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REVIEW ARTICLE

Response of *Saccharomyces cerevisiae* to changes in external osmolarity

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Overview

A few years ago, knowledge about the molecular and genetic details of osmoregulatory processes was confined to prokaryotes (reviewed by Csonka, 1989; Csonka & Hanson, 1991). Recently, however, numerous reports have been published describing genes, or their products, involved in the response of eukaryotic organisms to changes in the external osmolarity. Because of its genetic tractability, the budding yeast *Saccharomyces cerevisiae* has been the organism of choice for the isolation and characterization of components that may have functional counterparts in higher eukaryotes. In addition, *S. cerevisiae* is used in industrial processes (e.g. dough leavening), where tolerance to high salt and sugar as well as other stresses is essential (Reed & Nagodawithana, 1991). This has led to an increasingly better understanding of how yeast senses and responds to osmotic and ionic stress (previously reviewed by Mager & Varela, 1993).

Sensing of and responding to osmotic stress

Hyperosmotic stress

The earliest event upon transfer of yeast cells to conditions of high osmolarity (equivalent to salt concentrations above about 0·3 M) is the almost instantaneous loss of cytoplasmic water. This sudden dehydration is partially compensated by a rapid influx of water from the vacuole (Blomberg & Adler, 1992; Latterich & Watson, 1992). This net loss of water results in a decrease or even abolishment of the turgor pressure exerted by the plasma membrane upon the cell wall, depending upon the severity of the stress (Blomberg & Adler, 1992). At a later stage, glycerol accumulates in the cytoplasm up to molar concentrations, this being a long-term osmoregulatory strategy widely used by fungi and other organisms (e.g. algae) in order to counteract cell dehydration. However, other mechanisms protecting yeast from changes in the external osmolarity are likely to exist (see below).

Two models can be envisioned as to how yeast senses osmostress: (i) intracellularly, e.g. by sudden increase in the concentration of a solute (e.g. Ca^{2+}); or (ii) extracellularly, e.g. by activation/inhibition of a plasma-membrane-bound receptor.

Recently, model (ii) has received experimental support through the identification of an osmosensing transmembrane protein, Sln1p (Maeda *et al.*, 1994). *SLN1* was initially isolated in a synthetic lethality screen with *UBR1*, a gene implicated in ubiquitin-dependent proteolytic processes (Ota & Varshavsky, 1993). *SLN1* is an essential gene for growth on rich medium, showing strong similarities with the 'two-component' regulatory systems that respond to a variety of environmental stimuli in prokaryotes (Ota & Varshavsky, 1993). These systems are typically composed of a membrane-bound 'sensor' and a cytosolic 'regulator' (Fig. 1a). Upon stimulation, histidine autophosphorylation of the sensor molecule is induced. Subsequently, the kinase transfers the phosphate group to an aspartate residue on the response regulator, modulating its activity (Fig. 1a).

Unlike the prokaryotic two-component systems, the eukaryotic homologues described so far do not relay the signal directly to the transcriptional machinery but rather to a regulator of a protein kinase cascade. This cascade may eventually regulate transcriptional activation (Hughes, 1994). In the *SLN1*-mediated pathway, the regulator corresponds to Ssk1p (Fig. 1b). High osmolarity appears to inhibit phosphorylation of Ssk1p by Sln1p (Maeda *et al.*, 1994). It has been proposed that the unphosphorylated form of Ssk1p activates a pair of serine-threonine kinases, Ssk2p and Ssk22p, two yeast homologues of mitogen-activated protein (MAP) kinase kinase

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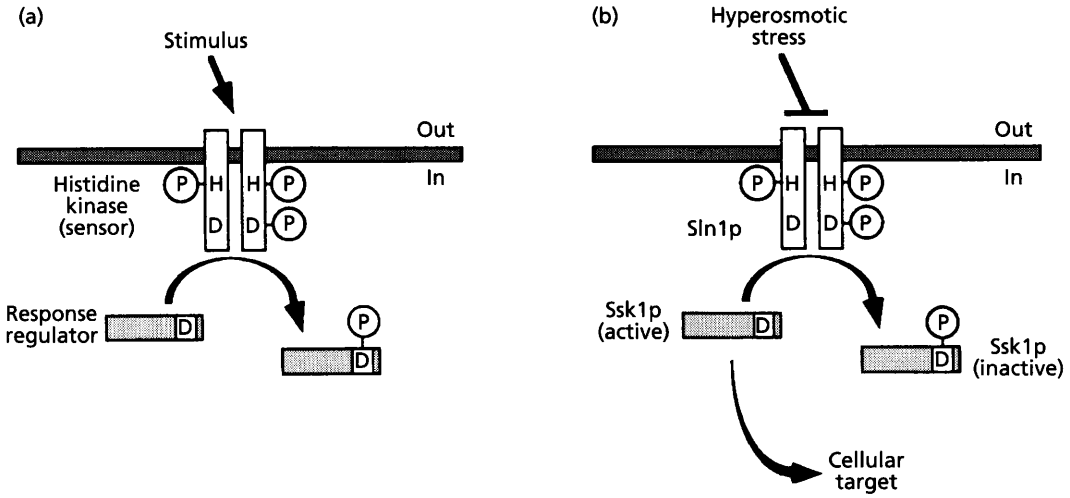

