Arch Microbiol (2008) 189:293–296
DOI 10.1007/s00203-007-0332-8

COMMENTARY

# Stress responses in yeasts: what rules apply?

**Pilar González-Párraga · Ruth Sánchez-Fresneda · María Martínez-Esparza · Juan-Carlos Argüelles**

Received: 2 October 2007 / Revised: 6 November 2007 / Accepted: 17 November 2007 / Published online: 8 December 2007


**Abstract** Living organisms have evolved a complex network of mechanisms to face the unforeseen nutritional and environmental circumstances imposed on their natural habitats, commonly termed "stress". To learn more about these mechanisms, several challenges are usually applied in the laboratory, namely nutrient starvation, heat shock, dehydration, oxidative exposures, etc. Yeasts are chosen as convenient models for studying stress phenomena because of their simple cellular organization and the amenability to genetic analysis. A vast scientific literature has recently appeared on the defensive cellular responses to stress. However, this plethora of studies covers quite different experimental conditions, making any conclusions open to dispute. In fact, the term "yeast stress" is rather confusing, since the same treatment may be very stressful or irrelevant, depending on the yeast. Customary expressions such as "gentle stress" (non-lethal) or "severe stress" (potentially lethal) should be precisely clarified. In turn, although prototypic yeasts share a common repertoire of signalling responsive pathways to stress, these are adapted to the specific ecological niche and biological activity of each particular species. What does "stress" really mean? Before we go any deeper, we have to define this uncertain meaning along with a proper explanation concerning the terms and conditions used in research on yeast stress.

**Keywords** Yeast · Stress responses · Trehalose · Heat shock · Oxidative stress · Hog1 · General response

Communicated by Erko Stackebrandt.

Dedicated to Prof. Rafael Sentandreu on the occasion of his 70th birthday and to celebrate his appointment as Emeritus Professor at the University of Valencia (Spain).

P. González-Párraga · R. Sánchez-Fresneda · J.-C. Argüelles (✉)
Área de Microbiología, Facultad de Biología,
Universidad de Murcia, Campus de Espinardo,
30071 Murcia, Spain
e-mail: arguelle@um.es

M. Martínez-Esparza
Departamento de Bioquímica y Biología Molecular
B e Inmunología, Universidad de Murcia,
30071 Murcia, Spain

## Introduction

Continuous and unforeseen biological and physical perturbations occur in the biosphere and organisms have to cope with them in a rapid and efficient way. These anomalous disturbances are usually known as "stress" and an intensive research effort has been put in by many laboratories in an attempt to mimic the naturally imposed conditions. Nevertheless, this concept is rather ambiguous, since it encompasses risks which might be termed as moderate, to other potentially life-threatening situations. Let us, first, then, clarify what we denote by the word stress. We will briefly discuss some particular examples taking as starting point the definition from a scientific dictionary, where stress is defined as "*an unusual environmental condition that causes physiological, emotional, behavioural or cognitive changes in an individual*" (*or in a population in the case of microorganisms*).

A lot of experimental work carried out on stress taking yeast as a biological model has long been performed using just a few species as reference. The best studied is the budding yeast *Saccharomyces cerevisiae*, the classical baker's or wine-making yeast, together with other closely related strains employed in brewing. The fission yeast *Schizosaccharomyces pombe* is also an interesting archetype, since

some molecular analyses have pointed to its higher genetic homology with mammals than in the case of budding yeast. In addition, the dimorphic pathogenic yeast *Candida albicans* holds both scientific and clinical interest. This commentary will mainly be directed at a comparative analysis of the responses developed by *S. cerevisiae* and *C. albicans* against oxidative stress and heat-shock challenges. Extensive and detailed analysis on the stress responses in fungi and the putative connection between stress and fungal virulence can be found in the following reviews (Estruch 2000; Ikner and Shiozaki 2005; Román et al. 2007).

