Control of yeast signal transduction

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Adaptation to changes in the environment

Availability of water is obviously central for life.

Water homeostasis is critical to maintain shape and turgor, to allow transmembrane transport processes and to ensure proper conditions for biochemical processes.

Therefore cells and organisms go a long way to ensure water homeostasis and adapt to changes in the intracellular water balance.
Cell volume changes

The higher the external osmolarity, the more cells shrink.
The more cells shrink, the longer it takes cells to start volume recovery.
Osmotic adaptation is mediated by the HOG (High Osmolarity Glycerol) signalling pathway

The HOG pathway monitors osmotic changes via two sensor systems.

The Sln1-Ypd1-Ssk1 phosphorelay system monitors turgor changes (plasma membrane stretching).

The Hkr1, Msb2 mucins monitor plasma membrane – cell wall changes.

Hyperosmotic stress activates MAPKKKs (Ste11, Ssk2/22), which activate MAPKK (Pbs2), which activates MAPK (p38/Hog1).

Protein phosphatases (Ptp2, Ptp3, Ptc1) are negative regulators.

Hog1 controls gene expression, cell cycle, translation, metabolism, transport processes....
Hog1 activity can be monitored in different ways

Hog1 activity is correlated to its phosphorylation state. phospho-Hog1 can be quantified by Western blotting using specific antibodies.

Active Hog1 accumulates in the nucleus (although it also has cytosolic targets), which can be quantified using a Hog1-GFP fusion.

This allows monitoring signalling in real-time and determining cell-to-cell variation.
Hog1 activation is transient

Western blotting monitoring Hog1 phosphorylation over time following hyperosmotic shock.

Hog1 phosphorylation is transient.
Observing Hog1 signalling

Cell array generated by optical tweezer technology in microfluidic device.

Moving cells repeatedly from high to low osmolarity and back: Hog1 shuttles in and out of the nucleus every time.

This technology allows monitoring signalling in real time but also allows determining population properties such as cell-to-cell variation, threshold and noise.
HOG pathway activation depends on stress intensity – single cell traces
HOG pathway activation depends on stress intensity – different profile and response delay at high stress

At lower stress levels, the amplitude increases.

At higher stress levels, the period of Hog1 activity increases....

....and the response is delayed....

...and eventually the amplitude drops again.
Correlating cell volume to Hog1 activation/nuclear accumulation

Experimental scenario in which cell volume drops to different levels and cells recover with different kinetics.

Lower volume after stress results in slower Hog1 nuclear entry – unknown adaptation mechanism that acts before Hog1 nuclear entry?

Hog1 exits from nucleus long before volume has recovered – volume/turgor change as signal rather than absolute volume/turgor?
The HOG pathway controls accumulation of glycerol at various levels

Glycerol serves as compatible solute and accumulates to more than 1M inside the cell.

Hog1 stimulates expression of genes encoding enzymes in glycerol production (GPD1, GPP1, GPP2).

Hog1 appears to activate Pfk26, which produces fructose-2,6-bisphosphate, an activator of glycolysis.

Gpd1 is phosphorylated/dephosphorylated in a Hog1-dependent manner.

Hog1 stimulates expression of STL1, which encodes an active glycerol uptake system.

Hog1 controls Fps1, the glycerol export channel.

We wish to understand the quantitative and temporal contributions of those mechanisms.
Hog1 controls the activity of the Fps1 glycerol facilitator

Fps1 mediates export of glycerol from the cell.

Fps1 closes following hyperosmotic shock and opens following hypo-osmotic shock (and both processes are critical for adaptation).

Fps1 is also an entry route for toxic arsenite and acetic acid.

Under such conditions, Hog1 clearly phosphorylates and controls Fps1 and...

...Fps1 seems to be the only Hog1 target under arsenite stress.

Hog1 may control Fps1 trafficking or stability but probably not rapid gating under osmostress.

Recently several proteins interacting with Fps1 have been identified.

Rgc1 and Rgc2 may control channel activity and are phosphorylated in a Hog1 dependent manner.