Fig. 1. Two-component systems. (a) Two-component systems consist of a 'sensor' and a 'response regulator'. Upon stimulation, the histidine autophosphorylation activity of the sensor molecule (shown as a homodimer) is activated. Subsequently, phosphotransfer to an aspartate residue of the sensor and/or of the response regulator takes place. This phosphorylation may then modulate the activity of both the sensor and the response regulator. The plasma membrane is depicted as a grey bar. (b) In yeast, the activity of the sensor molecule, Sln1p, is repressed by high osmolarity, preventing the phosphorylation and inactivation of the response regulator, Ssk1p (modified from Hughes, 1994).

kinases (MAPKKKs) of mammalian cells (Fig. 2). In turn, Ssk2p and Ssk22p appear to stimulate tyrosine phosphorylation on Hog1p, a yeast homologue of the mammalian MAP kinases (MAPKs), by Pbs2p, a MAPK kinase (MAPKK) homologue (Fig. 2; Brewster *et al.*, 1993; Maeda *et al.*, 1995). Recently, it has been found that Pbs2p binds to a second putative transmembrane protein, Sho1p (Maeda *et al.*, 1995). The interaction between a proline-rich motif of Pbs2p and the SH3 domain present in Sho1p seems to result in the activation of the MAPKK upon a shift to high osmolarity (Fig. 2). Deletions in *HOG1* or *PBS2* or the simultaneous disruption of *SSK2*, *SSK22* and *SHO1* produce an osmosensitive phenotype and (at least in the former cases) was shown to lead to the accumulation of lower amounts of glycerol in the cell, thus confirming the essential role played by the *SLN1*- and *SHO1*-mediated pathways [also known as the high-osmolarity glycerol (HOG) pathway] in sensing osmostress (Brewster *et al.*, 1993; Maeda *et al.*, 1994, 1995). This conclusion is further supported by the finding that *GPD1*, encoding the key enzyme for glycerol production, glycerol-3-phosphate dehydrogenase, is under the regulation of this osmosensing signal transduction pathway (Albertyn *et al.*, 1994). In addition, activation of several general stress-responsive genes, such as *CTT1* (encoding cytosolic catalase; Marchler *et al.*, 1993) and *HSP12* (Varela *et al.*, 1992, 1995), are triggered through the HOG pathway. In the latter case, gene activation by the HOG pathway is apparently mediated by the CCCCT-motif (STRE, stress-responsive element; Marchler *et al.*, 1993; Varela *et al.*, 1995; Mager & De Kruijff, 1995).

STREs are *cis*-acting elements occurring in the promoter regions of many stress-related genes (summarized by Varela *et al.*, 1995; Mager & De Kruijff, 1995) and responsive to a broad range of stresses, including high osmolarity. Detailed analysis of the *HSP12* promoter revealed that multiple STREs are required for full activation under stress circumstances (Varela *et al.*, 1995). Since the HOG pathway appears to be specifically involved in signalling increases in external osmolarity, it is thus likely that multiple stress-signalling pathways are targeted to the STRE. Tyrosine phosphorylation of Hog1p has been shown to occur within 1 min after the onset of the osmotic stress, and immediately afterwards *CTT1* mRNAs were found to accumulate (Schüller *et al.*, 1994). Combined with the dramatically reduced levels of transcription of *CTT1*, *HSP12* and *DDR2* (implicated in DNA damage repair) observed in *hog1* or *pbs2* mutants (Schüller *et al.*, 1994; Varela *et al.*, 1995), the data are consistent with the idea that osmostress-induced activation via STREs is directly triggered by Hog1p. Very recently, Msn2p and Msn4p, two Mig1p-like polypeptides (Estruch & Carlson, 1993), have been identified as STRE-binding factors (Ruis & Schüller, 1995). Consistent with this, Msn2p and Msn4p have been shown to be essential for the stress-inducibility of the *HSP12* and *HSP26* genes (Martínez-Pastor & Estruch, 1995). The possibility of Mig1p-like factors regulating the expression of stress-inducible genes is further strengthened by the presence of sequences resembling Mig1p-binding sites which overlap functional STREs in the *HSP12* promoter (Varela *et al.*, 1995).

Hypo-osmotic stress

Upon a downshift in the external osmolarity, the yeast cell ceases accumulating glycerol, and rather releases this polyol into the medium (Blomberg & Adler, 1992). Glycerol efflux appears to be mediated by a glycerol

