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MiniReview

Metabolic surprises in *Saccharomyces cerevisiae* during adaptation to saline conditions: questions, some answers and a model

Anders Blomberg *

Dept. of Cell and Molecular Biology, Microbiology Lundberg Laboratory, Göteborg University, Medicinaregatan 9c, 413 19, Göteborg, Sweden

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Abstract

This review describes the metabolic alterations and adaptations of yeast cells in response to osmotic stress. The basic theme of the cellular response is known to be exclusion of the extracellular stress agent salt and intracellular accumulation of the compatible solute glycerol. Molecular details of these basic processes are currently rather well known. However, analysis of expression changes during adaptation to salt has revealed a number of metabolic surprises. These include the induced expression of genes involved in glycerol dissimilation as well as trehalose turnover. The physiological rationale for these responses to osmotic stress is discussed. A model is presented in which it is hypothesised that the two pathways function as glycolytic safety valves during adaptation to stress.

Keywords: Yeast; Osmoregulation; Glycerol; Trehalose; Futile cycles

Sodium chloride is present in variable concentration in most natural environments. Thus, selection has strongly favoured variants that efficiently and adequately can adjust to various degrees of salinity. Recently, an extraordinary quantity of valuable molecular information has been obtained about how cells can sense and intracellularly transmit information about the availability in external water. Researchers in this area have conducted detailed studies on salinity-induced signal transduction cascades which are making an important contribution to many fields of biology. However, our understanding of the physiological role of the target genes of these signalling paths is, in many instances, meagre. Some of these salinity responders will be the focus of this review. For more detailed information about osmotic stress-imposed changes in signal transduction or its impact on the general physiology of yeast I encourage readers to explore other more focused reviews [1–5].

1. Osmotic challenge – some of the basics

Addition of high amounts of sugar or salt to a growing culture of yeast stops growth. This rapid impact on proliferation is explained by the fact that this treatment rapidly dehydrates cells, through a purely osmotic process (Fig. 1A). It is disputed whether the loss in turgor or in cell volume would be the main factor determining growth arrest, and considerable effort has been invested in determining which of these two factors is of most general importance. It should be realised, however, that the quantitative effect on cell turgor or volume by osmotic dehydration will differ between different species and is dependent on, among other cellular features, the elasticity of the cell wall [2]. Dehydration is a rapid process and, for most cell systems, it is completed within about a minute. It has long been envisaged that water efflux would be mediated solely through the lipid bilayer. However, since *Saccharomyces cerevisiae* has genes which encode proteins with homology to well-characterised water channels in other organisms [6], it is tempting to hypothesise these putative channels to be involved in water efflux.

Dehydrated cells must regain turgor and/or volume in order to survive and grow. Cells eventually die at extremes of saline stress [7]. However, at physiologically acceptable solute concentrations, cell proliferation resumes after a period of adaptation. The length of this adaptive phase can vary considerably, depending on a number of factors, including the type and concentration of stress solute, the strain of yeast, and the state of growth [8]. It is clear that the main theme in this cellular reprogramming is exclusion

* Corresponding author. E-mail: Anders.Blomberg@gmm.gu.se

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from the cell of a harmful solute like NaCl [9], and the intracellular accumulation of a more compatible solute; in yeast mainly the polyhydroxyalcohol, glycerol [1,2]. Great progress has been made in our understanding of the efflux systems for sodium in yeast, which appear to be largely governed by salt-stimulated production of the P-type ATPase Ena1p and the sodium-proton antiporter Nha1p [10,9].

The salt-stimulated accumulation of glycerol leads to lowered osmotic potential of the cytoplasm and re-entry of some of the lost water (Fig. 1B). The turgor and/or volume parameters of the cell would thus increase toward growth promoting levels. Different species of yeast employ different physiological strategies in this adaptive response. The marine yeast *Debaryomyces hansenii* alters the retention of glycerol via activation of an active uptake system for glycerol [11]. Other species without salt activated uptake systems for glycerol, like *S. cerevisiae*, increase their production of glycerol in order to maintain a proper level of accumulation. This latter strategy was in the past referred to as 'the American method' – plentiful production and wasteful use [1]. However, the identification and characterisation of a glycerol-specific channel protein in the plasma membrane of *S. cerevisiae*, Fps1p, which has been proven to be a regulated glycerol facilitator involved in the release of accumulated glycerol upon dilution stress [12,4], challenges this simplified view.

The key enzyme in glycerol production in *S. cerevisiae* appears to be the first enzyme redirecting the flux in glycolysis into glycerol, namely glycerol-3-phosphate dehydrogenase (GPD) (Fig. 2), since it was reported that this enzyme exhibits high controlling capacity in glycerol formation [13]. The two genes, *GPD1* and *GPD2*, encoding this enzyme activity were later cloned and characterised [14,15], although the only one induced by osmotic stress is *GPD1* [16].

