L-Thyroxine induces thermotolerance in yeast

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Abstract

The cellular stress response (CSR) is a universal inducible reaction modulated, among others, by heat, drugs, and hormones. We aimed to investigate the role of L-thyroxine (T4) on the heat shock (HS) response in *Saccharomyces cerevisiae*. The CSR was evaluated by determining growth and viability of post-logarithmic phase grown yeast cultures after HS at 53 °C for 30 min. We found that long-term T4 exposure can induce a dose-dependent and Hsp90 and H⁺ trafficking-related thermotolerance in yeast.

Keywords Heat shock response · Thyroxine · Heat shock proteins · Yeast · Thermotolerance

Introduction

Yeast cells are often exposed to rapid and severe changes of their external surroundings that can disturb their normal function, such as high temperature, pH extremes, diverse osmotic concentrations, and starvation (Brion et al. 2016). These harmful environmental conditions can trigger two types of cellular stress response (CSR), one corresponding to a specific stimulus involving the expression of genes related to the stressful condition, and one more general called environmental stress response (ESR) (Gasch et al. 2000). The CSR to adverse environmental/microenvironmental conditions is governed by evolutionary conserved endogenous multicomponent processes, including heat shock protein (Hsp) expression and ion homeostasis (Świeciło 2016). Physical, chemical, or physiological stimuli, such as heat, drugs, and hormones trigger and/or modulate CSR (Delitheos et al. 2010; Papamichael et al. 2006; Papamichael et al. 2013). The evolutionary conservation of the basic physiological and biochemical, cellular, and molecular mechanisms between yeasts and higher eukaryotes made the yeast an established experimental model for the pharmacological inves-

tigation of the CSR and the adaptive and protective mechanisms underlying drug resistance (Verghese et al. 2012). Additionally, although yeast lacks known homologs of nuclear receptors, it can be useful in modeling hormonal pathways, as it maintains sufficient homology with mammalian basal signaling and transcription machineries (Burshell et al. 1984; Hall et al. 1993; Caticha et al. 1994).

Hormones can exert their physiological activities not only via the well-established genomic ligand-nuclear receptor interactions, but also through extranuclear molecular mechanisms (Gururaj et al. 2006). Regarding thyroid hormones, besides the classical thyroid nuclear receptors, they can exert also “non-genomic” actions often related to regulation of cation trafficking via cell surface receptors and thyroid-binding proteins in the cytoplasm and mitochondria (Davis et al. 2008). L-Thyroxine (T4) was found, already since many years ago, to significantly stimulate respiration in *S. cerevisiae* (Gutenstein and Marx 1957). Hormones and/or synthetic hormonal analogs have been shown to regulate the CSR (Pantos et al. 2001; Pantos et al. 2003; Graham et al. 2009). In yeast, preconditioning with prednisolone displayed alterations in cell cycle and conferred thermotolerance to post-logarithmically grown cells during subsequent exposure to otherwise lethal condition, in contrast to 17-β estradiol which showed no influence on the stress response (Papamichael et al. 2006). Prednisolone binds to the glucocorticoid receptor located in the cytoplasm and forms the glucocorticoid/glucocorticoid receptor complex that translocates inside the nucleus and binds to specific DNA binding sites (glucocorticoid response elements) resulting in gene expression or inhibition (Stahn et al. 2007).

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Fig. 1 Induction of the thermotolerant phenotype by L-thyroxine (T4) in yeast is dependent upon the dosage and protein synthesis. **A** Growth curve of *S. cerevisiae* in the absence (○) or presence of 100 μmol l⁻¹ T4 (●). *n* = 3; * *p* < 0.05 as compared to control untreated cultures at time 0; # *p* < 0.05 as compared to cultures exposed to 100 μmol l⁻¹ T4 at time 0. **B–D** T4 differentially induces thermotolerance in yeast. Thermotolerance was assessed after heat shock (HS, 53 °C, 30 min) by determining under the optical microscope the proportion of viable cells (%), following vital staining with methylene blue in samples of yeast cultures incubated with various concentrations of T4 at 27 °C for 24 h (**B**, long-term; *n* = 4–10) or 2 h (**C**, short-term; *n* = 5–8) prior to HS, as well as during thermal preconditioning for 2 h at 37 °C (**D**; *n* = 5–8). * *p* < 0.05, *** *p* < 0.001 as compared to control untreated cultures ([T4] = 0). **E** T4 administration

during the HS in unconditioned (*n* = 4–10) or preconditioned (PRE; *n* = 4–8) cultures do not induce the thermotolerant phenotype. *** *p* < 0.001 as compared to unconditioned control cultures; # *p* < 0.05 significantly different from unconditioned cultures treated with 1000 μmol l⁻¹ T4 during HS. **F** Exposure for 2 h before HS to 0.35 mmol l⁻¹ cycloheximide, an inhibitor of de novo protein synthesis (gray bars), circumvents the thermotolerance induced by long-term incubation of normal cultures with 1000 μmol l⁻¹ T4 and by preconditioning in the absence (control) or presence of 100 μmol l⁻¹ T4 (closed bars). *n* = 4–5; * *p* < 0.05, *** *p* < 0.001 as compared to untreated control normal cultures; ## *p* < 0.01, ### *p* < 0.001 significantly different from viability in the presence of cycloheximide

This study aimed at exploring the role of T4 on the HS response in yeast. This was further investigated using the Hsp90 inhibitor geldanamycin (GA) and the H⁺-ATPase inhibitor omeprazole.