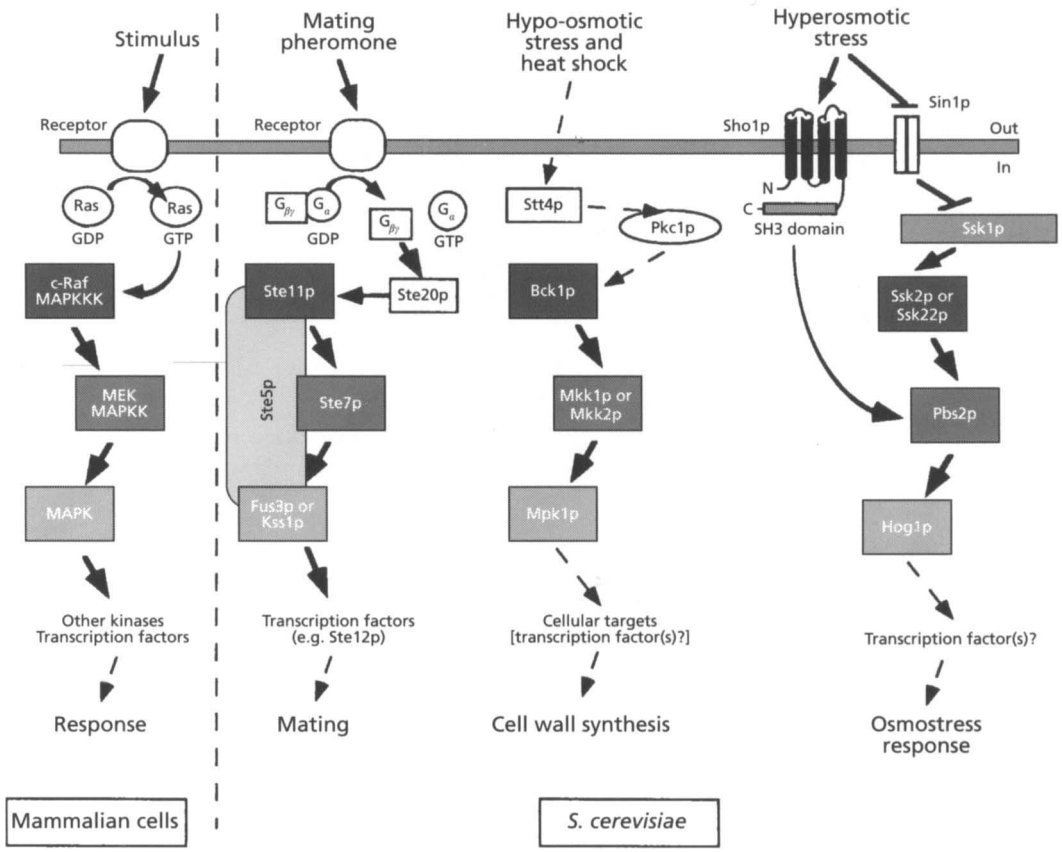

Fig. 2. **Mitogen-activated protein (MAP) kinase pathways. MAP kinase (MAPK) cascades are typically composed of a MAPK, a MAPK kinase (MAPKK) and a MAPKK kinase (MAPKKK). MAPKs and MAPKKKs correspond to serine/threonine protein kinases, whereas MAPKKs are serine/threonine-tyrosine-directed protein kinases. Identical shades of grey indicate homology among protein kinases. Components operating upstream of the MAPK cascade are given as boxes, rounded rectangles and ellipses with a white background, except for Sho1p and Ssk1p. The plasma membrane is depicted as a grey bar.**

facilitator, Fps1p, which is inactive in cells exposed to hyperosmotic stress (Luyten *et al.*, 1995). Fps1p, however, is apparently not regulated by the HOG pathway. Instead, the activity of Fps1p may be modulated by protein kinase C (encoded by *PKC1*), an enzyme thought to regulate a MAPK cascade involved in signalling hypo-osmotic stress (Thevelein, 1994; Gustin *et al.*, 1995).

The MAPK cascade regulated by Pkc1p (*PKC1* pathway) consists of a MAPKKK (Bck1p), a pair of functionally redundant MAPKKs (Mkk1p and Mkk2p), and a MAPK (Mpk1p) (Fig. 2; Torres *et al.*, 1991; Lee & Levin, 1992; Irie *et al.*, 1993; Lee *et al.*, 1993b; reviewed by Thevelein, 1994; Herskowitz, 1995). Besides hypo-osmotic stress, components of the *PKC1* pathway have been reported to signal heat stress and (lack of) nutrients, the latter in a cAMP- independent manner (Costigan & Snyder, 1994; Gustin *et al.*, 1995; Kamada *et al.*, 1995). Accordingly, mutations in this pathway produce lytic growth defects which are suppressed by osmotic stabilizers (e.g. sorbitol; Torres *et al.*, 1991; Costigan *et al.*, 1992; Lee & Levin, 1992; Levin & Bartlett-Heubusch, 1992; Paravicini *et al.*, 1992; Irie *et al.*, 1993; Lee *et al.*, 1993b; Martín *et al.*, 1993; Posas *et al.*, 1993; Shimizu *et al.*, 1994; Yoshida *et al.*, 1994a, b, 1995). Mutations in *PKC1* result in a lethal phenotype at all temperatures (Levin & Bartlett-Heubusch, 1992; Paravicini *et al.*, 1992). However, cells defective in the kinases operating downstream of Pkc1p exhibit a lethal growth defect at 37 °C (Lee & Levin, 1992; Irie *et al.*, 1993; Lee *et al.*, 1993b; Martín *et al.*, 1993). The more severe phenotype of *pkc1* cells has led to the conclusion that Pkc1p may have targets other than Bck1p. Nevertheless, the thermosensitive phenotype of these mutants is in agreement with the activation of the *PKC1* pathway by heat shock (Kamada *et al.*, 1995).

In addition to promoting protein denaturation, hypo-osmotic stress and heat stress may have similar effects on the structure of the cell wall and membrane fluidity. In both cases, the cell wall is weakened and the membrane stretches, probably due to an increase in turgor pressure (Kamada *et al.*, 1995). Interestingly, the *PKC1* pathway seems to regulate cell morphogenesis and growth by modulating the synthesis of enzymes involved in the cell wall assembly (Fig. 2; Costigan *et al.*, 1992; Mazzoni *et al.*, 1993; Roemer *et al.*, 1994; Shimizu *et al.*, 1994). Since

Pkc1p is a homologue of the mammalian Ca^{2+}-responsive, phospholipid-dependent protein kinases, it has been proposed that the *PKC1* pathway may be activated by stress through an influx of Ca^{2+} (Kamada *et al.*, 1995). This ion movement could be caused by the activation of mechanosensitive ion channels (responsive to membrane stretching) previously described by Gustin *et al.* (1988). This model may be supported by the apparent involvement of Ca^{2+} in the regulation of growth and stress tolerance in yeast (see below).

