

Osmoregulation at different stages of the yeast life cycle

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Experience is not what happens to you; it's what you
do with what happens to you

- Aldous Huxley

Abstract

The ability to adapt to changing and potentially harmful conditions in the surrounding environment is crucial for fitness and survival of all living cells; in particular unicellular organisms, since they are frequently exposed to stress factors such as heat, drought, nutritional starvation and toxic substances. The aim of this thesis is to determine how cells respond to osmotic and nutritional changes in the environment and how downstream targets of signalling cascades are regulated.

Water is fundamental to life, and all cells must be able to adapt to fluctuations in water availability to maintain cellular water homeostasis. In bakers' yeast *Saccharomyces cerevisiae*, the High Osmolarity Glycerol (HOG) pathway is activated upon conditions of high osmolarity, and the pathway coordinates the responses needed to counteract loss of volume and turgor pressure. These actions include glycerol accumulation, ion efflux and transcriptional and translational changes. In this thesis, the osmotic stress response is characterized using a conditional osmotic system. We show that the period of Hog1 activation affects the transcriptional output in a quantitative rather than qualitative way. The analysis also sheds light on an initial adaptation process involving regain of volume through accumulation of compatible osmolytes, which precedes Hog1 nuclear accumulation and the transcriptional response.

The *S. cerevisiae* aquaglyceroporin Fps1 plays an important role during osmotic stress as a regulator of the intracellular glycerol concentration. A decrease in external osmolarity leads to water inflow and cell swelling, and Fps1 activity is vital under this condition for rapid release of excessive glycerol to lower the cells' turgor pressure. During a hyperosmotic shock, glycerol flux through Fps1 must be decreased; if not, the cells have great difficulties to accumulate glycerol and hence show osmosensitivity. The exact mechanisms behind Fps1 regulation are still unknown, but regulatory domains on both cytoplasmic termini have been identified. Here, the importance of the Fps1 transmembrane core in restricting glycerol flux is described, and we show that the termini alone are not sufficient to regulate channel activity. We have also studied an orthodox aquaporin that is important for freeze and thaw resistance in the yeast *Pichia pastoris*. The activity of this aquaporin was shown to be regulated by a combination of phosphorylation and mechanosensitivity.

Finally, osmotic regulation throughout the yeast developmental pathways of sporulation and germination is briefly discussed. We have determined the transcriptional changes occurring during yeast spore germination and the analysis revealed a sequential upregulation of different subprograms that we can link to specific transcription factors. Although qualitatively similar responses, the transcriptional output of spores in response to glucose is not as pronounced as to rich growth medium, suggesting that spores can sense nutrient starvation early on in the quickening process.

Keywords: stress signalling, osmoregulation, aquaporins, *cerevisiae*, germination

Papers included

This thesis is based on the following papers, which are referred to by their roman numerals:

- I. **Geijer C**, Medrala-Klein D, Petelenz-Kurdziel E, Ericsson A, Smedh M, Krantz M, Goksör M, Nordlander B, Hohmann S: *Initiation of the transcriptional response to hyperosmotic shock correlates with the volume recovery*. Manuscript
- II. **Geijer C***, Ahmadpour D*, Filipsson C, Palmgren M, Medrala-Klein D, Tamas M, Hohmann S, Lindkvist-Petersson K: *Yeast aquaglyceroporins use the transmembrane core to control glycerol transport*. Manuscript for JBC
*) contributed equally
- III. Fischer G, Kosinska-Eriksson U, Aponte-Santamaría C, Palmgren M, **Geijer C**, Hedfalk K, Hohmann S, de Groot BL, Neutze R, Lindkvist-Petersson K: *Crystal structure of a yeast aquaporin at 1.15 angstrom reveals a novel gating mechanism*. PloS Biology. 7(6); (2009)
- IV. **Geijer C**, Joseph-Strauss D, Simchen G, Barkai N and Hohmann S: *Saccharomyces cerevisiae Spore Germination*. Dormancy and Resistance in Harsh Environments, Topics in Current Genetics, 2010, Volume 21/2010, 29-41
- V. **Geijer C**, Pirkov I, Wongsangnak W, Ericsson A, Nielsen J, Krantz M, Hohmann S: *Transcriptional regulation profiling of germination in Saccharomyces cerevisiae*. Manuscript for BMC Genomics

Table of Contents

1	Introduction.....	11
2	Osmosis and osmo-regulation.....	12
3	MAP kinase signalling pathways	14
3.1	High Osmolarity Glycerol (HOG) pathway.....	15
3.1.1	HOG pathway architecture.....	15
3.1.2	The transcriptional and translational responses to osmostress.....	17
3.1.3	Initial adaptation precedes Hog1 nuclear accumulation	18
3.1.4	Glycerol accumulation.....	20
3.1.5	Cell cycle block and cross-talk.....	20
3.1.6	Pathway termination	21
3.1.7	Other stressors that activates the Hog pathway.....	21
3.2	Cell Wall Integrity (CWI) pathway	22
3.2.1	The yeast cell wall.....	22
3.2.2	CWI pathway architecture.....	22
3.2.3	Activation of CWI pathway	24
3.2.4	Effectors downstream of CWI pathway	26
3.3	Glycerol concentrations determined by interplay between HOG and CWI pathways?.....	27
4	Aquaporins.....	28
4.1	The family of aquaporins.....	28
4.1.1	Structure of aquaporins	28
4.1.2	Aquaporins in all kingdoms of life.....	29
4.2	Yeast aquaglyceroporin Fps1	31
4.2.1	Discovery of Fps1	31
4.2.2	Fps1 is important for osmotic homeostasis	31
4.2.3	Deletion of Fps1 affects the composition of the cell wall and the plasma membrane.....	33
4.2.4	Fps1 is important during mating.....	33
4.2.5	Unregulated Fps1 mutants confer severe osmo-sensitivity.....	34

4.2.6	Fps1 transports arsenite and other small molecules.....	38
4.2.7	Fps1 regulators	38
4.2.8	The regulatory mechanisms of Fps1.....	41
4.3	Yeast aquaglyceroporin Yfl054	43
4.4	Yeast orthodox aquaporins Aqy1 and Aqy2	43
4.4.1	Aqy1 is involved in sporulation.....	44
4.4.2	<i>Pichia pastoris</i> orthodox aquaporin Aqy1	45
5	Osmostress during sporulation and germination.....	46
5.1	Developmental transitions of <i>S. cerevisiae</i>	46
5.2	The process of sporulation	47
5.2.1	Regulators of sporulation	47
5.2.2	Osmotic homeostasis during sporulation	48
5.3	The process of germination.....	48
5.3.1	Transcriptional response in germination.....	49
5.3.2	Osmotic homeostasis during germination	49
6	Concluding remarks and future perspectives	51
7	Acknowledgements.....	54
8	References	57

1 Introduction

No cell is an island; it exists in a context and is strongly dependent on and responsive to the surrounding environment. All cells, but in particular unicellular organisms, can be exposed to stressors in the environment such as heat, drought, irradiation and nutritional limitations. Cells must be able to adapt to changing and potentially harmful conditions to ensure fitness and survival of the organism.

Upon external stimuli (such as high temperature, salt stress or glucose starvation), intracellular signalling pathways become activated and transfer the signal into the cell to mediate changes at many different levels. For example, transcription is regulated such that mRNAs encoding proteins important for coping with the environmental changes are upregulated, whereas the levels of mRNAs encoding dispensable proteins are reduced. Changes of translational capacity are also important to ensure that critical proteins are produced. In addition, post-translational adjustments fine-tune the activity of proteins. These transitions in the setup of the cells' machinery ultimately lead to stress adaptation and resistance or optimal use of the available nutrients. In fundamental biological research, *Saccharomyces cerevisiae* has proven to be a powerful eukaryotic model organism; easy to grow and handle and amenable to genetics, cell and molecular biology. This thesis will deal with how the yeast cell responds to environmental queues; in particular how it adapts to altered water availability and how it develops from a dormant spore to a vegetative cell when glucose and nutrients become accessible. Aquaporins and aquaglyceroporins, membrane channel proteins that facilitate the movement of water and small solutes across the plasma membrane, are highly involved in osmo-stress adaptation. The expression of yeast aquaporins is also dependent on nutrient availability, which connects osmoregulation with yeast spore quickening; the process of germination.

It is important to understand how signalling pathways and their target proteins function in a cell to improve, for example, drug design and food preservation techniques, and to learn how to fight diseases such as cancer and obesity. The aim of my thesis is to shed light on how cells respond to osmotic and nutritional changes in the environment and how downstream targets of signalling cascades are regulated. In particular I focus on the transcriptional output and the role of aquaporins and aquaglyceroporins in the response to external stimuli.

2 Osmosis and osmo-regulation

Water potential is defined as the potential energy of water per unit volume relative to pure water, and quantifies the tendency of water to move from one area to another. Water molecules tend to travel from areas with high water potentials to areas with low water potentials; from pure water to solutions. Water traverses the cell's plasma membrane by passive diffusion mainly through the phospholipid bilayer, and through aquaporins (Preston and Agre 1991). The diffusion of water across a membrane down the water potential gradient is called osmosis, and reduces the difference in concentrations between the two sides of a membrane. Upon an increase in the external solute concentration (osmolarity), water spontaneously leaves the cell driven by the difference in water potential, which leads to an increase in concentration of other intracellular molecules. Vice versa, a decrease in the external osmolarity leads to water re-entry into the cell and subsequently a dilution of intracellular molecules.

A cell's ability to actively maintain a proper intracellular water balance is critical to uphold shape and strength, to allow transmembrane transport processes and to ensure appropriate conditions for biochemical processes. Upon loss of the cell's internal water, for example after severe diarrhoea, mammals risk death if hydration is not restored. Plants store water in the vacuole, and upon dehydration the organism loses shape and turgor pressure (the outward pressure that arises when the cytoplasm and vacuoles fill up with water and the cell plasma membrane presses against the cell wall), and eventually the plant will wither. Water stress of unicellular organisms includes exposure to sudden drought, rain fall, high concentrations of sugar during wine fermentation, and freezing during winter time. To cope with water stress, cells have developed different ways to recover water or to release excessive water and thereby regain water homeostasis. Cells that encounter an increase in external osmolarity (hyperosmotic condition) strive to re-establish the water balance by accumulating compatible osmolytes (Hohmann 2002). *S. cerevisiae* utilizes glycerol, but amino acids, ions, sugars and polyols can all act as compatible osmolytes. These molecules function to decrease the intracellular water potential and thereby driving water back in to the cell, without disturbing the processes of the cell. Some of these osmolytes may also have direct protective roles as antioxidants, as stabilizers for proteins or by providing redox balance (Yancey 2005). Once the cell has accumulated sufficient levels of compatible osmolytes, growth can resume in the high osmolarity condition. The opposite of a hyper-osmotic shock is a hypo-osmotic shock, where the external osmolarity decreases and water enters the cell (Levin 2005). To adapt to a

hypo-osmotic shock, it is crucial to rapidly lower the concentrations of molecules in the cell, which is efficiently done by releasing excessive compatible solutes (Martinac et al. 1987; Tamas et al. 1999) (Fig. 1).

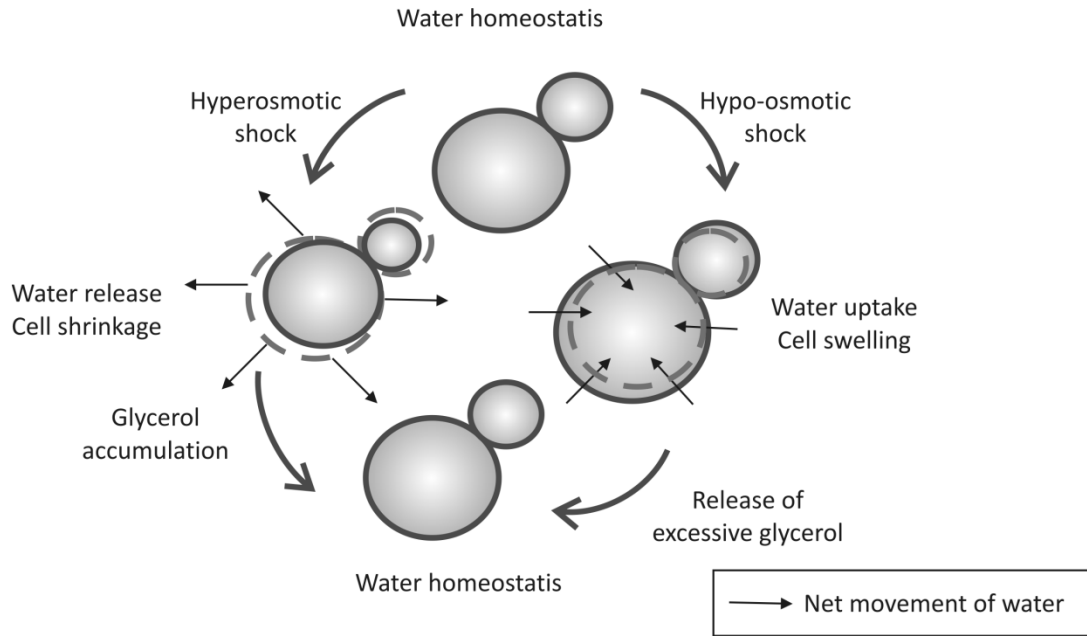


Fig. 1. Schematic view of osmoregulation in *S. cerevisiae*. Upon a hyperosmotic shock, water efflux leads to loss of turgor pressure and cell volume. Adaptation involves glycerol accumulation that enables water influx, volume recovery and restored turgor pressure. Once adapted, cells can continue growth and division. A hypo-osmotic shock results in rapid water inflow and cell swelling. Cells respond by releasing excessive glycerol to decrease turgor pressure and prevent bursting.

Central in the response to hyper- and hypo-osmotic shock in yeast is the transient activation of mitogen-activated protein kinase (MAPK) signalling cascades; the high osmolarity glycerol (HOG) pathway and the cell wall integrity (CWI) pathway, respectively (Hohmann 2002; Levin 2005).

3 MAP kinase signalling pathways

MAP kinase pathways are highly conserved in eukaryotes and function as signalling cascades that respond to environmental cues and control cell cycle progression, morphogenesis and stress responses. A MAP kinase cascade can amplify a signal initiated from the cell surface and convert a graded input to a sensitive, switch-like response (Marshall 1994; Robinson and Cobb 1997). The upstream activation mechanisms of the MAPK cascades are diverse and include mechanosensitive sensors, G-protein-coupled receptors and phosphorelay systems. The core of the MAPK pathway is a three-tiered cascade of protein kinases. A MAP kinase kinase kinase (MAPKKK) activates the MAP kinase kinase (MAPKK) by dual phosphorylation on a serine and a threonine. The activated MAPKK then phosphorylates the MAP kinase (MAPK) by phosphorylation on a threonine/serine and a tyrosine residue parted by a single amino acid. The MAPK typically has targets both in the cytosol and nuclei that become phosphorylated on a serine/threonine next to a proline (Marshall 1994; Tanoue and Nishida 2003).

MAPK pathways are highly intertwined with components shared between different pathways, and deletion of component(s) in one pathway may lead to unintentional activation, cross-talk, of other MAPK pathways. Different strategies are employed to ensure the specificity of signal transmission, including scaffold proteins, cross-pathway inhibition and kinetic insulation (occurs when one pathway is activated by a transient signal whereas the other pathway is activated by a slowly increasing input) (Schwartz and Madhani 2004; Behar et al. 2007). Recent work indicates that cross-talk and communication between pathways plays important roles in prioritising responses when the cell receives different, potentially conflicting signals (Furukawa et al. 2011).

The MAP kinase pathways in *S. cerevisiae* consist of: i) the mating pheromone pathway with the Fus3 MAPK (Leberer et al. 1997), ii) the pseudo-hyphal/invasive growth pathway including Kss1 (Liu et al. 1993; Roberts and Fink 1994), iii) the HOG pathway with the Hog1 kinase (Brewster et al. 1993; Hohmann 2002), and iv) the CWI pathway with Slt2 as effector kinase (Davenport et al. 1995; Kamada et al. 1995). There is another MAPK in yeast, Smk1, which plays a role in sporulation (Krisak et al. 1994). Although the sequence of Smk1 is similar to other MAPKs and the protein is activated by phosphorylation of a MAPK-like activation loop, Smk1 activation is not thought to require members of the MAPKK family (Wagner et al. 1997; Schaber et al. 2002).

3.1 High Osmolarity Glycerol (HOG) pathway

The *S. cerevisiae* HOG pathway controls the adaptive responses to hyperosmotic shock. These include accumulation of the osmolyte glycerol, regulation of the transcriptional response and imposing cell cycle delays to ensure adaptation to the new osmotic environment before continuing proliferation.

3.1.1 HOG pathway architecture

The HOG signalling system is activated via two partially redundant but mechanistically distinct branches: the Sho1 branch and the Sln1 branch (Fig. 2). Although any of the two branches alone is sufficient for signalling upon high-osmolarity conditions, the Sln1 branch has a lower activation threshold than the Sho1 branch. This allows cells to respond to a wide range of external osmolarities (Maeda et al. (1995).

The Sho1 branch senses a hyperosmotic challenge through two transmembrane mucins, Hkr1 and Msb2 (Tatebayashi et al. 2007). Hkr1 and Msb2 form complexes with Sho1, and localize at the plasma membrane where cell growth occurs, ie at the budneck, the growing bud and mating projections (Raitt et al. 2000; Reiser et al. 2000). Sho1 acts like a scaffold protein, important for the assembly of MAPKKK Ste11 and MAPKK Pbs2 as well as other proteins involved in the branch, including Cdc42, Ste20 and Opy2 (Maeda et al. 1995; Raitt et al. 2000; Reiser et al. 2000). Components of the Sho1 branch, such as Ste20, Ste50 and Ste11, are also involved in the pseudohyphal development pathway and in the pheromone response pathway (O'Rourke and Herskowitz 1998).

The Sln1 branch consists of the osmosensor and histidine kinase Sln1, the signal transmitter protein Ypd1 and the response regulator Ssk1 that together form a phosphorelay system, and the downstream MAPKKKs Ssk2 and Ssk22 (Stock et al. 2000). Hyperosmotic shock reduces in an unknown manner Sln1 activity which causes dephosphorylation of Ypd1 and Ssk1 (Posas et al. 1996; Reiser et al. 2003). Unphosphorylated Ssk1 allows the MAPKKKs Ssk2 and Ssk22 to activate themselves by auto-phosphorylation (Maeda et al. 1994).

The Sho1 and Sln1 branches converge by activating MAPKK Pbs2, which activates MAPK Hog1 by dual phosphorylation on two adjacent Threonine and Tyrosine residues, T174 and Y176 (Posas and Saito 1997). Hog1 activity is well correlated with its phosphorylation state, and Phospho-Hog1 can be quantified by Western blotting

using specific antibodies. Hog1 is rapidly but transiently phosphorylated upon a hyperosmotic shock. With stronger degrees of osmotic stress, the phosphorylation amplitude increases until it reaches a maximum. At even stronger stress levels, the period of Hog1 phosphorylation increases (Van Wuytswinkel et al. 2000; Krantz et al. 2004; Karlgren et al. 2005).