Thus, it appears as if the above-mentioned cellular features would be the most important mechanisms for adaptation to dehydration in yeast. Does that also mean that they are the only cellular stress responders? To find out, global analysis of changes in gene expression under osmotic stress has been conducted. Quantitative protein expression analysis by two-dimensional gel electrophoresis (2D-PAGE) separates more than 1000 cellular proteins on the basis of size (M_r) and charge differences (p*I*) [17]. Since it has been estimated that approximately 4000 genes are expressed under 'normal' growth conditions, two-dimensional gel electrophoresis provides an analytic tool for roughly 25% of the expressed genes. These analyses showed an astonishing cellular plasticity during adaptation to dehydration. During the adaptation to 0.7 M NaCl medium more than 200 proteins displayed significant expression changes [18]. However, when classifying expression changes according to their response magnitude, large changes in expression were almost exclusively apparent for induced proteins; at least 18 proteins exhibited salt-induced expression by a factor greater than 8 while only two proteins were repressed to the same extent. That means that the cell primarily responds to these conditions by adding new features to the cellular machinery and not by rapidly removing existing ones.

The pertinent question was then, of course, the biochemical identity of these highly responsive proteins. At least some of them should be expected to be functionally involved in the main osmoregulatory features already outlined above: glycerol accumulation and salt extrusion. This was indeed shown to be the case since one osmoresponsive protein was identified by microsequencing as Gpd1p. In addition, sequence analysis of another salt-induced protein revealed it to be encoded by the functionally uncharacterised open reading frame *YER062C*. Biochemical characterisation of the glycerol-3-phosphate phosphatase in *S. cerevisiae* clearly linked the salt-induced *YER062C* encoded protein (named Gpp2p) to this enzymatic activity and revealed a totally new protein class of small phosphatases [19]. The *YER062C*/*GPP2* gene was also identified among cDNAs for hyperosmolarity responsive (HOR) genes by differential screening [20]. An isogene to *GPP2* has also been characterised, namely *GPP1*, which was found less responsive to salt [19]. Thus, under these conditions of salt-stimulated increased glycerol production there is an upregulation of one of the isogenes in each of the enzymatic steps involved in glycerol synthesis; *GPD1* and *GPP2* (Fig. 2). Such a salt-increased production of these proteins is logical and in line with an increased glycerol flux.

2. Glycerol production – is that all that there is?

Another salt-induced protein was identified as the product of the *YML070W* gene [21]. The full length sequence of this salt-induced protein exhibits a high sequence similarity to an enzyme in the bacterium *Citrobacter freundii* that has been biochemically characterised as a dihydroxyacetone kinase (DAK). DAK enzymatic activity had earlier not been unequivocally identified in *S. cerevisiae* under standard growth conditions, but was clearly found to be under salt regulation [21], strongly indicating a link between the DAK enzymatic activity and the *YML070W* gene product (subsequently named Dak1p). This link has recently been confirmed by genetic and biochemical studies, where in addition it was found that its isogene *DAK2*/*YFL053W* seemed to be of less physiological importance (Norbeck, Molin and Blomberg, unpublished result). The functional role of the DAK protein in cellular physiology has been characterised in some other yeast species: DAK has been shown to be involved in the assimilation of methanol via the xylulose monophosphate cycle for formaldehyde fixation in *Hansenula polymorpha* [22]. Its role in *S. cerevisiae* was, however, not really clear, particularly under these stress conditions.

