by heat stress or may be constitutive proteins whose production is markedly increased as a response to such stress. In every system where heat shock has been investigated HSPs have been found (Lindquist, 1986). In some organisms production of HSPs has been associated with an increase in thermotolerance, i.e., an enhanced ability of organisms to survive exposure to otherwise lethal temperatures (Carper *et al.*, 1987; Plesset *et al.*, 1982; Plesofsky-Vig & Brambl, 1985).

The opportunistic fungus *Candida albicans* grows as a yeast-form that reproduces by budding on most media when incubated at room temperature (23–25 °C) (Odds, 1988). The yeast cells form germ tubes in media that contain germ-tube-inducing ingredients when shifted from 23 °C to 37 °C at pH 6.5–7.0 (Dabrowa & Howard, 1983). Such a temperature shift also evokes HSPs (Dabrowa & Howard, 1984; Zeuthen *et al.*, 1988). It has been suggested that HSPs might be involved in some aspects of growth and differentiation of cells (Carper *et al.*, 1987; LeJohn & Braithwaite, 1984; Lindquist, 1986; Schlesinger, 1986; Wanner *et al.*, 1985), yet the HSPs observed after a temperature shift from 23 °C to 37 °C would appear not to be involved in the germination of *C. albicans* in the warm-blooded host since the yeasts do not experience a temperature shift under these circumstances, although tissue invasion is often initiated by germ-tube emergence (Borg & Rùchel, 1988; Odds, 1988; Ray & Payne, 1988). However, it is well-known that forms of stress other than heat shock may evoke HSPs and it is, therefore, possible that HSPs produced by stimuli other than heat may play a role in germ-tube formation in a warm-blooded host (Jenkins *et al.*, 1988; LeJohn & Braithwaite, 1984). We have continued our study of the HSPs of *C. albicans* by assessing the stress response of cells shifted from 37 °C to supraoptimal temperatures (41–46 °C), temperature shifts that are not accompanied by germ-tube formation. These studies were made to observe the heat-shock response independent of morphogenetic changes and to compare the response to that evoked by other forms of stress.

Abbreviations: HSP, heat-shock protein; GPA, glucose/peptone agar; YNB, yeast nitrogen base (without amino acids); YNBG, YNG + 1% (w/v) glucose.

METHODS

Organisms. The *Candida albicans* strains used in the study were strain 300 (Dabrowa & Howard, 1984; National Collection of Pathogenic Fungi, NCPF 3153A), strain 300-SG (Howard *et al.*, 1986), strain 320 (Gibbons & Howard, 1986) and strain 336, a recent isolate (Zeuthen *et al.*, 1988). Strains 300, 320 and 336 formed germ tubes under standard test conditions (Zeuthen *et al.*, 1988) while strain 300-SG was germination deficient (Howard *et al.*, 1986). Stock cultures are preserved in the Fungus Collection, University of California, Los Angeles, CA, USA. Working cultures were maintained on GPA (see below) in a refrigerator and subcultured every month.

Media. Glucose/peptone agar (GPA) contained 2% (w/v) Bacto Dextrose (Difco), 1% (w/v) Bacto Peptone (Difco) and 2% (w/v) Bacto Agar (Difco). Yeast Nitrogen Base without amino acids (YNB) was prepared in accordance with the manufacturer's instructions (Difco). YNBG was YNB with 1% (w/v) Bacto Dextrose (Difco). Chemicals, including amino acids, were purchased from Sigma, Calbiochem or Eastman Kodak.

Analysis of the heat shock response. Cells were grown overnight in YNBG at 37 °C and used to inoculate 50 ml of fresh YNBG contained in 300 ml nepheloflasks with side-arms (Bellco Glass). The cultures were grown on a rotary shaker (New Brunswick) at 37 °C and growth was assessed at regular intervals by measuring optical density (OD) with a Klett-Summerson photoelectric colorimeter (red filter). Growth was interrupted and cultures used at the mid-exponential phase of growth (Howard *et al.*, 1986). Cells were harvested, washed in distilled water and resuspended at 1×10^8 cells ml⁻¹ in YNBG prewarmed to the different temperatures studied (40–46 °C). Proteins were labelled with L-[³⁵S]methionine (specific activity 45 TBq mmol⁻¹) to a final concentration of 3.7 MBq ml⁻¹ for a 15 min pulse. Labelling was stopped by addition of unlabelled DL-methionine (final concentration 650 µM). The cells were immediately placed on ice, washed in distilled water, and held as a pellet at -20 °C until they were prepared for electrophoresis.