## Oxidative stress

In aerobic organisms, the generation of toxic reactive oxygen species (ROS), i.e., $O_2^{\cdot -}$, $H_2O_2$ or $^{\cdot}OH$, is the consequence of internal metabolic oxidations or caused by the supply of external agents, this latter process being common practice in experimental assays (Estruch 2000). ROS are very harmful to essential cellular macromolecules (nucleic acids, lipids and proteins) and, to combat them, yeasts have evolved efficient defensive mechanisms, which involve the synthesis and/or activation of protective enzymes or molecules (Ikner and Shiozaki 2005). However, as regards the damage caused by a given specific oxidative compound, there may be strong differences between the two yeast species taken as a model. For example, in the case of the potential toxic effects of hydrogen peroxide, whereas cellular viability in *S. cerevisiae* is seriously compromised after low $H_2O_2$ exposures (1–5 mM), *C. albicans* exhibits noticeable resistance and higher non-physiological concentrations are required to cause a significant degree of cell-killing (25–100 mM, Pedreño et al. 2002; Álvarez-Peral et al. 2002, Table 1). In addition to individual features, these sharp contrasts could obey their respective intrinsic metabolisms. Thus, *S. cerevisiae* is mainly a fermentative yeast with a low respiratory rate (depending on the substrate), while the contribution of oxidative metabolism is more important in *C. albicans*. The natural habitat might also be relevant in explaining this divergent antioxidant capacity. In the course of an in vivo infection, *C. albicans* is exposed to the strongly oxidant action of ROS produced by phagocytes, while for the budding yeast the level of oxidant aggression should be theoretically lower (Vazquez-Torres and Balish 1997).

## Heat shock

Both mild and acute shifts in the regular growth temperatures of yeasts have been the classical methods followed to study the primary responses to heat stress. There is an ample body of experimental evidence on this topic, which has led to the definition of a family of heat-shock proteins (HSPs, Craig et al. 1993). In this case, variability is lower since, with insignificant exceptions, most of the yeasts are mesophilic microorganisms. Standard growth is compatible in an ample range of temperatures (between 20 and 40°C). Usually, unicellular yeast-like forms are found at around 30°C, and moderate temperature up-shifts (e.g., from 25–30°C to 37–42°C) are often not detrimental, but rather stimulatory of cell growth. Viability is, however, compromised after exposure to higher temperatures (>40–50°C). In fact, proliferating yeast cells are able to withstand a potentially lethal heat shock, provided they are previously submitted to a gentle temperature rise (37 or 42°C). This phenomenon is the well-studied "adaptive thermotolerance", which, it turns out, can be applied to other types of environmental threat (Argüelles 2000).

## The paradigm of trehalose storage

The protective role of the non-reducing disaccharide trehalose is another excellent paradigm of the striking differences existing between *S. cerevisiae* and *C. albicans* with respect to the stress response mechanism. In both species, a huge amount of intracellular trehalose is synthesised in resting cells or in exponential cultures subjected to sudden environmental challenge (Argüelles 2000). However, whereas in the opportunistic yeast, trehalose behaves as a specific protector against oxidative agents (Álvarez-Peral et al. 2002), this outcome seems less relevant in budding

**Table 1** Differential sensitivity of *S. cerevisiae* and *C. albicans* to oxidative stress triggered by exposure to hydrogen peroxide

| Oxidative treatment ($H_2O_2$) | | | | |
|---|---|---|---|---|
| Yeast | Gentle (mM) | Cell viability (%) | Severe (mM) | Cell viability (%) |
| *Saccharomyces cerevisiae* MCY1264 | 0.3–0.5 | 75–60 | 5–10 | <1–0.01 |
| *Candida albicans* CAI.4 | 0.5–5.0 | 80–95 | 25–100 | <10–1.0 |

Data on stress sensitivity to $H_2O_2$ were extrapolated from specific assays in rich liquid media appropriate for each yeast species. The oxidative exposure lasted for about 1 h. For more specific details, see the following references: Pedreño et al. 2002, 2007, together with some key citations included therein. Similar values of oxidative stress susceptibility are displayed by other standard laboratory strains from the two yeasts (see Estruch 2000; Ikner and Shiozaki 2005; Román et al. 2007)

cells, which rarely encounters high levels of oxidants in their habitat (Fig. 1). Furthermore, *S. cerevisiae* mutants, whose trehalose-mobilising enzyme (Nth1p) has been disrupted, are extremely sensitive to low $H_2O_2$ exposure, while the equivalent mutants in *C. albicans* show increased resistance even to elevated $H_2O_2$ concentrations (Pedreño et al. 2007).