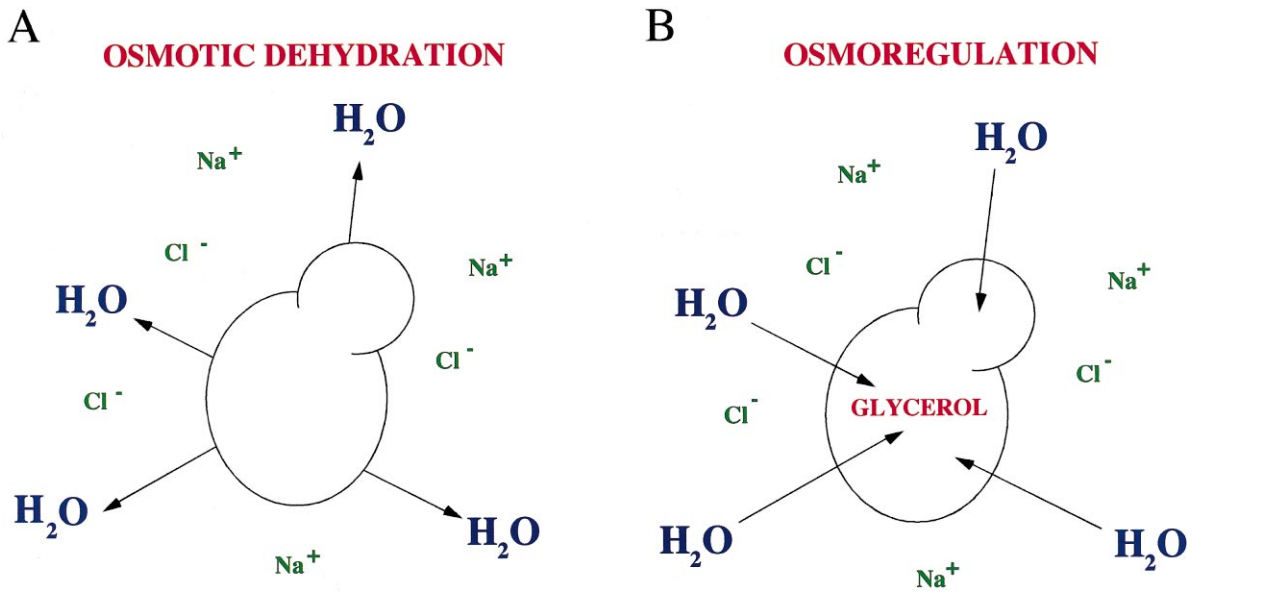

Fig. 1. Key features of the cellular response in yeast to saline conditions. A: The initial response of the cell is efflux of water, which leads to cell shrinkage and growth arrest. B: The main osmoregulatory strategy of the cell is (i) exclusion of the salt via ion pumps, and (ii) regain lost water, and thus turgor and/or volume, by increased production and accumulation of glycerol.

A salt regulated DAK activity has also been recorded in *Zygosaccharomyces rouxii*, in conjunction with an increased glycerol dehydrogenase (GLD) activity [23]. GLD catalyses the conversion of glycerol into dihydroxyacetone. Attempts were made to experimentally verify by in vitro activity measurements that a GLD also existed in *S. cerevisiae*, but without success [21]. Sequence analysis of the yeast genome did not reveal any homologue to the bacterial GLD counterpart. However, the bacterial GLD is involved in the catabolic utilisation of glycerol and strictly requires NADH. In contrast, one of the characterised fungal enzymes was from *Aspergillus niger*, and this fungal GLD is clearly not dependent on NADH but on NADPH. Sequencing of peptides for the GLD enzyme from *A. niger* enabled homology searches in the yeast genome, where a number of putative GLD-encoding genes was identified [21]. All these were annotated in the database as aldo-keto reductases, thus providing good candidates for a glycerol dehydrogenase in *S. cerevisiae*. Analysis at the transcript level clearly showed one of these, *GCY1*, to be a salt upregulated gene [21]. This salt-induced induction of both the kinase Dak1p and the putative dehydrogenase Gcy1p was a great surprise, since this would probably indicate that not only the path to glycerol was salt activated but that also the dissimilation of this important osmolyte was favoured under salt stress. What could be the reason for this potential glycerol turnover? A number of more or less intriguing hypotheses has been put forward, including a transhydrogenase activity converting one molecule of NADH to NADPH. However, it should be pointed out here that this glycerol turnover going from dihydroxyacetone phosphate, via glycerol-3-phosphate, glycerol, dihydroxyacetone and back again would be at the expense of one ATP (Fig. 2).

3. Trehalose – only stress protection?

Another metabolite, the disaccharide trehalose, accumulates during salt adaptation [24,25]. Trehalose accumulates in the cells in response to a number of stress conditions, and has been shown to protect cells against high temperatures by stabilising proteins [26] and against cellular desiccation by helping to both stabilise proteins [27] and to maintain membrane integrity [28]. However, trehalose does not accumulate under salt stress to levels that would have a major impact on the osmotic properties of the cells [29]; in this regard the increased amount of glycerol seems by far the most important factor.

Increased production of trehalose is reflected in the reported salt-induced expression of the enzymes in the biosynthetic pathway from glucose-6-phosphate to trehalose. Expression analysis during salt stress revealed a dramatic increase in the expression of *TPS1*, which encodes a trehalose-6-phosphate synthase [30,31,25,32]. It is interesting to observe that under quite a number of growth limiting conditions besides high salinity, e.g. heat and oxidative stress, the expression of the Tps1 enzyme is enhanced. In addition, under oxidative stress the expression of other enzymes in the path to trehalose is also induced [33]. This includes the two enzymes, Ugp1p and Pgm2p, that are involved in the production of UDP-glucose, which is one of the substrates for trehalose-6-phosphate production via the Tps1 protein. The *UGP1* and *PGM2* genes have