Effect of heavy metals on protein synthesis. Cells were grown in YNBG at 37 °C, harvested, washed and standardized as described in the preceding paragraph. About 1×10^8 cells ml⁻¹ were added to YNBG containing separately 10 µM-phenylmercuric acetate, and 1, 20 and 40 mM CdCl₂, SrCl₂ or SnCl₂. Proteins were labelled and stored for electrophoresis as described in the preceding paragraph.

Thermal death time. Cells were grown at 37 °C as described above. Growth was interrupted and cells used during the exponential and stationary phases of growth (Howard *et al.*, 1986). Cells were harvested at the desired phase of growth, washed in distilled water and resuspended at a concentration of 5×10^6 cells ml⁻¹ in YNBG prewarmed to 55 °C. Samples were removed at regular intervals, diluted and spread on GPA. Colonies (c.f.u.) were counted after 5–7 d incubation at room temperature. Both mid-exponential and stationary phase cells were tested.

Thermotolerance. Preliminary experiments established that *C. albicans* strain 300 survived exposure to temperatures in the range 40–46 °C. The lethal temperature of 55 °C used in the thermal-death time experiments (preceding paragraph) was arbitrarily chosen to be well above the range of tolerated supraoptimal temperatures.

The induction of thermotolerance was tested by shifting cells, grown and prepared as described above, from 37 °C to selected supraoptimal temperatures (40–46 °C) for 30 min. All media were prewarmed to the temperature studied. The temperature-exposed cells were shifted to the prewarmed YNBG at the lethal temperature (55 °C). Samples were withdrawn at intervals, diluted in distilled water and spread on GPA. Colonies were counted after 5–7 d incubation at room temperature. The results of each experimental treatment are expressed as a percentage of controls kept at 37 °C. To test for the requirement for protein synthesis in the induction of thermotolerance, 3.5 µg trichodermin ml⁻¹ was added to YNBG 20 min before a shift from 37 °C to 43 °C or to 45 °C. The trichodermin was left in the medium during the 30 min incubation at 43 °C or 45 °C. Cells preincubated at 43 °C or 45 °C in the presence of trichodermin were shifted to 55 °C. After the shift to 55 °C, samples were withdrawn at selected intervals, diluted and cultured as indicated previously. Trichodermin was a gift from W. O. Godtfredsen, Leo Pharmaceuticals, Ballerup, Denmark.

Polyacrylamide gel electrophoresis. Cells from experiments designed to study the stress response were washed twice and suspended in 0.4 ml 1 mM-phenylmethylsulphonyl fluoride in water. Glass beads (2 vols, 0.45 mm) were added to each tube. Cells were broken by mixing on a vortex mixer (Vortex-Genie) at maximum speed for six periods of 30 s each. Cells were kept on ice except during vortex mixing. Microscopic examination revealed that 90–95% of the cells were broken. Beads and cell wall debris were pelleted by centrifugation. The supernatant containing crude protein was added to an equal volume of 0.1 M-Tris/HCl (pH 6.8) containing 18% (v/v) glycerol, 1.8% (w/v) SDS, 0.18% 2-mercaptoethanol and 0.0018% bromophenol blue, and was immediately heated at 100 °C for 2 min. The radiolabelled proteins in the cell extracts were separated by one-dimensional SDS gel electrophoresis in 12% (w/v) polyacrylamide slab gels (Dabrowa & Howard, 1984). The time of electrophoresis was approximately 3 h. The amount of protein per lane was 10–15 µg. Proteins were separated at a constant current of 25 mA, fixed in methanol/acetic acid/water (5:4:1, by vol.) and stained by the silver nitrate method (Morrissey, 1981). After staining, the gels were dried onto GelBond PAG support paper (FMC BioProducts) and exposed to Kodak XAR-5 film at -70 °C. Molecular mass markers were from Pharmacia. Reagents for electrophoresis were obtained from BRL and Sigma.

