Biochimica et Biophysica Acta 1840 (2014) 1482–1491

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Yeast reveals unexpected roles and regulatory features of aquaporins and aquaglyceroporins ☆

Doryaneh Ahmadpour [a,1,2], Cecilia Geijer [a,1,3], Markus J. Tamás [a], Karin Lindkvist-Petersson [b], Stefan Hohmann [a,*]

[a] Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden
[b] Experimental Medical Science, Medical Structural biology, Lund University, Sweden

ARTICLE INFO

Article history:
Received 18 July 2013
Received in revised form 18 September 2013
Accepted 19 September 2013
Available online 26 September 2013

Keywords:
Aquaporin
Osmoregulation
Freeze tolerance
Stress resistance
Signal transduction
MAPK pathway

ABSTRACT

Background: The yeast *Saccharomyces cerevisiae* provides unique opportunities to study roles and regulation of aqua/glyceroporins using frontline tools of genetics and genomics as well as molecular cell and systems biology. *Scope of review: S. cerevisiae* has two similar orthodox aquaporins. Based on phenotypes mediated by gene deletion or overexpression as well as on their expression pattern, the yeast aquaporins play important roles in key aspects of yeast biology: establishment of freeze tolerance, during spore formation as well as determination of cell surface properties for substrate adhesion and colony formation. Exactly how the aquaporins perform those roles and the mechanisms that regulate their function under such conditions remain to be elucidated. *S. cerevisiae* also has two different aquaglyceroporins. While the role of one of them, Yfl054c, remains to be determined, Fps1 plays critical roles in osmoregulation by controlling the accumulation of the osmolyte glycerol. Fps1 communicates with two osmo-sensing MAPK signalling pathways to perform its functions but the details of Fps1 regulation remain to be determined.
Major conclusions: Several phenotypes associated with aqua/glyceroporin function in yeasts have been established. However, how water and glycerol transport contribute to the observed effects is not understood in detail. Also many of the basic principles of regulation of yeast aqua/glyceroporins remain to be elucidated.
General significance: Studying the yeast aquaporins and aquaglyceroporins offers rich insight into the life style, evolution and adaptive responses of yeast and rewards us with discoveries of unexpected roles and regulatory mechanisms of members of this ancient protein family. This article is part of a Special Issue entitled Aquaporins.

1. Introduction

The discovery of aquaporins 20 years ago has changed our view on water and solute movements in cells and organisms. The roles of water channels in human diseases [1,2] as well as in water, solute and gas transport in plants [3,4] have attracted much research interest in the last two decades. However, it was not immediately apparent why micro-organisms should have channels for water and glycerol. For a long time it was widely accepted that the large surface to volume-ratio of single-celled organisms should allow for sufficient transmembrane water and glycerol flux to satisfy their needs. Research performed in recent years shows that there are several, sometimes astonishing, explanations why micro-organisms possess aqua/glyceroporins. This is also reflected by the fact that sometimes even closely related micro-organisms possess different types and numbers of members of this ancient protein family. Purposes for aqua/glyceroporins in micro-organisms may include (1) adaptation to environments and conditions under which transmembrane water or glycerol flux actually becomes limiting; (2) developmental stages where water or glycerol transport becomes critical; (3) the ability to control water and glycerol flux through the plasma membrane. The yeast *Saccharomyces cerevisiae* offers opportunities to study all these aspects, some of which have potential to offer unexpected insight into cell biological, evolutionary and adaptive mechanisms.

The budding yeast *S. cerevisiae* is probably the best studied eukaryotic cell and more than 85% of the yeast genes have been functionally characterised [5]. This yeast is widespread in nature in cold, temperate and hot climates and is generally found in sugar containing plant material (flowers, fruits, sap) and in the soil in association with plants. Yeast spreads with wind, water and with help of insects. Wild yeast strains can grow both as dispersed single cells but they also have the ability to form pseudohyphae of attached cells, biofilms as well as colonies of ordered structure (fluffy colonies). The recent evolution of *S. cerevisiae*

☆ This Article is Part of a Special Issue Entitled Aquaporins.

* Corresponding author at: Department of Chemistry and Molecular Biology, University of Gothenburg, Box 462, SE-40530 Göteborg, Sweden. Tel.: +46 31 3608488; fax: +46 31 862599.
E-mail address: stefan.hohmann@gu.se (S. Hohmann).

[1] DA and CG contributed equally to this review.
[2] Present address: Department of Physics, University of Gothenburg, Sweden.
[3] Present address: Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

0304-4165/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.09.027

is affected by its association with human activity and even more recently domestication (beer, wine, bread, research). The *S. cerevisiae* genome has undergone a whole genome duplication ca 100 Ma ago. Subsequently most of the duplicated genes were lost but approximately 15% of this genome duplication is still apparent today [5,6]. It is thought that this genome duplication provided a selective advantage in high sugar containing environments and supported the development of an extreme metabolic adaptation: the fermentation of sugar to ethanol even in the presence of oxygen, high ethanol tolerance and the ability to consume the produced ethanol after the diauxic shift. Yeast is a sexual organism where haploids have one of two different mating types, a and α, which attract each other by mating pheromones to form diploid cells [7]. Both haploids and diploids exist as vegetative cells (see Fig. 1 for yeast cell types and morphological adaptations). Under nitrogen and carbon starvation diploid cells undergo meiosis immediately followed by the formation of four haploid spores, the survival structures of yeast [8]. Several of the features of *S. cerevisiae* described above bear importance for the discussion on the role and regulation of the aqua/glyceroporins.

The molecular and systems level mechanisms controlling yeast osmoadaptation have been very well studied in the last 20 years [9–11]. Hyperosmotic stress (Fig. 2) stimulates the HOG (High Osmolarity Glycerol) MAP kinase pathway (see also Fig. 5). The Hog1 MAPK controls numerous features of yeast osmoadaptation, including the production (at gene expression and metabolic level) as well as accumulation of the main osmolyte glycerol [12]. Hypo-osmotic shock (Fig. 2) as well as cell wall damage and stimuli leading to cell wall remodelling activate a different MAPK pathway with Slt2 as the effector protein kinase (see also Fig. 5), which controls expression of cell wall remodelling factors [13]. The two other yeast MAPKs, Fus3 and Kss1 control mating responses and morphological adaptations (such as pseudohyphal development), respectively [14]. The entire MAPK system closely communicates. This was recently illustrated in an experimental scenario, where yeast cells adapted to high osmolarity where treated with α-factor pheromone, resulting in the consecutive activation of Fus3, Slt2 and Hog1 [15].

The yeast *S. cerevisiae* possesses four proteins of the aqua/glyceroporin family [16,17]. Two of those are very similar orthodox aquaporins. Their expression, however, is differentially regulated and they appear to perform similar but also different functions. Possession of two aquaporin genes appears to be restricted to some of those yeasts that have undergone whole-genome duplication. All other yeasts seem to possess only one or even no orthodox aquaporin at all [18]. *S. cerevisiae* also has two aquaglyceroporins, which, however, do not seem to be closely related and they have different distribution pattern among other yeasts and fungi.

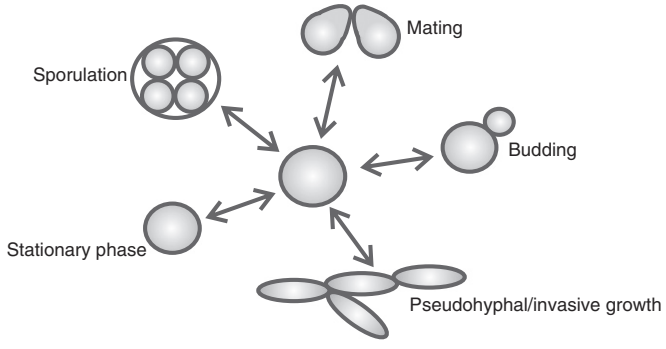

Fig. 1. Yeast cell types and morphological adaptations. Haploid as well as diploid yeast cells can multiply by budding. Under specific starvation conditions cells can form pseudohyphae and/or invade the substrate. Haploid cells of different mating types can mate and form a diploid cell. Under both nitrogen and carbon starvation yeast cells enter dormant, stress tolerant stages either as stationary phase cells or as spores following meiosis of diploid cells.

2. Orthodox aquaporins in yeast

S. cerevisiae possesses two paralogous genes, *AQY1* and *AQY2*, which encode orthodox aquaporins [16,17,19–21]. Aqy1 (305 amino acids) and Aqy2 (289) are 88% identical. From their discovery it has puzzled researchers that most laboratory strains, but even wine strains and wild yeasts, possess mutated forms of those genes and hence do not express functional aquaporins [16,17,19–24]. *S. cerevisiae* laboratory strains contain one of two alleles of *AQY1*; *AQY1-1* or *AQY1-2*. For instance, SK1, a strain often used in studies on meiosis and sporulation, possesses the *AQY1-1* allele, which encodes a functional aquaporin. On the other hand, the widely used strains S288C, W303-1A and BY4741 carry the *AQY1-2* allele, which has three point mutations and encodes a non-functional gene product [19,21–24]. *AQY2* was not even listed as a gene in the first genome annotation of the reference strain S288C because the open reading frame is interrupted by an 11 bp deletion. This deletion causes a premature stop codon and the gene product is non-functional [19,25]. One exception is strain Σ1278b, which is generally regarded as being close to wild yeasts and is used to study pseudohyphal development. This strain possesses functional versions of both Aqy1 and Aqy2 [19,21–23]. Taken together, it appeared that conditions not only in the laboratory but also in industry, and even in nature, exert selective pressure against functional aquaporins in yeast.