Materials and methods

Compounds

Omeprazole and cycloheximide were purchased from Sigma-Aldrich (Mo, U.S.A.). T4 was kindly provided by Uni-pharma Greece, (Biochemie, Austria). GA was kindly provided by the National Institutes of Health (USA). The compounds were readily dissolved and diluted in the minimal volume of distilled water (for T4 and cycloheximide), methanol (0.3–0.12 mM, for omeprazole), and dimethyl sulfoxide (0.13 mM, for GA) where appropriate and did not induce

any significant effects on any of the studied responses (data not shown). All compounds were kept in –4 to –6 °C.

Yeast strain and culture media

The budding yeast *Saccharomyces cerevisiae* ATCC® 2366™, also referred to as *Saccharomyces pastorianus*, was obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained on yeast agar [yeast extract peptone dextrose (YEPD), containing in w/v, 0.3% yeast extract, 0.5% mycological peptone, and 1% dextrose, supplemented with 1.5% bacteriological agar; Oxoid, UK].

Heat shock and thermal preconditioning

A single 2-day-old colony was inoculated into 5 ml YEPD, incubated at 27 °C for 2 h and subsequently cultured in YEPD at 27 °C for 24 h through to the post-logarithmic phase of growth. Yeast cells were then submitted to HS at 53 °C for

30 min. Thermal preconditioning was performed by shifting the post-logarithmic phase growing cultures to 37 °C for 2 h before HS and served as positive control (Papamichael et al. 2006).

Pharmacological treatment

T4 was administered at concentrations of 0.01 $\mu\text{mol l}^{-1}$ to 1000 $\mu\text{mol l}^{-1}$ during the 24-h incubation period at 27 °C (long-term administration), for 2 h at 27 °C (short-term administration) and during thermal preconditioning for 2 h at 37 °C prior to HS. T4 was also administered at a concentration of 1000 $\mu\text{mol l}^{-1}$ during HS for 30 min at 53 °C. Cycloheximide at 0.35 mmol l^{-1} was added for 2 h before HS, alone or in combination with T4. GA (90 $\mu\text{mol l}^{-1}$) and omeprazole (100 and 200 $\mu\text{mol l}^{-1}$) were added during the 24-h incubation before the HS.

Evaluation of cell viability after heat shock

Cell viability after HS was monitored using the vital exclusion dye methylene blue. Culture samples (0.1 ml) were appropriately diluted with full-strength Ringer's solution containing 0.1% methylene blue. An aliquot was loaded onto a Neubauer hemocytometer and examined under the optical microscope at 400 $\times$ magnification (Zeiss, Germany). Viability was determined by counting the blue-stained dead cells and their unstained viable counterparts and expressed as the percentage of the viable cells in each culture (Papamichael et al. 2006). Yeast cells were viable before exposure to HS independently of the treatment.

Statistical analysis

Results are expressed as mean $\pm$ S.E.M from 4 to 8 independent experiments. Multiple groups were compared using one-way analysis of variance (ANOVA) followed by Dunnett T3 or Scheffé post-hoc tests. The Student *t* test was used for direct comparisons. Statistical dependence between variables was measured by the non-parametric Spearman's rank correlation coefficient (ρ). All tests were performed using SPSS v.19, they were two-sided and $p < 0.05$ was regarded as statistically significant.

Results

Yeast cultures were exposed to T4 during the 24-h incubation period through to post-logarithmic phase of growth (long-term; Fig. 1A, B), for 2 h during the post-logarithmic phase (short-term; Fig. 1C), as well as during thermal preconditioning for 2 h at 37 °C (Fig. 1D) or HS for 30 min at 53 °C (Fig. 1E). Thermotolerance was evaluated in the cultures after exposure to HS by determining cell viability upon vital staining

Fig. 2 Induction of the thermotolerant phenotype by L-thyroxine (T4) in yeast is Hsp90 and H⁺ trafficking-related dependent. **A** The thermotolerant phenotype acquired after long-term exposure to 100 $\mu\text{mol l}^{-1}$ T4 was reversed by long-term co-administration of 90 $\mu\text{mol l}^{-1}$ GA ($n = 2-4$). * $p < 0.05$ as compared to control untreated cultures; ## $p < 0.01$ significantly different from viability in the presence of 100 $\mu\text{mol l}^{-1}$ T4 alone. **B** Long-term administration of omeprazole (OME) in yeast cultures growing in the presence of 100 $\mu\text{mol l}^{-1}$ T4 ($n = 5-7$) reduced the tolerant phenotype observed after HS upon long-term T4 exposure. * $p < 0.05$ as compared to untreated cultures, ## $p < 0.01$, ### $p < 0.001$ as compared to T4 alone