RESULTS

Heat-shock proteins

HSPs were produced by *C. albicans* strain 300 after a shift to temperatures a few degrees above the normal growth temperature of 37 °C (Fig. 1). HSPs could be demonstrated at elevated temperatures of 41 °C to 46 °C (data not shown). At 48 °C all protein synthesis stopped (data not shown). Exponential phase cells of strain 300 when shifted from 37 °C to 45 °C produced nine HSPs (Fig. 1). The apparent molecular masses were 98, 85, 81, 76, 72, 54, 34, 26 and 18 kDa. The values obtained in different experiments under the same conditions varied slightly but the variation of band positions among runs was less than 10%. The HSPs were not the only proteins synthesized at temperatures above 37 °C and a number of bands seen at 37 °C were also seen at the same intensity at 45 °C (Fig. 1). Three other strains of *C. albicans*, 300-SG, 320 and 336, synthesized HSPs of similar molecular mass to those synthesized by strain 300.

Studies on heavy metals

A brief exposure of mammalian and avian cells to heavy metals causes the synthesis of proteins of similar molecular mass to some HSPs (Atkinson & Dean, 1985; Li & Laszlo, 1985; Schlesinger, 1985). We asked whether such substances would evoke a stress response in *C. albicans*. A preliminary series of observations were made with CdCl₂, SrCl₂ and SnCl₂ (each at 1, 20 and 40 mM), and mercury as the organomercurial phenylmercuric acetate (10 µM). Only cadmium and mercury evoked the synthesis of new proteins and these were chosen for further work. Cells of strain 300 exposed to 20 mM-CdCl₂ produced a single new protein of about 135 kDa (Fig. 2; Table 2) and none of the stress proteins seen after heat shock or ethanol exposure (Zeuthen *et al.*, 1988). Treatment of cells with 10 µM-phenylmercuric acetate resulted in three new proteins (Fig. 3) one of which corresponded in size to an HSP (81 kDa; Table 2), another of which was similar in size to an HSP (84 kDa; Table 2), and one which was unique to exposure to the organomercurial (88 kDa; Table 2). Exposure of cells to levels of CdCl₂ greater than 40 mM resulted in cessation of protein synthesis and eventual death of the cells. Higher levels of phenylmercuric acetate were not studied.

Thermal death and induction of thermotolerance in C. albicans

A temperature of 55 °C was lethal for strain 300: 98% of the exponential phase cells died in 5 min after a shift from 37 °C to 55 °C; it took 25 min to kill a similar proportion of stationary phase cells (Fig. 4a).

The lethality of exposure to 55 °C was greatly reduced when cells were exposed to 45 °C for 30 min before transfer to 55 °C (Fig. 4b). The tolerance was induced to a more striking extent in stationary phase cells than it was in exponential phase cells (Fig. 4b). Induction of thermotolerance was tested over the range 40–46 °C. The time of incubation at each temperature was 30 min. Cells incubated at 40 °C for 30 min survived no better at 55 °C than did those incubated at 37 °C before the shift. Temperatures of 43–46 °C conferred tolerance on cells shifted to 55 °C (Fig. 5). For mid-exponential phase cells the most effective preincubation temperature was 45 °C or 46 °C. The survival curves of cells preincubated at these two temperatures were virtually identical and only the data for 46 °C are shown in Fig. 5. A temperature of 45 °C was selected for conditioning the cells in the subsequent experiments.

The time of preconditioning affected survival at 55 °C. Cells preincubated for 15 min at 45 °C survived better than cells not preconditioned but the best survival was obtained with cells preconditioned for 30 min (data not shown). Cells preincubated at 45 °C for longer periods were no better able to survive at 55 °C (data not shown).