A recent study [18] employing a range of wild yeast strains from different parts of the world appears to provide answers to this puzzle. Studying the genetic determination of freeze tolerance in yeast strains, Will et al. found that more than 90% of the phenotypic variation among strains can be explained by the two *AQY* loci. A link to freeze tolerance had been reported previously [26] and is discussed in more details below. Natural freeze/thawing regimes occur in climates with winter/summer seasons but not in tropical and sub-tropical climates and are also uncommon in the laboratory or industry (storing yeasts in frozen cultures is a rather recent development and commonly does not include repeated cycles of freezing and thawing). Here it appears that an opposite selective pressures comes into the picture: aquaporins render yeast cells sensitive to repeated osmotic cycles [20]. Those may occur in nature on high sugar containing substrate exposed to rain showers or during washing procedures in the laboratory. Hence, such conditions provide a selective pressure against possession of aquaporins and offer plausible explanations why aquaporin function has been lost by several independent mutation events in different yeast strains or strain families. Taken together, yeast aquaporins provide a compelling example for local adaptation as a driving force in population differentiation [18].

2.1. Aquaporins and yeast freeze tolerance

While searching among different *S. cerevisiae* strains for genetic determinants of freeze tolerance with the aim to improve industrial baker's yeast strains, the Thevelein lab observed a correlation between the degree of tolerance to freeze/thawing cycles and the level of expression of *AQY* genes [26]. Indeed, deletion of *AQY* genes diminished freeze tolerance while overexpression of the yeast as well as a human aquaporin improved freeze tolerance. Overexpression of aquaporins also improved freeze tolerance in two unrelated yeast species, *Candida albicans* (a human pathogen) as well as fission yeast, *Schizosaccharomyces pombe* [27,28]. Deletion of the single aquaporin gene in the yeast *Pichia pastoris*, *PpAQY1*, also causes freezing intolerance [29]. These observations are well in line with the fact that more than 90% of the phenotypic variation of freeze tolerance in natural yeast strains can be explained by different *AQY* alleles [18].

There are, however, several unresolved issues. First, it appears that the effect on freeze tolerance is restricted to very rapid freezing [30]. This not only limits the usefulness for industrial applications but also raises the questions if, and under which conditions, such rapid freezing may occur in nature. In addition, as discussed in detail below, the

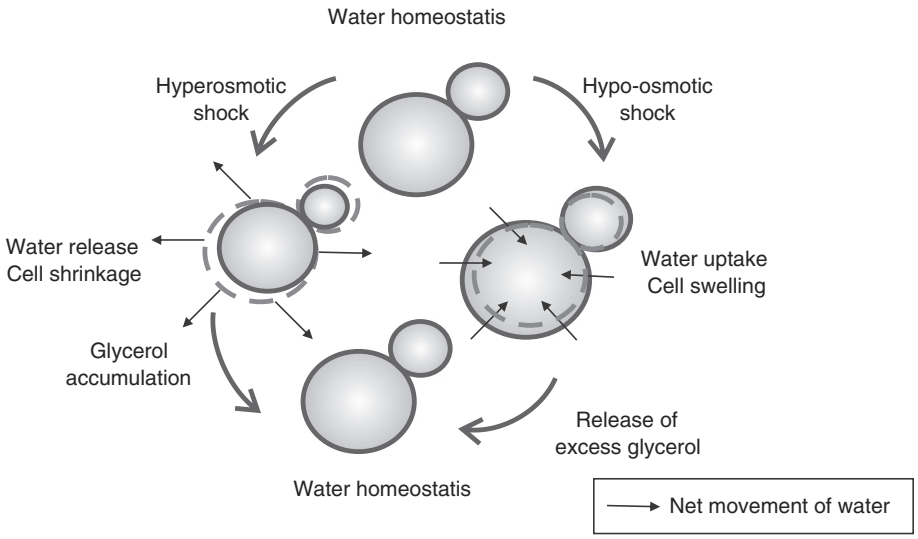

Fig. 2. Following a hyperosmotic shock (experimentally achieved by adding NaCl or sorbitol to the medium) yeast cells lose water and shrink within seconds. Cells adapt by accumulating glycerol. Following a hypo-osmotic shock cells take up water and swell and compensate by rapidly releasing glycerol.

control of expression of the *S. cerevisiae* aquaporins hints at different physiological effects and upregulation of *AQY* expression at low temperature has not been reported. In any case, the observations reported by the Thevelein and Gasch groups provide compelling support for a role of the yeast aquaporins in freeze tolerance.

This raises the question how aquaporins could improve tolerance to rapid freezing. The standard functional assay for aquaporin function makes use of transient expression in *Xenopus* oocytes and measurement of osmotic water movement (oocyte swelling) at 0 °C [31]. The low temperature is chosen because it limits free water flow through the plasma membrane. Water channels are specifically important for water transmembrane water flow at lower temperature even in yeast [32]. The reason for cell death at freezing temperature is due to formation of ice crystals inside the cell, which damage cell structure. Putting these facts together, one can phrase the following hypothesis: when the temperature drops below freezing, the fluid surrounding of the cell probably freezes first (because of lower solute content as compared to the cytoplasm), generating an outward-driving concentration gradient for liquid water. Aquaporins may then facilitate water efflux, which causes a further drop of the freezing point inside the cells with all its dissolved molecules to eventually completely prevent formation of ice crystals. In the absence of water channels, free diffusion through the plasma membrane may be too slow to reach this effect [30]. This picture would also explain why the aquaporin effect on freeze tolerance appears to be restricted to rapid freezing, because during slow freezing the free diffusion through the lipid bilayer may still be sufficiently fast.

2.2. Aqy1, a sporulation-specific aquaporin

Inspired by data from global gene expression analysis we investigated if Aqy1 might have some role during sporulation. Yeast sporulation is a complex process [8]: under nitrogen and carbon starvation diploid yeast cells initiate the meiotic programme to develop four haploid cells. Integrated into meiosis is then the developmental programme of spore formation and the four haploid cells develop into spores. Those not only serve as yeast gametes but also serve as survival structures. Yeast spores possess a specialised cell wall, have reduced water and highly elevated trehalose levels and are extremely stress tolerant [8].

The yeast SK1 strain is commonly used for sporulation studies because it sporulates under nitrogen starvation with almost 100% efficiency. SK1 possesses a functional *AQY1*. *AQY1* expression is strongly upregulated in sporulating SK1 diploid cells [33]. *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 (unpublished observations), while diploids show a sharp upregulation coinciding with ascus formation eight hours after shift to sporulation medium [33]. Addition of a fermentable carbon source to the starvation medium prevents expression of *AQY1* in both haploid and diploid cells (our unpublished observations). It seems therefore that there are at least two layers of regulation: stimulation of expression by glucose starvation and a ploidy-specific induction.

In our study [33] we were unable to establish a strong sporulation-specific phenotype associated with deletion of *AQY1* in the SK1 strain. However, in wild yeast strains, such as strain YPS163 isolated from soil under an oak tree, it appears that spore formation is strongly dependent in Aqy1 [18]. Hence it seems that yeast strains that are living in environments where possession of aquaporins is advantageous (the freeze/thawing effect) rely on Aqy1 for spore formation. On the other hand, strains that lack Aqy1 because they have adapted to osmotic cycles and are not exposed to freeze/thawing overcame this Aqy1 sporulation requirement and form spores even without Aqy1 [18].

It is not immediately obvious why Aqy1 would be important for sporulation process. Based on the observation that the Aqy1 protein only appears after the four spores have already separated from each other within the developing ascus [33] and based on the properties of yeast spores [8] we speculate the role of Aqy1 may be as follows. During spore formation the water content of spores is strongly reduced and water is partly replaced by trehalose, which can make up to 20% of the dry weight of spores. How this water leaves spores is not known. It may simply be turgor pressure that drives water out of developing spores. In contrast to the cell wall of vegetative cells, which is highly flexible [13], spore walls are very rigid [8]. Hence, trehalose accumulation in combination with an inflexible cell volume may generate the force to drive water outwards, and this process may be facilitated by Aqy1. Stationary yeast cells have many features in common with spores [34] and the stimulated expression of *AQY1* in haploid, starved cells, may hint at a similar role of Aqy1 in establishing dormancy of stationary cells. However, all this is pure speculation. It is also not known how yeast strains adapted to osmotic cycles and lacking Aqy1 overcame its requirement for sporulation.

2.3. Aqy2 is associated with morphological adaptations

As mentioned above, Aqy1 and Aqy2 are 88% identical but their genes are differentially regulated. While expression of *AQY1* is upregulated

under starvation conditions, expression of *AQY2* is highest in cells growing exponentially in rich medium [22]. Encouraged by reports that aquaporins may affect cell surface and colony structure properties (see below), we analysed the *AQY2* expression pattern [35]. It appears, that *AQY2* expression is down-regulated by hyperosmotic stress in a Hog1-dependent manner. Hog1 also negatively regulates the formation of so-called fluffy colonies (structured colonies high in extracellular matrix), formation of pseudohyphae and agar invasion. Two additional signalling pathways are involved in pseudohyphal development, invasion and colony morphology: the cAMP-dependent protein kinase (PKA) pathway and the pseudohyphal development Kss1 pathway [36]. Both also affect expression of *AQY2*: PKA has a positive effect on expression while Kss1 appears to have a negative effect [35]. Taken together, the expression pattern of *AQY2* and its dependence on regulatory pathways in many ways resembles that of *FLO11*. Flo11 is a cell surface glycoprotein and strictly required for pseudohyphal development, invasion, adhesion and formation of structured colonies [36].