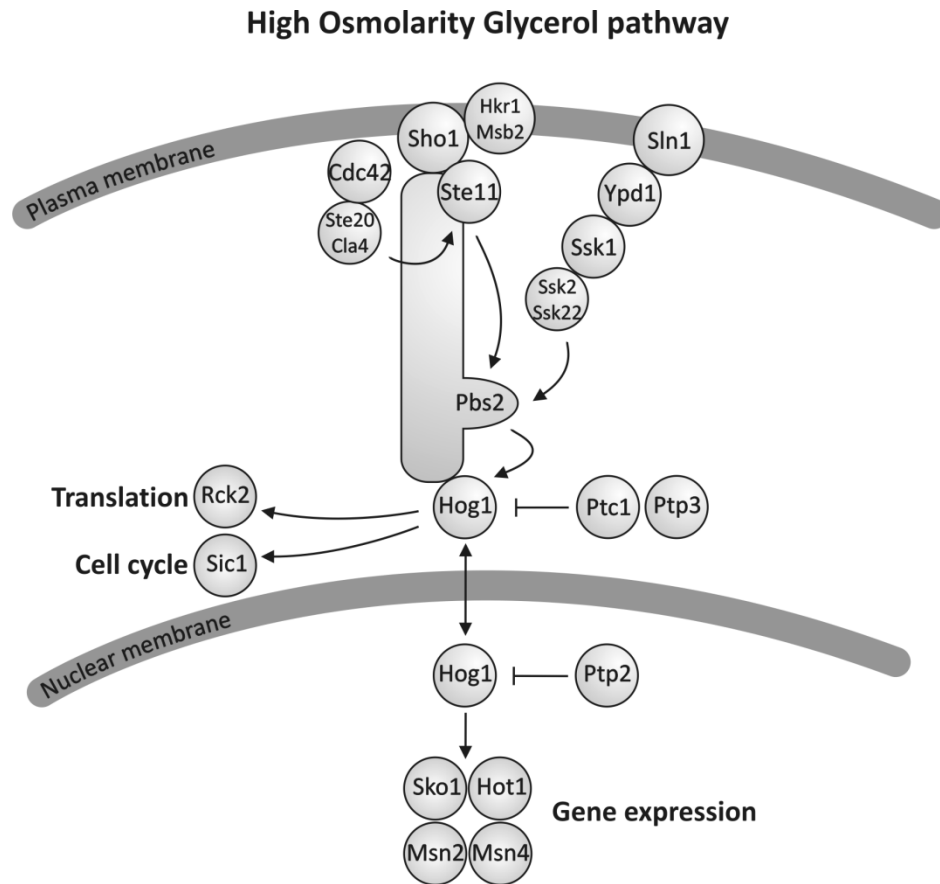


Fig. 2. Overview of the yeast HOG pathway. High osmolarity stress is sensed by the Sho1 and Sln1 branches that converge on and activate the MAPKK Pbs2. Pbs2 activates the MAPK Hog1, and Hog1 accumulates in the nucleus where it induces a transcriptional response. Hog1 also influences cell cycle progression and the translational machinery. Courtesy of Professor Stefan Hohmann, CMB, University of Gothenburg.

3.1.2 The transcriptional and translational responses to osmostress

Whereas Hog1 appears to be distributed evenly in the cytoplasm and nucleus during normal conditions, a hyperosmotic shock leads to a rapid accumulation of the MAP kinase in the nucleus (Fig. 2) (Ferrigno et al. 1998; Reiser et al. 1999a). Activated Hog1 in the nucleus stimulates transcription of numerous genes while it leads to downregulation of the expression of others (Posas et al. 2000; Rep et al. 2000). Similar to the phosphorylation profile of Hog1, the transcriptional response to osmostress is transient, and the period and the amplitude of the response depend on the strength of the osmotic shock (Posas et al. 2000). Also the timing of induction of stress-mRNAs depends on the magnitude of the stress; the stronger the stress level the later the peak (Posas et al. 2000; Rep et al. 2000).

Several microarray analyses on the transcriptional response to hyperosmotic shock have been reported (Gasch et al. 2000; Posas et al. 2000; Rep et al. 2000; Causton et al. 2001). These studies identified 200-400 upregulated genes, including genes for production of the compatible osmolyte glycerol, ion homeostasis, redox metabolism and general stress response genes (Albertyn et al. 1994; Marquez and Serrano 1996; Gasch et al. 2000; Krantz et al. 2004). Only a fraction of the osmo-induced genes requires Hog1 for upregulated expression, but these genes are often among those displaying the highest fold induction (Rep et al. 2000; O'Rourke and Herskowitz 2004). Some genes are upregulated but not fully induced in the absence of Hog1, and yet other genes are Hog1 independent (Rep et al. 1999; Rep et al. 2000). The transcriptional response reflects the change from proliferation to stress adaptation, and many genes down-regulated following osmotic shock encode proteins for ribosome biogenesis, the translational machinery and glycolysis (Gasch et al. 2000; Posas et al. 2000; Rep et al. 2000). The expression of those genes commonly correlates with the proliferation capacity of the cell.

Hog1 controls different steps during the transcription process, which together mediate the Hog1-dependent transcriptional changes observed upon a hyperosmotic shock. Stimulating transcription initiation is the best-characterized role of Hog1 in transcriptional regulation. Hog1 is recruited to target promoters through binding of several transcription factors, including Msn2, Msn4, Hot1, Sko1 and Smp1 (Rep et al. 2001; Proft and Struhl 2002). Moreover, nuclear retention of Hog1 is dependent on its association with these transcription factors (Reiser et al. 1999b). Hog1 is also important for recruitment of the Polymerase II, SAGA (Spt-Ada-Gcn5 acetyltransferase) and Mediator transcription complexes to osmo-responsive genes (Alepez et al. 2003; Zapater et al. 2007). Binding of Hog1 to chromatin is not

restricted to the promoter regions but also extends to the entire coding regions of stress-responsive genes, and Hog1 associates with elongating PolII and the transcriptional elongation complex (Proft et al. 2006). Recently, Hog1 was shown to interact also with the RSC (chromatin structure remodelling) complex and recruits it to coding regions of osmo-responsive genes. Chromatin remodelling is important for efficient polymerase progression and osmo-responsive gene expression, and RSC mutants display osmosensitivity (Mas et al. 2009).

Interestingly, most genes upregulated after a hyperosmotic shock are dispensable for tolerance to moderate osmotic stress, as there is little overlap between the list of upregulated genes and that of deletion mutants showing an osmosensitive phenotype (Warringer et al. 2003). Proteins required for the immediate response to hyperosmotic stress need to be present in the cell at the time of stress, and thus the transcriptional induction is more important for adaptation to than survival of an acute shock. An example is *ENA1*, encoding an ATPase sodium pump mediating Na⁺ and Li⁺ efflux. Expression of *ENA1* is repressed during normal conditions but upregulated upon hyperosmotic stress. *Ena1* is dispensable at the early stages of adaptation but plays an important role during long term growth at high osmolarity (Proft and Struhl 2004). Further, a strain expressing an artificially membrane-attached Hog1 that cannot enter the nucleus is not sensitive to moderate hyperosmotic stress (Westfall et al. 2008). This finding emphasizes the fact that transcriptional response is more important for fine-tuning and long-term adaptation than for the immediate stress response and survival.

Although the transcriptional response has a measurable impact for long-term stress survival, the early translational response to a hyperosmotic shock appears to be biologically more relevant than the transcriptional response (Warringer et al. 2010). Hyperosmotic shock causes repression of translationally active ribosomes, and relief of this repression is dependent on Hog1 (Teige et al. 2001; Warringer et al. 2010). Moreover, Hog1 activates Rck2, a cytoplasmic protein kinase that has been implicated in the regulation of translation, as well as transcription, in the response to osmotic stress (Bilsland-Marchesan et al. 2000; Teige et al. 2001; Warringer et al. 2010).

3.1.3 Initial adaptation precedes Hog1 nuclear accumulation

As described above, the period of Hog1 activation is dependent on the strength of the osmotic stress. Intriguingly, at severe osmotic shock (1.4M NaCl), Hog1 nuclear

accumulation and the transcriptional response are delayed (Van Wuytswinkel et al. 2000). A more systematic approach showed that the higher the salt concentration the slower the nuclear localization of Hog1 (Petelenz-Kurdziel E, unpublished observations). Water outflow following an osmotic shock leads to an increased concentration of ions in the nucleus and as a consequence, transcription factors are released from the chromatin. Within a few minutes, the ion concentration is again diminished, enabling these proteins to re-associate with chromatin. Cells lacking Hog1 show a significant delay in protein-chromatin binding upon salt stress (Proft and Struhl 2004). Nha1, a Na⁺/H⁺ antiporter that mediates the exchange of protons from the medium against Na⁺ in the cytosol, is activated by Hog1-dependent phosphorylation upon osmotic stress, and re-association of transcription factors and chromatin is delayed also in a *nha1*Δ mutant (Proft and Struhl 2004). Together, these findings suggest that some initial adaptation must take place to enable Hog1 nuclear localization and transcriptional response.

In paper I, we demonstrated this phenomenon in a more sophisticated way by using a conditional osmostress system that allowed us to change the period of Hog1 activation without altering the initial stress level. With this system we could determine that although Hog1 is phosphorylated with the same kinetics (Karlgrén et al. 2005), Hog1 nuclear accumulation and the onset and amplitude of the initial transcriptional response after hyperosmotic shock is dependent on how fast cells are able to adapt (i.e. regain volume). Cells that recover quickly initiate the transcriptional response fast whereas cells that adapt more slowly display a delayed transcriptional response. These results suggest that phosphorylated Hog1 in slowly adapting cells is either trapped in the cytosol or shuttles between the two compartments but cannot accumulate in the nucleus. The most crucial action of the HOG pathway in response to hyperosmotic stress is to increase the levels of glycerol which consequently drives water back into the cell (Hohmann 2002). It is likely that rapid accumulation of this compatible solute, together with ion export, constitute the most important contributions to the initial adaptation. In agreement with this hypothesis, cells deficient in glycerol production display prolonged Hog1 phosphorylation and a delayed transcriptional response after hyperosmotic shock (Siderius et al. 2000). Glycerol accumulation is accomplished by stimulation of both nuclear and cytoplasmic Hog1 targets and is discussed in detail below.

3.1.4 Glycerol accumulation

Accumulation of glycerol to counteract water efflux is a central feature of hyperosmotic adaptation in *S. cerevisiae* and is accomplished through three different mechanisms: production, prevention of outflow and uptake (Hohmann 2002). Rapid glycerol accumulation upon a hyperosmotic shock is influenced mainly by volume loss and prevention of glycerol efflux through the aquaglyceroporin Fps1 (further discussed in the Fps1 section of the thesis). After 30-45 min of stress, increased production becomes the major contributor for accumulation (Petelenz-Kurdziel E, manuscript in preparation). Glycerol is produced from the glycolytic intermediate dihydroxyacetone-phosphate (DHAP) that is converted to glycerol-3-phosphate. This reaction is catalysed by the NAD⁺-dependent glycerol-3-phosphate dehydrogenases Gpd1 and Gpd2 (Ansell et al. 1997; Pahlman et al. 2001). Glycerol-3-phosphate is converted into glycerol by the glycerol-3-phosphate phosphatases Gpp1 and Gpp2 (Norbeck et al. 1996; Pahlman et al. 2001). *gpd1Δ gpd2Δ* and *gpp1Δ gpp2Δ* double mutants cannot produce glycerol and these cells have consequently difficulties to adapt to high osmolarity conditions (Siderius et al. 2000; Klipp et al. 2005). There is also evidence that Hog1 directly controls central glycolytic metabolism via activation of phosphofructo-2-kinase to enhance glycerol production (Dihazi et al. 2004). Moreover, *STL1*, encoding a glycerol-proton symporter, is upregulated upon osmostress. Stl1 could potentially influence internal glycerol levels by active uptake from the surrounding medium. It appears though that the symporters' impact on glycerol accumulation in a wild type cell under osmostress is neglectable (Petelenz-Kurdziel E, manuscript in preparation).

3.1.5 Cell cycle block and cross-talk

Osmostress not only leads to accumulation of osmolytes, but also has profound effects on yeast proliferation. Active Hog1 is a negative regulator of cell cycle progression and has to be deactivated to allow cell division. Cell cycle arrest is mediated by activated Hog1 in G₁ through down-regulation of cyclin expression and phosphorylation of the CDK-inhibitor Sic1, and in G₂ through phosphorylation of the protein kinase Hsl1 (Escote et al. 2004; Clotet et al. 2006). The fact that Hog1 can activate check points in different phases of the cell cycle makes sense, since cells can be subjected to osmostress at any time and must be able to adapt before advancing into particularly sensitive stages of division (Clotet et al. 2006). Because of the role of Hog1 in cell cycle arrest, continuous activation of the HOG pathway is lethal. Such activation occurs upon expression of constitutively active forms of Hog1, Pbs2, Ssk2

and Ssk22 or deletion of *SLN1* (Hohmann 2002). Recent findings suggest that prolonged Hog1-activation also leads to induced cell death (Vendrell et al. 2011).

Under hyperosmotic stress a *pbs2Δ* or *hog1Δ* mutant exhibits signalling cross talk to the pheromone and pseudophyphal growth pathway leading to activation of pheromone response genes, cell cycle arrest and morphological aberrations (O'Rourke and Herskowitz 1998). Thus, HOG pathway signalling is also important for maintaining signal fidelity during high osmolarity stress.

3.1.6 Pathway termination

Hog1 is inactivated by de-phosphorylation when the cell has regained some of its volume and turgor pressure as a consequence of glycerol accumulation ((Krantz et al. 2004; Karlgren et al. 2005; Klipp et al. 2005). Efficient HOG pathway downregulation is important, both because a constitutively active Hog1 prevents cells from dividing (as discussed above), and because the cell must be able to respond again upon further increase in osmolarity. Hog1 activity is negatively controlled by protein phosphatases Ptp2, Ptp3 and Ptc1, but it seems that the main role of these phosphatases is to control and counteract pathway activation and reduce noise (while pathway deactivation is closely linked to actual osmotic adaptation) (Jacoby et al. 1997; Wurgler-Murphy et al. 1997; Young et al. 2002; Klipp et al. 2005).

3.1.7 Other stressors that activates the Hog pathway

Hog1 is activated also under other stress conditions, including heat and oxidative stress, acetic acid and arsenite exposure (Singh 2000; Rep et al. 2001; Winkler et al. 2002; Bilslund et al. 2004; Mollapour and Piper 2006; Thorsen et al. 2006). The phosphorylation profile of Hog1 depends on the stress, where hyper-osmotic shock leads to rapid phosphorylation whereas arsenite stress results in a slowly accumulating profile of phospho-Hog1. Arsenite-triggered Hog1-activation does not lead to nuclear accumulation nor Hog1-dependent transcriptional changes, indicating that the targets of Hog1 during arsenite stress are (mainly) cytosolic (Thorsen et al. 2006). How activated Hog1 can discriminate between different stresses, exert diverse outputs and only in some cases accumulate in the nucleus still needs to be determined. One possible scenario is that Hog1 can separate rapid stimulation from slow and accumulating activation, so called kinetic insulation, a mechanism postulated for achieving pathway specificity (Behar et al. 2007).

3.2 Cell Wall Integrity (CWI) pathway

In nature *S. cerevisiae* can be exposed to sudden hypo-osmotic shocks, for example upon rainfall. Rapid inflow of water causes cell swelling, perturbing the osmotic homeostasis and interfering with biochemical reactions by dilution of the cytoplasm. Yeasts, fungi and plant cells are surrounded by a strong yet elastic cell wall that protects from excessive swelling and bursting. Several signalling pathways are involved in the maintenance and regulation of the cell wall, among which the MAPK cell wall integrity (CWI) pathway plays a central role (Davenport et al. 1995; Kamada et al. 1995).

3.2.1 The yeast cell wall

The cell wall of *S. cerevisiae* consists of an inner layer of mainly glucan polymers and a small amount of chitin, and an outer layer made up of mostly glycosylated mannoproteins (Kapteyn et al. 1999; Smits et al. 1999). The inner layer is central for the mechanical strength and elasticity of the cell wall, while the outer layer plays a protective role against wall-degrading enzymes such as lyticase (Smits et al. 1999).

The yeast cell has a lower water potential than the surrounding medium also during normal growth conditions, and the cell wall is crucial for establishing a turgor pressure that counteracts water inflow and prevents the cell from bursting (Levin 2011). Yeast cells deficient of cell walls and mutants with weakened cell walls can be prevented from bursting by addition of an external osmotic stabilizer such as sorbitol. Further, the cell wall is required for yeast to maintain cell shape, as well as to establish new cell forms during growth (budding), mating (shmoo and zygote formation) and filamentation during pseudohyphal development (Levin 2011). During growth and in response to environmental stresses that activates the CWI pathway, the cell wall composition is optimized to stand tall against the given challenge (Cid et al. 1995; Smits et al. 1999).

3.2.2 CWI pathway architecture

Five different sensors have been identified to transmit signals from the cell surface to the components of the cell wall integrity pathway, namely Wsc1-3, Mid2 and Mtl1 (Verna et al. 1997; Ketela et al. 1999; Rajavel et al. 1999). These sensors are all transmembrane mucin proteins, localised to the plasma membrane and share structural features with a small C-terminal cytoplasmic domains, a single

transmembrane domain and a periplasmic domain rich in highly O-mannosylated Ser/Thr residues (Levin 2011). Wsc1 and Mid2 are the most important of these sensors, and a *wsc1* Δ *mid2* Δ double mutant requires an osmotic stabilizer to inhibit lysis (Rajavel et al. 1999). Deletion of *WSC1* leads to cell lysis at high temperature and confers difficulties to cope with hypo-osmotic shock, whereas *mid2* Δ mutants fail to survive pheromone treatment (Ono et al. 1994; Gray et al. 1997; Gualtieri et al. 2004). Overexpression of *MID2* can rescue lethality of *wsc1* mutants and vice versa, suggesting that the two sensors have partially overlapping roles (Ketela et al. 1999). The cytoplasmic domains of Wsc1 and Mid2 interact with Rom1/2, GDP/GTP exchange factors (GEFs) that activates the small G-protein Rho1 (Philip and Levin 2001). Rho1 is considered as the master regulator of CWI signalling. In the GTP bound state, Rho1 activates Pkc1 and triggers activation of the CWI MAPK cascade: the MAPKKK Bck1, the MAPKKs Mkk1 and Mkk2 and the MAPK Mpk1/Slt2 (Fig. 3) (Levin et al. 1990; Lee and Levin 1992; Irie et al. 1993; Lee et al. 1993; Martin et al. 1993). Cells lacking *PCK1* require osmotic stabilization (1M sorbitol) for growth at room temperature, whereas deletion of components in the MAPK cascade (*BCK1*, *MKK1* and *MKK2*, or *SLT2*) results in sorbitol-remediable cell lysis at elevated temperatures, which is a weaker phenotype (Lee and Levin 1992; Levin and Bartlett-Heubusch 1992; Levin 2005). The fact that *PKC1* deletion results in a more severe phenotype than the MAPK cascade elements indicates that Pkc1 activates also other cell wall-related pathways (Lee and Levin 1992).