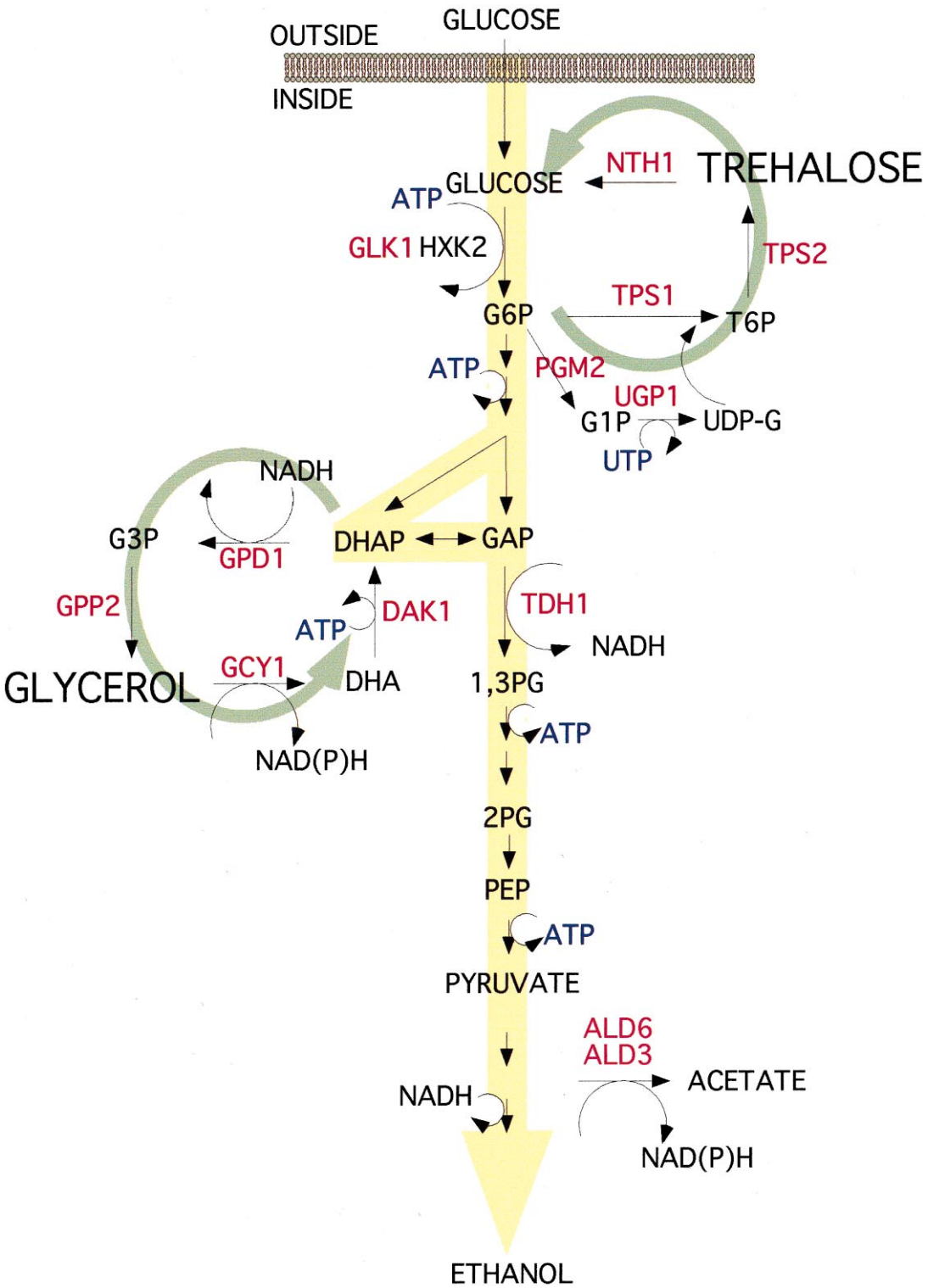

Fig. 2. The central metabolism of yeast depicting a model for stress-imposed activation of ATP futile cycles by trehalose and glycerol turnover (green circles). Salt-induced changes in gene expression during adaptation to salt are indicated in red and placed in their metabolic context. See text for further information.

recently also been shown to be induced during adaptation to salt (Rep, Krantz, Thevelein and Hohmann, unpublished result). Even the gene encoding the last enzyme in the pathway to trehalose, trehalose-6-phosphatase (*TPS2*), has been reported to exhibit salt-stimulated expression [30].

The upregulation of genes involved in trehalose synthesis is interesting and logical if the main purpose of this

cellular exercise is to produce and accumulate high amounts of trehalose. However, it has recently been argued that the main metabolic rationale for an increased flux in this path would be the enhanced accumulation of the metabolite trehalose-6-phosphate (T6P). This compound has inhibitory action on two of the three isoforms of the first enzymatic step in glycolysis, the hexokinases Hxk1p and Hxk2p [34]. T6P has thus been proposed to exhibit the potential to act as a gate keeper in glycolysis via feedback inhibition [35]. This hypothesis appears especially valid since a *tps1*Δ strain, with no production of trehalose-6-phosphate, exhibits unregulated glycolytic flux and accumulates high amounts of sugar phosphates [36].

However, there are three experimental observations that challenge the completeness of that picture. First, in a *nth1*Δ strain, where the dominant form of the trehalose-degrading enzyme trehalase (Nth1p) has been deleted, there was a dramatic increase in the accumulation of trehalose compared to the wild-type during adaptation to salt stress [25]. This indicates not only trehalose accumulation but also salt-stimulated trehalose turnover during salt adaptation, which in the *nth1*Δ mutant results in enhanced trehalose build-up. Secondly, further support for stress-stimulated trehalose turnover is found in the fact that not only the enzymes involved in the production of trehalose but also the *NTH1* encoded trehalase expression is salt induced [25]. Thirdly, under adaptation to salt the sugar kinase enzyme with the strongest inhibition by trehalose-6-phosphate, Hxk2p, exhibits dramatically decreased expression (Norbeck and Blomberg, unpublished data) while another sugar kinase, Glk1p, is strongly induced [20]. The striking thing is that the activity of the Glk1 protein is not inhibited at all by the metabolite trehalose-6-phosphate [34]. Thus, the consequence of Hxk2p being repressed and Glk1p being induced would be diminished flux control on glycolysis from trehalose-6-phosphate under these stress conditions. In summary, as for glycerol there is evidence for salt-stimulated turnover of the stress responsive metabolite trehalose. Once again this turnover would be at the expense of ATP (Fig. 2), since going from glucose, to glucose-6-phosphate, trehalose and back again to glucose will effectively consume three ATP molecules. The Ugp1 protein utilises UTP instead of ATP for the activation of the glucose moiety, but these two nucleotide pools communicate via the exchange factor Ura6p [37].