Two new proteins may link Fps1 to the control of mating and cell wall integrity signalling as well as lipid rafts.
Modelling of yeast osmoregulation

A model consisting of 35 ordinary differential equations for the signalling pathway, gene expression, metabolism, and volume/pressure changes. 70 parameters were initially fitted to a standard experiment.
The HOG pathway is controlled by feedback mechanisms

The transient character of Hog1 phosphorylation and nuclear accumulation indicates a rigorous feedback control system of the HOG pathway.

In fact, there appear to be two major mechanisms, one controlling basal signalling and one controlling adaptation after stress.

The mechanism that controls basal signalling and signalling thresholds is not known in detail.

The adaptation after stress is tightly linked to osmotic adaptation and volume recovery, and not due to an internal feedback loop.
The HOG pathway controls accumulation of glycerol at various levels

We wish to understand the quantitative and temporal contributions of Hog1-dependent mechanisms controlling glycerol accumulation:

- Control of Fps1 activity
- Control of gene expression (*GPD1*)
- Control of metabolism (via Pfk26)
- Control of expression of the Stl1 uptake system

We employed a combination of experimentation and modelling/simulation.
Experimentally determined time courses following addition of 0.4M NaCl:

A: extracellular glycerol
B: extracellular glucose
C: intracellular glycerol
D: extracellular ethanol
E: intracellular trehalose
F: optical density
G: phospho Hog1
H: Gpd1 protein levels

Symbols:
- black filled circles: wild type
- green empty circles: unstressed wild type
- blue filled squares: pfk26/27 mutant
- red empty squares: hog1 mutant
- pink filled triangles: hog1Δ HOG1-attached
- orange empty triangles: gpd1 Δ
- purple filled trapezoid: fps1 Δ FPS1- Δ1.
ODE model used

Topology of the ODE model presented. Symbols according to SBGN:

- Circles indicate metabolites
- Rectangles with rounded corners indicate proteins
- Rectangles with two sharp corners indicate genes
- Concave hexagons indicate perturbations varied in experiments (NaCl: hyperosmotic stress, I: \textit{hog1}\Delta, II: \textit{pfk26}\Delta \textit{pfk27}\Delta, III: \textit{hog1}\Delta \textit{HOG1}-attached, IV: \textit{gpd1}\Delta, V: \textit{fps1}\Delta \textit{FPS1}-\Delta1).
- Reactions and molecules are coloured according to module (brown: transport, blue: glycolysis, green: biomass, red: adaptation, yellow: biophysical).
Examples of model simulations

Simulations results and experimental data (data points) of intracellular glycerol levels.

Addition of 0.4M NaCl at time zero min.

A. Simulation results for a model variant without Hog1-Fps1 interaction, where the time course of glycerol accumulation is not reproduced satisfactorily for \(\text{hog1}\Delta\ HOG1\)-attached and \(\text{hog1}\Delta\) and abundance of open Fps1 is similar in all strains.

B. Time courses for a model in which active Hog1 negatively affects Fps1. In this scenario, glycerol time courses for \(\text{hog1}\Delta\ HOG1\)-attached and \(\text{hog1}\Delta\) are reproduced faithfully.
Calculating different contributions

Direct contribution to intracellular glycerol concentration.

A: Stacked contributions for all reactions for wild type and the \textit{gpd1\Delta} mutant reveals that loss of Gpd1-mediated production is partly rescued by increased Stl1-mediated import, increased basal production and reduced efflux.

B: Ternary plots that visualise the relative contribution of Fps1, Gpd1 and the sum of the other contributions. The relative contributions along a time course are plotted, time is indicated by colour.

For most strains, the glycerol concentration is initially strongly affected by changes in volume and flux through Fps1 is minimal.

As the abundance of Gpd1 increases and cells recover, Fps1 re-opens.

At the final stages, Gpd1 concentration decreases again and well adapted cells return to a state close to that before stress. Differences between strains that adapt well and strains where adaptation is impaired are apparent.
Response coefficients (RCs) illustrate time- and context dependence:

A: different temporal phases of osmoadaptation in wild type, normalised RCs of adaptation reactions on intracellular glycerol.