## Do yeasts possess a core stress response mechanism?

A broad experimental body points to the existence of a core stress response (CSR) in *S. pombe* and *S. cerevisiae*, which, in part, involves a differential regulation of stress-activated protein kinases pathways (SAPKs). This conserved mechanism ensures cross-protection against different environmental injuries (Smith et al. 2004, Fig. 1). Whereas in fission yeast, the Sty1-SAPK plays a predominant role in the response to a set of stress challenges, in budding yeast the adaptation to stress occurs through a variety of signaling pathways and transcription factors (Smith et al. 2004, Fig. 1). In addition to the zinc finger transcription factors Msn2/Msn4, a good example of SAPK in *S. cerevisiae* is the Hog1 (homologous to the Sty1), which mainly responds to alterations in osmolarity and regulates the transcription of core stress genes in response to this stress (Smith et al. 2004). In *C. albicans*, however, Hog1 plays a crucial function in cell protection against oxidative damage, in addition to being activated in response to high external osmotic pressure (Alonso-Monge et al. 2003; see below).

In contrast, a transcript profiling study (using positive stimuli for *S. cerevisiae*) suggests the absence of a general stress response (Enjalbert et al. 2003) and of stress-cross protection in *C. albicans* (Fig. 1). However, such an inference is not definitive, since protection against severe oxidative exposure to $H_2O_2$ is conferred by previous osmotic stress treatment, in a cross-defense mechanism that is Hog1-dependent (Smith et al. 2004). Moreover, in a recent report, Enjalbert et al. (2006) pointed out that *C. albicans* can mount a core transcriptional response to stress through the *HOG1* pathway. In fact, besides its function in osmotic adaptation and morphogenesis, the Hog1 MAP kinase in *C. albicans* is essential for resistance to acute oxidative stress (Alonso-Monge et al. 2003), a challenge presumably critical for pathogen survival during host tissue colonization (Vazquez-Torres and Balish 1997). Temperature upshifts (28–37°C) also increase the capacity to withstand a severe oxidative challenge (Álvarez-Peral et al. 2002)

## Final comment

Some general conclusions firmly established in the scientific literature concerning yeast stress responses, the protective role played by certain molecules as well as enzymes and/or signalling pathways, or the putative existence of a general stress core response, should be accepted with caution. They are usually founded on very specific experimental conditions and any finding is not necessarily extrapolable to other yeasts with different biological organizations, activities and habitats. Furthermore, some particular responses, whether at transcriptional or postranslational level, when elicited by a stressing agent may be dose-dependent. First of all, the conditions of a specific cell