4. Stress-imposed metabolic changes – a model

Do two ATP-dependent systems for glycerol and trehalose turnover make physiological sense during salt adaptation? At first sight it might appear inefficient of the cell to waste ATP under conditions of stress. However, I will argue below that under suddenly imposed stress conditions this is a highly appropriate metabolic response.

Actively proliferating cells that experience an osmotic dehydration will face rapid growth retardation, if not growth arrest. One of the consequences of this rapid decrease in macromolecular synthesis [18] is a dramatic decrease in the ATP demand from biosynthesis. It has recently been proposed by elegant theoretical considerations from models of glycolysis that under conditions of growth arrest, with low ATP demand, cells face the threat of substrate-accelerated death [35]. The reason for this is the 'turbo' design of glycolysis, where two molecules of ATP are invested in the upper part of the glycolysis pathway yielding a total outcome of four ATP molecules in the lower part, per glucose utilised. This increased net production of ATP will stimulate the flux in the upper part of glycolysis, and thus leads to an imbalance in the rates in the upper and lower part. The theoretical result in this non-regulated case is a dramatic and steady accumulation of the intermediates hexose phosphate and fructose-1,6-bisphosphate, probably leading ultimately to phosphate depletion and cell death. This scenario is supported by experimental data and has been proposed to be the mechanistic explanation for the lethality of the *tps1*Δ strain in glucose media [38].

A remedy to this problem was shown theoretically to be a decrease in activity in sugar kinase activity, either by decreased enzyme amounts or by feedback inhibition. Experimental support for this is found in the fact that a deletion of the *HXK2* gene suppresses the glucose lethality of the *tps1*Δ strain [39]. Increased production of the Glk1 protein during osmotic adaptation could have a similar consequence since this isoform has been shown to display a much lower V_{max} compared to Hxk2p [34]. To immediately stop glycolysis would be an alternative remedy to this ATP overproduction problem. However, there is a conflict, since cells need to osmoregulate by glycerol production and thus require a flux in glycolysis; both DHAP and NADH are substrates in the formation of glycerol. However, a metabolic change that would equally well counteract the stress-instigated metabolic ATP imbalance, would be to increase the ATP demand by introducing ATP futile cycles. Thus, I propose an osmo stress-imposed activation of ATP futile cycles via trehalose and glycerol turnover, as glycolytic safety valves, to avoid substrate-accelerated death under stress (Fig. 2). In fact, a similar model has been proposed for glycerol on the basis of the experimentally observed salt-stimulated 'energy spillage' during chemostat culturing of fungi [40].

There is experimental evidence for trehalose and glycerol turnover during cellular adaptation to different environmental stresses [41,25,33]. In addition, the expressions of trehalase and dihydroxyacetone kinase are induced by a number of other stresses besides osmotic dehydration [42,33,43]. Thus, the proposed salt stress-activated ATP

futile cycles might be of general importance during cellular stress adaptation. Trehalose turnover during heat stress has already been suggested as essential for stress adaptation, in the context of maintenance of a constant glucose concentration in the cytosol [41].

Occurrence of glycolytic safety valves does not, however, exclude the involvement of other control mechanisms for glycolytic flux during adaptation to osmotic stress, such as: (i) the trehalose-6-phosphate inhibition of hexokinase which will most probably be operational, at least during the first part of the adaptation period where Hxk2 is the predominant form of sugar kinase, and (ii) increased accumulation of glycerol and trehalose *per se* acts to drain the lower part of the glycolysis pathway from triose phosphates, which will also improve the ATP balance. In addition, accumulation of both glycerol and trehalose has clearly beneficial implications in the protection of cellular structures in responses to stress. The protein and membrane stabilising properties of trehalose have already been mentioned [28,26,27] and glycerol is clearly instrumental in adjusting the water potential of yeast cells [2]. However, it should also be stressed that glycerol appears to have unique properties when it comes to protein stabilisation based on its preferential exclusion from the protein surface [44]. The evolution of metabolic systems fine-tuned for sudden environmental changes could have occurred by simultaneous selection and optimisation of pathways that at the same time produces important stress protectants as well as provides glycolytic safety valves. In this context both glycerol and trehalose appear to be ideal cases.

It is interesting to note that these two futile cycles display one main difference, in that the glycerol path also involves redox-coupled reactions. The balance in the use of the two cycles could thus be linked to the overall redox state of the cell. In this context it will be of importance to biochemically characterise the cofactor and substrate requirements of the putative glycerol dehydrogenase Gcy1p and the other members of the aldo/keto reductase family, as well as the cofactor requirement for some other stress responders (see below).