The data in Table 1 show that induction of thermotolerance was dependent on protein synthesis during preincubation. A short preincubation at 43 °C or 45 °C extended the survival of the cells when shifted to 55 °C. The protection accorded by preincubation at 43 °C or 45 °C was markedly reduced when trichodermin was incorporated in the medium. Trichodermin at the level used inhibits protein synthesis (Shepherd *et al.*, 1980; Gopal *et al.*, 1982) but does not kill

Fig. 1. Autoradiogram of cellular proteins separated by SDS-PAGE (12%, w/v, polyacrylamide). Cells of *C. albicans* strain 300 were grown in YNBG at 37 °C and transferred to prewarmed YNBG. Lane 1, 37 °C; lane 2, 45 °C. Cells were pulse-labelled with L-[³⁵S]methionine for 15 min during the period 15–30 min after the shift. Values on the left represent molecular mass standards. The arrows indicate HSPs.

C. albicans and, after removal of the drug, cells resume growth. A role for HSPs in thermotolerance was supported further by the following results. Cells of strain 300 that had been grown at 37 °C were shifted to 45 °C for 30 min and then shifted back to 37 °C. The HSPs formed (see Fig. 1) persisted for 1 h at 37 °C but began to disappear after 2 h. By 2.5 h HSPs were not detectable in the cells by gel electrophoresis. Such cells were no longer thermotolerant and died at 55 °C as rapidly as cells that had not been preincubated at 45 °C (data not shown).

Ethanol has been reported to induce thermotolerance in *Saccharomyces cerevisiae* (Plesset *et al.*, 1982). In a series of six experiments we observed a slight induction of thermotolerance of *C. albicans* strain 300 by preliminary incubation of cells in 7% (v/v) ethanol for 30 min (Fig. 6). The cells were not killed by this exposure to 7% ethanol but they did synthesize stress proteins (Zeuthen *et al.*, 1988). Induction of thermotolerance by ethanol was modest compared to that evoked by preincubation at 45 °C (Fig. 6).

Fig. 2

Fig. 3

Fig. 2. Autoradiogram of cellular proteins separated in the same manner as those shown in Fig. 1. Cells of *C. albicans* strain 300 were grown in YNBG at 37 °C, then transferred to prewarmed YNBG (lane 1) or YNBG supplemented with 20 mM- CdCl_2 (lane 2). Cells were labelled with L-[^{35}S]methionine during the period 15–30 min after transfer to medium with (lane 2) or without (lane 1) cadmium. Values on the left represent molecular mass standards. The arrow indicates the stress protein.

Fig. 3. Autoradiogram of cellular proteins separated in the same manner as those shown in Fig. 1. Cells of *C. albicans* strain 300 were grown in YNBG at 37 °C, then transferred to prewarmed (37 °C) YNBG supplemented with 10 μM -phenylmercuric acetate and pulse-labelled with L-[^{35}S]methionine. (Lane 1, cells not exposed to mercury; lane 2, cells exposed to mercury; both pulse-labelled from 0–15 min during exposure to mercury. Lane 3, cells exposed to mercury and pulse-labelled from 15–30 min during exposure to mercury.) Values on the left represent molecular mass standards. Arrows indicating stress proteins are shown by lane 3.

Fig. 4. Effect of growth phase of *C. albicans* strain 300 on survival of cells shifted from 37 °C (a) or 45 °C (b) to 55 °C. About 5×10^6 cells ml^{-1} that had been grown at 37 °C were inoculated into YNBG and incubated for 30 min at 45 °C (b) before shift to 55 °C. Cells from the stationary phase of growth (○) and the exponential phase of growth (●) were tested. The results are from a representative experiment that was repeated several times with similar results.

Fig. 5

Fig. 6

Fig. 5. Survival of *C. albicans* strain 300 at 55 °C after a preliminary incubation at a lower temperature. About 5×10^6 cells ml^{-1} that had been grown at 37 °C were inoculated into YNBG. Cells were harvested in the exponential phase, resuspended in prewarmed media, and incubated for 30 min at 43 °C (□), 44 °C (○) or 46 °C (▲). The treated cells were then harvested and reincubated in fresh YNBG at 55 °C. Cells incubated at 37 °C (●) and shifted to 55 °C served as controls. The results are from a single experiment that was repeated several times with similar results.