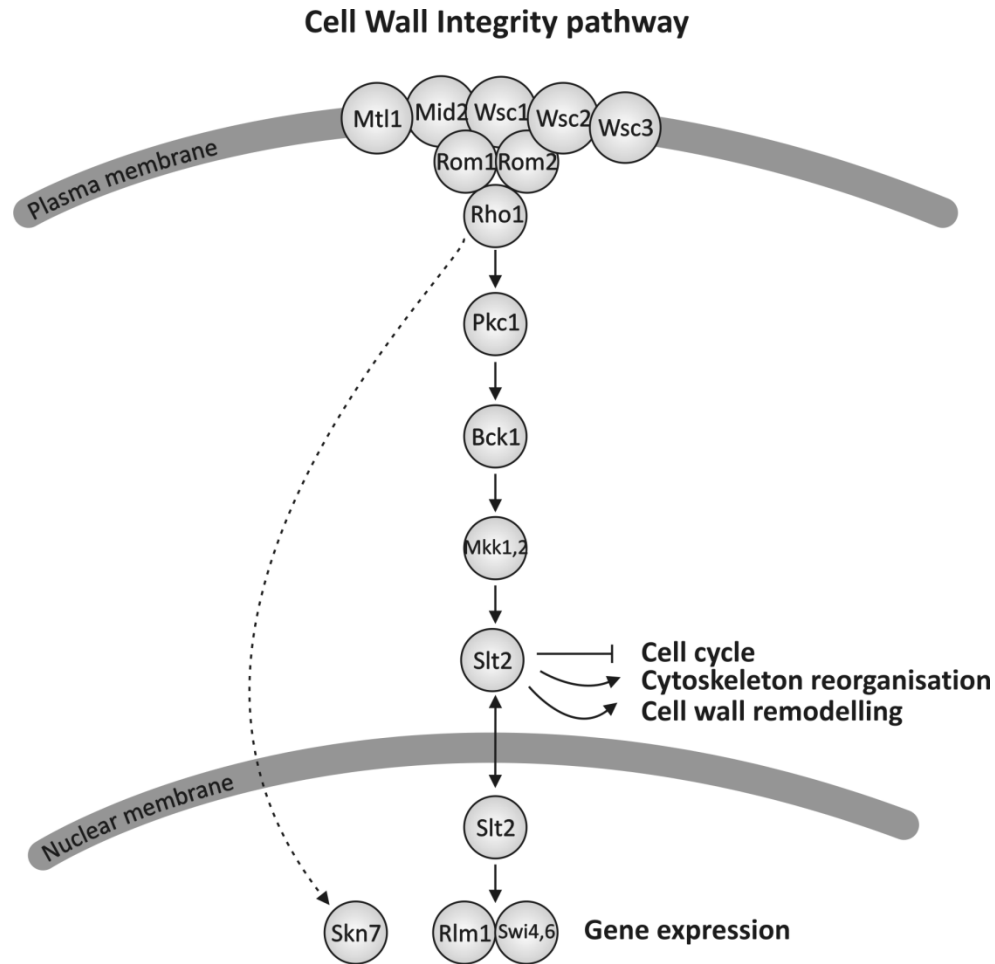


Fig. 3. Overview of the yeast CWI pathway. Membrane tension is sensed through the cell surface sensors Mid2, Mtl1 and Wsc1-3, of which Mid2 and Wsc1 are the most important. The sensors activate Rho1 and the downstream Pkc1-activated MAPK cascade: MAPKKK Bck1, MAPKK Mkk1 and Mkk2 and MAPK Slit2. Slit2 has nuclear targets including transcription factors Rlm1 and Swi4/6 and possibly also Skn7 (dashed arrow), and cytosolic targets that influence cytoskeleton reorganisation, cell cycle and cell wall remodelling. Courtesy of Professor Stefan Hohmann, CMB, University of Gothenburg.

3.2.3 Activation of CWI pathway

CWI signalling activity can be determined by monitoring the activation of Slit2 using antibodies that identify the dually phosphorylated form of Slit2 (de Nobel et al. 2000).

Another way is to indirectly measure CWI activity via *lacZ* reporter genes driven by the Rlm1 transcription factor that is under the control of CWI (Jung et al. 2002). Many different stresses affect the cell wall and consequently activate the CWI pathway:

3.2.3.1 Hypo-osmotic shock

Cells exposed to a hypo-osmotic shock experience swelling, cell wall extension, membrane stretching and transient depolarization of the actin cytoskeleton. To lower the internal turgor pressure and prevent bursting, cells release excessive compatible solutes through the aquaglyceroporin Fps1 (Davenport et al. 1995; Tamas et al. 1999; Gualtieri et al. 2004). The sudden decrease in external osmolarity induces a very rapid but transient activation of Slt2 (Davenport et al. 1995; Kamada et al. 1995). Besides CWI activation, hypo-osmotic shock is known to activate the Sln1 branch that in turn inhibits the HOG pathway and simultaneously stimulates the Skn7 transcription factor (Li et al. 1998; Hohmann 2002; Reiser et al. 2003). The role of the Sln1-Skn7 pathway is not well understood, and cells lacking or overexpressing *SKN7* are not sensitive to hyper- or hypo-osmotic stress (Brown et al. 1994; Ketela et al. 1998). Synthetic lethality of *skn7Δ slt2Δ* suggests that the Sln1-Skn7 and CWI pathways somehow work in parallel to control cell wall composition and integrity, whereas other studies suggest that Skn7 is (at least partly) under the control of CWI pathway (Brown et al. 1994; Ketela et al. 1998; Ketela et al. 1999).

3.2.3.2 Cell cycle and heat shock

The CWI pathway is periodically regulated throughout the cell cycle. Cell growth is most highly polarized at the time of bud emergence, and cells experience heavy cell wall stress with a consequential peak in CWI activation at this time point (Cid et al. 1995). Growth at elevated temperatures (37-39°C) also causes stress on the cell wall which results in persistent activation of the CWI pathway, and null mutants in many of the CWI pathway components lyse with a small bud when cultivated at high temperature (Kamada et al. 1995). High temperature results in a slow increase in Slt2 activation (compared to the rapid Slt2 activation upon hypo-osmotic shock), suggesting that the CWI pathway is not sensing heat directly but rather respond to a secondary effect (Kamada et al. 1995; Beese et al. 2009). It has been shown that a rise in temperature results in elevated levels of both glycerol and trehalose (Singer and Lindquist 1998; Siderius et al. 2000). These molecules are required during heat stress, as mutants that cannot produce glycerol or trehalose are more temperature sensitive

than wild type cells (Singer and Lindquist 1998; Siderius et al. 2000; Mensonides et al. 2005). Increased glycerol and trehalose levels upon heat shock lead to increased turgor pressure in cells, and it is likely the increased turgor that activates the CWI pathway. Accordingly, prevention of trehalose production releases turgor pressure and diminishes CWI activation (Mensonides et al. 2005).

3.2.3.3 Harmful substances

Agents that affect cell wall biogenesis and function include caffeine, lyticase, zymolyase, Congo red and calcofluor white (Ketela et al. 1999; de Nobel et al. 2000; Martin et al. 2000; Levin 2011). Rapamycin, which depolarizes the actin cytoskeleton, also activates the CWI pathway (Torres et al. 2002). Moreover, CWI signalling is important for modulating the response to arsenite and arsenate (Matia-Gonzalez and Rodriguez-Gabriel 2011) (Ahmadpour D, manuscript in preparation), which will be further discussed in the Fps1 section of this thesis.

3.2.3.4 Plasma membrane stretch

Plasma membrane stretch is likely the underlying physical stress that activates the (plasma membrane) sensors of the CWI pathway (Kamada et al. 1995; Mensonides et al. 2005; Levin 2011). Membrane stretch can be imagined under heat stress and hypo-osmotic shock (increased turgor pressure), upon weakening of the cell wall by stress agents and upon morphological changes during cell cycle and conjugation (Errede et al. 1995). In addition, mutants defective in cell wall biogenesis display high level of Slt2-phosphorylation (de Nobel et al. 2000). The spherical and swollen state of these mutants, which is due to the cell wall defects, may create a stress condition that mimics that of a hypo-osmotic stress (Ram et al. 1998).

3.2.4 Effectors downstream of CWI pathway

CWI activation ensures that the cell wall is remodelled and/or repaired by regulating both nuclear and cytoplasmic targets (Fig. 3) (Levin 2005). These changes include, but are not limited to, β -glucan synthesis at the site of wall remodelling, elevated levels of chitin, increase in a number of cell wall proteins and changes in how the cell wall polymers are connected with each other (Delley and Hall 1999; Kapteyn et al. 1999).

Microarray analyses of the transcriptional response after treatment with cell wall-perturbing agents Zymolyase and Congo Red reveal that a large fraction of the upregulated genes encode proteins for cell wall remodelling, and the majority of these genes are controlled by Slt2 and the transcription factor Rlm1 (Garcia et al. 2004). Other transcription factors involved in cell wall stress response include Swi4, Swi6, Msn2 and the redundant Msn4, Hsf1 and Skn7 (Li et al. 1998; Jung and Levin 1999; Garcia et al. 2004). Genome-wide analyses of mutants with cell wall defects identified ~80 common upregulated genes, a list that largely overlaps with the genes induced by the agents described above (Boorsma et al. 2004). Cytoplasmic roles of CWI include regulation of the actin cytoskeleton and stress-induced relocalisation of chitin synthase III (Chs3p), which is required for synthesis of chitin to strengthen the cell wall (Delley and Hall 1999; Valdivia and Schekman 2003). In addition, a cell wall integrity checkpoint has been identified, which ensures completion of cell wall remodelling before mitosis (Suzuki et al. 2004).

3.3 Glycerol concentrations determined by interplay between HOG and CWI pathways?

An increase in glycerol concentration is crucial for adaptation to high osmolarity conditions. A hypo-osmotic shock, on the other hand, leads to rapid glycerol efflux to prevent cells from bursting. As mentioned briefly above, the protein responsible for releasing glycerol in *S. cerevisiae* is the glycerol-transporting aquaglyceroporin Fps1. HOG and CWI pathways controls Fps1 activity upon arsenite stress (Maciaszczyk-Dziubinska et al. 2010) (Ahmadpour D, manuscript in preparation), and it is possible that both pathways, directly or indirectly, also regulate glycerol flux through Fps1 during basal conditions and upon osmostress. Fps1 function has been studied in our lab over many years, and the next section will summarize the field of aquaporins and Fps1 research.

4 Aquaporins

4.1 The family of aquaporins

Water diffusion through the membrane lipid bilayer alone cannot explain the fast water movement observed in certain cells, such as mammalian red blood cells and renal tubules. For that reason, aquaporins were predicted to exist long before their discovery in the early 1990's. The first aquaporin discovered was human aquaporin (AQP) 1, and in 2003 Peter Agre was awarded the Nobel Prize in Chemistry for the discovery of aquaporins and the elucidation of their structures and functions (Agre et al. 1987; Preston and Agre 1991). Aquaporins, also called Major Intrinsic Proteins (MIPs), are commonly divided into two sub-groups; orthodox aquaporins and aquaglyceroporins. Orthodox aquaporins allow water to freely pass biological membranes while preventing the passage of ions and other solutes. Aquaglyceroporins also, or preferably, mediate facilitated diffusion of small solutes such as glycerol and other polyols, urea and arsenite (Park and Saier 1996; Borgnia et al. 1999; Wysocki et al. 2001). Aquaporin activity can be regulated by pH changes, phosphorylation, trafficking and mechanosensitive gating (Thorsen et al. 2006; Tornroth-Horsefield et al. 2010).

4.1.1 Structure of aquaporins

Aquaporins are typically small (25-34 kDa), hydrophobic integral membrane proteins composed of four identical subunits, with each monomer acting as a water or solute channel. Each subunit consists of six transmembrane α -helices connected with five loops, and amino and carboxyl termini facing the cytosol. Loop B and loop E are hydrophobic and fold into the pore, forming a seventh pseudo-transmembrane helix (Fig. 4). Loop B and E also contain the highly conserved Asn-Pro-Ala (NPA) motif, which overlap at the centre of the channel to form a size-exclusion filter (de Groot and Grubmuller 2001; Gonen and Walz 2006). Water molecules travel through the pore of the channel in a single file, and the orientation of the water molecules ensures that only water passes the pore and that passage of protons in form of H_3O^+ is prevented. The ar/R (aromatic/arginine) constriction site of orthodox aquaporins supports the passage of water molecules while excluding other substrates. In aquaglyceroporins, this constriction site is larger in diameter, enabling also glycerol and other larger molecules to permeate the pore (Savage et al. 2003; Gonen and Walz 2006).

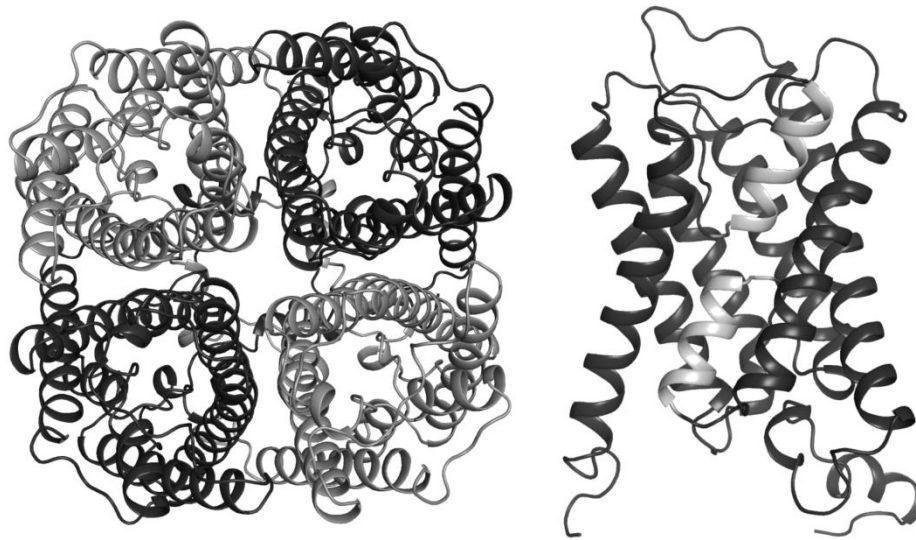


Fig. 4. Overview of the structure of aquaporins. Aquaporins are tetramers composed of four identical subunits, with each monomer acting as a water or solute channel. Each subunit consists of six transmembrane α -helices connected with five loops, and both the amino and carboxyl termini are facing the cytosol. Loop B and loop E fold into the pore, forming a seventh pseudo-transmembrane helix. Courtesy of Dr. Urszula Eriksson, CMB, University of Gothenburg.

4.1.2 Aquaporins in all kingdoms of life

Aquaporins are present in most organisms, ranging from bacteria to human. There are organisms completely lacking aquaporins, and hence aquaporins are not a prerequisite for life (Tanghe et al. 2006). Nevertheless, deletion of aquaporins causes phenotypes in many organisms. One example is the rodent parasite *Plasmodium berghei*, a close relative to the malaria parasite *Plasmodium falciparum*. Deletion of the *P. berghei*'s only aquaporin PbAQP, a transporter of both water and glycerol, severely affects growth of the parasite and increases survival of infected mice compared to mice carrying wild-type parasites (Promeneur et al. 2007).

Higher organisms typically contain multiple aquaporins. Humans possess thirteen aquaporins that differ with respect to localization, expression pattern as well as in their regulation and selectivity. AQP 0, 1, 2, 4, 5, 6 and 8 are orthodox aquaporins, AQP 3, 7, 9, 10 belong to the aquaglyceroporin group, while AQP11 and 12 belong to a new subfamily called supraaquaporins (King et al. 2004; Gonen and Walz 2006;

Yakata et al. 2007; Carbrej and Agre 2009; Ishibashi 2009). The physiological roles of the human aquaporins are diverse. For example, AQP0 serves both as a water channel and a structural protein in the eye lens (Harries et al. 2004). AQP1 and AQP2 are expressed in the kidney and play critical roles in urine concentration, while AQP4 is the most important aquaporin of the brain and spinal cord (Jung et al. 1994; Takata et al. 2004). AQP7 transports glycerol produced in adipocytes into the blood stream and AQP9 mediates uptake of glycerol into the liver (Hara-Chikuma and Verkman 2006). Dysfunctional regulation and/or activity of aquaporins are involved in several diseases. For example, a mutation in AQP2 causes nephrogenic diabetes insipidus, a condition characterized by excretion of large amounts of diluted urine and consequently body dehydration, whereas mice deficient of AQP7 suffer from severe increase in body fat mass (Deen et al. 1994; Hara-Chikuma and Verkman 2006).

Aquaporin-mediated water transport in plants is important during various processes, such as osmo-adaptation, cell elongation and seed germination (Maurel et al. 2008). Plant genomes encode numerous aquaporins; 35 different AQP genes have been identified in *Arabidopsis thaliana* (Alexandersson et al. 2005). The plant aquaporins are divided into four subgroups based on sequence similarity, namely Plasma membrane Intrinsic Proteins (PIPs), Tonoplast Intrinsic Proteins (TIPs), Nodulin-26 like Intrinsic Proteins (NIPs) and Small basic Intrinsic Proteins (SIPs). Most of the studied plant aquaporins transport water, but a subset of aquaporins also transport small solutes such as glycerol, urea, ammonia and hydrogen peroxide (Maurel et al. 2008). Some plant aquaporins also mediate uptake of metalloids such as arsenite and antimony. Arsenite uptake in food crops is highly unwanted, yet plant accumulation of arsenite is a potential strategy to efficiently remove the metalloid from farmlands (Zhao et al. 2010).

Many, but not all, microbes possess aquaporins. For example, *E. coli* possess one aquaporin (AqpZ), and one aquaglyceroporin (GlpF), whereas gram-positive bacterium *Lactococcus lactis* express one aquaporins with dual specificity for water and glycerol (Borgnia and Agre 2001; Froger et al. 2001). The number of aquaporins in different species of yeasts and filamentous fungi seems to vary a lot; several yeasts possess only one aquaporin whereas others have up to five (Pettersson et al. 2005). *S. cerevisiae* has two aquaglyceroporins (Fps1 and Yfl054) and two orthodox aquaporins (Aqy1 and Aqy2) (Van Aelst et al. 1991; Bonhivers et al. 1998; Laize et al. 2000).

4.2 Yeast aquaglyceroporin Fps1

4.2.1 Discovery of Fps1

The aquaglyceroporin Fps1 in *Saccharomyces cerevisiae* was initially identified as a suppressor of the growth defect of the *fdp1* mutant, and the name Fps1 stands for *fdp1* suppressor (Van Aelst et al. 1991). The *FDP1* gene is now called *TPS1* and encodes trehalose-6-phosphate synthase. Lack of Tps1 results in an imbalance in glycolysis and causes a growth defect on fermentable carbon sources (Banuelos and Fraenkel 1982). The suppressive effect of the plasmid-borne *FPS1* is probably due to *FPS1* overexpression resulting in increased glycerol leakage. This in turn leads to enhanced glycerol production. Overexpression of *GPD1* also suppresses the growth defect of *TPS1* deletion mutant on glucose, showing that enhanced glycerol production indeed rescues the *tps1* Δ mutant from glycolytic deregulation (Luyten et al. 1995).

4.2.2 Fps1 is important for osmotic homeostasis

A few years after the discovery of Fps1, the channel protein was demonstrated to mediate glycerol flux over the plasma membrane (Luyten et al. 1995; Sutherland et al. 1997). This was later substantiated by Tamas and co-workers who showed that Fps1 plays a central role in yeast osmoregulation by controlling intracellular glycerol levels (Tamas et al. 1999). Fps1 can mediate passive diffusion of glycerol in both directions over the plasma membrane, yet the main physiological role of Fps1 seems to be to control glycerol homeostasis by releasing excessive intracellular glycerol. The diffusion rate of glycerol through the Fps1 channel is regulated to allow the cell to adjust to changes in external osmolarity. Within seconds after a hyper-osmotic shock, the activity of Fps1 diminishes to allow glycerol to accumulate (Luyten et al. 1995; Tamas et al. 1999). Once the cell has accumulated glycerol and adapted to the high osmolarity condition, or when cells are shifted to hypo-osmotic conditions, Fps1 activity increases again to release glycerol and turgor pressure (Tamas et al. 1999). Cells lacking Fps1 are sensitive to hypo-osmotic shock due to the inability to quickly release turgor pressure to prevent bursting (Tamas et al. 1999). Whereas wild type cells release up to 75% of the glycerol accumulated during growth on high osmolarity within minutes after a transfer to hypo-osmotic conditions, *fps1* Δ cells remain loaded with >75% of the original level of glycerol still 30min after the osmotic shift (Luyten et al. 1995; Tamas et al. 1999).