B: Hog1-dependent regulation of Pfk26/27 activity affects pyruvate concentration levels stronger than those of glycerol, indicating that the role of this regulation is in stabilisation of flux towards lower glycolysis.

C: Stl1-dependent import of glycerol is negligible in most strains except for the gpd1Δ mutant.

D: Prediction of time courses for glycerol accumulation in stl1Δ and gpd1Δ stl1Δ mutants. Comparison to simulation results for wild type and gpd1Δ illustrates the role of Stl1.
Investment in adaptation

Yeast osmoadaptation requires rerouting of glycolytic flux resulting in a reduced growth rate.

A: Experimentally monitored doubling times before and after stress. The investment for glycerol accumulation is reflected in a decrease in growth rate

B: Simulation results indicate that glycerol accumulation is facilitated by a decrease in carbon flux towards biomass generating reactions. Fluxes are depicted for -30, 20, 90 minutes (stress at 0).
Conclusions for HOG pathway regulation and control of glycerol accumulation

The HOG-pathway is controlled by different feedback mechanisms, one depending on osmotic adaptation.

Single cell analyses reveal new features of HOG control and its relation to volume/turgor regulation.

The HOG-pathway controls glycerol accumulation in different ways.

Initially closing of Fps1 plays the most important role, subsequently increased production become more important.

The control of glycolysis via Pfk26 may be more important for stabilising flux towards pyruvate than for glycerol production.

Hog1 appears to control Fps1 under osmostress – but we do not know how.

The Stl1 uptake system becomes important when glycerol production is impaired.
Yeast MAPK pathways

MAPK pathways form a complex network coordinating cell growth, morphogenesis, differentiation and proliferation in response to external stimuli.

Yeast has four distinct "pathways" (defined by signal-output), which communicate intensely with each other.
Budding yeast has four aqua(glycero)porins

**Aqy1** – aquaporin in resting cells

**Aqy2** – aquaporin associated with morphological development

**Fps1** – aquaglyceroporin with long cytoplasmic extensions, transports glycerol and arsenite, controlled by the Hog1 (p38) MAPK (stability, trafficking, gating?)

**Yfl054** – aquaglyceroporin of unknown function

Recent work from the Gasch lab potentially explains the great variability between strains of the presence of active aquaporins:

Presence of aquaporins seems to confer an advantage to survive freezing....

....but a disadvantage in high osmolarity conditions.
Yeast life cycle and life style

Though unicellular, yeast has different cell types:

Haploids of mating type a and alpha, diploids and spores (meiotic products, haploid)

Both haploids and diploids form vegetative cells that can be studied

In Nature, yeast lives in (micro)colonies of variable morphology on plants

Yeast also develops filaments and invades its substrate

Resting stages: spores and stationary (G0) cells
Aqy1 is a resting stage aquaporin

Shift of diploid yeast cells to starvation conditions instigates the meiosis and the spore formation programme.

Under these conditions expression of AQY1 mRNA and protein are strongly upregulated.

The Aqy1 protein appears at the same time point as spores become visible.

**mRNA**

**Protein**

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<th>Protein</th>
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Shift to 1% KAc at time 0

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Aqy2 is associated with yeast morphological developments

Morphological features of yeast cells and colonies include:

- Filamentous/pseudohyphal growth (attached chains of cells)
- Invasive growth (substrate/agar invasion)
- Colony fluffiness
- Mat formation, i.e. spreading out of a colony
- Plastic adhesion and biofilm formation

Many of those features are also associated with a change in cell shape (round versus elongated)

Invasion and pseudohyphae formation are essential virulence features in e.g. *Candida* and *Cryptococcus*
Aqy2 expression affects agar invasion

Overexpression of Aqy2 results in strong agar invasion
Aqy2 affects colony fluffiness

Deletion of AQY2 diminishes colony fluffiness as compared to wild type.
Aqy2 expression affects plastic adhesion

Deletion, but especially AQY2 overexpression affect the yeast cell’s ability to adhere to hydrophobic surfaces
Morphological developments are stimulated by Kss1 and PKA and repressed by Hog1
Osmostress and active Hog1 represses morphological developments, and deletion of \textit{HOG1} strongly activates those pseudohyphae formation.