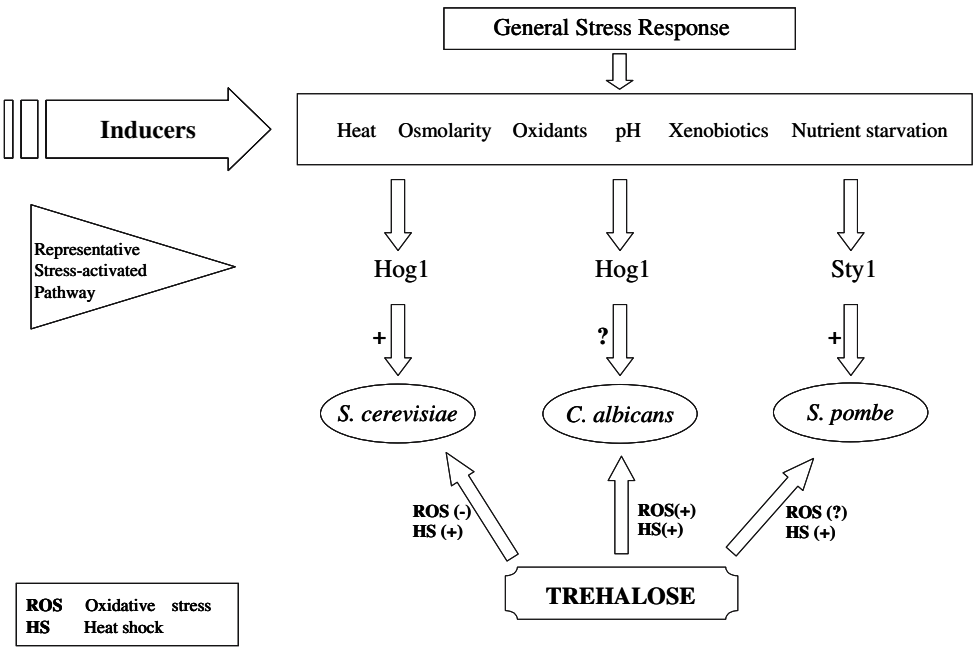


**Fig. 1** Schematic representation of the hypothetical existence of core stress responses in *S. cerevisiae*, *S. pombe* and *C. albicans*, as well as the specific role played by the non-reducing disaccharide trehalose in cell protection against oxidative stress (ROS) and heat shock (HS) in these three prototypic yeasts. Symbols: *plus* presence; *minus* absence; *question mark* unclear or contradictory evidence

stress in each specific cell type must be properly defined. Before starting any game, the rules should be correctly established.

**Acknowledgments** We are indebted to Dr. E. Hidalgo (Universitat Pompeu i Fabra, Barcelona) for her critical reading of the manuscript and useful comments. The experimental research is supported by grant BIO-BMC 06/01-003 from Dirección General de Investigación (Comunidad de Murcia, Spain). We also thank the financial contract provided by Cespa-Ingeniería Urbana, SA.

## References

Alonso-Monge R, Navarro-García F, Román E, Negredo AI, Eisman B, Nombela C, Plá J (2003) The Hog1 mitogen-activated protein kinase is essential in the oxidative stress response and chlamydospore formation in *Candida albicans*. Eukaryot Cell 2:351–361

Alvarez-Peral FJ, Zaragoza O, Pedreño Y, Argüelles JC (2002) Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in *Candida albicans*. Microbiology 148:2599–2606

Argüelles JC (2000) Physiological roles of trehalose in bacteria and yeasts: a comparative analysis. Arch Microbiol 174:217–224

Craig EA, Gambill D, Nelson RJ (1993) Heat shock proteins: Molecular chaperones of protein biogenesis. Microbiol Rev 57:402–414

Enjalbert B, Nantel A, Whiteway M (2003) Stress-induced general expression in *Candida albicans*: absence of a general stress response. Mol Biol Cell 14:1460–1467

Enjalbert B, Smith DA, Cornell MJ, Alam I, Nicholls S, Brown AJB, Quinn J (2006) Role of the Hog1 stress-activated protein kinase in the global transcriptional response to stress in the fungal pathogen *Candida albicans*. Mol Biol Cell 17:1018–1032

Estruch F (2000) Stress-controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. FEMS Microbiol Rev 24:469–486

Ikner A, Shiozaki K (2005) Yeast signaling pathways in the oxidative stress response. Mutat Res 569:13–27

Pedreño Y, Gimeno-Alcañiz JV, Matallana E, Argüelles JC (2002) Response to oxidative stress caused by $H_2O_2$ in *Saccharomyces cerevisiae* mutants deficient in trehalase genes. Arch Microbiol 177:494–499

Pedreño Y, González-Párraga P, Martínez-Esparza M, Sentandreu R, Valentín E, Argüelles JC (2007) Disruption of the *Candida albicans ATC1* gene encoding a cell-linked acid trehalase decreases hypha formation and infectivity without affecting resistance to oxidative stress. Microbiology 153:1372–1381

Román E, Arana DM, Nombela C, Alonso-Monge R, Pla J (2007) MAP kinase pathways as regulators of fungal virulence. Trends Microbiol 15:181–190

Smith DA, Nicholls S, Morgan BA, Brown AJB, Quinn J (2004) A conserved stress-activated protein kinase regulates a core stress response in the human pathogen *Candida albicans*. Mol Biol Cell 15:4179–4190

Vázquez-Torres A, Balish E (1997) Macrophages in resistance to candidiasis. Microbiol Mol Biol Rev 61:170–192