Fps1 is evidently important also during non-stress conditions for fine-tuning the intracellular glycerol concentration, as cells lacking Fps1 contain up to twelve times as much glycerol as wild type cells. Glycerol overload in *fps1Δ* cells results in reduced fitness and poor recovery from stationary phase (Tamas et al. 1999; Beese et al. 2009). The necessity of a cell to fine-tune its glycerol levels is even more evident during anaerobic growth, where intracellular glycerol levels are high and glycerol metabolism is strictly required as a redox sink for excess cytosolic NADH (Ansell et al. 1997). A mutant lacking Fps1 accumulates high amounts of glycerol under these conditions, and grows much slower than wild type, probably due to inappropriate osmotic homeostasis (Tamas et al. 1999). Moreover, deletion of *FPS1* confers temperature sensitivity (Beese et al. 2009). Increased temperatures lead to higher intracellular glycerol levels, and this phenotype is probably also a result of intracellular glycerol overload (Fig. 5) (Siderius et al. 2000; Beese et al. 2009).

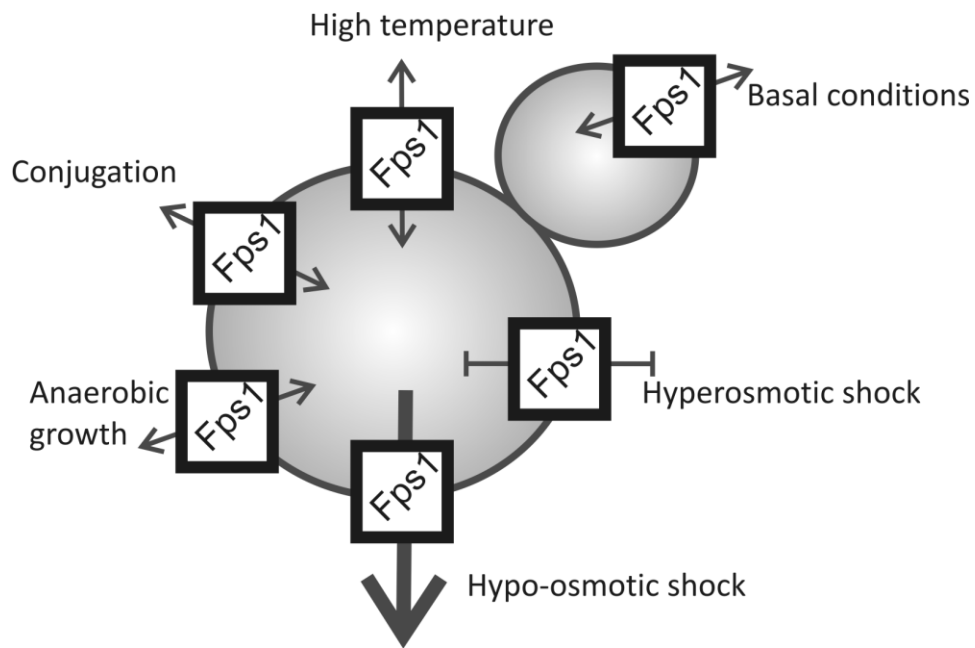


Fig. 5. Glycerol flux through Fps1. Fps1 is an important regulator of the intracellular glycerol concentration during basal conditions and upon osmotic stress and high temperature.

4.2.3 Deletion of Fps1 affects the composition of the cell wall and the plasma membrane

Since cells lacking Fps1 hyper-accumulate glycerol, they are constantly under high turgor pressure and cell wall stress. The cell compensates for this by building fortified cell walls. Consequently, cells lacking Fps1 display decreased zymolyase (cell wall degrading enzyme) sensitivity and calcofluor white hypersensitivity (Beese et al. 2009) (Andersson M, Geijer C unpublished observations). Calcofluor white binds to chitin in the cell wall, thereby interfering with the assembly of growing walls. The higher the chitin content of the wall, the more sensitive the cells become to calcofluor (Elorza et al. 1983; Ketela et al. 1999). CWI signalling is elevated in *fps1* Δ cells, most probably due to the increase in turgor pressure in this mutant (Tamas et al. 1999; Mollapour et al. 2009). An *fps1* Δ *slt2* Δ double mutant displays a synthetically lethal phenotype, suggesting that increased turgor pressure together with weakened cell walls is a detrimental combination (Philips and Herskowitz 1997; Tamas et al. 1999). Similarly, deletion of *FPS1* in combination with deletion of other genes involved in cell wall maintenance, for example *CHS1*, *FKS1*, *SKT5* and *SMI1*, also result in synthetic lethality (SGD). In addition, deletion of *FPS1* causes altered lipid composition of the plasma membrane, which can influence the permeability of glycerol through the membrane (Sutherland et al. 1997; Toh et al. 2001). However, the impact of these changes on glycerol diffusion and cell physiology is not well understood.

4.2.4 Fps1 is important during mating

The ratio between intra- and extracellular glycerol has proved important also upon local degradation of the cell wall during mating between haploid cells of opposite mating type. The process of mating is an ordered set of events and controlled by two MAPK pathways; the mating pheromone pathway and the cell wall integrity pathway (during polarized projection formation) (Buehrer and Errede 1997). In response to mating pheromone cells arrest in G₁ and cell-cell contact is promoted by morphological changes, so called shmoo formation. The cell walls become irreversibly attached to each other, followed by a thinning of the walls beginning in the centre of the region and proceeding outwards. The plasma membranes then fuse to allow the cytoplasm to mix and nuclei to fuse, forming a diploid zygote that can resume vegetative growth. Thinning of the cell wall is a quick process in wild type cells, but does not occur in mutants defective in cell wall fusion. Instead, these mutants adhere to each other as prezygotes with the cell walls still intact, shaped like dumbbells (Trueheart et al. 1987). Deletion of *FPS1* substantially aggravates the mild

cell wall fusion defect of *fus1Δ fus2Δ* mutants (Philips and Herskowitz 1997). Fus1 and Fus2 localize to the growing tip of mating projections (Trueheart et al. 1987; Nelson et al. 2004). Fus1 physically interacts with both Pkc1 and Sho1 and has been suggested to play an important role in the coordinated regulation of multiple kinase pathways (Nelson et al. 2004). Deletion of *GPD1* results in lower intracellular glycerol concentrations and suppresses the *fps1Δ* mating problem, indicating that the fusion defect of *fps1Δ* results from the inability to control the osmotic balance between the two mating partners (Philips and Herskowitz 1997). Further, cells expressing a hyperactive Pkc1 variant show fusion defects similar to that of *fps1Δ* mutants. This suggests that activated Pkc1 can inhibit cell fusion upon osmotic disequilibrium (Philips and Herskowitz 1997). If the Pkc1-mediated inhibition holds true, a plausible mechanism for the effect of *FPS1* deletion on mating would be that *fps1Δ* cells overloaded with glycerol halt cell wall fusion at this CWI controlled checkpoint.

4.2.5 Unregulated Fps1 mutants confer severe osmo-sensitivity

Following a hyperosmotic shock, flux through Fps1 is rapidly decreased to ensure retention and accumulation of the glycerol produced by the yeast cell (Luyten et al. 1995; Tamas et al. 1999; Hohmann 2002). The importance of regulated flux through aquaglyceroporins in yeast during osmostress is evident from numerous Fps1 mutant variants unable to decrease glycerol transport, conferring cells inability to efficiently accumulate glycerol and adapt to high osmolarity conditions (Fig. 6) (Tamas et al. 1999).

Whereas most aquaporins and aquaglyceroporins are 250-280 amino acids long, Fps1 consists of 669 amino acids. The size difference is due to long N- and C-terminal extensions, ~250 and ~150 amino acids, respectively. Truncation analysis showed that most, but not all, of the N-terminus appears to be dispensable for regulation and function (Tamas et al. 1999; Tamas et al. 2003). Mutational analysis of the N-terminus has shown that a regulatory domain consisting of twelve amino acids (225LYQNPQTPTVLP236) close to the first transmembrane (TM) domain is crucial for channel regulation. Deletion of or mutations within this domain cause unregulated glycerol flux through the channel and sensitivity to hyperosmotic stress (Fig. 6). Not only the amino acid sequence of the regulatory domain, but also the position of the domain is important; both an increase and decrease in the distance between TM1 and the regulatory domain result in osmo-sensitivity (Tamas et al. 2003). Sequence alignment of Fps1 from *S. cerevisiae* and Fps1-like proteins from other yeasts revealed a highly conserved 32 amino acid stretch (Fps1 amino acids

218-249 comprising the twelve amino acids mentioned above), suggesting that this region is important for channel regulation (Pettersson et al. 2005).

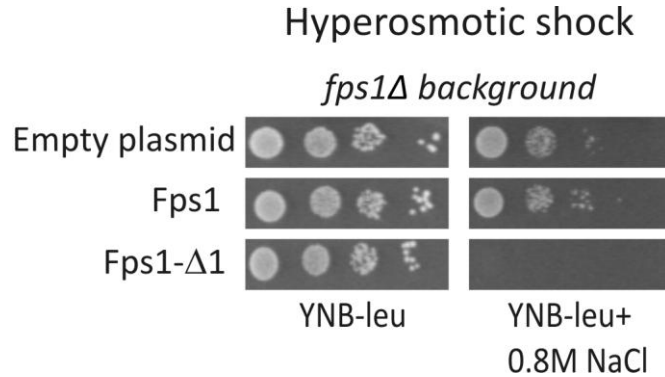


Fig. 6. Growth phenotypes of cells expressing Fps1, unregulated Fps1 and empty plasmid. Serial dilution drop tests of *fps1Δ* cells pregrown in YNB medium and spotted onto plates with NaCl (hyperosmotic shock) and without NaCl (control). Cells expressing the unregulated Fps1-Δ1 mutant (deleted of the N-termini (Tamas et al. 1999)) display poor growth on salt plates due to glycerol leakage. Courtesy of Doryaneh Ahmadpour, CMB, University of Gothenburg.

Like in the case for the N-terminal extension, parts of the C-terminus can be removed without causing apparent phenotypes. However, twelve amino acids (535HESPVNWSLPVY546) close to the sixth transmembrane domain are important for Fps1 regulation (Hedfalk et al. 2004; Pettersson et al. 2005). Screens for mutations causing unregulated Fps1 revealed additional important residues close to TM1 and in the B loop. All these mutations face the cytosolic side of the protein (Karlgrén et al. 2004). In opposite, a screen for intragenic suppressor mutations that suppress unregulated Fps1 activity of a mutant lacking the N-terminal regulatory domain identified four residues on the extracellular side of the protein (Fig. 7). Cells expressing these *FPS1* alleles survive osmotic up and down shocks, but the glycerol efflux rate is diminished, suggesting that the basal transport capacity is affected (Tamas et al. 2003).

Fps1 topology map

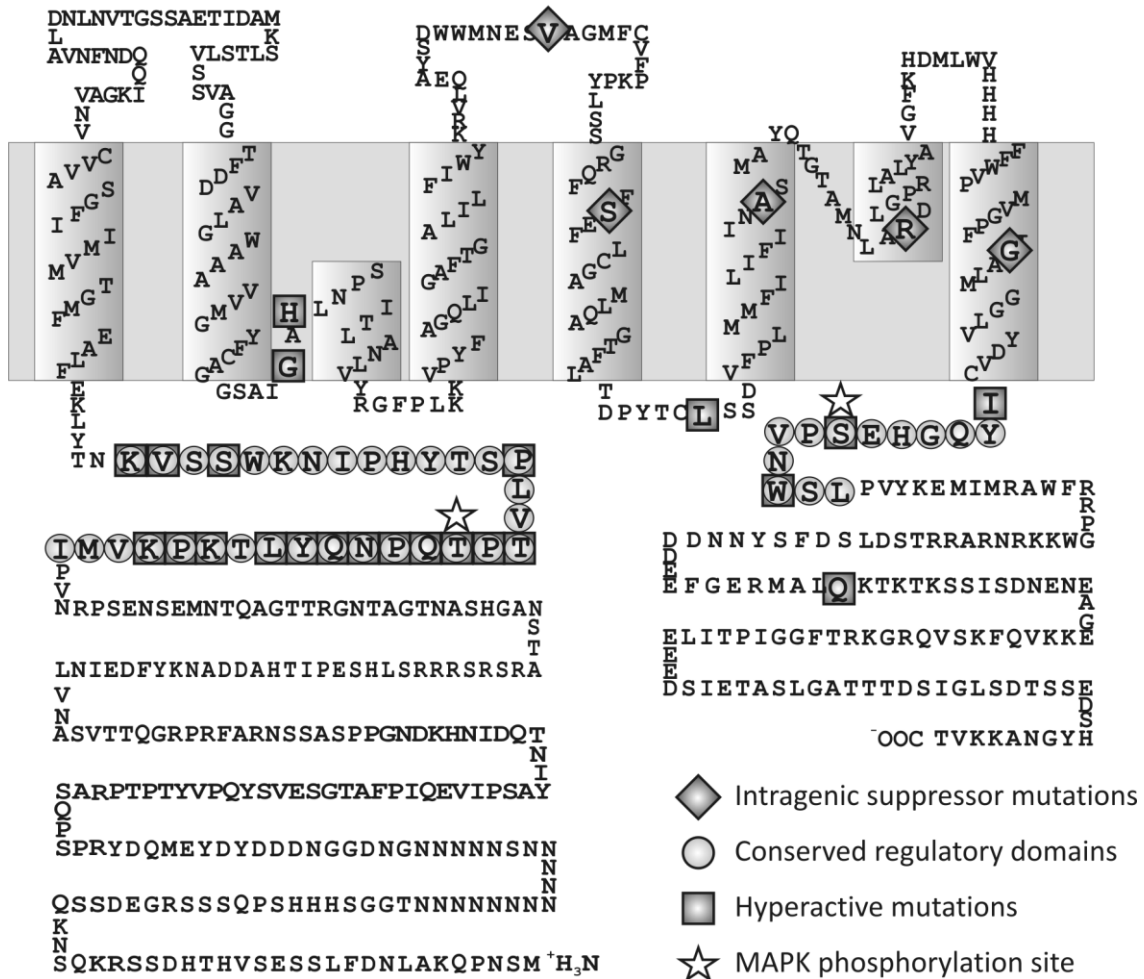


Fig. 7. Topology map of Fps1. Aquaglyceroporin Fps1 has unusually long hydrophilic N- and C-terminal extensions. Both termini contain domains that are crucial for regulating flux through the channel. Important amino acids identified in screens for unregulated channels, intrinsic suppressor mutations, MAPK phosphorylation sites and the conserved regulatory domains are highlighted. Model revised by courtesy of Dr. Kristina Hedfalk and Mikael Andersson, CMB, University of Gothenburg.

The glycerol transport rate through unregulated Fps1 mutants is higher than through wild type Fps1, and thus, these Fps1 mutants are said to be hyperactive in comparison to Fps1. During conditions of high osmolarity, the activity of both Fps1 and hyperactive Fps1 mutants seems to be downregulated compared to basal

conditions (Tamas et al. 1999; Tamas et al. 2003; Karlgren et al. 2004) (Fig. 8). It is possible that such mutations cause hyperactive channels by affecting the basal transport capacity, but that the activity of these channels is still (at least somewhat) regulated. The data might also be interpreted such that cell shrinking reduces the capacity for uptake (Karlgren et al. 2004). Further research is needed to elucidate the exact regulatory mechanisms of Fps1 during osmotic stress.

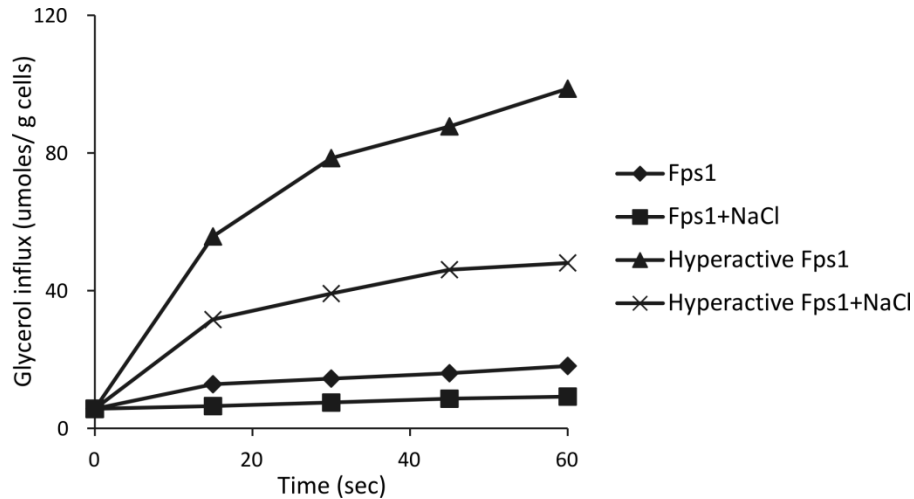


Fig. 8. Uptake profiles of 100mM radiolabelled glycerol. Influx of glycerol into yeast *fps1* cells expressing different *FPS1* alleles as a measure for Fps1 channel activity, before and after an osmotic shift to 0.8M NaCl. Courtesy of Professor Markus Tamas, CMB, University of Gothenburg.

Different Fps1 point mutations and truncations result in altered expression levels as judged by western blot employing a C-terminal *myc* tag. The different expression levels do not seem to affect the phenotypes observed in functional assays such as serial dilution drop tests, neither for hypo- or hyperosmotic shocks (Tamas et al. 2003; Hedfalk et al. 2004; Karlgren et al. 2004) paper II). This could be explained by altered accessibility to the C-terminally fused *myc* tag in mutant proteins or, rather, that Fps1 usually is present in excess in the plasma membrane and fewer copies are sufficient for full function (Karlgren et al. 2004).

4.2.6 Fps1 transports arsenite and other small molecules

Arsenic is a toxic metalloid found in nature mostly as arsenite (As(III)) or arsenate (As(V)). Tolerance to arsenic in yeast is mediated mainly by Acr2, a cytosolic arsenate reductase that converts arsenate to arsenite, and by the plasma membrane transporter Acr3, which mediates arsenite efflux (Wysocki et al. 1997; Mukhopadhyay and Rosen 1998). Ycf1, an ABC-transporter that transports arsenite and antimonite (Sb(III)) into the vacuole when in complex with glutathione, is also important for de-toxification (Ghosh et al. 1999). Arsenate enters cells through inorganic phosphate (Pi) transporters, whereas arsenite crosses the plasma membrane through hexose permeases and Fps1 (Bun-ya et al. 1996; Wysocki et al. 2001; Liu et al. 2004). *fps1*Δ mutants survive growth on arsenite-containing plates better than wild type cells, whereas cells expressing hyperactive mutants of Fps1 display increased sensitivity to arsenite (Wysocki et al. 2001). Intriguingly, wild type, *bog1*Δ and *ycf1*Δ cells are all more tolerant to arsenite upon overexpression of *FPS1*, probably because of increased efflux of arsenite through Fps1 in such strains (Maciaszczyk-Dziubinska et al. 2010). It appears that the arsenate-sensitivity of *fps1*Δ cells also can be explained by a role of Fps1 as an efflux channel for arsenite, which is converted from arsenate by action of Acr2 (Maciaszczyk-Dziubinska et al. 2010).

Recently, naturally-occurring fusion proteins consisting of an arsenite-transporting aquaglyceroporin and an arsenate reductase domain were found in actinobacteria *Frankia alni* and *Salinispora tropica* (Wu et al. 2010). In these organisms, arsenate is reduced to arsenite in close vicinity to an arsenite efflux channel. Perhaps one of the long cytoplasmic domains of Fps1 serves as a docking site for Acr2 in *S. cerevisiae*.