Although yeast aquaporins have been associated with cell surface properties and formation of fluffy colonies some of the observation appears to be contradictory. Carbrey et al. reported that deletion of aquaporin genes in Σ1278 (the lab strain with functional aquaporins) increases cell surface hydrophobicity, plastic adhesion and yeast cell clumping [19]. We observed that deletion of *AQY2* diminishes colony fluffiness and overexpression of *AQY2* increases plastic adhesion and agar invasion [35]. The Palkova lab reported upregulation of expression of *AQY1* (not *AQY2*) during formation of fluffy colonies and a correlation between the expression of *AQY1* (and *FLO11*) and the degree of fluffiness of colonies, but no effect of deletion of *AQY1* [37,38]. Since *AQY1* and *AQY2* are highly similar and given the available information on aquaporin gene expression in yeast, those authors may actually have observed *AQY2* expression in their experiments.

How can one integrate those pieces of information into a picture of the physiological role of Aqy2? Cell surface properties of yeast cells are determined by those of the cell wall that surrounds them. The cell wall is an "organelle" consisting of polysaccharides (mainly glucans and mannans) as well as glycoproteins, such as Flo11 [13]. When the carbohydrate moieties in the cell wall are hydrated, the cell wall has low tendency to attract water and the cell surface may be hydrophobic. On the other hand, if the carbohydrates are under-hydrated they may attract water and hence the cell surface may be more hydrophilic. Water channels may serve a role in water exchange between the cytoplasm and the cell wall and thereby contribute to hydration of cell wall carbohydrates and hence hydrophobicity. This concept is purely speculative and not supported by experimental data.

In the context of a yeast colony in nature, i.e. a structured "fluffy" colony with extracellular matrix, water exchange between the cytosol and the cell wall/extracellular matrix may be critically important to ensure transport of nutrients and metabolic waste products within the colony and between the colony and its substrate [39]. In such colonies, yeast cells collaborate to shape their environment and hence the conditions for the individual and, more importantly, the population. It appears that aquaporins, in particular Aqy2, may play roles in communication of yeast cell in communities and how they affect their environment. A quite fascinating possibility. It would be interesting to learn if similar roles for aquaporins exist in other microorganisms.

2.4. Pichia pastoris Aqy1 is regulated by phosphorylation and mechano-sensitivity

Pichia pastoris, a methylotrophic yeast, is frequently used for protein overproduction, including membrane proteins [40]. This yeast possesses one orthodox aquaporin (and one aquaglyceroporin) referred to as PpAqy1, which is abundantly expressed during fermentation in methanol-containing medium [29]. PpAqy1 has earned quite some fame recently: its structure has been resolved to a staggering 0.88 Å resolution, making it the presently highest resolution membrane protein structure [41]. At this resolution individual water molecules become visible inside the channel allowing for detailed mechanistic insight into water transport through aquaporins and the exclusion of protons [41].

The crystal structure of PpAqy1 also revealed gating mechanisms: the extended N-terminus of this aquaporin caps the pore [29,42]. Like all aquaporins, PpAqy1 assembles as tetramers in the membrane. The cytoplasmic N-terminus folds as a coiled helical bundle and intertwines with the aquaporin monomers. Of particular importance are two amino acids in the N-terminus; Pro29 that introduces a kink and thereby allows Tyr31 to block the water channel [29,42]. The N-terminus seems to be 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 parts of the sequence of the N-terminus (including Pro29 and Tyr31) are conserved among yeast aquaporins, suggesting that the gating mechanism may be a common characteristic [29,42]. Ser107 in loop B, may also be important in regulating the activity of PpAqy1, as replacement with aspartate (which mimics a phosphorylated serine) resulted in increased water transport activity. Molecular dynamics simulations suggest that PpAqy1 could be subject to mechano-sensitive gating [29,42]. Membrane tension has previously been suggested to regulate Aqy1 and Aqy2 in *S. cerevisiae* [43]. During rapid temperature or osmolarity changes, mechano-sensitive gating would indeed be an effective way to quickly increase or decrease aquaporin activity, whereas phosphorylation through signalling pathways could provide scope for long-term regulation. However, protein kinases that phosphorylate yeast aquaporins have not yet been identified.

P. pastoris was chosen as a host system for the expression of, among others, mammalian aquaporins. However, first attempts to express human aquaporins resulted instead in purification of the endogenous PpAqy1 aquaporin, which became a success story on its own [29]. In order to use *P. pastoris* for heterologous aquaporin production the *PpAQY1* gene was deleted [44].

3. Aquaglyceroporins in *S. cerevisiae*

S. cerevisiae possesses two aquaglyceroporins encoded by the *FPS1* and *YFL054C* genes, respectively [17]. Fps1 and Yfl054 are not closely related to each other and do not appear to be paralogues. Fps1-like proteins, characterised by conserved regulatory sequences in the N- and C-terminal extensions, only appear to exist within confined groups of yeasts closely related to *S. cerevisiae* [17] (and re-analysed for this review). Yfl054-like proteins, characterised by very long N-terminal and shorter C-terminal extensions as well as sequence similarity, seem to occur over a wider range of organisms within the fungal kingdom [17] (and re-analysed for this review).

Aquaglyceroporins have a broader substrate specificity than orthodox aquaporins. Fps1 transports glycerol ($HCOH)_3H_2$), arsenite ($As(OH)_3$), antimonite ($Sb(OH)_3$), boric acid ($B(OH)_3$) and acetic acid (CH_3COOH) [45–47]. Loss of Fps1 confers resistance to acrolein and allyl alcohol and it compromises the use of acetamide as a nitrogen source, suggesting that also these compounds may be transported by Fps1 [48]. Similar to orthodox aquaporins, Fps1 and probably also other aquaglyceroporins function as homotetramers [49]. On the other hand, the highly conserved, channel constriction-forming NPA (Asn- Pro- Ala) motifs in the B and E loops seem to be less strictly conserved among yeast and fungal aquaglyceroporins. For instance, in Fps1 NPA is replaced by NPS (Asn- Pro- Ser) and NLA (Asn-Leu- Ala), respectively. While Fps1 appears to function well with canonical NPA motifs the *E. coli* glycerol facilitator GlpF does not tolerate NPS and NLA, suggesting that other structural features in Fps1 compensate for these alterations, such as for instance a more flexible pore structure [50]. A feature that distinguishes fungal aquaglyceroporins from most other members of the family is their size: they are significantly larger due to unusually long N- and C-terminal extensions. As outlined below, both termini are critically involved in regulation of transport in Fps1.

Little is known about the function of Yfl054 in *S. cerevisiae* or any of its homologues in other yeasts or fungi. It has been suggested that Yfl054 mediates passive diffusion of glycerol in the presence of ethanol [51]. However, Yfl054 does not seem to mediate glycerol entry under normal conditions, and neither glycerol accumulation nor export is affected by deletion of *YFL054c* during hyper- and hypo-osmotic stress (Tamas et al., unpublished data). It has also been reported that deletion of *FPS1* or *YFL054c* results in increased passive diffusion of ethanol [51], which can be a result of altered membrane composition. Global gene expression studies show that the *YFL054c* mRNA level is increased during entry into stationary phase as well as during sporulation [52], data that we confirmed (unpublished results). The level of the *YFL054c* transcript is rapidly down-regulated during germination induced by glucose with or without other nutrients (unpublished results), suggesting that the gene might be glucose repressed. However, the physiological significance of these observations remains unclear.

Fps1, however, has attracted wider interest and is studied intensely at different levels. It appears to be a major player in the control of glycerol accumulation and hence turgor and osmotic regulation. Fps1 appears to be regulated and may integrate signals from different osmoregulatory pathways, as discussed in more details below.

3.1. Fps1 function

The name *FPS1* stands for "fdp1 suppressor" since it was first discovered as a multi-copy suppressor of the growth defect of the *fdp1* (*tps1*) mutant on fermentable sugars [53]. Tps1 encodes trehalose-phosphate synthase and is required for proper coordination of glucose phosphorylation with glycolytic capacity in a manner that is not completely understood [54,55]. Deletion of *TPS1* causes inability to grow on glucose and this defect can be suppressed by deletion of *HXK2* [56], which encodes the main hexokinase, or by stimulating the production of glycerol [57]. Deletion of *HXK2* apparently reduces glucose phosphorylation and thereby restores glycolytic metabolism. The enhancement of glycerol production probably confers its effect by recovering inorganic phosphate, which is required further downstream in glycolysis [55]. Glycerol is produced in yeast by a two-step pathway from the glycolytic intermediate dihydroxyacetonephosphate. These two steps are catalysed by glycerol-3-phosphate dehydrogenase (encoded by the differentially expressed paralogues *GPD1* and *GPD2*) and glycerol-3-phosphatase (encoded by *GPP1* and *GPP2*). Overexpression of *GPD1* increases glycerol production capacity and suppresses the growth defect on glucose of the *tps1* mutant. Overexpression of *FPS1* has a similar effect, although it is not completely clear why it leads to enhanced glycerol production [55,57].

At the time of discovery of Fps1 about 20 years ago it was widely accepted, based on over-interpretation of some earlier observations [58], that the yeast plasma membrane is freely permeable for glycerol and that no transporter would be required for this purpose. However, it rather appears that the yeast plasma membrane is impermeable for glycerol and that the major part of passive glycerol diffusion through the plasma membrane is mediated by Fps1 [51]. In addition, yeast possesses a glycerol-H^+ symport system for active uptake of glycerol, Stl1, which is required for utilisation of glycerol as carbon source as well as for glycerol uptake from the surrounding medium during adaptation to osmotic stress [59] and cold temperatures [60]. The gene, *STL1*, is strongly upregulated under osmostress in a Hog1-dependent manner [61]. Stl1 is structurally unrelated to Fps1 and belongs to the family of hexose transporters with twelve transmembrane domains (Stl1: Sugar Transporter Like).

3.1.1. Fps1 glycerol transport and osmoregulation

As a channel protein, Fps1 mediates bidirectional transport of glycerol across the plasma membrane. However, all presently known phenotypes of Fps1 relate to glycerol export (Fig. 3).

