A Systems Biology analysis of combinatorial stress responses in *Candida albicans*

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Abstract

Living organisms must adapt to environmental changes which often happen simultaneously. Previous studies have focused on the response to individual stresses alone. In contrast, little is known about responses to combinatorial stress conditions. Here, we study the combinatorial stress responses (osmotic, oxidative and nitrosative) in a major fungal pathogen of humans, *Candida albicans.* A number of physiological and molecular techniques were used to monitor the amounts and activities of key proteins and molecules. Some of the results were used to develop a mathematical model that predicts hyperosmotic responses in *C. albicans*, which was validated through other experimentations. Our modelling of combinatorial stress responses formed some testable hypotheses to design further experiments.

Osmosensing

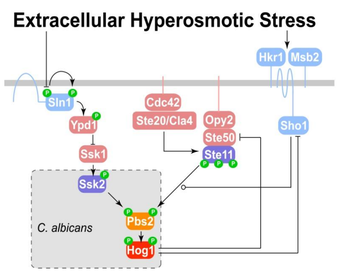


Figure 1. Osmosensing networks

Being the best characterised stress, osmotic stress responses are investigated the first. We have developed an Ordinary Differential Equation model to describe osmosensing in *C. albicans.* This model encompasses the signalling pathways and some of the known molecular mechanisms involved in adaptation. In particular, hyperosmotic stress activates Hog1 which induces internal glycerol accumulation, and it triggers closure of aqua glycerol channels to decrease glycerol efflux. The model is calibrated using in-house generated experimental data.

The signalling network in *Saccharomyces cerevisiae* comprises two branches that culminate at Hog1 phosphorylation. The two branches posses different kinetic properties in *S. cerevisiae*. The Sln1 branch responds to all osmolarity, exhibits relatively faster responses with higher maximum Hog1 activities. The Sho1 branch does not respond to lower signal, displays slower responses with lower maximum responses [1]. Comparatively, less is known in the *Candida* species. Currently, it is established that osmotic stress only activates Hog1 through Ssk2 in *C. albicans* [2]. However, the molecular mechanisms for monitoring osmotic changes remain elusive.

Once activated, the phosphorylated form of Hog1 induces internal glycerol accumulation at various steps in *S. cerevisiae*. These include upregulating expression of the genes encoding glycerol biosynthetic enzymes (Gpd1, Gpd2 and Gpp2) and glycerol/H+ symport system (Stl1, actively uptake glycerol), increasing activity of Pfk26 to diverge metabolic flux into glycerol production by phosphorylation, and targeting glycerol efflux channel Fps1 for degradation [3, 4]. Induced internal glycerol helps restore the turgor pressure which resumes the cell size. Similar mechanisms may be conserved in *C. albicans.*

Model construction

The duration of Hog1 signal increases with elevations in NaCl concentration. However, the amplitude saturates beyond 0.5M NaCl.

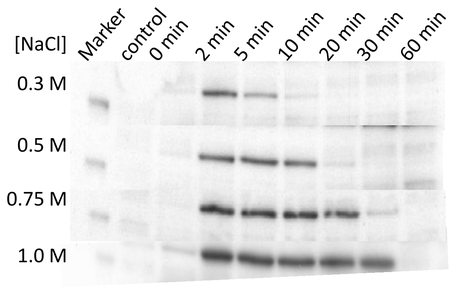
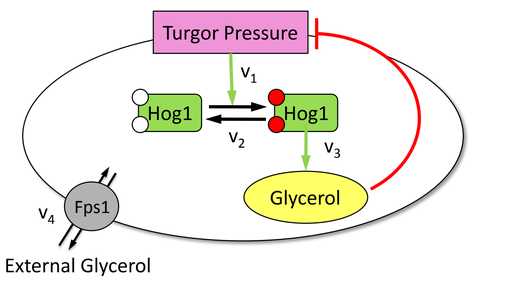
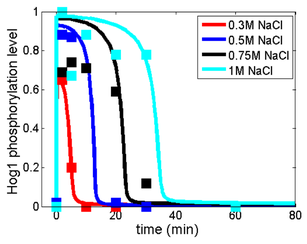


Figure 2 Dynamics of Hog1-phosphorylation in *Candida albicans*
Cells were grown to exponential phase in YPD-T (pH 7.4) and exposed to osmotic stress (NaCl) at different concentrations. Cells were harvested at the times indicated and total protein extracts prepared. Hog1 activation was detected using a phospho-specific antibody.



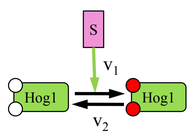


A simple model was constructed to simulate the Hog1 activation. The model is able to predict the experimental observations.

Figure 3 A simple model and simulations of osmosensing

Underpinning hypothesis Hog1 exercises an integral control over glycerol production rate. This is valid if the time scales of HOG signalling (relatively fast, ~100 sec) and ensuing adaptation mechanisms (often slower, ~2000 sec) are separated. It is tested in the next section.

