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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

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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*

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* 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

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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.

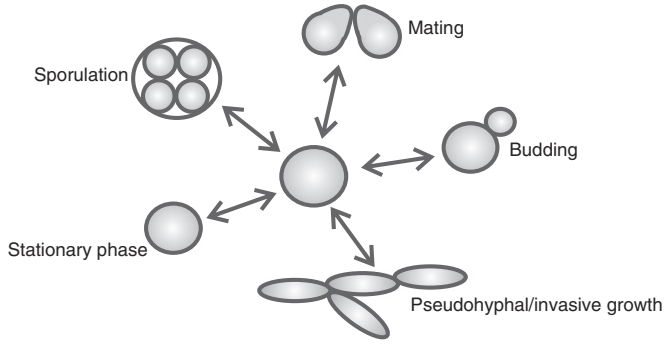

Fig. 1. Yeast cell types and morphological adaptations. Haploid as well as diploid yeast cells can multiply by budding. Under specific starvation conditions cells can form pseudohyphae and/or invade the substrate. Haploid cells of different mating types can mate and form a diploid cell. Under both nitrogen 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