Stress-signalling pathways and substrate specificity

The widespread occurrence in eukaryotic cells of highly homologous protein kinase cascades is intriguing (Kosako *et al.*, 1993; Lange-Carter *et al.*, 1993; Cano & Mahadevan, 1995; Herskowitz, 1995). From yeast to mammals, MAPK cascades transduce signals triggered by diverse environmental stimuli, such as nutrients, mating pheromones, plant hormones (ethylene), growth factors, heat shock, UV irradiation, hyperosmotic- and hypo-osmotic stress (Dubois & Bensaude, 1993; Kosako *et al.*, 1993; Lange-Carter *et al.*, 1993; Blumer & Johnson, 1994; Costigan & Snyder, 1994; Galcheva-Gargova *et al.*, 1994; Han *et al.*, 1994; Hughes, 1994; Rouse *et al.*, 1994; Terada *et al.*, 1994; Thevelein, 1994; Cano & Mahadevan, 1995; Gustin *et al.*, 1995; Herskowitz, 1995; Shiozaki & Russell, 1995). Although the significance of the reiteration of such protein kinases 'modules' is presently not known (see Herskowitz, 1995), it has been suggested that the sequential activation of kinases serves two purposes, namely (i) rapid signal amplification and (ii) multiple signal integration via interaction (cross-talk) among components of different pathways (Maeda *et al.*, 1995).

In yeast, diverse MAPK cascades operate in parallel, being activated by different stimuli (Fig. 2). Substrate-specificity is apparently achieved by anchoring proteins, such as Ste5p (Fig. 2), which confine the kinases of a particular pathway to a defined multiprotein complex, thereby avoiding promiscuous activation (Cano & Mahadevan, 1995; Herskowitz, 1995). Other possible anchoring proteins regulating protein kinases as well as phosphatases may correspond to the 14-3-3 proteins. Like the components of the MAPK cascades, the 14-3-3 proteins are highly conserved throughout evolution (Ferl *et al.*, 1994; Aitken, 1995). This family of acidic proteins binds and possibly regulates diverse protein kinases (e.g. protein kinase C and Raf, a mammalian homologue of MAPKKK), whereas other 14-3-3 isotypes participate in DNA-binding complexes in plants (Ferl *et al.*, 1994; Aitken, 1995). With the recent elucidation of the structure of the 14-3-3 dimers, it became clear that these polypeptides may act as 'adapter' proteins (Liu *et al.*, 1995; Xiao *et al.*, 1995). The formation of either hetero- or homodimers may bring together different regulatory proteins, allowing a productive interaction between the enzyme (kinase or phosphatase) and the protein substrate (Fig. 3a; Jones *et al.*, 1995). Moreover, the negatively charged pocket formed by the 14-3-3 dimer may associate with cytoskeleton- and membrane-bound proteins, anchoring the multiprotein complex to a circumscribed location in the cell (Jones *et al.*, 1995). As to the yeast 14-3-3 homologues Bmh1p and Bmh2p (van Heusden *et al.*, 1992, 1995), it is as yet not clear whether their function is related to regulation of protein kinases/phosphatases or direct interaction with the transcriptional machinery. Notably, we have recently found that disruption of either *BMH1* or *BMH2* causes a Na^+- and Li^+-sensitive phenotype (J. C. S. Varela and others, unpublished results). The intolerance to these ions however appeared not to be due to a general osmotic defect, suggesting that the response to high salt is more complex than a simple osmoregulatory process (see below).

Sensing of and responding to sodium stress

Upon transfer to high salt (NaCl), the toxic Na^+ ions enter the cell via the yeast K^+ channels (encoded by *TRK1* and *TRK2*) and probably via other cation transporters (e.g. *HOL1*; Fig. 3b; Rodríguez-Navarro & Ramos, 1984; Gaber *et al.*, 1988, 1990; Ko *et al.*, 1990; Wright *et al.*, 1996). The toxicity of Na^+ to the yeast cell may be due to the interference of this ion with cellular processes that require K^+ (Haro *et al.*, 1993). Indeed, overexpression of genes (e.g. *HAL1*) improving the K^+/Na^+ ratio appear to enhance salt tolerance (Gaxiola *et al.*, 1992).

In order to prevent extensive uptake of Na^+, the K^+ transport system shifts from a low-affinity to a high-affinity state in NaCl-containing media (Fig. 3b; Rodríguez-Navarro & Ramos, 1984; Haro *et al.*, 1993). This shift in the affinity to K^+ is determined by Trk1p, whereas Trk2p seems to be exclusively required for the low-affinity mode (Gaber *et al.*, 1988; Ko *et al.*, 1990). The excess of Na^+ ions which have entered the cell are then pumped out by a P-type ATPase encoded by *ENA1–ENA4* (Fig. 3b; reviewed by Rodríguez-Navarro *et al.*, 1994). However, only Ena1p appears to be salt-responsive (Garciadeblás *et al.*, 1993).