Fps1 has also been implicated in the movement of a number of additional molecules across the plasma membrane, including ethanol, acetic acid, urea, metylamine, boron, acrolein, allyl alcohol and small aliphatic amides (Nozawa et al. 2006; Mollapour and Piper 2007; Beitz et al. 2009; Teixeira et al. 2009; Shepherd and Piper 2010).

4.2.7 Fps1 regulators

There are several lines of evidence suggesting that both the HOG and CWI MAPK pathways control Fps1 activity under normal and different types of stress conditions.

4.2.7.1 HOG pathway

Hog1 appears to be associated with the N-terminal extension of Fps1 in unstressed cells (Mollapour and Piper 2007). Under normal conditions the glycerol uptake rate is increased in a *hog1*Δ mutant compared to wild type, suggesting that Fps1 is not as tightly regulated in this mutant (Thorsen et al. 2006). Hence, Fps1 activity seems negatively regulated by Hog1 under non-stress conditions (Fig. 9). On the other hand, inactivation of Fps1 during hyperosmotic shock appears to be independent of the HOG pathway, since glycerol transport through Fps1 under salt stress is lower in both wild type and *hog1*Δ cells (Luyten et al. 1995).

On western blots Fps1 appears in two forms displaying different mobility, of which the slower form is phosphorylated Fps1 (Thorsen et al. 2006). It is known that MAP kinases phosphorylate their substrates at TP/SP motifs, and amino acids T231-P232 in the N-terminal regulatory domain of Fps1 is an important site for regulation as mutation of T231 renders the channel hyperactive (Tamas et al. 1999; Tamas et al. 2003; Tanoue and Nishida 2003). The N-terminal domain is phosphorylated by Hog1 *in vitro*, and the T231A mutation abolishes this phosphorylation. The Fps1-T231A mutant lacks the phosphorylated form in Westerns, demonstrating that this threonine is phosphorylated also *in vivo*. Strikingly, phosphorylation of T231 is still present in *hog1*Δ cells, suggesting that Hog1 is not the only kinase responsible for phosphorylating Fps1 (Thorsen et al. 2006). Other mutations in the N-terminal regulatory domain that result in Fps1 hyperactivity also eliminate phosphorylation of Fps1 (Tamas M, unpublished results). It is therefore likely that these mutations cause structural modifications of this domain that prevent phosphorylation on T231, and that lack of this phosphorylation causes hyperactive Fps1.

As mentioned above, the HOG pathway is activated also upon arsenite stress. *hog1*Δ mutants are hypersensitive to arsenite and display increased arsenite uptake (Thorsen et al. 2006). Both these phenotypes are suppressed by deletion of *FPS1*, indicating that Fps1 mediates a higher arsenite transport rate in *hog1*Δ cells. In addition, Fps1 becomes phosphorylated in response to arsenite stress, and the phosphorylation is at least partly Hog1-dependent (Thorsen et al. 2006). Taken together, these data indicate that Fps1 is regulated by the Hog1 kinase under arsenite stress. Further, *hog1*Δ mutants show enhanced Fps1-dependent acetic acid uptake. Following acetic acid addition, Fps1 becomes phosphorylated by the Hog1 kinase. This phosphorylation appears to lead to ubiquitination, removal from the plasma membrane, endocytosis and degradation (Mollapour and Piper 2007). Interestingly, Fps1 is neither ubiquitinated nor degraded when Hog1 is activated by osmotic stress (Mollapour and

Piper 2007), indicating that the regulatory mechanisms differ under different stress conditions.

4.2.7.2 CWI pathway

Activation of the CWI pathway is important for modulating the response to arsenite and arsenate, as cells lacking Slt2 and upstream kinases are hypersensitive to both these toxic compounds (Matia-Gonzalez and Rodriguez-Gabriel 2011) (Ahmadpour D, manuscript in preparation). Unlike in wild type cells, overexpression of Fps1 does not suppress the arsenite sensitivity of *slt2*Δ mutants. Furthermore, Slt2 appears to bind Fps1 in vivo. These results suggest that Slt2 is an activator of Fps1 (Fig. 9) (Ahmadpour D, manuscript in preparation). The Fps1 C-terminal regulatory domain contains a second putative MAPK phosphorylation site: S537. Mutation of S537 to alanine does not affect the cell's ability to adapt to high osmolarity conditions and thus Fps1-S537A behaves as a regulated channel during osmostress (Filipsson C, unpublished results). On the other hand, expression of Fps1-S537A sensitizes cells to arsenite, and it is possible that phosphorylation of S537 is important for efflux of arsenite (Ahmadpour D, manuscript in preparation). As Fps1 is needed for glycerol efflux under hypo-osmotic shock, Slt2 is perhaps a positive regulator of Fps1 activity also under these conditions, although Tamas and co-authors provided evidence that deletion of *SLT2* does not affect glycerol accumulation or the kinetics of glycerol release (Tamas et al. 1999). Further research is needed to clarify these matters.

4.2.7.3 Rgc1 and Rgc2

Two members of the family of pleckstrin homology (PH) domain proteins, named Regulator of Glycerol Channel 1 and 2 (Rgc1, 2), have recently been identified as positive regulators of Fps1 (Yu et al. 2004; Beese et al. 2009). Rgc2 (Ask10) was previously identified as a multi-copy activator of Skn7-dependent transcription, but the connection between Rgc2 and the Sln1-Skn7 and CWI pathways is not thoroughly investigated (Page et al. 1996). The *rgc1*Δ *rgc2*Δ double mutant displays temperature sensitivity and like the *fps1*Δ mutant, the *rgc1*Δ *rgc2*Δ double mutant is under constitutive cell wall stress and has fortified walls. The quadruple mutant *rgc1*Δ *rgc2*Δ *gpd1*Δ *gpd2*Δ which is incapable of producing glycerol can grow at high temperatures, confirming that glycerol overload causes the temperature sensitivity for *rgc1*Δ *rgc2*Δ (Beese et al. 2009). In the *rgc1*Δ *rgc2*Δ double mutant Fps1 is in its closed conformation because this strain is unable to export glycerol following a hypo-osmotic shock. However, *FPS1* overexpression relieves temperature sensitivity,

indicating that the tight regulation of Fps1 is lost upon overexpression. The *RGC1, 2* deletion mutant displays elevated levels of Fps1, perhaps because the cell tries to compensate for the low diffusion rate through Fps1. If *FPS1* is expressed from high copy plasmid the protein levels are even higher (Beese et al. 2009). Hog1 seems to act upstream of Rgc1, 2, and basal phosphorylation of Rgc2 is Hog1-dependent. It is possible that Hog1 negatively regulates Fps1 by inhibiting the positive regulators Rgc1 and Rgc2 (Beese et al. 2009) (Fig. 9).

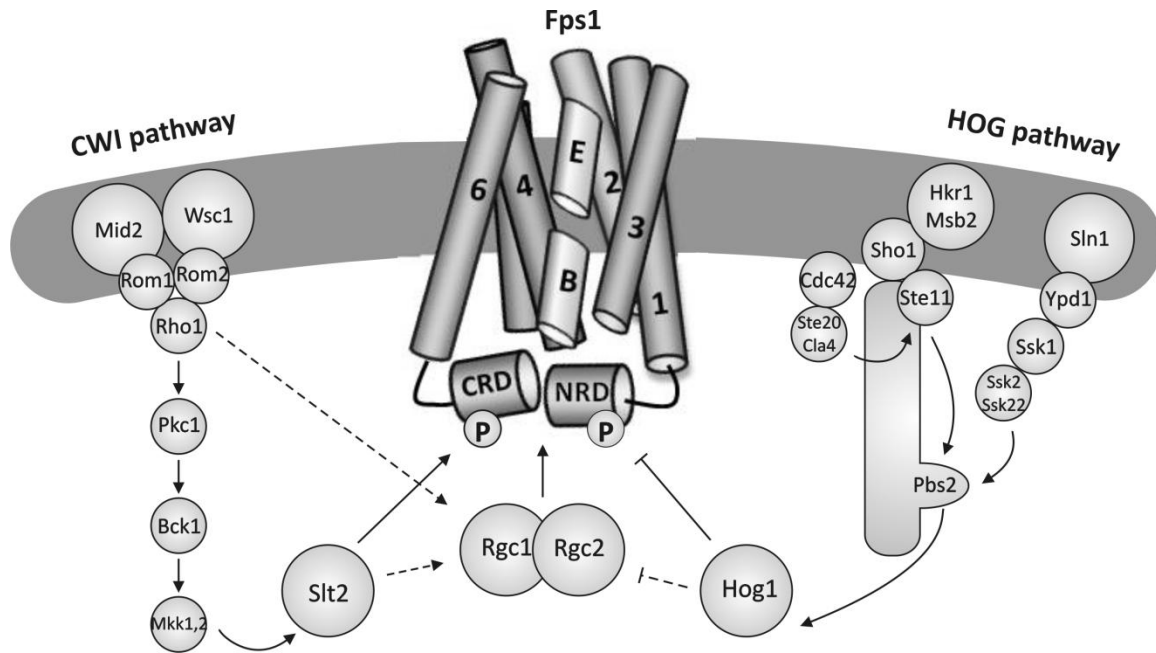


Fig. 9. Fps1 regulation. The activity of Fps1 is negatively regulated by Hog1 during basal conditions and upon arsenite and acetic acid stress. Phosphorylation of amino acid T231 on the N-terminal regulatory domain (NRD) is important for regulated flux through Fps1, and this phosphorylation is at least partly Hog1 dependent. Slf2 is likely an activator of Fps1, and phosphorylation of S537 on the C-terminal regulatory domain (CRD) may be important during arsenite efflux. Rgc1 and Rgc2 are also positive regulators of Fps1. Hog1 may negatively regulate Fps1 by inhibiting Rgc1,2. A genetic screen also links Rho1 and/or Slf2 to Rgc2. Dashed lines indicate potential regulation.

4.2.8 The regulatory mechanisms of Fps1

Although a great deal of effort has been put into elucidating the complex regulation of Fps1, our understanding of the mechanisms is still far from complete. Attempts to over-express, purify and crystallize Fps1 for determination of its three dimensional structure have so far been unsuccessful.

We know that the N- and C-terminal regulatory domains are important for regulation, and that phosphorylation of the N-terminal regulatory domain inhibits flux through Fps1, but exactly how this regulation occurs is not understood ((Tamas et al. 2003; Karlgren et al. 2004; Thorsen et al. 2006; Mollapour and Piper 2007). It has been suggested that both domains can dip into the pore and block the channel (Tamas et al. 2003; Hedfalk et al. 2004). In paper II, we demonstrate the importance of the transmembrane domains of Fps1 for restricting glycerol flux through Fps1, and show that the termini alone are not sufficient for regulating Fps1 activity. In a screen for Fps1 intragenic suppressor mutations, mutation of glycine 519 to serine in the centre of TM6 was found to overrule the hyperactivity caused by a point mutation in the N-terminal regulatory domain (N228A). Cells expressing Fps1-N228AG519S are tolerant to hyper and hypo-osmotic shocks and accumulate glycerol with a rate similar to cells expressing wild type Fps1, suggesting that glycerol flux is restricted in this Fps1 mutant. Based on these results we propose a model of Fps1 regulation where the terminal regulatory domains influence the aquaglyceroporin pore properties such that glycerol flux through the channel is regulated. Small modifications of the N- (or C-) terminal regulatory domains due to changes in phosphorylation status would enable a fine-tuned increase and decrease in glycerol flux as appropriate. Deletion or mutation of the regulatory domains abolishes the proposed termini influence on the pore, causing strongly increased glycerol flux. This two-step mechanism, where phosphorylation of the termini influences helix positioning and pore structure of Fps1, would enable fine-tuning of the glycerol transport capacity, which in turn is important for fitness and survival of the organism in diverse osmotic environments.

A *gpd1Δgpd2Δ* double mutant that is unable to produce glycerol cannot grow at elevated osmolarity caused by salt or by various polyols (glycerol, xylitol, sorbitol etc). In xylitol-containing medium, expression of hyperactive aquaglyceroporins (such as Fps1-N228A) mediates uptake and intracellular accumulation of xylitol as compatible solute (substituting glycerol), thereby allowing growth. Expression of Fps1 wild type protein does not support growth under these conditions (Karlgren et al. 2005). It is possible that hyperactive Fps1 mutants have a wider pore and hence can accommodate larger molecules than the wild type protein, enabling flux of not only glycerol ((HCOH)₃H₂) but also the larger xylitol molecule ((HCOH)₅H₂). Accordingly, most hyperactive Fps1 mutants mediate xylitol uptake in these assays, and one Fps1 mutant, Fps1-Q592stop+S246P, readily transports the even bigger polyol sorbitol ((HCOH)₆H₂) (Karlgren et al. 2004). If this hypothesis holds true, the tighter pore

could also explain the moderate glycerol transport rate of Fps1 compared to hyperactive Fps1 mutants (Tamas et al. 1999).

Rapid osmotic changes result in immediate water influx or efflux. To limit cellular damage during osmo-shocks, regulation of Fps1 activity should ideally be instantaneous. One possible scenario is that Fps1 is regulated by mechanosensitive gating; open upon membrane stretch during hypo-osmotic conditions and closed following hyperosmotic shock when cells lose volume. Regulation by phosphorylation is likely slower than mechanosensitive gating and might be more appropriate for fine-tuning aquaglyceroporin activity under basal conditions and during arsenite and acetic acid stress. Further analysis is needed to determine whether this hypothesis holds true, and if so, how this fits with the observation that Fps1 is closed in *rgc1*Δ *rgc2*Δ mutants.

4.3 Yeast aquaglyceroporin Yfl054

Little has been reported on the second aquaglyceroporin in *S. cerevisiae*, Yfl054, except that Yfl054 seems to mediate glycerol diffusion in the presence of ethanol (Oliveira et al. 2003). Yfl054-like putative proteins exist in many other yeasts, and a conserved stretch of amino acids in the long (~350 amino acids in *S. cerevisiae*) N-terminal extension implies a functionally important domain (Pettersson et al. 2005). Global gene expression studies show that the *YFL054* mRNA is upregulated during entry into stationary phase as well as under the process of sporulation (Gasch et al. 2000) (Pirkov I, unpublished results). The level of the *YFL054* transcript is rapidly down-regulated during germination induced by glucose with or without other nutrients (Pirkov I, unpublished results), suggesting that the gene might be glucose repressed. Most high-throughput studies have so far been performed on glucose medium, which may explain why so few phenotypes have yet been reported for deletion of *YFL054*.

4.4 Yeast orthodox aquaporins Aqy1 and Aqy2

S. cerevisiae possesses two paralogous genes, *AQY1* and *AQY2*, encoding orthodox aquaporins (Bonhivers et al. 1998; Carbrey et al. 2001). Given the small size of microorganisms and their large surface-to-volume ratio, there seems to be no obvious need for aquaporins to enhance water permeability for cellular water homeostasis, as simple diffusion through the plasma membrane should be sufficient (Tanghe et al. 2006). In fact, most vineyard and laboratory strains of *S. cerevisiae* (BY4741, W303-1A etc) contain mutated alleles of both *AQY1* and *AQY2* and the gene products cannot

transport water across the plasma membrane. Wild yeast strains and strains that have undergone less extensive domestication in the laboratory express functional Aqy1 and in many cases also functional Aqy2, indicating that aquaporins confer an advantage in nature but not in a winery or laboratory environment. Several phenotypes conferred by deletion or overexpression of the yeast aquaporins have been reported, yet the physiological roles for the proteins are still not fully elucidated. Overexpression of Aqy1 or Aqy2 confers resistance to repeated cycles of rapid freezing and thawing (Tanghe et al. 2002; Tanghe et al. 2004), paper III). The plasma membrane is probably highly impermeable for water at low temperature, and it is believed that rapid export of water through aquaporins protects cells from detrimental intracellular water crystallization during freezing (Tanghe et al. 2002; Tanghe et al. 2004). In a survey of phenotypic variation in *Saccharomyces* collected from different environments, several freeze tolerant strains were isolated from oak soil in North-eastern United States, while freeze sensitive strains were isolated from warm places and vineyards. It turns out that the freeze tolerant strains possess functional Aqy1 and Aqy2, which explain >90% of the phenotypic variation between freeze tolerant and freeze sensitive strains (Will et al. 2010). On the other hand, cells lacking aquaporins survive better cycles of hypo- and hyperosmotic washes than cells with aquaporins, and loss of both *AQY* genes confer cells a growth advantage in high osmolarity conditions (Bonhivers et al. 1998; Will et al. 2010). It seems that the antagonistic effect of aquaporins activity, beneficial during wintry conditions and damaging during high sugar conditions in wine fermentations, has promoted yeasts from different environments to keep or lose the orthodox aquaporins, respectively (Will et al. 2010).

Yeast cells without orthodox aquaporins have a more hydrophobic cell surface, and both Aqy1 and Aqy2 seem to play a role in “fluffy” or “structured” colony morphology formation (Carbrey et al. 2001; Kuthan et al. 2003; Furukawa et al. 2009). It is difficult to explain how aquaporins can have such effects on cell surface properties and colony morphology. Perhaps they mediate water transport between the cytosol and the cell wall and thereby influences cell surface hydrophobicity, and/or control water flow in yeast colonies, which is critical for nutrient and metabolic waste product transport (Furukawa et al. 2009).

4.4.1 Aqy1 is involved in sporulation

Aqy1 has also been associated with sporulation (Sidoux-Walter et al. 2004; Will et al. 2010). In the SK1 strain, which possesses a functional *AQY1* variant, expression is

strongly upregulated in sporulating diploid cells (Sidoux-Walter et al. 2004). *AQY1* expression is also upregulated in haploid cells starved for essential nutrients and glucose, but the pattern of expression is different from that in diploid sporulating cells. In haploids, *AQY1* is upregulated already after one hour in starvation medium, while diploids show a sharp upregulation coinciding with asci formation eight hours after shift to sporulation medium. Addition of a fermentable carbon source to the starvation medium prevents expression of *AQY1* in both haploid and diploid cells (unpublished observations). It seems that there are at least two layers of regulation: stimulation of expression by glucose starvation and a ploidy specific induction. Interestingly, diploids lacking Aqy1 in the oak soil YPS163 strain background exhibit severe sporulation defects (Will et al. 2010), whereas laboratory strains (including SK1) seem to carry suppressing mutations that make aquaporins less important during sporulation in these backgrounds (Will et al. 2010).

4.4.2 *Pichia pastoris* orthodox aquaporin Aqy1

In paper III we describe that deletion of the orthodox aquaporin gene in the yeast *Pichia pastoris*, *PpAQY1*, confers freezing sensitivity similar to that observed for *S. cerevisiae*. The crystal structure of PpAqy1 reveals that the extended N-terminus of this aquaporin caps the pore. The N-terminus is important for regulating the water flux through the channel, since mutation or deletion of the N-terminal extension confers increased water flux. The extended length and part of the sequence of the N-terminus is conserved among other yeast aquaporins, suggesting that the gating mechanism may be a common characteristic. Structural features, molecular dynamics simulations and functional studies suggest that PpAqy1 could be subjected to mechanosensitive gating. In fact, membrane tension has also been suggested to regulate Aqy1 and Aqy2 in *S. cerevisiae* (Soveral et al. 2008). During rapid temperature or osmolarity changes, mechanosensitive gating would indeed be an effective way to quickly increase or decrease aquaporin activity.