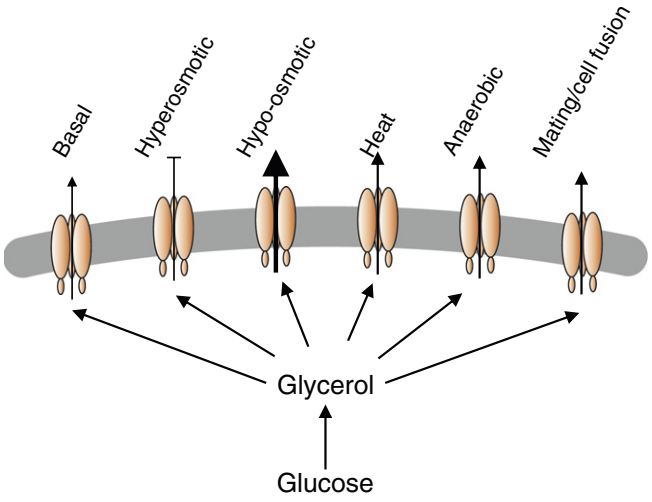

Fig. 3. Fps1-mediated glycerol export under different conditions. Mutants lacking *FPS1* or expressing constitutively active Fps1 show phenotypes under these conditions as outlined in the text.

Fps1 plays a central role in yeast osmoregulation by mediating the regulated export of glycerol that is produced by yeast [46,57,62]. As mentioned above, *S. cerevisiae* adapts to hyperosmotic stress by accumulating glycerol, which it produces as a by-product of alcohol fermentation. Osmostress-induced glycerol accumulation is controlled by the HOG pathway at the level of gene expression, metabolism and transport [10–12]. It appears that within seconds after a hyper-osmotic shock, Fps1 is inactivated (or 'closed') to ensure intracellular retention and accumulation of glycerol [46]. The importance of Fps1 inactivation during hyper-osmotic conditions is best illustrated by the phenotypes of cells expressing constitutively open or unregulated Fps1 mutants. Such cells are sensitive to high osmolarity, excrete glycerol and overproduce it in an attempt to compensate for glycerol loss [46,63]. When yeast cells are shifted from high to low external osmolarity, Fps1 is rapidly activated (or 'opened') allowing fast glycerol release. Mutants lacking Fps1 poorly recover from such a hypo-osmotic shock [46]. In fact, combination of deletion of *FPS1* with mutations that weaken the yeast cell wall causes synthetic lethality and cell lysis [46,64] illustrating the importance for Fps1 for cell stability under changing external osmolarity.

Glycerol export, and therefore Fps1, appear to be important under a range of different physiological scenarios (Fig. 3). Glucose-growing cells produce glycerol as metabolic by-product and when lacking Fps1 accumulate glycerol. This glycerol overload appears to cause reduced fitness and poor recovery from stationary phase of *fps1*Δ cells [46,65]. Yeast cells produce particular high amounts of glycerol when fermenting glucose in the absence of oxygen, when glycerol production is strictly required to re-oxidise excess NADH [66–68]. Mutants lacking Fps1 hyper-accumulate intracellular glycerol and grow poorly under anaerobic conditions [46].

Fps1 activity and glycerol release are also important for cell fusion during yeast mating [69]. Cell fusion of two haploid mating partners involves local degradation of the cell wall. The cell fusion defect of *fps1Δ* cells is caused by an inability of these mutants to maintain their osmotic balance; decreasing intracellular glycerol levels by deleting *GPD1* partially suppressed the cell fusion defect of *fps1Δ* mutants whereas glycerol hyper-accumulation (*GPD1* overexpression) exacerbated the defect. The fusion defect could also be partially suppressed by increasing extracellular osmolarity. Recent work illustrates nicely that glycerol release during mating is tightly regulated. When yeast cells adapted to high osmolarity are exposed to mating pheromone, glycerol is released through Fps1 during a later stage of the mating response, apparently stimulated by the Slt2 MAPK (see further), which in turn causes a compensating osmotic response by the cell via activation of Hog1 [15].

Additional phenotypes associated with Fps1-dependent control of intracellular glycerol levels and osmolarity have been reported. Since cells lacking Fps1 hyper-accumulate glycerol, they constantly appear to be 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 [65] (and our own unpublished observations). Loss of Fps1 also affects membrane/cellular lipid composition. *Fps1Δ* cells have lower phospholipid and ergosterol content whereas the glycolipid content is higher compared to wild type cells [62,70]. Also the effect of Fps1 on membrane lipid composition is probably a consequence of altered intracellular glycerol levels and/or altered turgor pressure. Deletion of *FPS1* confers high temperature sensitivity [65]. Increase in temperature leads to higher intracellular glycerol levels, and the temperature sensitive phenotype of *fps1Δ* cells is probably a result of glycerol overload [65,71]. Increased glycerol accumulation can also be beneficial for cells. Glycerol is well known as cryoprotectant and cells with higher intracellular glycerol levels acquire freeze tolerance and retain high leavening ability in dough after freeze storage. The *fps1Δ* mutant shows enhanced high freeze tolerance [72,73].

3.1.2. Metalloid transport and tolerance

Arsenic and antimony are two toxic metalloids that are naturally present in the environment. Arsenic is particularly abundant in certain areas of the world where it contaminates the drinking water and soil thereby affecting the health of millions of people [74–76]. These metalloids have a long history of usage as chemotherapeutic agents, and are also employed today in modern anticancer and antiprotozoan therapies [77,78]. However, their cellular entry routes remained unknown for a long time until we demonstrated that Fps1 mediates the passage of trivalent arsenite and antimonite across the plasma membrane [45]. *Fps1Δ* cells accumulate less arsenite and tolerate higher levels of arsenite and antimonite than wild type cells. Cells growing under high osmolarity, when Fps1 is down-regulated, tolerate higher levels of these metalloids while cells expressing a constitutively open Fps1 exhibited higher intracellular arsenic accumulation and increased sensitivity to both arsenite and antimonite [45]. Following this first demonstration of aquaporin-mediated metalloid transport in a eukaryotic organism metalloid-transporting aquaporins were also found in plants and mammals [79–81]. Aquaglyceroporins probably recognize these metalloids in the form of $As(OH)_3$ and $Sb(OH)_3$ [82,83], which are structurally similar to glycerol [84]. Taken together, it appears that aquaglyceroporins are, together with phosphate and hexose transporters, one main entry route for arsenite and antimonite in yeast and other eukaryotic cells.

3.1.3. Acetic acid transport and tolerance

Acetic acid is toxic to yeast cells as many other weak acids [85]. It is produced by yeast and other micro-organisms as a by-product of sugar fermentation and hence occurs very commonly in the natural environment of yeast. Fps1 apparently facilitates the entry of uncharged, undissociated acetic acid (CH_3COOH) into yeast cells. Loss of Fps1 essentially eliminates acetic acid accumulation in cells at low pH. Cells exposed to the same acetic acid concentration at neutral pH, when the acetic acid in the medium is almost completely in the dissociated anionic form (CH_3COO^-), exhibit decreased cellular accumulation of acetate. Deletion of *FPS1* improves acetic acid resistance whereas expression of unregulated Fps1 renders cells more sensitive. These observations establish a role of Fps1 in acetic acid transport and tolerance [86].

3.2. Regulation of Fps1

3.2.1. Roles of the unique termini and transmembrane domains

As outlined above, Fps1 is rapidly inactivated (or 'closed') following a hyperosmotic shock to ensure glycerol retention whereas a hypo-osmotic shock activates Fps1 to mediate glycerol efflux [46,57,63]. The unique N- and C-terminal cytoplasmic extensions (Fig. 4) appear to play critical roles in regulation of Fps1. These extensions have variable length among Fps1 orthologues (which, as mentioned above, only occur in closely related yeasts) but the N-terminal extension (250 amino acids in ScFps1) is always longer than the C-terminal extension (150 amino acids). Even among closely related yeasts conservation of the extensions is poor and restricted to rather short elements close to the first and sixth (last) transmembrane domain [17] (Fig. 4). These conserved elements seem to contribute to channel regulation.

Deletion or mutation within a short domain consisting of twelve amino acids (^{225}LYQNPQTPTVLP^{236}) close to the first transmembrane (TM) domain cause unregulated glycerol flux and sensitivity to hyperosmotic stress [63] as well as increased arsenite transport and sensitivity [45] and increased sensitivity to acetic acid [86]. Not only the amino acid sequence but also the distance of the domain to TM1 appears to be important [63]. Deletion of a stretch of twelve well-conserved amino acids (^{535}HESPVNWSLPVY^{546}) in the C-terminus close to the sixth transmembrane domain also causes inability of cells to retain glycerol during hyper-osmotic stress and confers osmo-sensitivity [87].

When studying by heterologous expression and domain swapping the Fps1 orthologue of the filamentous yeast *Ashbya gossypii*, we noted that the N- and C-terminal extensions of ScFps1 could not confer regulation on AgFps1 and that AgFps1 expressed in *S. cerevisiae* was apparently unregulated. We concluded that (a) probably additional features of Fps1 are involved in regulation and (b) additional proteins are probably required for regulation, which do not seem to recognise the heterologous channel [88]. To identify other regions within Fps1 implicated in regulation, we employed different genetic screens. Random mutations that render Fps1 unregulated revealed several important residues close to TM1 and in the B loop (Fig. 4). All these mutations face the cytosolic side of the protein [89]. A screen for intragenic mutations that suppress unregulated Fps1 activity caused by deletion of the N-terminal regulatory domain, identified four residues on the extracellular side of the protein (Fig. 4). Cells expressing these *FPS1* alleles survived osmotic up- and down-shocks, but the glycerol efflux rate through Fps1 was strongly diminished, suggesting that these mutations rather affect basal transport capacity than specific regulatory features [63]. Suppression of Fps1 hyperactivity caused by a point mutation (Gln228Ala), however, revealed a more informative mutation of Gly519 to Ser in the centre of TM6. Cells expressing Fps1-Gln228Ala-Gly519Ser are tolerant to hyper- and hypo-osmotic shocks and accumulate and release glycerol with a rate similar to cells expressing wild type Fps1, suggesting that glycerol flux is regulated in this Fps1 mutant [88]. These observations are consistent with a scenario where the N-terminal regulatory domain folds back and influences transmembrane helix positioning and pore structure of Fps1 to fine-tune glycerol transport, somewhat in analogy to the gating mechanisms proposed for PpAqy1 (see above).