Parameter calibration



$$\frac{dH}{dt} = \frac{k_1 \cdot S \cdot (1-H)}{K_1 + (1-H)} - \frac{k_2 \cdot H}{K_2 + H}$$

1. the signal S fluctuates around a mean value by a small amplitude $S = S_0 + A \cdot \cos(\omega t)$
2. H fluctuates around a steady-state solution by a small deviation $H = H_0 + \delta H$

$$\delta H(t) = \left(\delta H_0 - \frac{BC}{B^2+\omega^2}\right)e^{-Bt} + \frac{C}{\sqrt{B^2+\omega^2}}\sin(\omega t + \varphi) \qquad B = \frac{k_1 \cdot K_1 \cdot S_0}{(K_1+1-H_0)^2} + \frac{k_2 \cdot K_2}{(K_2+H_0)^2} \qquad C = \frac{k_1 \cdot (1-H_0)}{K_1 + (1-H_0)}A$$

Osmotic Response Memory

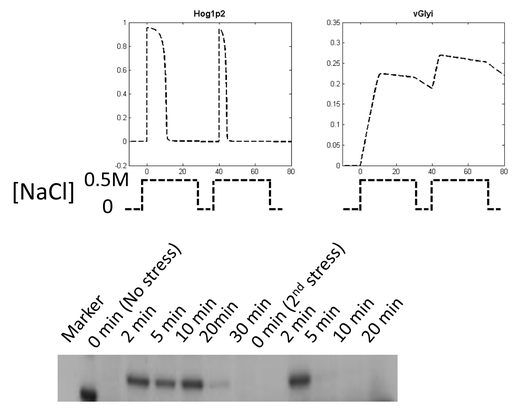


Figure 4 Repeated osmotic stress experiment

This model predicts that a *C. albicans* cell mounts a smaller response to the second identical osmotic shock as a result of integral control (i.e. elevated glycerol production is retained in a longer time-scale than that of signalling). This was tested by carrying out a repeated salt insult experiment. *C. albicans* cells first adapted to a hyperosmotic stress (0.5M NaCl). After a brief period (10 min) of no stress, these cells were subjected to a second identical hyperosmotic stress (0.5M NaCl).

The Hog1-P response is much shorter following the second shock. This validates the existence of an osmotic stress response memory which arises from Hog1's integral control.

OS and XS Transcriptional Responses

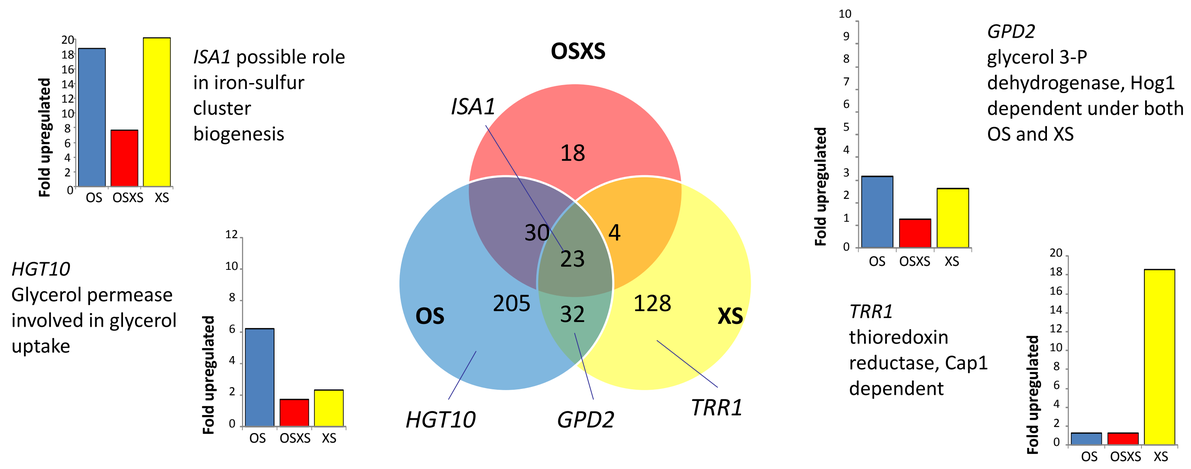


Figure 5 Numbers of genes upregulated >2.5 fold following 10 min stress
Transcript profiling by custom arrays was done in triplicates, compared to a no-stress control
OS = 1 M NaCl, XS = 5mM H_2O_2, OSXS = combination of both

C. albicans responds to both osmotic and oxidative stress by rapid regulation of expression of >100 genes. A similar strength of response was not observed under osmotic and oxidative combinatorial condition. Those annotated as oxidative stress response genes were all found in oxidative stress alone – not in combinatorial conditions, indicating that the normal oxidative response is inhibited when cells undergo combinatorial conditions. A greater overlap was observed between osmotic and combinatorial stress. A range of activation assays of key proteins (e.g. Hog1, Cap1) are now being carried out to determine which pathways are active.

Conclusions and Beyond

We developed and validated a simple model of hyperosmotic response of *C. albicans* through experimentations. Hog1 has an integral control which leads to a osmotic response memory. Transcriptional analysis indicate that only expression of a few genes are specifically regulated for the combinatorial condition. Normal oxidative response is not observed under combinatorial stress whereas osmotic responses are retained.

Significant synergism has been observed between osmotic and oxidative conditions, in an analysis of the lethality using normalised isobologram. When doses are low, the combinatorial condition leads to elongated adaptation time. Oxidative stress induces generation of NADPH through pentose phosphate pathway, a competing metabolic process with glycerol production that is required for osmotic adaptations. This competition is conjectured to explain the synergism. Formulations of oxidative and combinatorial stresses are now being developed and will guide designs of future experiments to investigate the biology behind.

ACKNOWLEDGEMENTS:

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REFERENCES :

1. Macia *et al* (2009). Dynamic signaling in the Hog1 MAPK pathway relies on high basal signal transduction. *Sci Signal* 2(63), ra13.
2. Cheetham *et al* (2007). A single MAPKKK regulates the Hog1 MAPK pathway in the pathogenic fungus *Candida albicans*. *Mol Bio Cell* 18, 4603-4614.
3. Westfall *et al* (2008) Stress resistance and signal fidelity independent of nuclear MAPK function. *Proc Natl Acad Sci U S A* 105(34): 12212-12217.
4. Hohmann (2009) Control of high osmolarity signalling in the yeast *Saccharomyces cerevisiae*. *FEBS Lett* 583(24): 4025-4029.