5 Osmostress during sporulation and germination

5.1 Developmental transitions of *S. cerevisiae*

S. cerevisiae is commonly regarded as a single-celled micro-organism and proliferates by a mitotic asymmetric division process called budding. Upon changes in nutrient availability yeast can alter its morphology and growth pattern. For example, nitrogen limitation can induce pseudohyphal growth, where cells remain attached to each other and/or invasive growth, where cells penetrate into the growth substrate. Upon starvation, yeast enters stationary phase where cells do not divide and rest in a G_0 like state. Although the preferred ploidy state of *S. cerevisiae* is diploid, the life cycle of yeast involves both haploid and diploid stages. Stimulated by starvation, diploid cells go through meiosis and gametogenesis followed by sporulation. Haploid spores of mating type **a** and α exposed to a fermentable carbon source and nutrients germinate to resume growth and then mate to form diploids (Fig. 10). Entry into and exit from spore dormancy and osmotic adaptation during these developmental transitions is discussed below.

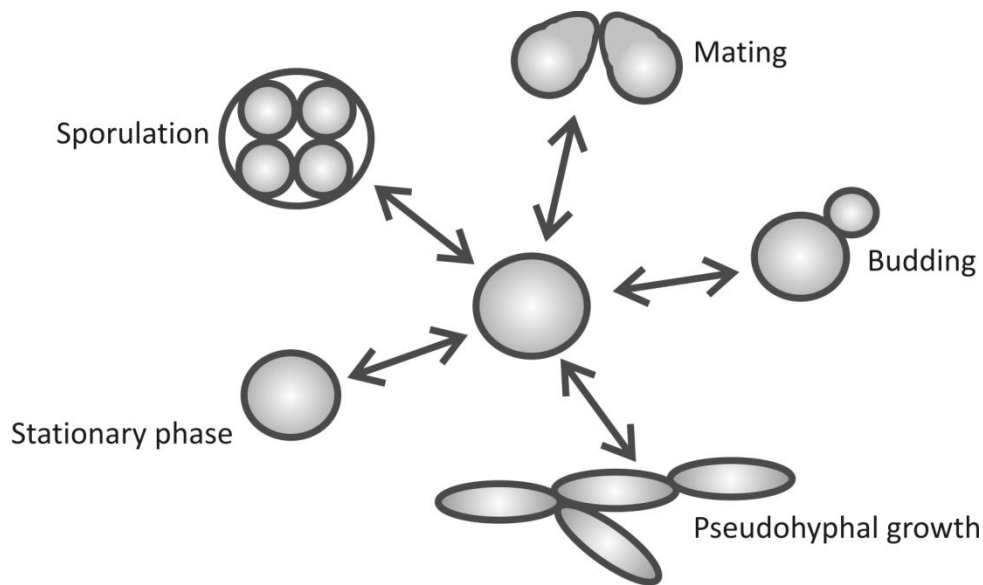


Fig. 10. Morphological transitions in *S. cerevisiae*. In response to changes in nutrient availability, yeast cells adapt their morphology and growth pattern. Developmental decisions can be gradual (pseudohyphal growth) or involve a commitment step at which cells become irreversibly destined to complete the transition (sporulation).

5.2 The process of sporulation

Meiosis and subsequent sporulation of diploid yeast cells is stimulated by absence of a fermentable carbon source combined with nitrogen starvation. As energy is needed for the cell to complete meiosis/sporulation, the presence of a non-fermentable carbon source is required (Neiman 2011). In the laboratory, sporulation is typically stimulated by transferring cells from rich medium to 1% KAc, which contains no nitrogen source and provides acetate as the sole carbon source.

Gametogenesis of yeast consists of two overlapping processes; meiosis and spore morphogenesis. When transferred to sporulation medium, cells exit the mitotic cell cycle at G₁ and enter the meiotic prophase where DNA is synthesised, meiotic recombination occurs and the synaptonemal complex is formed. Subsequently cells go through meiosis I (chromosome segregation) and meiosis II (chromatid separation). Once chromatids have separated, the forespore membranes are formed around each of the four haploid nuclei within the mother cell cytoplasm, protective four-layered spore walls are assembled and finally the spores mature (Neiman 2005). The end product of meiosis, starting from one diploid cell, is an ascus (derived from the mother cell) with four haploid spores, two of mating type **a** and two of mating type *a*.

5.2.1 Regulators of sporulation

The behaviour as haploids or diploids is controlled by regulators encoded by the mating type genes (*MATa* and *MATα*); haploids can undergo mating with a cell of opposite mating type but cannot undergo meiosis while diploid cells cannot mate but have the ability to initiate meiosis and sporulation (Neiman 2011). The sporulation process is characterised by sequential transcription of four sets of genes; early, middle, mid-late, and late (Chu et al. 1998). Most of the products of the early genes are involved in meiotic prophase. The transcription factor Ime1 appears to be the major regulator of this class (Mitchell et al. 1990; Chu et al. 1998; Primig et al. 2000). Another important player is the protein kinase Ime2, which has a role throughout meiosis and has multiple targets (Mitchell et al. 1990). The middle gene products are required for meiotic nuclear division and spore formation, and the transcription factor Ndt80 is highly important for upregulation of these genes (Chu and Herskowitz 1998). The mid-late class genes encode proteins necessary for formation of the outer layer of the spore wall, and the late genes are thought to have a role in spore maturation (Chu et al. 1998). The transcription factors regulating expression of

these two later classes remain to be discovered. It has been reported that expression of mid-late and late sporulation genes is abolished in cells lacking the MAPK Smk1 (Krisak et al. 1994). Smk1 is also believed to be required for controlling the assembly of the spore wall in a temporal order (Krisak et al. 1994; Wagner et al. 1999). In conclusion, although meiosis and spore formation are distinct processes they are tightly intertwined and different check points ensure the correct order of events.

5.2.2 Osmotic homeostasis during sporulation

As described in the section on yeast orthodox aquaporins, Aqy1 is expressed upon sporulation and required in some strain backgrounds for efficient spore formation (Sidoux-Walter et al. 2004; Will et al. 2010). It is not immediately obvious why Aqy1 would be important under the sporulation process since water can diffuse rather freely over the plasma membrane. This said, it should be noted that the water content of spores is far lower than that of vegetative cells. Spores produce large amounts of trehalose and concentrate their cell material during spore formation, which is thought to be important for the high stress tolerance of spores. Aqy1 might facilitate water efflux during spore formation, which could be driven against the water concentration gradient by the pressure imposed by the rigid spore wall (Sidoux-Walter et al. 2004). If cells lacking aquaporins cannot efficiently remove intracellular water, this might impact spore fitness and resistance to harsh environments. The largely uncharacterized aquaglyceroporin Yfl054 seems also to be upregulated in spores (Pirkov I, unpublished results). More work is needed to understand in detail the role of Aqy1 and Yfl054 in spore formation and such work may need the use of non-laboratory strains that require Aqy1 for sporulation (Will et al. 2010).

5.3 The process of germination

Dormant spores in the appropriate environment can remain viable for months and years. Upon availability of water, a fermentable carbon source and nutrients, spores exit dormancy and germinate. This process is described in detail in paper IV.

Briefly, *S. cerevisiae* spore germination is the multi-step developmental route on which dormant spores re-enter the mitotic cell cycle and resume vegetative growth. Germinating spores gradually lose their resistance to heat, chemicals and cell wall degrading enzymes as the outer protective cell walls layers are locally degraded at the site where the first bud later appears (Herman and Rine 1997; Kono et al. 2005). Swelling and elongation of the tightly packed spore is also observed. Protein synthesis

initiates minutes after the onset of germination, closely followed by or in parallel to RNA synthesis (Rousseau and Halvorson 1973). Later, the germinating spore enters the mitotic cell cycle, DNA synthesis begins and the first bud appears (Rousseau and Halvorson 1973; Kono et al. 2005).

5.3.1 Transcriptional response in germination

As described in paper V, germination initiation is accompanied by rapid and massive transcriptional changes with sequential onset of different transcriptional subprograms. The transcriptional response resembles that of cells exiting stationary phase, and reflects the shift to glucose metabolism and glucose repression, resumption of growth and the release from stress during germination (Radonjic et al. 2005). The main trigger for exit from dormancy is a fermentable carbon source, which suffices to induce early germination events such as spore wall degradation and swelling (Herman and Rine 1997). In our hands, germination induced by rich medium with glucose or by glucose alone resulted in qualitatively very similar transcriptional responses, but the response to rich medium was more pronounced. Interestingly, spores germinated in glucose upregulate genes for amino acid biosynthetic processes within the first 30min after germination onset, which strongly suggests that spores can sense and respond to amino acid starvation early on in the process (paper V). From about two hours into germination, the glucose transcriptional response differs significantly from the rich medium-response. This correlates well with the observation that spores cannot enter the mitotic cell cycle without essential nutrients in addition to glucose (Joseph-Strauss et al. 2007).

Little is known about the translational response of germinating spores. However, protein synthesis has been shown to be essential for dormancy exit, as treatment of spores with the protein synthesis inhibitor cycloheximide results in a complete inhibition of germination (Herman and Rine 1997). Determining which mRNAs are translated throughout germination by identifying mRNAs bound to polysomes would most probably be an informative complement to conventional microarray analysis (Warringer et al. 2010).

5.3.2 Osmotic homeostasis during germination

Germination involves different morphological changes including cell wall remodelling, swelling and elongation, suggesting that the CWI pathway is activated during the process. Indeed, an intact actin cytoskeleton is essential for dormancy exit, and

germinating spores that express a constitutively active Rho1 are abnormally elongated (Kono et al. 2005). Late germination events include conjugation of sibling spores of opposite mating type or budding followed by mother-daughter mating which are morphological events where CWI signalling is known to be important (Joseph-Strauss et al. 2007; Levin 2011).

6 Concluding remarks and future perspectives

In this thesis I have investigated how cells respond to osmotic stress, both at the transcriptional level and with respect to the role and regulation of the aquaglyceroporin Fps1.

We (and others) have recognised that an initial adaptation is needed to allow activated Hog1 to accumulate in the nucleus and to initiate a transcriptional response. The reason why Hog1 nuclear accumulation at high stress levels is delayed remains unknown. Different testable explanations exist: Hog1 might be physically trapped in the cytosol as massive water outflow following stress result in jammed molecules. Alternatively, Hog1 shuttles between cytosol and nucleus but does not accumulate, perhaps because transcription factors are released from DNA and consequently cannot anchor Hog1 (Proft and Struhl 2004). One could distinguish between these two scenarios by using fluorescence recovery after photobleaching (FRAP) (Houtsmuller 2005). This technique employs GFP tagged to the protein of interest, where the GFP-molecules are photo-destroyed in the nucleus and subsequent measurement over time of the repopulation of GFP in the nucleus. If the Hog1-GFP signal does not reach the initial level within a given time frame, this suggests that Hog1-GFP is trapped in the cytosol.

The importance of Hog1 cytoplasmic targets is becoming increasingly clear. These targets include proteins for translational regulation, ion export and also glycerol accumulation (Albertyn et al. 1994; Proft and Struhl 2004; Warringer et al. 2010). Decrease of glycerol efflux through Fps1 upon hyperosmotic stress is important for glycerol accumulation, but the role of Hog1 in restricting Fps1 activity during osmostress has been difficult to determine (Luyten et al. 1995). Although Fps1 has been studied for many years, our understanding of the complex regulation of Fps1 is still far from complete. Elucidating the regulatory mechanisms of Fps1 most probably requires determination of the three-dimensional structure of the channel in combination with mutational analysis and growth and transport assays.

Fps1 plays an important role in regulating intracellular glycerol levels during osmostress, mating and high temperature growth, as well as under non-stress conditions. It is likely that the activity of Fps1 is continuously monitored and regulated by different signalling pathways. Screening for proteins that physically

interact with Fps1 is one way to identify these signalling pathways. Using a Split-ubiquitin membrane-based yeast two-hybrid system where interaction between two proteins results in activation of reporter genes (Stagljar et al. 1998), we have identified two proteins of particular interests that physically interact with Fps1; Zeo1 and Mdg1 (Zoltowska S and Geijer C, unpublished results). Zeo1 has been described as a peripheral membrane protein and a physical interactor of Mid2, one of the major sensors of the cell wall integrity pathway. Zeo1 somehow dampens the signal from Mid2, and deletion of *ZEO1* leads to hyper-phosphorylation of Slt2 (Green et al. 2003). Interestingly, double deletion of *FPS1* and *MID2* leads to severe temperature sensitivity with lysis of cells with small buds (indicative for mutants in cell wall integrity pathway) (Andersson M and Geijer C, unpublished observations). It is possible that lack of the Mid2-generated signal results in decreased Slt2 activation and insufficient cell wall fortification at high temperature. If so, cell lysis is the likely consequence of weak cell walls in combination with increased turgor pressure. Intriguingly, additional deletion of *ZEO1* partly rescues the temperature sensitivity of the *fps1Δ mid2Δ* double mutant, suggesting that Zeo1 not exclusively acts on Mid2 (Andersson M and Geijer C, unpublished observations). Mdg1, the second identified interactor of Fps1, is a poorly characterized peripheral membrane protein shown to play a role during mating (Leberer et al. 1996). To elucidate why Fps1 interacts with Zeo1 and Mdg1 will hopefully highlight the role and regulation of Fps1 during mating and cell wall stress.

Other screening methods than the Split ubiquitin system might be more appropriate when studying transient protein interactions between Fps1 and kinases and phosphatases. The lab of Gustav Ammerer has determined physical interaction of both Hog1 and Slt2 with Fps1, using the M-TRACK protein proximity assay (interactions that were missed in the split ubiquitin assay) (unpublished observations). This assay is based on a histone methyl-transferase and its highly specific substrate, the N-terminal fragment of histone 3. Fusing these proteins to proteins of interest (that do interact) permits methylation of histone 3, which can be detected and quantified by western blot. Another potential method is the protein photo cross-linking technique, where certain amino acids of Fps1 can be modified such that they will covalently bind interacting proteins upon light stimulation (Hino et al. 2005). Purification of Fps1 together with the covalently attached interactors would allow identification of these proteins using MS or, if tagged proteins, on a western blot.

In this thesis I have also studied yeast entry into and exit from spore dormancy. While the process of sporulation has been intensely studied over many years we

focused in paper IV and V on germination and the transcriptional response of spores to nutrients and glucose. The cycle of sporulation and germination differs from stationary phase entry and exit in that it encompasses the transition from a diploid mother to haploid spores that mate to form diploids again upon quickening. The mating response with strong upregulation of genes for conjugation is initiated between one and two hours into germination, well before mitotic cell cycle initiation and budding (Joseph-Strauss et al. 2007) (paper V). Curiously, *STE5*, which encodes a pheromone-response scaffold protein important for mating, is upregulated a few minutes into germination (unpublished results), indicating that quickening spores might assemble the MAPK mating pheromone pathway at this stage of the process. Further research is needed to determine if this is in fact the case.

It has proved rather complicated to identify mutants defective in germination, and so far only a handful of proteins have been appointed an essential role in the process (Herman and Rine 1997; Deutschbauer et al. 2002; Kloimwieder and Winston 2011). Germinating spores can mate and become diploids even before the first bud appears, allowing a defective spore close to a neighbouring wild-type spore to be rescued by mating into a heterozygous diploid. This obstacle can be avoided by separating spores using a micro-manipulator. While dissecting spores from heterozygote mutants, we realized that also spores defective in essential genes such as *TAF5*, *TIF5* and *CDC37* (encoding essential proteins for transcription, translation and division, respectively) can germinate and complete several cell cycles before they stop dividing. Wild type spores that lack the *kanMX* marker gene, but that descend from heterozygous diploids carrying *kanMX*, can germinate and form micro-colonies of >100 cells (7 divisions) on geneticin containing plates (unpublished observations). This phenotypic lag observed strongly suggests that dormant spores are packed with mRNA and/or proteins that were synthesised prior to closure of pro-spore membranes during sporulation, and these mRNAs/proteins influence life of the daughter cells for many generations. Not only are heterozygous diploids unsuitable in screens aiming to identify germination mutants, but the phenomena of maternal acting should probably be accounted for also when designing screens for vegetative cells. An alternative, more promising method is to use spores that contain temperature sensitive alleles of genes of interest (Herman and Rine 1997). If germination is abolished at high temperature in such a mutant, this suggests that the gene product is essential for spore dormancy exit.

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8 References

- Agre P, Saboori AM, Asimos A, Smith BL (1987) Purification and partial characterization of the Mr 30,000 integral membrane protein associated with the erythrocyte Rh(D) antigen. *J Biol Chem* 262:17497-17503
- Albertyn J, Hohmann S, Thevelein JM, Prior BA (1994) GPD1, which encodes glycerol-3-phosphate dehydrogenase, is essential for growth under osmotic stress in *Saccharomyces cerevisiae*, and its expression is regulated by the high-osmolarity glycerol response pathway. *Mol Cell Biol* 14:4135-4144
- Alepuz PM, de Nadal E, Zapater M, Ammerer G, Posas F (2003) Osmostress-induced transcription by Hot1 depends on a Hog1-mediated recruitment of the RNA Pol II. *EMBO J* 22:2433-2442
- Alexandersson E, Fraysse L, Sjoval-Larsen S, Gustavsson S, Fellert M, Karlsson M, Johanson U, Kjellbom P (2005) Whole gene family expression and drought stress regulation of aquaporins. *Plant Mol Biol* 59:469-484
- Ansell R, Granath K, Hohmann S, Thevelein JM, Adler L (1997) The two isoenzymes for yeast NAD⁺-dependent glycerol 3-phosphate dehydrogenase encoded by GPD1 and GPD2 have distinct roles in osmoadaptation and redox regulation. *EMBO J* 16:2179-2187
- Banuelos M, Fraenkel DG (1982) *Saccharomyces carlsbergensis* fdp mutant and futile cycling of fructose 6-phosphate. *Mol Cell Biol* 2:921-929
- Beese SE, Negishi T, Levin DE (2009) Identification of positive regulators of the yeast *fps1* glycerol channel. *PLoS Genet* 5:e1000738
- Behar M, Dohlman HG, Elston TC (2007) Kinetic insulation as an effective mechanism for achieving pathway specificity in intracellular signaling networks. *Proc Natl Acad Sci U S A* 104:16146-16151
- Beitz E, Becker D, von Bulow J, Conrad C, Fricke N, Geadkaew A, Krenc D, Song J, Wree D, Wu B (2009) In vitro analysis and modification of aquaporin pore selectivity. *Handb Exp Pharmacol*:77-92
- Bilsland-Marchesan E, Arino J, Saito H, Sunnerhagen P, Posas F (2000) Rck2 kinase is a substrate for the osmotic stress-activated mitogen-activated protein kinase Hog1. *Mol Cell Biol* 20:3887-3895
- Bilsland E, Molin C, Swaminathan S, Ramne A, Sunnerhagen P (2004) Rck1 and Rck2 MAPKAP kinases and the HOG pathway are required for oxidative stress resistance. *Mol Microbiol* 53:1743-1756
- Bonhivers M, Carbrey JM, Gould SJ, Agre P (1998) Aquaporins in *Saccharomyces*. Genetic and functional distinctions between laboratory and wild-type strains. *J Biol Chem* 273:27565-27572
- Boorsma A, de Nobel H, ter Riet B, Bargmann B, Brul S, Hellingwerf KJ, Klis FM (2004) Characterization of the transcriptional response to cell wall stress in *Saccharomyces cerevisiae*. *Yeast* 21:413-427