3.2.2. Hog1 is a negative regulator of Fps1 activity

There is ample evidence that the Hog1 MAPK controls Fps1 under osmotic stress as well as arsenite and acetic acid exposure. However, the presently available evidence does not provide a coherent picture and the underlying mechanisms by which Hog1 controls Fps1 may differ under different conditions.

The N-terminal regulatory domain contains a conserved MAPK phosphorylation site at position Thr231 (Fig. 4). Upstream from this region, around position 175, there is a potential MAPK docking site (SRRRSRSR) whose function has, however, not been confirmed by mutational studies nor is it conserved in Fps1 orthologous. Fps1 is phosphorylated in vivo and mutation of Thr231 to alanine (T231A) leads to loss of Fps1 phosphorylation and to an unregulated channel with high basal transport activity [90]. Hog1 phosphorylates Fps1 on residue Thr231 in vitro and deletion of *HOG1* reduces in vivo Fps1 phosphorylation by ca 25%. These observations indicate that Hog1 controls basal Fps1 activity and that Hog1 is probably not the only (MAP) kinase that phosphorylates Fps1 on Thr231 [90] (Figs. 4 and 5). However, it has

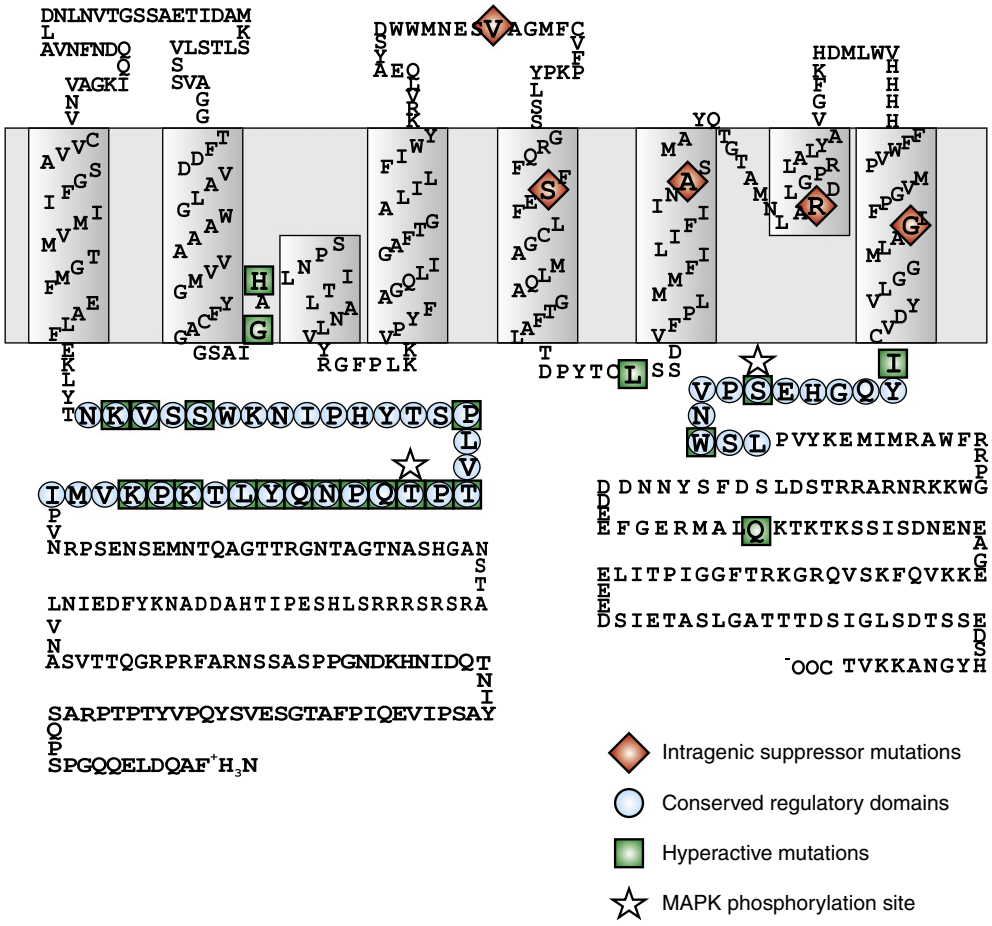

Fig. 4. Topology map of Fps1 highlighting (1) six transmembrane domains, (2) the long N- and C-terminal extensions, (3) residues in those extensions conserved among Fps1 proteins from different yeasts and (4) various sites studied by mutation as explained in the text.

not been demonstrated that Hog1 directly phosphorylates Fps1 under hyperosmotic stress in vivo. Moreover, other proteins appear to be involved in Hog1-mediated Fps1 control, such as Rgc1 and 2 (see further), and Rgc2 is phosphorylated in a Hog1-dependent manner [65]. Taken together, it is presently not known if Hog1 controls Fps1 directly nor if it mediates osmostress-induced closure.

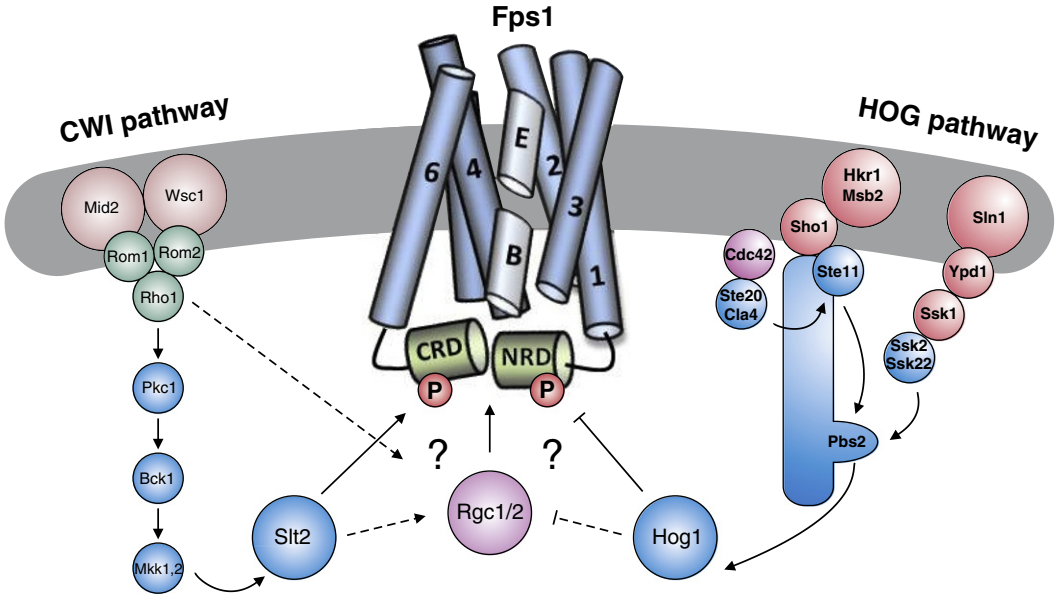

Fig. 5. Fps1 communicates with the two yeast osmosensing pathways, the HOG pathway as well as the cell wall integrity Slt2 pathway. Blue proteins are protein kinases, red proteins are part of sensing complexes and green indicates a G-protein signalling complex. The mechanisms by which Hog1, Slt2 and the Fps1 activators Rgc1 and 2 functional together to control Fps1 and how in turn Fps1 affects the signalling pathways are not fully understood.

Hog1 is also phosphorylated and activated in response to arsenite although in contrast to osmostress-induced Hog1 activation it appears that phospho-Hog1 does not enter the nucleus nor stimulate a transcriptional response under these conditions. Fps1 phosphorylation on Thr231 increases during arsenite exposure and this phosphorylation appears to be critical for restricting arsenite influx and for arsenite tolerance. However, also under these conditions Fps1 phosphorylation appears to be only partly Hog1-independent [90]. A direct connection between activated Hog1 and the activity status of Fps1 under arsenite stress could so far not be established.

Hog1 is also activated upon exposure to acetic acid and under these conditions directly phosphorylates Fps1 on Thr231. In response to acetic acid, Fps1 is probably also phosphorylated on Ser537 located within the C-terminal regulatory domain, but this phosphorylation appears to be independent of Hog1. Acetic acid induced phosphorylation appears to target Fps1 for ubiquitination, endocytosis and vacuolar degradation. This mechanism of Fps1 down-regulation may prevent acetic acid influx and enhance tolerance. Consistent with this model, mutations that abolish endocytosis of Fps1 increase acetic acid sensitivity [86]. Interestingly, Fps1 destabilisation does not occur during hyperosmotic stress, and increasing extracellular osmolarity prior to acetic acid treatment prevents Fps1 degradation [86]. Fps1 is also not degraded during arsenite-exposure and even cells exposed to arsenite for up to 24 h still exhibit Fps1-dependent metalloid transport [90,91]. Thus, Fps1 appears to be subject to different modes of regulation in response to various stress conditions. The mechanistic details remain to be elucidated.