Fig. 6. Induction of thermotolerance in *C. albicans* strain 300 by preincubation at 45 °C or exposure to 7% (v/v) ethanol. Cells were grown to exponential phase in YNBG at 37 °C and shifted to 55 °C. ●, No preliminary treatment of cells; ▲, cells incubated for 30 min at 45 °C before the shift to 55 °C; ○, cells exposed to 7% (v/v) ethanol before the shift to 55 °C. The experiment was repeated five times with similar results.

DISCUSSION

Cells of *C. albicans* respond to supraoptimal temperatures with an obviously altered pattern of protein synthesis. At 45 °C nine new proteins were synthesized. Five of the high molecular mass proteins, 98, 85, 81, 76 and 72 kDa, are similar in molecular size to the highly conserved families

Table 1. *Importance of protein synthesis in the induction of thermotolerance in C. albicans*

About 5×10^6 cells ml^{-1} of *C. albicans* strain 300 were grown to exponential phase in YNBG, harvested, washed and distributed to culture vessels. After the time periods in min indicated in parentheses, samples were removed, diluted and plated on GPA. Colonies were counted after incubating plates for 7 d at room temperature. The temperature shifts were as shown and trichodermin was added to one vessel (T) before preincubation at 43 °C and 45 °C. Results are from a representative experiment.

Temperature shift (time in min)	c.f.u. (percentage of control)
37 °C → 37 °C (30)	100
37 °C → 43 °C (30)	98
37 °C → 45 °C (30)	98
37 °C → 55 °C (8)	<0.2*
37 °C → 43 °C (30) → 55 °C (8)	52
37 °C → 45 °C (30) → 55 °C (8)	64
37 °C → 43 °C (30, T†) → 55 °C (8)	<0.2*
37 °C → 45 °C (30, T†) → 55 °C (8)	6

* Below the limit of detection.

† To test the requirement for protein synthesis, 3.5 µg trichodermin ml^{-1} was added 20 min before the shift to 43 °C or 45 °C.

Table 2. *Induction of stress proteins by different forms of stress*

+, Induction; −, lack of induction.

Band (kDa)	Condition				
	23 °C → 37 °C	37 °C → 45 °C	Ethanol*	Mercury (as phenylmercuric acetate)	Cadmium (as CdCl_2)
135	−	−	−	−	+
98	−	+	−	−	−
88	−	−	−	+	−
84–85	+	+	−	+	−
80–82	−	+	+	+	−
76	−	+	−	−	−
70–73	+	+	−	−	−
68	+	−	+	−	−
64	+	−	+	−	−
54–56	+	+	−	−	−
46–48	+	−	+	−	−
38–43	+	−	+	−	−
34	−	+	+	−	−
26	−	+	−	−	−
22	+	−	−	−	−
18	+	+	−	−	−

* Determined visually from previously published gels (Dabrowa & Howard, 1984; Zeuthen *et al.*, 1988).

of HSPs at about 90, 80 and 70 kDa (Ashburner & Bonner, 1979; Dabrowa & Howard, 1984; Ingolia *et al.*, 1982; Kelly & Schlesinger, 1982; Welch & Feramisco, 1984). In addition, an HSP similar to the 34 kDa protein that has been recorded among the heat-stress response proteins of *Neurospora crassa* (Plesofsky-Vig & Brambl, 1985) was observed. The synthesis of HSPs in *C. albicans* was not accompanied by as severe a depression of normal protein synthesis as that seen in *N. crassa* (Plesofsky-Vig & Brambl, 1985) or as seen in *C. albicans* exposed to ethanol stress (Zeuthen *et al.*, 1988). Some cellular proteins were synthesized to the same extent at 45 °C as they had been at 37 °C (Fig. 1). Our efforts to completely suppress normal protein synthesis whilst allowing HSP synthesis, by exposure of cells to temperatures higher than 46 °C did not work and at 48 °C all protein synthesis stopped.

The proteins synthesized in response to different forms of stress may be determined by the nature of the stress (Gottesman, 1984). It is also known that some proteins associated with one

Table 3. *HSPs produced by a germination competent strain (300) and a germination deficient strain (300-SG) of C. albicans*

Results from a temperature shift of from 23 °C to 37 °C (Dabrowa & Howard, 1984) and from 37 °C to 45 °C (present study) were combined for this comparison. Only HSP bands were recorded.