Disruption of *TRK1* or *ENA1* confers a non-osmosensitive, Na^+-sensitive phenotype (Haro *et al.*, 1991, 1993; Garciadeblás *et al.*, 1993). This hypersensitivity to Na^+ resembles phenotypes exhibited by calcineurin-deficient strains as well as yeast containing defects in *ERG6*, *HOL1* and the 14-3-3 genes (see above; Gaber *et al.*, 1990; Nakamura *et al.*, 1993; Mendoza *et al.*, 1994; Welihinda *et al.*, 1994; Wright *et al.*, 1996; J. C. S. Varela and others, unpublished results). Calcineurin is a protein phosphatase (type 2B) requiring Ca^{2+} and calmodulin for enzyme activation. In yeast, calcineurin is a heterodimer consisting of a catalytic subunit, encoded by a pair of homologous genes (*CNA1* and *CNA2*), and a regulatory subunit, encoded by *CNB1* (Cyert *et al.*, 1991; Kuno *et al.*, 1991; Liu *et al.*, 1991; Cyert & Thorner, 1992). Calcineurin-deficient (*cnb1*) strains are unable to activate *ENA1* gene expression (Mendoza *et al.*, 1994). In addition, *cnb1* cells fail to shift the K^+ uptake system from the low-affinity to the high-affinity state (Mendoza *et al.*, 1994). The recently found salt-hypersensitive phenotype displayed by *bmh1* null mutant cells also appeared to be correlated with the loss of salt-induction of *ENA1* (J. C.

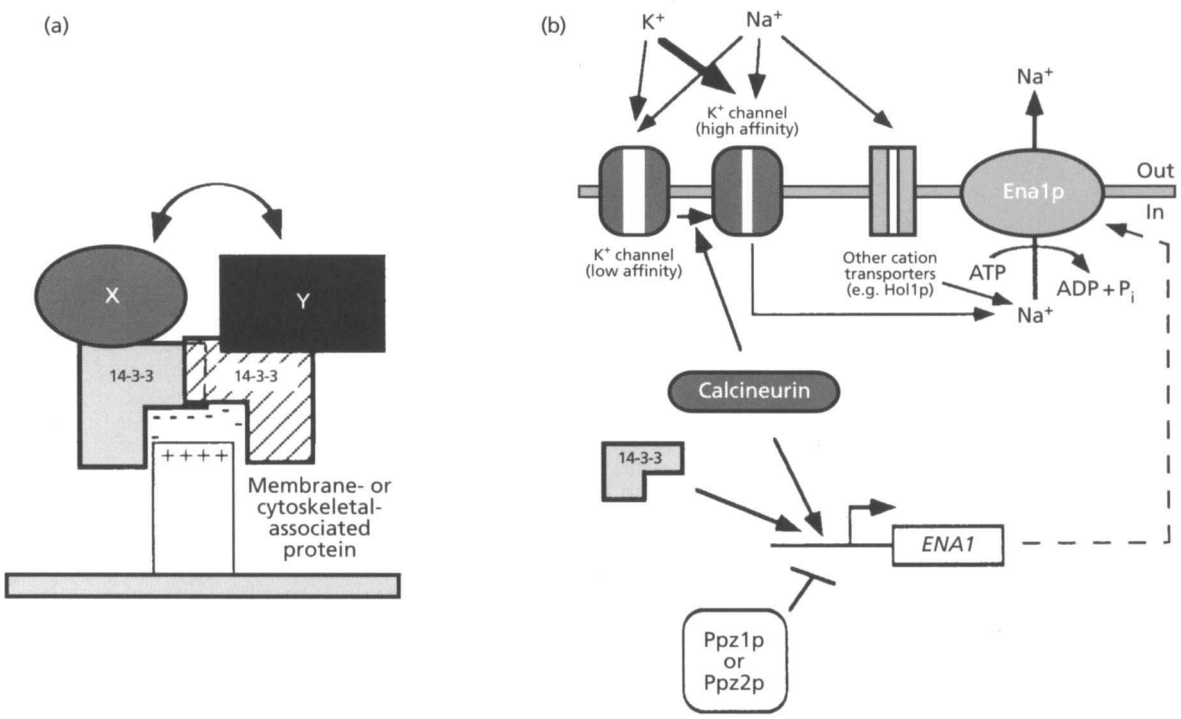

Fig. 3. Possible function of the 14-3-3 proteins. (a) 14-3-3 proteins as 'adaptors'. The mammalian 14-3-3 proteins can form homo- and heterodimers (as shown) and associate with several regulatory proteins (depicted as 'X' and 'Y'). The 14-3-3 dimers form a negatively charged pocket, which may bind to membrane- and cytoskeletal-associated proteins. This model reflects the proposed function of the 14-3-3 dimers, not only as an important determinant of the cellular location of regulatory proteins (e.g. the mammalian protein kinase Raf), but also as a scaffold for protein–protein interaction (and regulation) between signalling molecules (adapted from Jones *et al.*, 1995). (b) Possible function of the yeast 14-3-3 homologues (Bmh1p and Bmh2p). In yeast, the 14-3-3 homologue Bmh1p seems to play an important role in the regulation of *ENA1* gene expression, together with the protein phosphatases calcineurin, Ppz1p and Ppz2p. *ENA1* encodes a P-type ATPase that pumps the toxic Na^+ ions from the cytoplasm back into the medium. Na^+ may enter the cell through different cation transporters, such as Hol1p and the K^+ channels. Upon a shift to high salt, the transport system for K^+ shifts from low affinity to high affinity. This event is mediated by calcineurin. So far, it is not known whether the yeast 14-3-3 homologues and calcineurin operate in the same or independent pathways (see text for details). Arrows pointing from and to cations represent ionic fluxes; in all other cases, they indicate positive regulatory interactions.