3.2.3. Positive regulators

Two paralogous members of the family of pleckstrin homology (PH) domain proteins, named Regulator of Glycerol Channel (Rgc1 and 2), have recently been identified as positive regulators of Fps1 [65] (Fig. 5). Deletion of *RGC1* and *2* causes permanent cell wall stress, high temperature sensitivity, glycerol hyper-accumulation and generation of fortified cells, similar to the situation in an *fps1Δ* mutant. The *rgc1Δ rgc2Δ gpd1Δ gpd2Δ* mutant, which is unable to produce glycerol, can grow at high temperatures, confirming that glycerol overload causes the temperature sensitivity in *rgc1Δ rgc2Δ* [65]. In the *rgc1Δ rgc2Δ* double mutant, Fps1 is probably in its inactive (closed) form since this strain is unable to export glycerol. The mutant displays elevated levels of Fps1, perhaps as an attempt to compensate for the low glycerol diffusion rate. However, the intensity of the typical Fps1 spots in the plasma membrane does not appear to be altered in an *rgc1Δ rgc2Δ* mutant probably because the fluorescent protein is cleaved from the stabilized Fps1 and digested in the vacuole [65]. The cytoplasmic distribution of Rgc2 very rapidly re-localizes into punctate spots that appear near the cell surface in response to hypo-osmotic shock but do not co-localizes with Fps1. Physical interaction between Rgc1/2 and Fps1 could not be established [65] suggesting that regulation of Fps1 by Rgc1/2 is indirect (Fig. 5). Hog1 appears to act upstream of Rgc1/2, and basal phosphorylation of Rgc2 is Hog1-dependent. In addition to its direct effects on Fps1, it is possible that Hog1 negatively regulates Fps1 also by inhibiting the positive regulators Rgc1 and Rgc2 [65].

Hog1 appears to contribute to inactivation of Fps1 under arsenite stress in order to prevent arsenite uptake. Hence, it was surprising to identify *FPS1* as a multi-copy suppressor of the arsenite sensitivity of the *hog1Δ* mutant. *FPS1* overexpression improves arsenite tolerance by elevating its efflux [91]. We speculated that when Fps1 is present in multiple copies in the cell, it may partially escape Hog1-dependent regulation. One candidate kinase to regulate Fps1-mediated glycerol efflux is the MAPK Slt2. Slt2 is activated by hypo-osmolarity [92], a condition that triggers rapid activation (or opening) of Fps1 to allow glycerol export [46]. In fact, a recent paper provides strong evidence that Slt2 stimulates opening of Fps1 [15]. When yeast cells adapted to high osmolarity are exposed to mating pheromone, Slt2 activation (which is required for formation of mating projections) appears to cause Fps1-mediated glycerol release and subsequent Hog1 activation to re-establish osmotic pressure.

While overexpression of *FPS1* improves arsenite tolerance of wild type cells, this is not the case in *slt2Δ* mutants or in cells expressing a kinase-dead version of Slt2 (our unpublished data). Cells lacking *SLT2* are impaired in Fps1-dependent arsenite export. Mutation of Ser537 to alanine in the C-terminal domain abolishes Fps1 dependent arsenite export to the same extent as deletion of *SLT2*. These observations are consistent with the idea that Slt2 positively affects the export function of Fps1 by phosphorylating Ser537 (Figs. 4 and 5). Hence, it appears that Hog1 may negatively affect Fps1 activity via a phosphorylation site in the N-terminal extension (Thr231) and Slt2 may activate Fps1 via a site in the C-terminal extension (Ser537) (Fig. 5). However, evidence that Fps1 and Slt2 interact in vivo and that Slt2 phosphorylates Fps1 directly has not yet been reported.

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 and attempts to determine 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 regulatory domains affects Fps1-dependent transport, but exactly how this regulation occurs is not understood. It has been suggested that the regulatory domains may dip into the pore and block the channel [63,87]. Also the transmembrane domains contribute to controlling glycerol flux through Fps1 [88]. Phosphorylation and folding of the N- and/or C-terminal regulatory domains may influence helix positioning and pore structure of Fps1, either via direct interactions between the termini and intracellular loops and/or by triggering conformational changes that fine-tune the transport capacity of the protein.

We previously speculated that Fps1 may be regulated by mechanosensitive gating [46]. Rapid osmotic changes result in immediate water influx or efflux, and to limit cellular damage during osmoshocks, regulation of Fps1 activity should ideally be instantaneous. According to this idea, Fps1 would open upon membrane stretch during hypo-osmotic conditions and close following hyperosmotic shock when cells lose volume. Regulation by phosphorylation is likely to be slower than mechanosensitive gating and might be more appropriate for fine-tuning aquaglyceroporin activity under basal conditions and during arsenite and acetic acid stress.

4. Concluding remarks

Over the last years we have learned a great deal about the importance of aquaporins in yeast. It appears that they play roles in freeze tolerance, establishment of dormant survival forms and in determination of cell surface properties for substrate adhesion and formation of cell communities. In all instances the role of aquaporins is supported by phenotypes observed in deletion mutants or aquaporin overexpressing strains and in most cases also by the gene expression pattern and the signalling pathways involved. What we do not know are the underlying mechanisms by which aquaporins confer all those effects. We have presented a number of ideas how transmembrane water transport might contribute to the observed phenomena but these are hypotheses that require experimental testing. Interestingly, all explanations that we offer propose aquaporin-mediated water efflux, not influx. We also do not know if the *S. cerevisiae* water channels are regulated/gated in rapid ways as has been proposed for PpAqy1. Intuitively, one would expect that Aqy1 and 2 are controlled at the level of their activity by gating and/or trafficking but these aspects have so far not been studied.

Bacteria use aquaglyceroporins (glycerol facilitators) for the uptake of glycerol as carbon source. Yeast, at least *S. cerevisiae*, uses a sugar transporter like protein for this purpose. The Fps1 aquaglyceroporin instead plays a central role in controlling glycerol accumulation during osmoadaptation. The use of glycerol as compatible solute as well as Fps1-like proteins in osmoregulation appears to be a speciality of a

confined group of yeasts and may be founded on the high capacity glycolytic metabolism of these organisms. Fps1-like proteins appear to be highly efficient for controlling glycerol accumulation and release since yeast accepts as trade-off an entry route for toxic substances such as metalloids and acetic acid, and established mechanisms to deal with the problem.

We still know rather little molecular and cell biological detail about the regulation of Fps1. It appears that Fps1 is located at the cross road of the two main osmosensing signalling pathways, the HOG pathway and cell wall integrity Slt2 pathway (Fig. 5). Fps1 appears to receive signals from those pathways and it very clearly affects (probably indirectly through its role in glycerol accumulation) signalling through them. But if Hog1 and Slt2 control Fps1 directly or with the help of other proteins and how such control is exerted at the level of Fps1 activity remains to be established. Several Fps1-interacting proteins have recently been identified (some unpublished) and their exact roles need to be elucidated. Despite many years of effort, production, purification and crystallisation of Fps1 have not succeeded so far but a structure may be needed to fully understand the mechanisms regulating Fps1. We believe that studying the details of Fps1 function and regulation will prove rewarding and reveal interesting biological insight with relevance well beyond yeasts.