**Colony Fluffiness**

- \textit{HOG1} deletion activates colony fluffiness.

**Agar Invasion and Plastic Adhesion**

- Hog1Δ/hog1Δ and \textit{pbs2Δ active-HOG1/ pbs2Δ active-HOG1} show increased agar invasion and plastic adhesion.

**Graphical Data**

- A graph showing the activity of different strains under osmotic stress conditions.

- The graph compares WT, aqy2Δ, hog1Δ, and aqy2Δ hog1Δ strains in YPD media with and without 1 M KCl and 1.5 M sorbitol.

- Quantitative data for colony formation, agar invasion, and plastic adhesion is presented in the graph.
Osmotic stress represses AQY2 expression

Osmotic stress diminishes AQY2 mRNA levels
Osmotic stress diminishes AQY2 promoter activity
Aqy2 is not visible in cells under osmotic stress
Hog1 (p38) MAPK represses AQY2 expression

Low or absent Hog1 activity (hog1Δ, kinase-dead Hog1, inhibited Hog1, non-activated Hog1) leads to increased AQY2 promoter activity.

Increased Hog1 activity (constitutively active Hog1, absence of Hog1 phosphatases) leads to diminished AQY2 promoter activity.
PKA stimulates morphological developments as well as AQY2 expression
AQY2 expression is controlled by PKA via the Sfl1 repressor

Deletion of the TPK2 gene for a catalytic PKA subunit diminishes AQY2 promoter activity. This effect is mediated by phosphorylation of the Sfl1 transcriptional repressor.

The Hog1 effect on AQY2 expression may act through Tpk2.
The Filamentous Growth Pathway affects morphological developments as well as AQY2 expression.
The Hog1 effect on AQY2 expression can partially be explained by cross-talk with the Filamentation Pathway.

The Kss1 MAPK may both stimulate and repress AQY2 expression.

Kss1 partly mediates Hog1 effects on AQY2 expression.
AQY2 expression is controlled by yeast morphology-controlling pathways

Hyper-osmotic stress

FG pathway

HOG pathway

cAMP

PKA pathway

Osmotic homeostasis
Invasive growth
Fluffy morphology
Cell surface properties

Glucose

Nitrogen starvation

Hyper-osmotic stress

Gpr1

Msb2

Sho1

Sln1

Gpa2

Ras2

Ira1/2

Cdc42

Ste50

Ste20

Ypd1

Ssk1

Pde1/2

CAMP

Bcy1

Ste11

Pde

Ste7

Pbs2

Hog1

Ptp2

Ptp3

Tpk1

Tpk2

Sfl1

Flo8

Filamentation

Filamentation

Transcription factors

Osmoadaptation

Tpk3

Sfi1

Dig1

Tec1

Ste12

Kss1

Kss1-P

Kss2/Ssk22

Osmoadaptation
Conclusions on the role of Aqy2

Deletion and overexpression of Aqy2 confers phenotypes on growth and surface characteristics and morphological developments.

Yeast Aqy2 production is controlled by the same signalling pathways that control yeast cell surface properties, growth behaviour and colony morphology.

The expression pattern and regulatory pathways are similar - though not identical - to those of the yeast Muc1/Flo11 mucin, which is critical for those morphological developments.

So how could an aquaporin affect cell surface properties, growth behaviour and colony morphology?

Speculation: Aqy2 may mediate water transport between the cytosol and the cell wall (where Muc1 is located) to control cell surface hydrophobicity.

Speculation: Aqy2 may also be important to control water flow in yeast colonies, which is critical for nutrient and metabolic waste product transport.

Some of these aspects remind of the proposed role of human aquaporins in tumour cell migration and invasion.
Conclusions on roles of MAPK network – a decision making device

The MAPK system may be a decision making device

Cross-talk may have the purpose of taking decisions: are osmotic conditions appropriate to enter into morphological programmes