The model proposed here has yet to be theoretically evaluated and experimentally verified. However, some experimental support is found in the observation that overexpression of *GPD1* suppresses a *tps1*Δ strain [12]. A possible explanation for this suppression would thus be increased glycerol turnover, resulting in an enhanced ATP futile cycle, that would relieve some of the ATP problems of the *tps1*Δ mutant. The implication would be that this effect in *tps1*Δ by *GPD1* overexpression would not be effective in a *tps1*Δ *dak1*Δ *dak2*Δ strain, where the ATP futile cycle linked to glycerol turnover can not be operational. The model also raises the question of what is being sensed in metabolism and thus would trigger the response. The ATP concentration is a good candidate, since the ATP imbalance is at the core of the problem. In addition, the theoretical modelling indicates that in unguarded glycolysis the concentration of ATP falls to very low levels [35]. An important regulatory circuit in yeast is the RAS-PKA (protein kinase A) pathway, which has been implicated in overall nutrient sensing in this organism [45]. The intracellular concentration of cAMP plays a central role in this pathway, and since cAMP is produced from ATP, the concentration of ATP could be directly reflected in the cyclic AMP pool. This secondary signalling molecule binds to the negative regulatory subunit of the PKA complex, Bcy1p, releasing it from the complex and thus allowing high PKA activity. Low ATP could thus result in low cAMP, which would lead to low overall PKA activity. In this context it is interesting to note that the expressions of *TPS1* and *GCY1*, two key components in the two proposed futile cycles, appear solely dependent on the cellular level of PKA and are both induced by low levels of PKA [32].

5. More surprises – links to the cellular redox state

Are there any more metabolic surprises revealed by expression analyses? Some intriguing examples of differential expression of isogenes in glycolysis are not fully understood. It is surprising that the minor form of glyceraldehyde-3-phosphate dehydrogenase is strongly upregulated [31,32]. The salt-induced expression of this isogene, *TDH1*, is particularly intriguing in light of the fact that the other two isogenes, *TDH2* and *TDH3*, are two of the most highly expressed genes in the cell [17]. Both these proteins have been rather extensively studied [46] and their biochemistry is well understood. However, the *TDH1* induction clearly points to gaps in our understanding of the functional relations of these three isogenic products. It would be surprising if the increased expression of Tdh1p only reflected a generally increased need for enhanced overall activity, since this enzymatic step in glycolysis is believed to have an over-capacity even under basal growth conditions, and nothing indicates an increased overall glycolytic flux during osmotic stress.

Another enzymatic activity influenced by osmotic stress, and that also takes part in the cellular redox balance, is acetaldehyde dehydrogenase. This enzymatic activity is believed to be encoded by a large family including six gene members, two of which are regulated during adaptation to osmotic stress (*ALD3* and *ALD6*) [31,32]. The main cytoplasmic form of this enzyme under normal growth conditions is Ald6p, which has been functionally characterised and shown to involve the production of NADPH. However, under osmotic shock there is a reported increase in the NADH-dependent ALD activity [13]. This could in fact be the result of the increased production of the Ald3p (YMR169c) protein, which would then indicate another cofactor requirement for this isoform.

6. Final note – increasing complexity

Our understanding of the molecular physiology of the osmotic stress response in yeast has undergone rather extraordinary refinements during the last decade. The picture we have today of salt-instigated yeast physiology is more complex and far from the simplified 'glycerol in, salt out' description of the eighties. The understanding of the metabolic complexity has mainly originated from genetic analysis of mutants and expression analysis by proteomics. However, the recent advent of DNA microarray technology [47] and its use in future large-scale transcript analysis during saline stress conditions will most certainly identify new and intriguing responders to osmotic stress. To properly interpret the complete molecular repertoire involved in these cellular programs during adaptation to stress, will be a tremendous but also very exciting challenge. A good understanding of the cellular implications of the currently identified salt responses, some of which have been discussed in this review, will be an important prerequisite in that future pursuit. Furthermore, many of the stress responders to be identified in the future will belong to the large family of functionally uncharacterised genes, which ultimately has to be placed in a cellular context. The model presented here will hopefully provide a framework within which additional future functional information can be placed. The data presented here also underscore the importance of solid biochemical studies into the detailed functional differences between differentially expressed isogenes. Taken together, studies in adaptive responses to osmotic stress will most likely also in the future provide ample examples of metabolic surprises. Proper theoretical models and a better understanding of metabolic networks in operation in yeast will then be instrumental, if generated data are not only to formulate questions but also to provide some answers.