References

[1] L.S. King, D. Kozono, P. Agre, From structure to disease: the evolving tale of aquaporin biology, Nat. Rev. Mol. Cell Biol. 5 (2004) 687–698.
[2] A.S. Verkman, Aquaporins in clinical medicine, Annu. Rev. Med. 63 (2012) 303–316.
[3] C. Maurel, V. Santoni, D.T. Luu, M.M. Wudick, L. Verdoucq, The cellular dynamics of plant aquaporin expression and functions, Curr. Opin. Plant Biol. 12 (2009) 690–698.
[4] C. Maurel, L. Verdoucq, D.T. Luu, V. Santoni, Plant aquaporins: membrane channels with multiple integrated functions, Annu. Rev. Plant Biol. 59 (2008) 595–624.
[5] D. Botstein, G.R. Fink, Yeast: an experimental organism for 21st Century biology, Genetics 189 (2011) 695–704.
[6] B. Dujon, Yeast evolutionary genomics, Nat. Rev. Genet. 11 (2010) 512–524.
[7] J.E. Haber, Mating-type genes and MAT switching in *Saccharomyces cerevisiae*, Genetics 191 (2012) 33–64.
[8] A.M. Neiman, Sporulation in the budding yeast *Saccharomyces cerevisiae*, Genetics 189 (2011) 737–765.
[9] H. Saito, F. Posas, Response to hyperosmotic stress, Genetics 192 (2012) 289–318.
[10] S. Hohmann, Control of high osmolarity signalling in the yeast *Saccharomyces cerevisiae*, FEBS Lett. 583 (2009) 4025–4029.
[11] S. Hohmann, Osmotic stress signaling and osmoadaptation in yeasts, Microbiol. Mol. Biol. Rev. 66 (2002) 300–372.
[12] E. Petelenz-Kurdziel, C. Kuehn, B. Nordlander, D. Klein, K.K. Hong, T. Jacobson, P. Dahl, J. Schaber, J. Nielsen, S. Hohmann, E. Klipp, Quantitative analysis of glycerol accumulation, glycolysis and growth under hyper osmotic stress, PLoS Comput. Biol. 9 (2013) e1003084.
[13] D.E. Levin, Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway, Genetics 189 (2011) 1145–1175.
[14] R.E. Chen, J. Thorner, Function and regulation in MAPK signaling pathways: lessons learned from the yeast *Saccharomyces cerevisiae*, Biochim. Biophys. Acta 1773 (2007) 1311–1340.
[15] R. Baltanas, A. Bush, A. Couto, L. Durrieu, S. Hohmann, A. Colman-Lerner, Pheromone-induced morphogenesis improves osmoadaptation capacity by activating the HOG MAPK pathway, Sci. Signal. 6 (2013) ra26.
[16] G. Soveral, C. Prista, T.F. Moura, M.C. Loureiro-Dias, Yeast water channels: an overview of orthodox aquaporins, Biol. Cell 103 (2010) 35–54.
[17] N. Pettersson, C. Filipsson, E. Becit, L. Brive, S. Hohmann, Aquaporins in yeasts and filamentous fungi, Biol. Cell 97 (2005) 487–500.
[18] J.L. Will, H.S. Kim, J. Clarke, J.C. Painter, J.C. Fay, A.P. Gasch, Incipient balancing selection through adaptive loss of aquaporins in natural *Saccharomyces cerevisiae* populations, PLoS Genet. 6 (2010) e1000893.
[19] J.M. Carbrey, M. Bonhivers, J.D. Boeke, P. Agre, Aquaporins in *Saccharomyces*: characterization of a second functional water channel protein, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 1000–1005.
[20] M. Bonhivers, J.M. Carbrey, S.J. Gould, P. Agre, Aquaporins in *Saccharomyces*. Genetic and functional distinctions between laboratory and wild-type strains, J. Biol. Chem. 273 (1998) 27565–27572.
[21] V. Laize, F. Tacnet, P. Ripoche, S. Hohmann, Polymorphism of *Saccharomyces cerevisiae* aquaporins, Yeast 16 (2000) 897–903.
[22] V. Meyrial, V. Laize, R. Gobin, P. Ripoche, S. Hohmann, F. Tacnet, Existence of a tightly regulated water channel in *Saccharomyces cerevisiae*, Eur. J. Biochem. 268 (2001) 334–343.
[23] V. Laize, R. Gobin, G. Rousselet, C. Badier, S. Hohmann, P. Ripoche, F. Tacnet, Molecular and functional study of AQY1 from *Saccharomyces cerevisiae*: role of the C-terminal domain, Biochem. Biophys. Res. Commun. 257 (1999) 139–144.
[24] J.E. Karpel, L.F. Bisson, Aquaporins in *Saccharomyces cerevisiae* wine yeast, FEMS Microbiol. Lett. 257 (2006) 117–123.
[25] J.M. Carbrey, B.P. Cormack, P. Agre, Aquaporin in *Candida*: characterization of a functional water channel protein, Yeast 18 (2001) 1391–1396.
[26] A. Tanghe, P. Van Dijck, F. Dumortier, A. Teunissen, S. Hohmann, J.M. Thevelein, Aquaporin expression correlates with freeze tolerance in baker's yeast, and overexpression improves freeze tolerance in industrial strains, Appl. Environ. Microbiol. 68 (2002) 5981–5989.
[27] A. Tanghe, G. Kayingo, B.A. Prior, J.M. Thevelein, P. Van Dijck, Heterologous aquaporin (AQY2-1) expression strongly enhances freeze tolerance of *Schizosaccharomyces pombe*, J. Mol. Microbiol. Biotechnol. 9 (2005) 52–56.
[28] A. Tanghe, J.M. Carbrey, P. Agre, J.M. Thevelein, P. Van Dijck, Aquaporin expression and freeze tolerance in *Candida albicans*, Appl. Environ. Microbiol. 71 (2005) 6434–6437.
[29] G. Fischer, U. Kosinska-Eriksson, C. Aponte-Santamaria, M. Palmgren, C. Geijer, K. Hedfalk, S. Hohmann, B.L. de Groot, R. Neutze, K. Lindkvist-Petersson, Crystal structure of a yeast aquaporin at 1.15 angstrom reveals a novel gating mechanism, PLoS Biol. 7 (2009) e1000130.
[30] A. Tanghe, P. Van Dijck, D. Colavizza, J.M. Thevelein, Aquaporin-mediated improvement of freeze tolerance of *Saccharomyces cerevisiae* is restricted to rapid freezing conditions, Appl. Environ. Microbiol. 70 (2004) 3377–3382.
[31] G.M. Preston, T.P. Carroll, W.B. Guggino, P. Agre, Appearance of water channels in *Xenopus oocytes* expressing red cell CHIP28 protein, Science 256 (1992) 385–387.
[32] G. Soveral, A. Veiga, M.C. Loureiro-Dias, A. Tanghe, P. Van Dijck, T.F. Moura, Water channels are important for osmotic adjustments of yeast cells at low temperature, Microbiology 152 (2006) 1515–1521.
[33] F. Sidoux-Walter, N. Pettersson, S. Hohmann, The *Saccharomyces cerevisiae* aquaporin Aqy1 is involved in sporulation, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17422–17427.
[34] J.V. Gray, G.A. Petsko, G.C. Johnston, D. Ringe, R.A. Singer, M. Werner-Washburne, "Sleeping beauty": quiescence in *Saccharomyces cerevisiae*, Microbiol. Mol. Biol. Rev. 68 (2004) 187–206.
[35] K. Furukawa, F. Sidoux-Walter, S. Hohmann, Expression of the yeast aquaporin Aqy2 affects cell surface properties under the control of osmoregulatory and morphogenic signalling pathways, Mol. Microbiol. 74 (2009) 1272–1286.
[36] P.J. Cullen, G.F. Sprague Jr., The regulation of filamentous growth in yeast, Genetics 190 (2012) 23–49.
[37] V. St'ovicek, L. Vachova, M. Kuthan, Z. Palkova, General factors important for the formation of structured biofilm-like yeast colonies, Fungal Genet. Biol. 47 (2010) 1012–1022.
[38] M. Kuthan, F. Devaux, B. Janderova, I. Slaninova, C. Jacq, Z. Palkova, Domestication of wild *Saccharomyces cerevisiae* is accompanied by changes in gene expression and colony morphology, Mol. Microbiol. 47 (2003) 745–754.
[39] Z. Palkova, L. Vachova, Life within a community: benefit to yeast long-term survival, FEMS Microbiol. Rev. 30 (2006) 806–824.
[40] K. Hedfalk, Further advances in the production of membrane proteins in *Pichia pastoris*, Bioengineered 4 (2013).
[41] U. Kosinska Eriksson, G. Fischer, R. Friemann, G. Enkavi, E. Tajkhorshid, R. Neutze, Subangstrom resolution X-ray structure details aquaporin–water interactions, Science 340 (2013) 1346–1349.
[42] S. Tornroth-Horsefield, K. Hedfalk, G. Fischer, K. Lindkvist-Petersson, R. Neutze, Structural insights into eukaryotic aquaporin regulation, FEBS Lett. 584 (2010) 2580–2588.
[43] G. Soveral, A. Madeira, M.C. Loureiro-Dias, T.F. Moura, Membrane tension regulates water transport in yeast, Biochim. Biophys. Acta 1778 (2008) 2573–2579.
[44] F. Oberg, K. Hedfalk, Recombinant production of the human aquaporins in the yeast *Pichia pastoris*, Mol. Membr. Biol. 30 (2013) 15–31.
[45] R. Wysocki, C.C. Chery, D. Wawrzycka, M. Van Hulle, R. Cornelis, J.M. Thevelein, M.J. Tamas, The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in *Saccharomyces cerevisiae*, Mol. Microbiol. 40 (2001) 1391–1401.
[46] M.J. Tamas, K. Luyten, F.C. Sutherland, A. Hernandez, J. Albertyn, H. Valadi, H. Li, B.A. Prior, S.G. Kilian, J. Ramos, L. Gustafsson, J.M. Thevelein, S. Hohmann, Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation, Mol. Microbiol. 31 (1999) 1087–1104.
[47] A. Nozawa, J. Takano, M. Kobayashi, N. von Wiren, T. Fujiwara, Roles of *BOR1*, *DUR3*, and *FPS1* in boron transport and tolerance in *Saccharomyces cerevisiae*, FEMS Microbiol. Lett. 262 (2006) 216–222.
[48] A. Shepherd, P.W. Piper, The Fps1p aquaglyceroporin facilitates the use of small aliphatic amides as a nitrogen source by amidase-expressing yeasts, FEMS Yeast Res. 10 (2010) 527–534.
[49] S.E. Beese-Sims, J. Lee, D.E. Levin, Yeast Fps1 glycerol facilitator functions as a homotetramer, Yeast 28 (2011) 815–819.
[50] R.M. Bill, K. Hedfalk, S. Karlgren, J.G. Mullins, J. Rydstrom, S. Hohmann, Analysis of the pore of the unusual major intrinsic protein channel, yeast Fps1p, J. Biol. Chem. 276 (2001) 36543–36549.
[51] R. Oliveira, F. Lages, M. Silva-Graca, C. Lucas, Fps1p channel is the mediator of the major part of glycerol passive diffusion in *Saccharomyces cerevisiae*: artefacts and re-definitions, Biochim. Biophys. Acta 1613 (2003) 57–71.
[52] A.P. Gasch, P.T. Spellman, C.M. Kao, O. Carmel-Harel, M.B. Eisen, G. Storz, D. Botstein, P.O. Brown, Genomic expression programs in the response of yeast cells to environmental changes, Mol. Biol. Cell 11 (2000) 4241–4257.
[53] L. Van Aelst, S. Hohmann, F.K. Zimmermann, A.W. Jans, J.M. Thevelein, 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 (1991) 2095–2104.
[54] B. Teusink, M.C. Walsh, K. van Dam, H.V. Westerhoff, The danger of metabolic pathways with turbo design, Trends Biochem. Sci. 23 (1998) 162–169.