- Borgnia M, Nielsen S, Engel A, Agre P (1999) Cellular and molecular biology of the aquaporin water channels. *Annu Rev Biochem* 68:425-458
- Borgnia MJ, Agre P (2001) Reconstitution and functional comparison of purified GlpF and AqpZ, the glycerol and water channels from *Escherichia coli*. *Proc Natl Acad Sci U S A* 98:2888-2893
- Brewster JL, de Valoir T, Dwyer ND, Winter E, Gustin MC (1993) An osmosensing signal transduction pathway in yeast. *Science* 259:1760-1763
- Brown JL, Bussey H, Stewart RC (1994) Yeast Skn7p functions in a eukaryotic two-component regulatory pathway. *EMBO J* 13:5186-5194
- Buehrer BM, Errede B (1997) Coordination of the mating and cell integrity mitogen-activated protein kinase pathways in *Saccharomyces cerevisiae*. *Mol Cell Biol* 17:6517-6525
- Bun-ya M, Shikata K, Nakade S, Yompakdee C, Harashima S, Oshima Y (1996) Two new genes, PHO86 and PHO87, involved in inorganic phosphate uptake in *Saccharomyces cerevisiae*. *Curr Genet* 29:344-351
- Carbrey JM, Agre P (2009) Discovery of the aquaporins and development of the field. *Handb Exp Pharmacol*:3-28
- Carbrey JM, Bonhivers M, Boeke JD, Agre P (2001) Aquaporins in *Saccharomyces*: Characterization of a second functional water channel protein. *Proc Natl Acad Sci U S A* 98:1000-1005
- Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES, Young RA (2001) Remodeling of yeast genome expression in response to environmental changes. *Mol Biol Cell* 12:323-337
- Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, Herskowitz I (1998) The transcriptional program of sporulation in budding yeast. *Science* 282:699-705
- Chu S, Herskowitz I (1998) Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. *Mol Cell* 1:685-696
- Cid VJ, Duran A, del Rey F, Snyder MP, Nombela C, Sanchez M (1995) Molecular basis of cell integrity and morphogenesis in *Saccharomyces cerevisiae*. *Microbiol Rev* 59:345-386
- Clotet J, Escote X, Adrover MA, Yaakov G, Gari E, Aldea M, de Nadal E, Posas F (2006) Phosphorylation of Hsl1 by Hog1 leads to a G2 arrest essential for cell survival at high osmolarity. *EMBO J* 25:2338-2346
- Davenport KR, Sohaskey M, Kamada Y, Levin DE, Gustin MC (1995) A second osmosensing signal transduction pathway in yeast. Hypotonic shock activates the PKC1 protein kinase-regulated cell integrity pathway. *J Biol Chem* 270:30157-30161
- de Groot BL, Grubmuller H (2001) Water permeation across biological membranes: mechanism and dynamics of aquaporin-1 and GlpF. *Science* 294:2353-2357
- de Nobel H, Ruiz C, Martin H, Morris W, Brul S, Molina M, Klis FM (2000) Cell wall perturbation in yeast results in dual phosphorylation of the Slr2/Mpk1 MAP kinase and in an Slr2-mediated increase in FKS2-lacZ expression, glucanase resistance and thermotolerance. *Microbiology* 146 (Pt 9):2121-2132
- Deen PM, Verdijk MA, Knoers NV, Wieringa B, Monnens LA, van Os CH, van Oost BA (1994) Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science* 264:92-95

- Delley PA, Hall MN (1999) Cell wall stress depolarizes cell growth via hyperactivation of RHO1. *J Cell Biol* 147:163-174
- Deutschbauer AM, Williams RM, Chu AM, Davis RW (2002) Parallel phenotypic analysis of sporulation and postgermination growth in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 99:15530-15535
- Dihazi H, Kessler R, Eschrich K (2004) High osmolarity glycerol (HOG) pathway-induced phosphorylation and activation of 6-phosphofructo-2-kinase are essential for glycerol accumulation and yeast cell proliferation under hyperosmotic stress. *J Biol Chem* 279:23961-23968
- Elorza MV, Rico H, Sentandreu R (1983) Calcofluor white alters the assembly of chitin fibrils in *Saccharomyces cerevisiae* and *Candida albicans* cells. *J Gen Microbiol* 129:1577-1582
- Errede B, Cade RM, Yashar BM, Kamada Y, Levin DE, Irie K, Matsumoto K (1995) Dynamics and organization of MAP kinase signal pathways. *Mol Reprod Dev* 42:477-485
- Escote X, Zapater M, Clotet J, Posas F (2004) Hog1 mediates cell-cycle arrest in G1 phase by the dual targeting of Sic1. *Nat Cell Biol* 6:997-1002
- Ferrigno P, Posas F, Koepf D, Saito H, Silver PA (1998) Regulated nucleo/cytoplasmic exchange of HOG1 MAPK requires the importin beta homologs NMD5 and XPO1. *EMBO J* 17:5606-5614
- Froger A, Rolland JP, Bron P, Lagree V, Le Caherec F, Deschamps S, Hubert JF, Pellerin I, Thomas D, Delamarche C (2001) Functional characterization of a microbial aquaglyceroporin. *Microbiology* 147:1129-1135
- Furukawa K, Furukawa T, Hohmann S (2011) Efficient construction of homozygous diploid strains identifies genes required for the hyper-filamentous phenotype in *Saccharomyces cerevisiae*. *PLoS One* 6:e26584
- Furukawa K, Sidoux-Walter F, Hohmann S (2009) Expression of the yeast aquaporin Aqy2 affects cell surface properties under the control of osmoregulatory and morphogenic signalling pathways. *Mol Microbiol* 74:1272-1286
- Garcia R, Bermejo C, Grau C, Perez R, Rodriguez-Pena JM, Francois J, Nombela C, Arroyo J (2004) The global transcriptional response to transient cell wall damage in *Saccharomyces cerevisiae* and its regulation by the cell integrity signaling pathway. *J Biol Chem* 279:15183-15195
- Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* 11:4241-4257
- Ghosh M, Shen J, Rosen BP (1999) Pathways of As(III) detoxification in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 96:5001-5006
- Gonen T, Walz T (2006) The structure of aquaporins. *Q Rev Biophys* 39:361-396
- Gray JV, Ogas JP, Kamada Y, Stone M, Levin DE, Herskowitz I (1997) A role for the Pkc1 MAP kinase pathway of *Saccharomyces cerevisiae* in bud emergence and identification of a putative upstream regulator. *EMBO J* 16:4924-4937
- Green R, Lesage G, Sdicu AM, Menard P, Bussey H (2003) A synthetic analysis of the *Saccharomyces cerevisiae* stress sensor Mid2p, and identification of a Mid2p-interacting protein, Zeo1p, that modulates the PKC1-MPK1 cell integrity pathway. *Microbiology* 149:2487-2499

- Gualtieri T, Ragni E, Mizzi L, Fascio U, Popolo L (2004) The cell wall sensor Wsc1p is involved in reorganization of actin cytoskeleton in response to hypo-osmotic shock in *Saccharomyces cerevisiae*. *Yeast* 21:1107-1120
- Hara-Chikuma M, Verkman AS (2006) Physiological roles of glycerol-transporting aquaporins: the aquaglyceroporins. *Cell Mol Life Sci* 63:1386-1392
- Harries WE, Akhavan D, Miercke LJ, Khademi S, Stroud RM (2004) The channel architecture of aquaporin 0 at a 2.2-Å resolution. *Proc Natl Acad Sci U S A* 101:14045-14050
- Hedfalk K, Bill RM, Mullins JG, Karlgren S, Filipsson C, Bergstrom J, Tamas MJ, Rydstrom J, Hohmann S (2004) A regulatory domain in the C-terminal extension of the yeast glycerol channel Fps1p. *J Biol Chem* 279:14954-14960
- Herman PK, Rine J (1997) Yeast spore germination: a requirement for Ras protein activity during re-entry into the cell cycle. *EMBO J* 16:6171-6181
- Hino N, Okazaki Y, Kobayashi T, Hayashi A, Sakamoto K, Yokoyama S (2005) Protein photo-cross-linking in mammalian cells by site-specific incorporation of a photoreactive amino acid. *Nat Methods* 2:201-206
- Hohmann S (2002) Osmotic stress signaling and osmoadaptation in yeasts. *Microbiol Mol Biol Rev* 66:300-372
- Houtsmuller AB (2005) Fluorescence recovery after photobleaching: application to nuclear proteins. *Adv Biochem Eng Biotechnol* 95:177-199
- Irie K, Takase M, Lee KS, Levin DE, Araki H, Matsumoto K, Oshima Y (1993) MKK1 and MKK2, which encode *Saccharomyces cerevisiae* mitogen-activated protein kinase-kinase homologs, function in the pathway mediated by protein kinase C. *Mol Cell Biol* 13:3076-3083
- Ishibashi K (2009) New members of mammalian aquaporins: AQP10-AQP12. *Handb Exp Pharmacol*:251-262
- Jacoby T, Flanagan H, Faykin A, Seto AG, Mattison C, Ota I (1997) Two protein-tyrosine phosphatases inactivate the osmotic stress response pathway in yeast by targeting the mitogen-activated protein kinase, Hog1. *J Biol Chem* 272:17749-17755
- Joseph-Strauss D, Zenvirth D, Simchen G, Barkai N (2007) Spore germination in *Saccharomyces cerevisiae*: global gene expression patterns and cell cycle landmarks. *Genome Biol* 8:R241
- Jung JS, Bhat RV, Preston GM, Guggino WB, Baraban JM, Agre P (1994) Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. *Proc Natl Acad Sci U S A* 91:13052-13056
- Jung US, Levin DE (1999) Genome-wide analysis of gene expression regulated by the yeast cell wall integrity signalling pathway. *Mol Microbiol* 34:1049-1057
- Jung US, Sobering AK, Romeo MJ, Levin DE (2002) Regulation of the yeast Rlm1 transcription factor by the Mpk1 cell wall integrity MAP kinase. *Mol Microbiol* 46:781-789
- Kamada Y, Jung US, Piotrowski J, Levin DE (1995) The protein kinase C-activated MAP kinase pathway of *Saccharomyces cerevisiae* mediates a novel aspect of the heat shock response. *Genes Dev* 9:1559-1571
- Kapteyn JC, Van Den Ende H, Klis FM (1999) The contribution of cell wall proteins to the organization of the yeast cell wall. *Biochim Biophys Acta* 1426:373-383

- Karlgren S, Filipsson C, Mullins JG, Bill RM, Tamas MJ, Hohmann S (2004) Identification of residues controlling transport through the yeast aquaglyceroporin Fps1 using a genetic screen. *Eur J Biochem* 271:771-779
- Karlgren S, Pettersson N, Nordlander B, Mathai JC, Brodsky JL, Zeidel ML, Bill RM, Hohmann S (2005) Conditional osmotic stress in yeast: a system to study transport through aquaglyceroporins and osmostress signaling. *J Biol Chem* 280:7186-7193
- Ketela T, Brown JL, Stewart RC, Bussey H (1998) Yeast Skn7p activity is modulated by the Sln1p-Ypd1p osmosensor and contributes to regulation of the HOG pathway. *Mol Gen Genet* 259:372-378
- Ketela T, Green R, Bussey H (1999) *Saccharomyces cerevisiae* mid2p is a potential cell wall stress sensor and upstream activator of the PKC1-MPK1 cell integrity pathway. *J Bacteriol* 181:3330-3340
- King LS, Kozono D, Agre P (2004) From structure to disease: the evolving tale of aquaporin biology. *Nat Rev Mol Cell Biol* 5:687-698
- Klipp E, Nordlander B, Kruger R, Gennemark P, Hohmann S (2005) Integrative model of the response of yeast to osmotic shock. *Nat Biotechnol* 23:975-982
- Kloimwieder A, Winston F (2011) A Screen for Germination Mutants in *Saccharomyces cerevisiae*. *G3 (Bethesda)* 1:143-149
- Kono K, Matsunaga R, Hirata A, Suzuki G, Abe M, Ohya Y (2005) Involvement of actin and polarisome in morphological change during spore germination of *Saccharomyces cerevisiae*. *Yeast* 22:129-139
- Krantz M, Nordlander B, Valadi H, Johansson M, Gustafsson L, Hohmann S (2004) Anaerobicity prepares *Saccharomyces cerevisiae* cells for faster adaptation to osmotic shock. *Eukaryot Cell* 3:1381-1390
- Krisak L, Strich R, Winters RS, Hall JP, Mallory MJ, Kreitzer D, Tuan RS, Winter E (1994) SMK1, a developmentally regulated MAP kinase, is required for spore wall assembly in *Saccharomyces cerevisiae*. *Genes Dev* 8:2151-2161
- Kuthan M, Devaux F, Janderova B, Slaninova I, Jacq C, Palkova Z (2003) Domestication of wild *Saccharomyces cerevisiae* is accompanied by changes in gene expression and colony morphology. *Mol Microbiol* 47:745-754
- Laize V, Tacnet F, Ripoche P, Hohmann S (2000) Polymorphism of *Saccharomyces cerevisiae* aquaporins. *Yeast* 16:897-903
- Leberer E, Chenevert J, Leeuw T, H Marcus D, Herskowitz I, Thomas DY (1996) Genetic interactions indicate a role for Mdg1p and the SH3 domain protein Bem1p in linking the G-protein mediated yeast pheromone signalling pathway to regulators of cell polarity. *Mol Gen Genet* 252:608-621
- Leberer E, Thomas DY, Whiteway M (1997) Pheromone signalling and polarized morphogenesis in yeast. *Curr Opin Genet Dev* 7:59-66
- Lee KS, Irie K, Gotoh Y, Watanabe Y, Araki H, Nishida E, Matsumoto K, Levin DE (1993) A yeast mitogen-activated protein kinase homolog (Mpk1p) mediates signalling by protein kinase C. *Mol Cell Biol* 13:3067-3075
- Lee KS, Levin DE (1992) Dominant mutations in a gene encoding a putative protein kinase (BCK1) bypass the requirement for a *Saccharomyces cerevisiae* protein kinase C homolog. *Mol Cell Biol* 12:172-182

- Levin DE (2005) Cell wall integrity signaling in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 69:262-291
- Levin DE (2011) Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway. *Genetics* 189:1145-1175
- Levin DE, Bartlett-Heubusch E (1992) Mutants in the *S. cerevisiae* PKC1 gene display a cell cycle-specific osmotic stability defect. *J Cell Biol* 116:1221-1229
- Levin DE, Fields FO, Kunisawa R, Bishop JM, Thorner J (1990) A candidate protein kinase C gene, PKC1, is required for the *S. cerevisiae* cell cycle. *Cell* 62:213-224
- Li S, Ault A, Malone CL, Raitt D, Dean S, Johnston LH, Deschenes RJ, Fassler JS (1998) The yeast histidine protein kinase, Sln1p, mediates phosphotransfer to two response regulators, Ssk1p and Skn7p. *EMBO J* 17:6952-6962
- Liu H, Styles CA, Fink GR (1993) Elements of the yeast pheromone response pathway required for filamentous growth of diploids. *Science* 262:1741-1744
- Liu Z, Boles E, Rosen BP (2004) Arsenic trioxide uptake by hexose permeases in *Saccharomyces cerevisiae*. *J Biol Chem* 279:17312-17318
- Luyten K, Albertyn J, Skibbe WF, Prior BA, Ramos J, Thevelein JM, Hohmann S (1995) Fps1, a yeast member of the MIP family of channel proteins, is a facilitator for glycerol uptake and efflux and is inactive under osmotic stress. *EMBO J* 14:1360-1371
- Maciaszczyk-Dziubinska E, Migdal I, Migocka M, Bocer T, Wysocki R (2010) The yeast aquaglyceroporin Fps1p is a bidirectional arsenite channel. *FEBS Lett* 584:726-732
- Maeda T, Takekawa M, Saito H (1995) Activation of yeast PBS2 MAPKK by MAPKKs or by binding of an SH3-containing osmosensor. *Science* 269:554-558
- Maeda T, Wurgler-Murphy SM, Saito H (1994) A two-component system that regulates an osmosensing MAP kinase cascade in yeast. *Nature* 369:242-245
- Marquez JA, Serrano R (1996) Multiple transduction pathways regulate the sodium-extrusion gene PMR2/ENA1 during salt stress in yeast. *FEBS Lett* 382:89-92
- Marshall CJ (1994) MAP kinase kinase kinase, MAP kinase kinase and MAP kinase. *Curr Opin Genet Dev* 4:82-89
- Martin H, Arroyo J, Sanchez M, Molina M, Nombela C (1993) Activity of the yeast MAP kinase homologue Slt2 is critically required for cell integrity at 37 degrees C. *Mol Gen Genet* 241:177-184
- Martin H, Rodriguez-Pachon JM, Ruiz C, Nombela C, Molina M (2000) Regulatory mechanisms for modulation of signaling through the cell integrity Slt2-mediated pathway in *Saccharomyces cerevisiae*. *J Biol Chem* 275:1511-1519
- Martinac B, Buechner M, Delcour AH, Adler J, Kung C (1987) Pressure-sensitive ion channel in *Escherichia coli*. *Proc Natl Acad Sci U S A* 84:2297-2301
- Mas G, de Nadal E, Dechant R, Rodriguez de la Concepcion ML, Logie C, Jimeno-Gonzalez S, Chavez S, Ammerer G, Posas F (2009) Recruitment of a chromatin remodelling complex by the Hog1 MAP kinase to stress genes. *EMBO J* 28:326-336
- Matia-Gonzalez AM, Rodriguez-Gabriel MA (2011) Slt2 MAPK pathway is essential for cell integrity in the presence of arsenate. *Yeast* 28:9-17

- Maurel C, Verdoucq L, Luu DT, Santoni V (2008) Plant aquaporins: membrane channels with multiple integrated functions. *Annu Rev Plant Biol* 59:595-624
- Mensonides FI, Brul S, Klis FM, Hellingwerf KJ, Teixeira de Mattos MJ (2005) Activation of the protein kinase C1 pathway upon continuous heat stress in *Saccharomyces cerevisiae* is triggered by an intracellular increase in osmolarity due to trehalose accumulation. *Appl Environ Microbiol* 71:4531-4538
- Mitchell AP, Driscoll SE, Smith HE (1990) Positive control of sporulation-specific genes by the IME1 and IME2 products in *Saccharomyces cerevisiae*. *Mol Cell Biol* 10:2104-2110
- Mollapour M, Piper PW (2006) Hog1p mitogen-activated protein kinase determines acetic acid resistance in *Saccharomyces cerevisiae*. *FEMS Yeast Res* 6:1274-1280
- Mollapour M, Piper PW (2007) Hog1 mitogen-activated protein kinase phosphorylation targets the yeast Fps1 aquaglyceroporin for endocytosis, thereby rendering cells resistant to acetic acid. *Mol Cell Biol* 27:6446-6456
- Mollapour M, Shepherd A, Piper PW (2009) Presence of the Fps1p aquaglyceroporin channel is essential for Hog1p activation, but suppresses Slt2(Mpk1)p activation, with acetic acid stress of yeast. *Microbiology* 155:3304-3311
- Mukhopadhyay R, Rosen BP (1998) *Saccharomyces cerevisiae* ACR2 gene encodes an arsenate reductase. *FEMS Microbiol Lett* 168:127-136
- Neiman AM (2005) Ascospore formation in the yeast *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 69:565-584
- Neiman AM (2011) Sporulation in the budding yeast *Saccharomyces cerevisiae*. *Genetics* 189:737-765
- Nelson B, Parsons AB, Evangelista M, Schaefer K, Kennedy K, Ritchie S, Petryshen TL, Boone C (2004) Fus1p interacts with components of the Hog1p mitogen-activated protein kinase and Cdc42p morphogenesis signaling pathways to control cell fusion during yeast mating. *Genetics* 166:67-77
- Norbeck J, Pahlman AK, Akhtar N, Blomberg A, Adler L (1996) Purification and characterization of two isoenzymes of DL-glycerol-3-phosphatase from *Saccharomyces cerevisiae*. Identification of the corresponding GPP1 and GPP2 genes and evidence for osmotic regulation of Gpp2p expression by the osmosensing mitogen-activated protein kinase signal transduction pathway. *J Biol Chem* 271:13875-13881
- Nozawa A, Takano J, Kobayashi M, von Wiren N, Fujiwara T (2006) Roles of BOR1, DUR3, and FPS1 in boron transport and tolerance in *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* 262:216-222
- O'Rourke SM, Herskowitz I (1998) The Hog1 MAPK prevents cross talk between the HOG and pheromone response MAPK pathways in *Saccharomyces cerevisiae*. *Genes Dev* 12:2874-2886
- O'Rourke SM, Herskowitz I (2004) Unique and redundant roles for HOG MAPK pathway components as revealed by whole-genome expression analysis. *Mol Biol Cell* 15:532-542
- Oliveira R, Lages F, Silva-Graca M, Lucas C (2003) Fps1p channel is the mediator of the major part of glycerol passive diffusion in *Saccharomyces cerevisiae*: artefacts and re-definitions. *Biochim Biophys Acta* 1613:57-71