Band (kDa)	Strain	
	300	300-SG
98	+	+
84-85	+	+
80-82	+	+
76	+	+
70-73	+	+
68	+	+
64	+	-
54-56	+	+
46-48	+	-
38-43	+	+
34	+	+
26	+	+
33	+	+
18	+	+

stimulant can be evoked by other stresses. A summary of the proteins synthesized by *C. albicans* in response to five different stimulants is shown in Table 2. Nine HSPs were detected in each of the temperature shifts but only four were common to both temperature shift regimes. Thus, five distinctive HSPs were found in each of the temperature shifts designed to be environmentally stressful. Four of the six proteins formed in response to ethanol stress are also HSPs. It is therefore not unexpected that exposure to ethanol induces partial thermotolerance. Such cross-protection between different types of stress has been reported in *Saccharomyces cerevisiae* (Plesset *et al.*, 1982), in *N. crassa* (Guy *et al.*, 1986; Kapoor & Lewis, 1987), and in prokaryotic cells (Jenkins *et al.*, 1988). The degree of thermotolerance afforded by ethanol exposure was modest in comparison to that provided by preincubation at a supraoptimal temperature (Fig. 6).

The search for the function of stress proteins has not been highly productive. Much of the current data suggest a structural role rather than a catalytic activity for HSPs (Schlesinger, 1986), although the exact function of stress proteins has yet to be defined (Schlesinger, 1986). HSPs and perhaps other stress-response proteins may also be involved in cell cycle control and differentiation (Carper *et al.*, 1987; Lindquist, 1986; Schlesinger, 1986; Wanner *et al.*, 1985). LeJohn & Braithwaite (1984) have indicated that while nutritional shock proteins might be involved in sporulation of *Achlya klebsiana*, HSPs were probably not. The fact that 12 of the 14 HSPs formed by strain 300 (Table 2; Dabrowa & Howard, 1984; Zeuthen *et al.*, 1988) were also formed by the germination-defective variant, strain 300-SG (Table 3), makes it unlikely that they play a role in regulation of germination. This supposition is further supported by the fact that a temperature shift does not take place before tissue invasion by *C. albicans* as it does in dimorphic zoopathogens such as *Histoplasma capsulatum* (Schlesinger, 1986; Lambowitz *et al.*, 1983).

Early work on phase-specific proteins in *C. albicans* was initiated both to discover potential regulatory molecules and to use proteins expressed at 37 °C as potential probes for antibody responses of patients (Ahrens *et al.*, 1983; Brown & Chaffin, 1981; Brummel & Soll, 1982; Dabrowa & Howard, 1984; Dabrowa *et al.*, 1970; Manning & Mitchell, 1980; Syverson *et al.*, 1975). The proteins noted in those studies were, for the most part, produced in response to heat or nutritional shock (Dabrowa & Howard, 1984). That result would not of itself detract from the usefulness of such stress-response proteins as markers of an immune response. Indeed, it has been reported that a major immunogenic protein in *Schistosoma mansoni* is homologous to HSP-70 (Hedstrom *et al.*, 1987). We tried a pilot study of the interaction of nine HSPs reported herein with a polyclonal rabbit anti-*Candida* serum. A representative SDS-PAGE gel of proteins

was Western-blotted with the serum. The results were inconclusive, however, because the serum detected both HSPs and proteins normally synthesized by the cells. Perhaps probes more carefully prepared by adsorption or constructed from monoclonal antibodies will be more revealing.

Germination of the blastoconidia of *C. albicans* takes place at 37 °C, at a neutral pH, and in response to a variety of nutritional signals (Dabrowa & Howard, 1983). Two of the nutritional stimulants of germ-tube formation are glucose starvation and nitrogen limitation (Dabrowa & Howard, 1983; Lee *et al.*, 1975). Both of these are forms of nutritional stress and may modulate the pattern of protein synthesis (Dabrowa & Howard, 1988). The results of studies by LeJohn & Braithwaite (1984) clearly suggest that nutrition-shock proteins are involved in regulating sporulation of the water mould *Achlya klebsiana*. A similar role for proteins whose synthesis is regulated by nutritional constraints in *C. albicans* has yet to be demonstrated.

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