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References

[1] Brown, A.D. (1978) Compatible solute and extreme water stress in eukaryotic micro-organisms. Adv. Microb. Phys. 17, 181–242.
[2] Blomberg, A. and Adler, L. (1992) Physiology of osmotolerance in fungi. Adv. Microb. Phys. 33, 145–212.
[3] Varela, J.C.S. and Mager, W.H. (1996) Response of *Saccharomyces cerevisiae* to changes in external osmolarity. Microbiology 142, 721–731.
[4] Hohmann, S. (1997) Shaping up: the response of yeast to osmotic stress. In: Yeast Stress Responses (Hohmann, S. et al., Eds.), pp. 101–146. Springer, New York.
[5] Gustin, M.C., Albertyn, J., Alexander, M. and Davenport, K. (1998) MAP kinase pathways in the yeast *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. 62, 1264–1300.
[6] Bonhivers, M., Carbrey, J.M., Gould, S.J. and Agre, P. (1998) Aquaporins in Saccharomyces. Genetic and functional distinctions between laboratory and wild-type strains. J. Biol. Chem. 273, 27565–27572.
[7] Blomberg, A. (1997) The osmotic hypersensitivity of the yeast Saccharomyces cerevisiae is strain and growth media dependent: quantitative aspects of the phenomenon. Yeast 13, 529–539.
[8] Blomberg, A., Larsson, C. and Gustafsson, L. (1988) Microcalorimetric monitoring of growth of *Saccharomyces cerevisiae*: Osmotolerance in relation to physiological state. J. Bacteriol. 170, 4562–4568.
[9] Serrano, R., Marquez, J.A. and Rios, G. (1997) Crucial factors in salt stress tolerance. In: Yeast Stress Responses (Hohmann, S. et al., Eds.), pp. 147–170. Springer, New York.
[10] Prior, C., Potier, S., Souciet, J.L. and Sychrova, H. (1996) Characterization of the NHA1 gene encoding a Na+/H+-antiporter of the yeast Saccharomyces cerevisiae. FEBS Lett. 387, 89–93.
[11] Adler, L., Blomberg, A. and Nilsson, A. (1985) Glycerol metabolism and osmoregulation in the salt-tolerant yeast *Debaryomyces hansenii*. J. Bacteriol. 162, 300–306.
[12] Luyten, K., Albertyn, J., Skibbe, W.F., Prior, B.A., Ramos, J., Thevelein, J.M. and 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.
[13] Blomberg, A. and Adler, L. (1989) Roles of glycerol and glycerol-3-phosphate dehydrogenase (NAD^+) in acquired osmotolerance of *Saccharomyces cerevisiae*. J. Bacteriol. 171, 1087–1092.
[14] Larsson, K., Ansell, R., Eriksson, P. and Adler, L. (1993) A gene encoding *sn*-glycerol 3-phosphate dehydrogenase (NAD^+) complements an osmosensitive mutant of *Saccharomyces cerevisiae*. Mol. Microbiol. 10, 1101–1111.
[15] Albertyn, J., Hohmann, S., Thevelein, J.M. and Prior, B.A. (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.
[16] Eriksson, P., André, L., Ansell, R., Blomberg, A. and Adler, L. (1995) Cloning and characterisation of *GPD2*, a second gene encoding *sn*-glycerol 3-phosphate dehydrogenase (NAD^+) in *Saccharomyces cerevisiae*, and its comparison with *GPD1*. Mol. Microbiol. 17, 95–107.
[17] Norbeck, J. and Blomberg, A. (1997) Two-dimensional electrophoretic separation of yeast proteins using a non-linear wide range (pH 3–10) immobilized pH gradient in the first dimension; reproducibility and evidence for isoelectric focusing of alkaline ($pI > 7$) proteins. Yeast 13, 529–539.
[18] Blomberg, A. (1995) Global changes in protein synthesis during adaptation to 0.7 M NaCl medium of *Saccharomyces cerevisiae*. J. Bacteriol. 177, 3563–3572.
[19] Norbeck, J., Påhlmann, A.-K., Akhtar, N., Blomberg, A. and 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, 13875–13883.
[20] Hirayama, T., Maeda, T., Saito, H. and Shinozaki, K. (1995) Cloning and characterization of seven cDNAs for hyperosmolarity-responsive (*HOR*) genes of *Saccharomyces cerevisiae*. Mol. Gen. Genet. 249, 127–138.

[21] Norbeck, J. and Blomberg, A. (1997) Metabolic and regulatory changes associated with growth of *Saccharomyces cerevisiae* in 1.4 M sodium chloride: Evidence for osmotic induction of glycerol dissimilation via the dihydroxyacetone pathway. J. Biol. Chem. 272, 5544–5554.

[22] Kato, N., Yoshikawa, H., Tanaka, K., Shimao, M. and Sakazawa, C. (1988) Dihydroxyacetone kinase from a methylotrophic yeast, *Hansenula polymorpha* CBS 4732. Arch. Microbiol. 150, 155–159.

[23] van Zyl, P.J., Prior, B.A. and Kilian, S.G. (1991) Regulation of glycerol metabolism in *Zygosaccharomyces rouxii* in response to osmotic stress. Appl. Microbiol. Biotechnol. 36, 369–374.

[24] Meikle, A.J., Chudek, J.A., Reed, R.H. and Gadd, G.M. (1991) Natural abundance ^{13}C nuclear magnetic resonance spectroscopic analysis of acyclic polyol and trehalose accumulation by several yeast species in response to salt stress. FEMS Microbiol. Lett. 82, 163.

[25] Parrou, J.L., Teste, M.-A. and Francois, J. (1997) Effects of various types of stress on the metabolism of reserve carbohydratesin *Saccharomyces cerevisiae*: genetic evidence for a stress-induced recycling of glycogen and trehalose. Microbiology 143, 1891–1900.