[55] J.M. Thevelein, S. Hohmann, Trehalose synthase: guard to the gate of glycolysis in yeast? Trends Biochem. Sci. 20 (1995) 3–10.
[56] S. Hohmann, M.J. Neves, W. de Koning, R. Alijo, J. Ramos, J.M. Thevelein, The growth and signalling defects of the *ggs1* (*fdp1*/*byp1*) deletion mutant on glucose are suppressed by a deletion of the gene encoding hexokinase PII, Curr. Genet. 23 (1993) 281–289.
[57] K. Luyten, J. Albertyn, W.F. Skibbe, B.A. Prior, J. Ramos, J.M. Thevelein, S. Hohmann, 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 (1995) 1360–1371.
[58] C. Gancedo, J.M. Gancedo, A. Sols, Glycerol metabolism in yeasts. Pathways of utilization and production, Eur. J. Biochem. 5 (1968) 165–172.
[59] C. Ferreira, F. van Voorst, A. Martins, L. Neves, R. Oliveira, M.C. Kielland-Brandt, C. Lucas, A. Brandt, A member of the sugar transporter family, Stl1p is the glycerol/H + symporter in *Saccharomyces cerevisiae*, Mol. Biol. Cell 16 (2005) 2068–2076.
[60] J. Tulha, A. Lima, C. Lucas, C. Ferreira, *Saccharomyces cerevisiae* glycerol/H + symporter Stl1p is essential for cold/near-freeze and freeze stress adaptation. A simple recipe with high biotechnological potential is given, Microb. Cell Fact. 9 (2010) 82.
[61] M. Rep, M. Krantz, J.M. Thevelein, S. Hohmann, 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 (2000) 8290–8300.
[62] F.C. Sutherland, F. Lages, C. Lucas, K. Luyten, J. Albertyn, S. Hohmann, B.A. Prior, S.G. Kilian, Characteristics of Fps1-dependent and -independent glycerol transport in *Saccharomyces cerevisiae*, J. Bacteriol. 179 (1997) 7790–7795.
[63] M.J. Tamas, S. Karlgren, R.M. Bill, K. Hedfalk, L. Allegri, M. Ferreira, J.M. Thevelein, J. Rydstrom, J.G. Mullins, S. Hohmann, A short regulatory domain restricts glycerol transport through yeast Fps1p, J. Biol. Chem. 278 (2003) 6337–6345.
[64] A.H. Tong, G. Lesage, G.D. Bader, H. Ding, H. Xu, X. Xin, J. Young, G.F. Berriz, R.L. Brost, M. Chang, Y. Chen, X. Cheng, G. Chua, H. Friesen, D.S. Goldberg, J. Haynes, C. Humphries, G. He, S. Hussein, L. Ke, N. Krogan, Z. Li, J.N. Levinson, H. Lu, P. Menard, C. Munyana, A.B. Parsons, O. Ryan, R. Tonikian, T. Roberts, A.M. Sdicu, J. Shapiro, B. Sheikh, B. Suter, S.L. Wong, L.V. Zhang, H. Zhu, C.G. Burd, S. Munro, C. Sander, J. Rine, J. Greenblatt, M. Peter, A. Bretscher, G. Bell, F.P. Roth, G.W. Brown, B. Andrews, H. Bussey, C. Boone, Global mapping of the yeast genetic interaction network, Science 303 (2004) 808–813.
[65] S.E. Beese, T. Negishi, D.E. Levin, Identification of positive regulators of the yeast fps1 glycerol channel, PLoS Genet. 5 (2009) e1000738.
[66] S. Bjorkqvist, R. Ansell, L. Adler, G. Liden, Physiological response to anaerobicity of glycerol-3-phosphate dehydrogenase mutants of *Saccharomyces cerevisiae*, Appl. Environ. Microbiol. 63 (1997) 128–132.
[67] R. Ansell, K. Granath, S. Hohmann, J.M. Thevelein, L. Adler, 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 (1997) 2179–2187.
[68] E. Albers, C. Larsson, G. Liden, C. Niklasson, L. Gustafsson, Influence of the nitrogen source on *Saccharomyces cerevisiae* anaerobic growth and product formation, Appl. Environ. Microbiol. 62 (1996) 3187–3195.
[69] J. Philips, I. Herskowitz, Osmotic balance regulates cell fusion during mating in *Saccharomyces cerevisiae*, J. Cell Biol. 138 (1997) 961–974.
[70] T.H. Toh, G. Kayingo, M.J. van der Merwe, S.G. Kilian, J.E. Hallsworth, S. Hohmann, B.A. Prior, Implications of *FPS1* deletion and membrane ergosterol content for glycerol efflux from *Saccharomyces cerevisiae*, FEMS Yeast Res. 1 (2001) 205–211.
[71] M. Siderius, O. Van Wuytswinkel, K.A. Reijenga, M. Kelders, W.H. Mager, The control of intracellular glycerol in *Saccharomyces cerevisiae* influences osmotic stress response and resistance to increased temperature, Mol. Microbiol. 36 (2000) 1381–1390.
[72] S. Izawa, M. Sato, K. Yokoigawa, Y. Inoue, Intracellular glycerol influences resistance to freeze stress in *Saccharomyces cerevisiae*: analysis of a quadruple mutant in glycerol dehydrogenase genes and glycerol-enriched cells, Appl. Microbiol. Biotechnol. 66 (2004) 108–114.
[73] S. Izawa, K. Ikeda, K. Maeta, Y. Inoue, Deficiency in the glycerol channel Fps1p confers increased freeze tolerance to yeast cells: application of the fps1delta mutant to frozen dough technology, Appl. Microbiol. Biotechnol. 66 (2004) 303–305.
[74] M. Banerjee, N. Banerjee, P. Bhattacharjee, D. Mondal, P.R. Lythgoe, M. Martinez, J. Pan, D.A. Polya, A.K. Giri, High arsenic in rice is associated with elevated genotoxic effects in humans, Sci. Rep. 3 (2013) 2195.
[75] D. Beyersmann, A. Hartwig, Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms, Arch. Toxicol. 82 (2008) 493–512.
[76] D.K. Nordstrom, Public health. Worldwide occurrences of arsenic in ground water, Science 296 (2002) 2143–2145.
[77] F. Frezard, C. Demicheli, R.R. Ribeiro, Pentavalent antimonials: new perspectives for old drugs, Molecules 14 (2009) 2317–2336.
[78] P.J. Dilda, P.J. Hogg, Arsenical-based cancer drugs, Cancer Treat. Rev. 33 (2007) 542–564.
[79] S.V. Isayenkov, F.J. Maathuis, The *Arabidopsis thaliana* aquaglyceroporin AtNIP7;1 is a pathway for arsenite uptake, FEBS Lett. 582 (2008) 1625–1628.
[80] G.P. Bienert, M. Thorsen, M.D. Schussler, H.R. Nilsson, A. Wagner, M.J. Tamas, T.P. Jahn, A subgroup of plant aquaporins facilitate the bi-directional diffusion of As(OH)3 and Sb(OH)3 across membranes, BMC Biol. 6 (2008) 26.
[81] Z. Liu, J. Shen, J.M. Carbrey, R. Mukhopadhyay, P. Agre, B.P. Rosen, Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 6053–6058.
[82] A. Ramirez-Solis, R. Mukopadhyay, B.P. Rosen, T.L. Stemmler, Experimental and theoretical characterization of arsenite in water: insights into the coordination environment of As–O, Inorg. Chem. 43 (2004) 2954–2959.
[83] O.I. Sanders, C. Rensing, M. Kuroda, B. Mitra, B.P. Rosen, Antimonite is accumulated by the glycerol facilitator GlpF in *Escherichia coli*, J. Bacteriol. 179 (1997) 3365–3367.
[84] A. Porquet, M. Filella, Structural evidence of the similarity of Sb(OH)3 and As(OH)3 with glycerol: implications for their uptake, Chem. Res. Toxicol. 20 (2007) 1269–1276.
[85] M.C. Teixeira, N.P. Mira, I. Sa-Correia, A genome-wide perspective on the response and tolerance to food-relevant stresses in *Saccharomyces cerevisiae*, Curr. Opin. Biotechnol. 22 (2011) 150–156.
[86] M. Mollapour, P.W. Piper, Hog1 mitogen-activated protein kinase phosphorylation targets the yeast Fps1 aquaglyceroporin for endocytosis, thereby rendering cells resistant to acetic acid, Mol. Cell. Biol. 27 (2007) 6446–6456.
[87] K. Hedfalk, R.M. Bill, J.G. Mullins, S. Karlgren, C. Filipsson, J. Bergstrom, M.J. Tamas, J. Rydstrom, S. Hohmann, A regulatory domain in the C-terminal extension of the yeast glycerol channel Fps1p, J. Biol. Chem. 279 (2004) 14954–14960.
[88] C. Geijer, D. Ahmadpour, M. Palmgren, C. Filipsson, D.M. Klein, M.J. Tamas, S. Hohmann, K. Lindkvist-Petersson, Yeast aquaglyceroporins use the transmembrane core to restrict glycerol transport, J. Biol. Chem. 287 (2012) 23562–23570.
[89] S. Karlgren, C. Filipsson, J.G. Mullins, R.M. Bill, M.J. Tamas, S. Hohmann, Identification of residues controlling transport through the yeast aquaglyceroporin Fps1 using a genetic screen, Eur. J. Biochem. 271 (2004) 771–779.
[90] M. Thorsen, Y. Di, C. Tangemo, M. Morillas, D. Ahmadpour, C. Van der Does, A. Wagner, E. Johansson, J. Boman, F. Posas, R. Wysocki, M.J. Tamas, The MAPK Hog1p modulates Fps1p-dependent arsenite uptake and tolerance in yeast, Mol. Biol. Cell 17 (2006) 4400–4410.
[91] E. Maciaszczyk-Dziubinska, I. Migdal, M. Migocka, T. Bocer, R. Wysocki, The yeast aquaglyceroporin Fps1p is a bidirectional arsenite channel, FEBS Lett. 584 (2010) 726–732.
[92] K.R. Davenport, M. Sohaskey, Y. Kamada, D.E. Levin, M.C. Gustin, A second osmosensing signal transduction pathway in yeast. Hypotonic shock activates the PKC1 protein kinase-regulated cell integrity pathway, J. Biol. Chem. 270 (1995) 30157–30161.