- Ono T, Suzuki T, Anraku Y, Iida H (1994) The MID2 gene encodes a putative integral membrane protein with a Ca(2+)-binding domain and shows mating pheromone-stimulated expression in *Saccharomyces cerevisiae*. *Gene* 151:203-208
- Page N, Sheraton J, Brown JL, Stewart RC, Bussey H (1996) Identification of ASK10 as a multicopy activator of Skn7p-dependent transcription of a HIS3 reporter gene. *Yeast* 12:267-272
- Pahlman AK, Granath K, Ansell R, Hohmann S, Adler L (2001) The yeast glycerol 3-phosphatases Gpp1p and Gpp2p are required for glycerol biosynthesis and differentially involved in the cellular responses to osmotic, anaerobic, and oxidative stress. *J Biol Chem* 276:3555-3563
- Park JH, Saier MH, Jr. (1996) Phylogenetic characterization of the MIP family of transmembrane channel proteins. *J Membr Biol* 153:171-180
- Pettersson N, Filipsson C, Becit E, Brive L, Hohmann S (2005) Aquaporins in yeasts and filamentous fungi. *Biol Cell* 97:487-500
- Philip B, Levin DE (2001) Wsc1 and Mid2 are cell surface sensors for cell wall integrity signaling that act through Rom2, a guanine nucleotide exchange factor for Rho1. *Mol Cell Biol* 21:271-280
- Philips J, Herskowitz I (1997) Osmotic balance regulates cell fusion during mating in *Saccharomyces cerevisiae*. *J Cell Biol* 138:961-974
- Posas F, Chambers JR, Heyman JA, Hoeffler JP, de Nadal E, Arino J (2000) The transcriptional response of yeast to saline stress. *J Biol Chem* 275:17249-17255
- Posas F, Saito H (1997) Osmotic activation of the HOG MAPK pathway via Ste11p MAPKKK: scaffold role of Pbs2p MAPKK. *Science* 276:1702-1705
- Posas F, Wurgler-Murphy SM, Maeda T, Witten EA, Thai TC, Saito H (1996) Yeast HOG1 MAP kinase cascade is regulated by a multistep phosphorelay mechanism in the SLN1-YPD1-SSK1 "two-component" osmosensor. *Cell* 86:865-875
- Preston GM, Agre P (1991) Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family. *Proc Natl Acad Sci U S A* 88:11110-11114
- Primig M, Williams RM, Winzeler EA, Tevzadze GG, Conway AR, Hwang SY, Davis RW, Esposito RE (2000) The core meiotic transcriptome in budding yeasts. *Nat Genet* 26:415-423
- Proft M, Mas G, de Nadal E, Vendrell A, Noriega N, Struhl K, Posas F (2006) The stress-activated Hog1 kinase is a selective transcriptional elongation factor for genes responding to osmotic stress. *Mol Cell* 23:241-250
- Proft M, Struhl K (2002) Hog1 kinase converts the Sko1-Cyc8-Tup1 repressor complex into an activator that recruits SAGA and SWI/SNF in response to osmotic stress. *Mol Cell* 9:1307-1317
- Proft M, Struhl K (2004) MAP kinase-mediated stress relief that precedes and regulates the timing of transcriptional induction. *Cell* 118:351-361
- Promeneur D, Liu Y, Maciel J, Agre P, King LS, Kumar N (2007) Aquaglyceroporin PbAQP during intraerythrocytic development of the malaria parasite *Plasmodium berghei*. *Proc Natl Acad Sci U S A* 104:2211-2216
- Radonjic M, Andrau JC, Lijnzaad P, Kemmeren P, Kockelkorn TT, van Leenen D, van Berkum NL, Holstege FC (2005) Genome-wide analyses reveal RNA polymerase

- II located upstream of genes poised for rapid response upon *S. cerevisiae* stationary phase exit. *Mol Cell* 18:171-183
- Raitt DC, Posas F, Saito H (2000) Yeast Cdc42 GTPase and Ste20 PAK-like kinase regulate Sho1-dependent activation of the Hog1 MAPK pathway. *EMBO J* 19:4623-4631
- Rajavel M, Philip B, Buehrer BM, Errede B, Levin DE (1999) Mid2 is a putative sensor for cell integrity signaling in *Saccharomyces cerevisiae*. *Mol Cell Biol* 19:3969-3976
- Ram AF, Kapteyn JC, Montijn RC, Caro LH, Douwes JE, Baginsky W, Mazur P, van den Ende H, Klis FM (1998) Loss of the plasma membrane-bound protein Gas1p in *Saccharomyces cerevisiae* results in the release of beta1,3-glucan into the medium and induces a compensation mechanism to ensure cell wall integrity. *J Bacteriol* 180:1418-1424
- Reiser V, Ammerer G, Ruis H (1999a) Nucleocytoplasmic traffic of MAP kinases. *Gene Expr* 7:247-254
- Reiser V, Raitt DC, Saito H (2003) Yeast osmosensor Sln1 and plant cytokinin receptor Cre1 respond to changes in turgor pressure. *J Cell Biol* 161:1035-1040
- Reiser V, Ruis H, Ammerer G (1999b) Kinase activity-dependent nuclear export opposes stress-induced nuclear accumulation and retention of Hog1 mitogen-activated protein kinase in the budding yeast *Saccharomyces cerevisiae*. *Mol Biol Cell* 10:1147-1161
- Reiser V, Salah SM, Ammerer G (2000) Polarized localization of yeast Pbs2 depends on osmostress, the membrane protein Sho1 and Cdc42. *Nat Cell Biol* 2:620-627
- Rep M, Albertyn J, Thevelein JM, Prior BA, Hohmann S (1999) Different signalling pathways contribute to the control of GPD1 gene expression by osmotic stress in *Saccharomyces cerevisiae*. *Microbiology* 145 (Pt 3):715-727
- Rep M, Krantz M, Thevelein JM, Hohmann S (2000) The transcriptional response of *Saccharomyces cerevisiae* to osmotic shock. Hot1p and Msn2p/Msn4p are required for the induction of subsets of high osmolarity glycerol pathway-dependent genes. *J Biol Chem* 275:8290-8300
- Rep M, Proft M, Remize F, Tamas M, Serrano R, Thevelein JM, Hohmann S (2001) The *Saccharomyces cerevisiae* Sko1p transcription factor mediates HOG pathway-dependent osmotic regulation of a set of genes encoding enzymes implicated in protection from oxidative damage. *Mol Microbiol* 40:1067-1083
- Roberts RL, Fink GR (1994) Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. *Genes Dev* 8:2974-2985
- Robinson MJ, Cobb MH (1997) Mitogen-activated protein kinase pathways. *Curr Opin Cell Biol* 9:180-186
- Rousseau P, Halvorson HO (1973) Macromolecular synthesis during the germination of *Saccharomyces cerevisiae* spores. *J Bacteriol* 113:1289-1295
- Savage DF, Egea PF, Robles-Colmenares Y, O'Connell JD, 3rd, Stroud RM (2003) Architecture and selectivity in aquaporins: 2.5 Å X-ray structure of aquaporin Z. *PLoS Biol* 1:E72

- Schaber M, Lindgren A, Schindler K, Bungard D, Kaldis P, Winter E (2002) CAK1 promotes meiosis and spore formation in *Saccharomyces cerevisiae* in a CDC28-independent fashion. *Mol Cell Biol* 22:57-68
- Schwartz MA, Madhani HD (2004) Principles of MAP kinase signaling specificity in *Saccharomyces cerevisiae*. *Annu Rev Genet* 38:725-748
- Shepherd A, Piper PW (2010) The Fps1p aquaglyceroporin facilitates the use of small aliphatic amides as a nitrogen source by amidase-expressing yeasts. *FEMS Yeast Res* 10:527-534
- Siderius M, Van Wuytswinkel O, Reijenga KA, Kelders M, Mager WH (2000) The control of intracellular glycerol in *Saccharomyces cerevisiae* influences osmotic stress response and resistance to increased temperature. *Mol Microbiol* 36:1381-1390
- Sidoux-Walter F, Pettersson N, Hohmann S (2004) The *Saccharomyces cerevisiae* aquaporin Aqy1 is involved in sporulation. *Proc Natl Acad Sci U S A* 101:17422-17427
- Singer MA, Lindquist S (1998) Multiple effects of trehalose on protein folding in vitro and in vivo. *Mol Cell* 1:639-648
- Singh KK (2000) The *Saccharomyces cerevisiae* Sln1p-Ssk1p two-component system mediates response to oxidative stress and in an oxidant-specific fashion. *Free Radic Biol Med* 29:1043-1050
- Smits GJ, Kapteyn JC, van den Ende H, Klis FM (1999) Cell wall dynamics in yeast. *Curr Opin Microbiol* 2:348-352
- Soveral G, Madeira A, Loureiro-Dias MC, Moura TF (2008) Membrane tension regulates water transport in yeast. *Biochim Biophys Acta* 1778:2573-2579
- Stagljar I, Korostensky C, Johnsson N, te Heesen S (1998) A genetic system based on split-ubiquitin for the analysis of interactions between membrane proteins in vivo. *Proc Natl Acad Sci U S A* 95:5187-5192
- Stock AM, Robinson VL, Goudreau PN (2000) Two-component signal transduction. *Annu Rev Biochem* 69:183-215
- Sutherland FC, Lages F, Lucas C, Luyten K, Albertyn J, Hohmann S, Prior BA, Kilian SG (1997) Characteristics of Fps1-dependent and -independent glycerol transport in *Saccharomyces cerevisiae*. *J Bacteriol* 179:7790-7795
- Suzuki M, Igarashi R, Sekiya M, Utsugi T, Morishita S, Yukawa M, Ohya Y (2004) Dynactin is involved in a checkpoint to monitor cell wall synthesis in *Saccharomyces cerevisiae*. *Nat Cell Biol* 6:861-871
- Takata K, Tajika Y, Matsuzaki T, Aoki T, Suzuki T, Abduxukur A, Hagiwara H (2004) Molecular mechanisms and drug development in aquaporin water channel diseases: water channel aquaporin-2 of kidney collecting duct cells. *J Pharmacol Sci* 96:255-259
- Tamas MJ, Karlgren S, Bill RM, Hedfalk K, Allegri L, Ferreira M, Thevelein JM, Rydstrom J, Mullins JG, Hohmann S (2003) A short regulatory domain restricts glycerol transport through yeast Fps1p. *J Biol Chem* 278:6337-6345
- Tamas MJ, Luyten K, Sutherland FC, Hernandez A, Albertyn J, Valadi H, Li H, Prior BA, Kilian SG, Ramos J, Gustafsson L, Thevelein JM, Hohmann S (1999) Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. *Mol Microbiol* 31:1087-1104

- Tanghe A, Van Dijck P, Colavizza D, Thevelein JM (2004) Aquaporin-mediated improvement of freeze tolerance of *Saccharomyces cerevisiae* is restricted to rapid freezing conditions. *Appl Environ Microbiol* 70:3377-3382
- Tanghe A, Van Dijck P, Dumortier F, Teunissen A, Hohmann S, Thevelein JM (2002) Aquaporin expression correlates with freeze tolerance in baker's yeast, and overexpression improves freeze tolerance in industrial strains. *Appl Environ Microbiol* 68:5981-5989
- Tanghe A, Van Dijck P, Thevelein JM (2006) Why do microorganisms have aquaporins? *Trends Microbiol* 14:78-85
- Tanoue T, Nishida E (2003) Molecular recognitions in the MAP kinase cascades. *Cell Signal* 15:455-462
- Tatebayashi K, Tanaka K, Yang HY, Yamamoto K, Matsushita Y, Tomida T, Imai M, Saito H (2007) Transmembrane mucins Hkr1 and Msb2 are putative osmosensors in the SHO1 branch of yeast HOG pathway. *EMBO J* 26:3521-3533
- Teige M, Scheickl E, Reiser V, Ruis H, Ammerer G (2001) Rck2, a member of the calmodulin-protein kinase family, links protein synthesis to high osmolarity MAP kinase signaling in budding yeast. *Proc Natl Acad Sci U S A* 98:5625-5630
- Teixeira MC, Raposo LR, Mira NP, Lourenco AB, Sa-Correia I (2009) Genome-wide identification of *Saccharomyces cerevisiae* genes required for maximal tolerance to ethanol. *Appl Environ Microbiol* 75:5761-5772
- Thorsen M, Di Y, Tangemo C, Morillas M, Ahmadpour D, Van der Does C, Wagner A, Johansson E, Boman J, Posas F, Wysocki R, Tamas MJ (2006) The MAPK Hog1p modulates Fps1p-dependent arsenite uptake and tolerance in yeast. *Mol Biol Cell* 17:4400-4410
- Toh TH, Kayingo G, van der Merwe MJ, Kilian SG, Hallsworth JE, Hohmann S, Prior BA (2001) Implications of FPS1 deletion and membrane ergosterol content for glycerol efflux from *Saccharomyces cerevisiae*. *FEMS Yeast Res* 1:205-211
- Tornroth-Horsefield S, Hedfalk K, Fischer G, Lindkvist-Petersson K, Neutze R (2010) Structural insights into eukaryotic aquaporin regulation. *FEBS Lett* 584:2580-2588
- Torres J, Di Como CJ, Herrero E, De La Torre-Ruiz MA (2002) Regulation of the cell integrity pathway by rapamycin-sensitive TOR function in budding yeast. *J Biol Chem* 277:43495-43504
- Trueheart J, Boeke JD, Fink GR (1987) Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. *Mol Cell Biol* 7:2316-2328
- Wagner M, Briza P, Pierce M, Winter E (1999) Distinct steps in yeast spore morphogenesis require distinct SMK1 MAP kinase thresholds. *Genetics* 151:1327-1340
- Wagner M, Pierce M, Winter E (1997) The CDK-activating kinase CAK1 can dosage suppress sporulation defects of smk1 MAP kinase mutants and is required for spore wall morphogenesis in *Saccharomyces cerevisiae*. *EMBO J* 16:1305-1317
- Valdivia RH, Schekman R (2003) The yeasts Rho1p and Pkc1p regulate the transport of chitin synthase III (Chs3p) from internal stores to the plasma membrane. *Proc Natl Acad Sci U S A* 100:10287-10292

- Van Aelst L, Hohmann S, Zimmermann FK, Jans AW, Thevelein JM (1991) A yeast homologue of the bovine lens fibre MIP gene family complements the growth defect of a *Saccharomyces cerevisiae* mutant on fermentable sugars but not its defect in glucose-induced RAS-mediated cAMP signalling. *EMBO J* 10:2095-2104
- Van Wuytswinkel O, Reiser V, Siderius M, Kelders MC, Ammerer G, Ruis H, Mager WH (2000) Response of *Saccharomyces cerevisiae* to severe osmotic stress: evidence for a novel activation mechanism of the HOG MAP kinase pathway. *Mol Microbiol* 37:382-397
- Warringer J, Ericson E, Fernandez L, Nerman O, Blomberg A (2003) High-resolution yeast phenomics resolves different physiological features in the saline response. *Proc Natl Acad Sci U S A* 100:15724-15729
- Warringer J, Hult M, Regot S, Posas F, Sunnerhagen P (2010) The HOG pathway dictates the short-term translational response after hyperosmotic shock. *Mol Biol Cell* 21:3080-3092
- Vendrell A, Martinez-Pastor M, Gonzalez-Novo A, Pascual-Ahuir A, Sinclair DA, Proft M, Posas F (2011) Sir2 histone deacetylase prevents programmed cell death caused by sustained activation of the Hog1 stress-activated protein kinase. *EMBO Rep* 12:1062-1068
- Verna J, Lodder A, Lee K, Vagts A, Ballester R (1997) A family of genes required for maintenance of cell wall integrity and for the stress response in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 94:13804-13809
- Westfall PJ, Patterson JC, Chen RE, Thorner J (2008) Stress resistance and signal fidelity independent of nuclear MAPK function. *Proc Natl Acad Sci U S A* 105:12212-12217
- Will JL, Kim HS, Clarke J, Painter JC, Fay JC, Gasch AP (2010) Incipient balancing selection through adaptive loss of aquaporins in natural *Saccharomyces cerevisiae* populations. *PLoS Genet* 6:e1000893
- Winkler A, Arkind C, Mattison CP, Burkholder A, Knoche K, Ota I (2002) Heat stress activates the yeast high-osmolarity glycerol mitogen-activated protein kinase pathway, and protein tyrosine phosphatases are essential under heat stress. *Eukaryot Cell* 1:163-173
- Wu B, Song J, Beitz E (2010) Novel channel enzyme fusion proteins confer arsenate resistance. *J Biol Chem* 285:40081-40087
- Wurgler-Murphy SM, Maeda T, Witten EA, Saito H (1997) Regulation of the *Saccharomyces cerevisiae* HOG1 mitogen-activated protein kinase by the PTP2 and PTP3 protein tyrosine phosphatases. *Mol Cell Biol* 17:1289-1297
- Wysocki R, Bobrowicz P, Ulaszewski S (1997) The *Saccharomyces cerevisiae* ACR3 gene encodes a putative membrane protein involved in arsenite transport. *J Biol Chem* 272:30061-30066
- Wysocki R, Chery CC, Wawrzycka D, Van Hulle M, Cornelis R, Thevelein JM, Tamas MJ (2001) The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in *Saccharomyces cerevisiae*. *Mol Microbiol* 40:1391-1401
- Yakata K, Hiroaki Y, Ishibashi K, Sohara E, Sasaki S, Mitsuoka K, Fujiyoshi Y (2007) Aquaporin-11 containing a divergent NPA motif has normal water channel activity. *Biochim Biophys Acta* 1768:688-693

- Yancey PH (2005) Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J Exp Biol* 208:2819-2830
- Young C, Mapes J, Hanneman J, Al-Zarban S, Ota I (2002) Role of Ptc2 type 2C Ser/Thr phosphatase in yeast high-osmolarity glycerol pathway inactivation. *Eukaryot Cell* 1:1032-1040
- Yu JW, Mendrola JM, Audhya A, Singh S, Keleti D, DeWald DB, Murray D, Emr SD, Lemmon MA (2004) Genome-wide analysis of membrane targeting by *S. cerevisiae* pleckstrin homology domains. *Mol Cell* 13:677-688
- Zapater M, Sohrmann M, Peter M, Posas F, de Nadal E (2007) Selective requirement for SAGA in Hog1-mediated gene expression depending on the severity of the external osmostress conditions. *Mol Cell Biol* 27:3900-3910
- Zhao FJ, McGrath SP, Meharg AA (2010) Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. *Annu Rev Plant Biol* 61:535-559