[26] Singer, M.A. and Lindquist, S. (1998) Multiple effects of trehalose on protein folding in vitro and in vivo. Mol. Cell. 1, 639–648.

[27] Allison, S.D., Chang, B., Randolph, T.W. and Carpenter, J.F. (1999) Hydrogen bonding between sugar and protein is responsible for inhibition of dehydration-induced protein unfolding. Arch. Biochem. Biophys. 356, 289–298.

[28] Crowe, J.H., Crowe, L.M. and Chapman, D. (1984) Preservation of membranes in anhydrobiotic organisms: the role of trehalose. Science 223, 701–703.

[29] Ölz, R., Larsson, K., Adler, L. and Gustafsson, L. (1993) Energy flux and osmoregulation of *Saccharomyces cerevisiae* grown in chemostat under NaCl stress. J. Bacteriol. 175, 2205–2213.

[30] Winderickx, J.H.d.W.J., Crauwels, M., Hino, A., Hohmann, S., Van Dijck, P. and Thevelein, J.M. (1996) Regulation of genes encoding subunits of the trehalose synthase complex in *Saccharomyces cerevisiae*: novel variations of STRE-mediated transcription control? Mol. Gen. Genet. 252, 470–482.

[31] Akhtar, N., Blomberg, A. and Adler, L. (1997) Osmoregulation and protein expression in a *pbs2*Δ mutant of *Saccharomyces cerevisiae* during adaptation to hypersaline stress. FEBS Lett. 403, 173–180.

[32] Norbeck, J. and Blomberg, A. (1999) The level of cAMP dependent protein kinase A activity strongly affects osmotolerance and osmo instigated gene expression changes in *Saccharomyces cerevisiae*. Yeast, in press.

[33] Godon, C., Lagniel, G., Lee, J., Buhler, J.-M., Kieffer, S., Perrot, M., Boucherie, H., Toledano, M.B. and Labarre, J. (1998) The H2O2 stimulon in *Saccharomyces cerevisiae*. J. Biol. Chem. 273, 22480–22489.

[34] Blazquez, M.A., Lagunas, R., Gancedo, C. and Gancedo, J.M. (1993) Trehalose-6-phosphate, a new regulator of yeast glycolysis that inhibits hexokinases. FEBS Lett. 329, 51–54.

[35] Teusink, B., Walsh, M.C., van Dam, K. and Westerhoff, H.V. (1998) The danger of metabolic pathways with turbo design. TIBS 23, 162–169.

[36] Neves, M.J., Hohmann, S., Bell, W., Dumortier, F., Luyten, K., Ramos, J., Cobbaert, P., de Koning, W., Kaneva, Z. and Thevelein, J.M. (1995) Control of glucose influx into glycolysis and pleiotropic effects studied in different isogenic sets of *Saccharomyces cerevisiae* mutants in trehalose biosynthesis. Curr. Genet. 27, 110–122.

[37] Jong, A., Yeh, Y. and Ma, J.J. (1993) Characteristics, substrate analysis, and intracellular location of Saccharomyces cerevisiae UMP kinase. Arch. Biochem. Biophys. 304, 197–204.

[38] Van Aelst, T., Hohmann, S., Zimmermann, F.K., Jans, A.W.H. and Thevelein, J.M. (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.

[39] Hohmann, S., Neves, M.J., de Koning, W., Alijo, R., Ramos, J. and Thevelein, J.M. (1993) 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, 281–289.

[40] Jennings, D.H. and Burke, R.M. (1990) Compatible solutes - the mycological dimension and their role as physiological buffering agents. N. Phytol. 116, 277.

[41] Hottiger, T., Schmutz, P. and Wiemken, A. (1987) Heat-induced accumulation and cycling of trehalose in *Saccharomyces cerevisiae*. J. Bacteriol. 169, 5518–5522.

[42] Zähringer, H., Burgert, M., Holzer, H. and Nwaka, S. (1997) Neutral trehalase Nth1p of *Saccharomyces cerevisiae* encoded by the *NTH1* gene is a multiple stress responsive protein. FEBS Lett. 412, 615–620.

[43] Jelinsky, S.A. and Samson, L.D. (1999) Global responses of Saccharomyces cerevisiae to an alkylating agent. Proc. Natl. Acad. Sci. USA 96, 1486–1491.

[44] Timasheff, S.N. (1995) Solvent stabilization of protein structure. Methods Mol. Biol. 40, 253–269.

[45] de Winde, J.H., Thevelein, J.M. and Winderickx, J. (1997) From feast to famine: asaptation to nutrient depletion in yeast. In: Yeast Stress Responses (Hohmann, S. et al., Eds.), pp. 7–52. Springer, New York.

[46] McAlister, L. and Holland, M.J. (1985) Isolation and characterization of yeast strains carrying mutations in the glyceraldehyde-3-phosphate dehydrogenase genes. J. Biol. Chem. 260, 15013–15018.

[47] DeRisi, J.L., Lyer, V.R. and Brown, P.O. (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278, 680–686.