with methylene blue. Long-term incubation with T4 had no effect on the yeast growth profile (Fig. 1A) but resulted in significant dose-dependent (Spearman's $\rho = 0.831$, $p < 0.001$) increases in yeast viability after HS (Fig. 1B). The presence of 100 and 1000 $\mu\text{mol l}^{-1}$ T4 for 24 h induced maximal protection against the lethal effects of HS, the viability after HS being $82.7 \pm 2.4\%$ and $90.9 \pm 3.1\%$, respectively (Fig. 1B). On the contrary, short-term exposure of yeast cells to T4 for 2 h prior to HS failed to induce any significant ($\rho = 0.131$, $p > 0.05$) alterations in yeast viability after HS, as compared to the respective control untreated cultures (Fig. 1C), indicating the inability of T4 to induce the adaptive thermotolerant phenotype in post-logarithmically growing

normal yeast populations. However, incubation with 0.01–1000 $\mu\text{mol l}^{-1}$ T4 during thermal preconditioning at 37 °C for 2 h prior to HS dose-dependently induced resistance to the subsequent severe thermal shock ($\rho=0.515$, $p<0.001$). In this case, the viability was significantly increased to $89.1 \pm 3.0\%$ in the presence of 1000 $\mu\text{mol l}^{-1}$ T4 compared to $71.9 \pm 3.7\%$ obtained in the absence of the hormone (Fig. 1D). Administration of 1000 $\mu\text{mol l}^{-1}$ T4 during the HS did not induce the thermotolerant phenotype (Fig. 1E).

Administration of 0.35 mmol l^{-1} cycloheximide, a de novo protein synthesis inhibitor, for 2 h before HS partially circumvented the acquisition of thermotolerance by long-term treatment with 1000 $\mu\text{mol l}^{-1}$ T4 (Fig. 1F). Moreover, cycloheximide reversed the increased viability of yeast cells after HS acquired by preconditioning in the presence or absence of 100 $\mu\text{mol l}^{-1}$ T4 (Fig. 1F). Thus, it was confirmed that T4-induced thermotolerance is dependent on de novo protein synthesis, as previously also shown for thermal preconditioning (Papamichael et al. 2006).

Co-administration of 90 $\mu\text{mol l}^{-1}$ GA led to a significant decrease in thermotolerance induced by long-term exposure to 100 $\mu\text{mol l}^{-1}$ T4 from 83 ± 2.9 to $42.8 \pm 2.6\%$ (Fig. 2A). The T4-induced yeast viability was also significantly reduced by co-administration of 100 or 200 $\mu\text{mol l}^{-1}$ omeprazole from 89.0 ± 4.9 to $42.8 \pm 6.9\%$ or $36.7 \pm 4.2\%$, respectively (Fig. 2B).

Discussion

This pilot study showed that long-term T4 treatment of transcriptionally active, exponentially and postlogarithmic phase-growing cells before HS resulted to a dose-dependent

induction of thermotolerance in yeast. The T4-induced thermotolerant phenotype was related to the Hsp90 activity and cation trafficking regulation by the membrane H^+ -ATPase (Fig. 3). Although the exact mechanisms underlying this response remains to be elucidated, these may be related to activation of stress signal pathways similar to the universal adaptive mechanisms of the ESR that can explain the observed cross protection against a stressful environmental condition, such as high temperature.

We have previously shown that short-term preconditioning of *S. cerevisiae* with GA conferred thermal resistance (Papamichael et al. 2006). This could be explained by the inhibition of Hsp90 which has been associated with the acquisition of the heat-resistant phenotype (Ortega et al. 2014). However, upon long-term treatment, GA can induce cell cycle arrest both in yeast (Papamichael et al. 2006) and in cancer cell lines (Ui et al. 2014). We observed a significant decrease in thermotolerance induced by long-term exposure to T4 upon co-administration with GA, suggesting the contribution of the Hsp90 complex in T4-induced acquisition of thermotolerance.

The electrical membrane potential and ionic homeostasis participate in CSR in yeast (Coote et al. 1994). Short-term exposure to omeprazole was found to act as a preconditioning pharmacological agent and confer resistance to HS in *S. cerevisiae* (Vovou et al. 2004), probably via the transient inhibition of the plasma membrane H^+ -ATPase and the induction of the small Hsp30 (Piper et al. 1997). Nevertheless, our study clearly demonstrated that long-term co-administration of omeprazole can significantly reduce the T4-induced thermotolerance suggesting the implication of the membrane H^+ -ATPase in the adaptive response induced by long-term exposure to T4. Omeprazole has been reported to inhibit the

Fig. 3 Proposed mechanisms of L-thyroxine (T4) induction of thermotolerance in yeast

peripheral deiodination of thyroid hormones (Atterwill et al. 1989), whereas repeated administration of the various proton pump inhibitors increased thyroxine-metabolizing activity in rats (Masubuchi et al. 1997).

These results provide the first evidence for the involvement of T4 in the evolutionary conserved adaptive and protective mechanisms against lethal thermal insults in transcriptionally active eukaryotic cell

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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