S. Varela and others, unpublished results). Although epistatic interactions between *CNB1* and *BMH1* remain to be investigated, these results suggest that calcineurin and the 14-3-3 proteins participate in the regulation of ion homeostasis in yeast (Fig. 3b).

Deregulation of the K^+ channels or other ion transporters may also account for the increased levels of Na^+ found in *erg6* and *hol1* cells exposed to high salt (Gaber *et al.*, 1990; Welihinda *et al.*, 1994). *ERG6* encodes a putative *S*-adenosylmethionine-dependent methyltransferase that appears to be essential for the maintenance of suitable levels of ergosterol and other sterols in cell membranes (Welihinda *et al.*, 1994). On the other hand, gain-of-function mutations in *HOL1* result in increased uptake of Na^+ and other monovalent cations (Gaber *et al.*, 1990; Wright *et al.*, 1996). Therefore, it has been suggested that alterations in the structure of ion transporters, either due to mutations in the primary sequence or changes in the lipid composition of cell membranes, may influence the activity and specificity of these transport proteins (Welihinda *et al.*, 1994). This assumption is supported by the observation that Na^+ uptake but not Na^+ efflux is increased in *erg6* and *hol1* mutants (Gaber *et al.*, 1990; Welihinda *et al.*, 1994).

The importance of exclusion and extrusion of Na^+ ions for acquisition of NaCl tolerance is further emphasized by the hyper-resistant phenotype exhibited by cells lacking the protein phosphatases Ppz1p and Ppz2p (Posas *et al.*, 1995b). This enhanced NaCl resistance seems to be the consequence of increased *ENA1* gene expression, resulting in a more efficient efflux of Li^+, a Na^+ analogue in transport studies (Rodríguez-Navarro & Ortega, 1982; Posas *et al.*, 1995b). Remarkably, the Ppz protein phosphatases appear to interact with the *PKC1* pathway (Lee *et al.*, 1993a; Posas *et al.*, 1995b). *ppz1 ppz2* cells show a lytic defect at 37 °C very similar to that of *bck1*, *mkk1 mkk2* and *mpk1* cells (see above). However, when a *mpk1* null mutation is combined with a double disruption of *PPZ1* and *PPZ2*, cell lysis occurs at any temperature (Lee *et al.*, 1993a). This more severe phenotype has been taken to imply that the Ppz phosphatases and Mpk1p converge on a common downstream target, though

functioning in independent pathways. A possible functional link between this MAPK and the Ppz phosphatases is supported by the fact that the NaCl-hyperresistant phenotype caused by the disruption of *PPZ1* (see above) does not occur in a *mpk1* genetic background (Posas *et al.*, 1995b).

Stress tolerance versus growth

Cross-protection and similarities among stress responses

The thermosensitive, NaCl-hyper-resistant phenotype showed by *ppz1 ppz2* cells is a provocative example that tolerance to a form of stress is not always accompanied by an enhanced survival upon exposure to other stressful conditions. Nevertheless, it is apparent that the yeast responses to heat shock, osmostress and nutrient depletion share similarities. Apart from heat shock and osmostress, the heat-shock proteins Hsp12 and Hsp26 and the cytosolic catalase Ctt1p are induced by other stresses, such as nutrient depletion (Kurtz *et al.*, 1986; Praekelt & Meacock, 1990; Varela *et al.*, 1992, 1995; Marchler *et al.*, 1993). Similar observations were extended to *HSP104*, *DDR2* and *TPS2* (Gounalaki & Thireos, 1994; Schüller *et al.*, 1994). Hsp104 is a highly conserved stress-inducible protein, which, apparently by catalysing an ATPase-driven protein re-folding (Parsell *et al.*, 1994), is protective against several severe forms of stress (Sanchez *et al.*, 1992). The heat-responsive Ddr2p and Tps2p, on the other hand, accumulate upon exposure to DNA-damaging reagents and entry into stationary phase, respectively (Kobayashi & McEntee, 1993; Vuorio *et al.*, 1993; Gounalaki & Thireos, 1994). Interestingly, the latter protein has been identified as one of the three subunits of the trehalose synthase/phosphatase complex (De Virgilio *et al.*, 1993; Vuorio *et al.*, 1993). The stress-inducibility of *TPS2* is in keeping with enhanced trehalose levels in heat-shocked cells, stationary-phase cells and cells growing on high-salt media (Wiemken, 1990; André *et al.*, 1991) and the proposed protective effect of this carbohydrate on membranes and proteins (Wiemken, 1990; Hottiger *et al.*, 1994; Iwahashi *et al.*, 1995).

The overlap among stress responses is often confirmed by the observation that previous exposure to a stress increases tolerance against a challenge by another form of stress (Mager & Moradas Ferreira, 1993). Two factors may contribute to this cross-protection, namely (i) the existence of common transcriptional regulatory mechanisms, such as *cis*-acting elements responsive to a broad range of stressful conditions (STREs; Mager & De Kruijff, 1995); and (ii) the interplay between the proliferative state of the cell and stress tolerance (see below).

Nutrient signalling, growth control and stress tolerance

For an organism to survive successfully, a tight control between growth and response to (osmo)stress must be accomplished. Initiation of growth in media lacking essential nutrients is lethal (Granot & Snyder, 1991). This is further demonstrated by the rapid loss of viability and the extreme stress-sensitivity of mutants failing to arrest at the G1 phase of the cell cycle upon nutrient starvation (e.g. Thompson-Jaeger *et al.*, 1991). It is evident by now that the RAS-adenylate cyclase pathway plays a major part in the nutrient regulation of enzyme activities and gene expression in yeast (reviewed by Thevelein, 1994). It could be argued, therefore, that cAMP signals nutrient availability and regulates cell proliferation as well as acquisition of stress tolerance (Costigan & Snyder, 1994; Engelberg *et al.*, 1994). On the other hand, since the Ras-adenylate cyclase pathway is repressed by glucose, it has been suggested that in cells grown on rapidly fermentable sugars an alternative pathway, referred to as the fermentable-growth-medium (FGM)-induced pathway, is operative (Fig. 4; Thevelein, 1994).

How nutrient signalling is translated into control of cell proliferation and/or acquisition of tolerance to (osmo)stress is largely unknown. However, there is evidence suggesting that protein phosphorylation by PKA and other nutrient-signalling pathways regulate not only growth-related processes but also the response of yeast to several forms of stress. That PKA may play a role in integrating growth control with stress (cross-)protection is strengthened by the finding that high PKA activity reduces or abolishes transcriptional activation of several stress-inducible genes (Boorstein & Craig, 1990; Praekelt & Meacock, 1990; Marchler *et al.*, 1993; Varela *et al.*, 1995). This negative effect is apparently exerted on the CCCCT-motif (Marchler *et al.*, 1993; Varela *et al.*, 1995). Repression is only relieved upon stress by activation of stress-sensing pathways, such as the HOG pathway (Schüller *et al.*, 1994; Varela *et al.*, 1995), and/or by the failure of activating nutrient-sensing pathways, such as the FGM pathway (see Thevelein, 1994). Recent genetic evidence suggests that the antagonistic effects of PKA and the HOG pathway converge on a common cellular target (Schüller *et al.*, 1994). An attractive model predicts that this common target corresponds to the STRE-binding factor(s) (Fig. 4). The identification of Msn2p and Msn4p as STRE-binding proteins (see above) may soon address the validity of the proposed model.

Convergence of pathways on PKA cellular targets is not restricted to the HOG pathway. Epistasis studies have indicated that the protein kinases Yak1p and Sch9p and the catalytic subunits of PKA may phosphorylate overlapping targets (Fig. 4; Hartley *et al.*, 1994). Evidence exists suggesting that Sch9p, Yak1p and PKA operate in independent pathways and that Sch9p and PKA effects on cell proliferation are counteracted by Yak1p. Interestingly, overexpression of *YAK1* enhances tolerance to heat shock in wild-type cells (Hartley *et al.*, 1994). Although the mechanism for this increase in stress tolerance is yet to be understood, it highlights once more the link between a slow-growing or non-proliferative physiological state (growth control) and resistance to stress (Fig. 4).

A final example of an interaction between PKA and a stress-responsive pathway is provided by the caffeine-

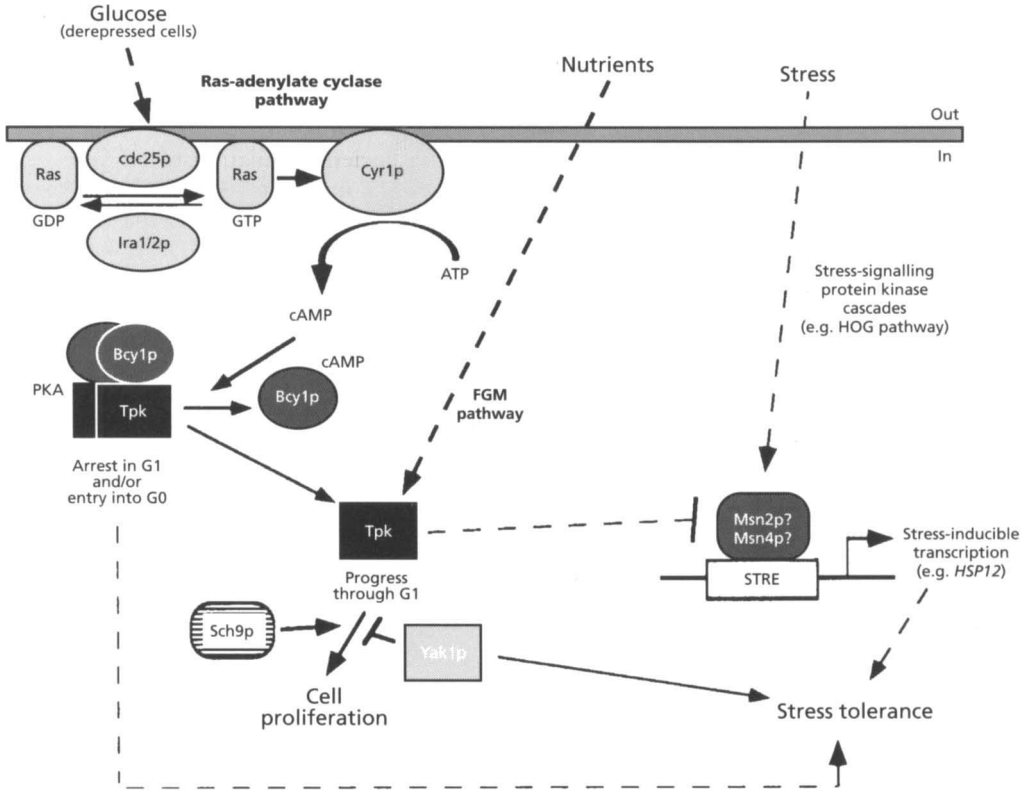

Fig. 4. Growth control and stress tolerance. Addition of glucose to derepressed cells activates the Ras-adenylate cyclase pathway (Thevelein, 1994). During this transition period from respiration to fermentation, cdc25p stimulates the exchange of GDP for GTP on the Ras proteins. This exchange is negatively regulated by Ira1p and Ira2p. The GTP-bound form of Ras activates Cyr1p (= adenylate cyclase), producing a transient cAMP peak. The rise in cAMP levels leads to release of the catalytic PKA subunits (encoded by three functionally redundant genes, *TPK1*, *TPK2* and *TPK3*) from the cAMP-bound form of Bcy1p. The Tpk catalytic subunits are then able to phosphorylate their cellular targets. The Ras-adenylate cyclase pathway is repressed by glucose. It has therefore been postulated that an alternative signal transduction pathway, the fermentable-growth-medium (FGM)-induced pathway, signals nutrients in glucose-grown cells. According to this model, basal levels of cAMP in cells fermenting glucose may permit the activation of the free catalytic subunits (Tpk) of protein kinase A (PKA) by a component of the FGM pathway. Activation of PKA by nutrients stimulates mitosis (progress through the G1 phase of the cell cycle) and cell growth and represses the expression of stress genes. On the other hand, stressful conditions activate stress-signalling pathways (e.g. HOG pathway), stimulating transcription of stress-inducible genes and overcoming the negative effect exerted by PKA. PKA activity and stress tolerance are apparently modulated by other protein kinases (e.g. Yak1p) and phosphatases (Ppz1p and Ppz2p?) (see text for details).

sensitive phenotype of *ppz1 ppz2* cells (Posas *et al.*, 1993). Caffeine is a known inhibitor of cAMP phosphodiesterases, mimicking mutations that result in high PKA activity (Parsons *et al.*, 1988). A possible interaction between Ppz1p/Ppz2p and PKA is further supported by the ability of PKA to phosphorylate Ppz1p *in vitro* and the hypersensitivity to heat shock shared by *ppz1 ppz2* and *bcy1* cells (Toda *et al.*, 1987; Posas *et al.*, 1993, 1995a). As a result, it has been suggested that the Ppz phosphatases may offset the effect of PKA phosphorylation and that PKA may regulate the activity of these phosphatases (Posas *et al.*, 1993, 1995a). The significance of this postulate concerning the role of Ppz1p/Ppz2p in the acquisition of NaCl tolerance is as yet unclear (see above). It should be noted, however, that the caffeine-sensitivity of *ppz1 ppz2* cells can be suppressed by osmotic stabilizers, a phenotype associated with mutations in the *PKC1* pathway (see above). On the other hand, Epstein & Cross (1994) and Di Como *et al.* (1995) have shown that *BCK2*, a multicopy suppressor of *pkc1* and *mpk1* lytic phenotype (Lee *et al.*, 1993a), regulates *CLN1* and *CLN2* gene expression. Interestingly enough, Bck2p seems to operate in the same pathway as the Ppz phosphatases (Lee *et al.*, 1993a), thereby providing another indication that yeast integrates the regulation of cell morphogenesis and cell cycle with the acquisition of stress tolerance.

Ca^{2+} signalling: a novel determinant of cell proliferation and stress tolerance?

Although nutrient signalling via PKA appears to be a key event for the modulation of the different stress responses, it is increasingly obvious that Ca^{2+} intracellular levels [see model (i)] may influence cell proliferation and stress tolerance as well (Cunningham & Fink, 1994b). Indeed, activation of calcineurin by Ca^{2+} and calmodulin (a Ca^{2+}-

binding protein) appears to negatively regulate cell growth (Cunningham & Fink, 1994a). This negative effect may be modulated by the *PKC1* pathway, a MAPK cascade presumably activated by Ca^{2+} (see above). This idea is supported by the lethality of the *pkc1* and *mpk1* growth defects in a calcineurin-deficient strain and the suppression of the *pkc1* and *mpk1* lytic phenotype by a constitutively active calcineurin (Garrett-Engele *et al.*, 1995). Moreover, calmodulin and the calmodulin-dependent protein kinase II have recently been shown to be required for induced thermotolerance (Iida *et al.*, 1995).

Final remarks

Although different stresses elicit different cellular responses in order to ensure survival and adaptation to the new growth conditions, a few common themes are observed. Firstly, protein kinase cascades, MAPK cascades in particular, are used to signal diverse forms of stress in yeast and mammals. Secondly, these cascades may also be responsive to growth-related stimuli, such as nutrients or growth factors. Thirdly, transcriptional regulation of stress-inducible genes may be mediated by a closely related family of transcription factors binding to the CCCCT motif (STRE), in conjunction with the previously described heat-shock transcription factor. Finally, it is apparent that an overlap between growth-related cellular processes and response to stress occur and that PKA, among other protein kinases and phosphatases, plays a central role in signal integration.

This interplay between growth control and stress tolerance is not surprising if one considers that the main goal of a stress response is adaptation to and growth under different, though potentially lethal, environmental conditions. Whether the response to stress (e.g. activation of stress-inducible genes) is due to the activation of stress-responsive pathways and/or the failure to activate pathways sensing favourable growth conditions (e.g. FGM pathway) remains unclear. A major role for both mechanisms in regulating growth and stress tolerance is a likely possibility. Further developments in the field, such as the characterization of the STRE-binding factor(s) and the pathways interacting with these transcription factors, will be crucial to understand how the equilibrium between growth and stress tolerance is achieved.

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