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REVIEW ARTICLE

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Learning from yeasts: intracellular sensing of stress conditions

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Abstract One intriguing challenge in modern biology is to understand how cells respond to, and distinguish between different stressing stimuli. Evidence accumulated in recent years indicates that a network of signaling pathways extends from the plasma membrane to the very core of the cell nucleus to transduce environmental changes into a graded transcriptional response. Although many steps still remain unclear, studies on the stress-activated protein kinase (SAPK) pathways and related mechanisms provide insight into the biochemistry that regulates signal transmission and leads to outcomes such as cell adaptation and differentiation. This review focuses on selected topics of current interest related to the sensing of stress signals in cells of the fission yeast *Schizosaccharomyces pombe*. Because signaling pathways appear to be evolutionarily well conserved, yeasts may be useful models to learn how higher eukaryotes sense and respond to stresses at the cellular level.

Keywords Signal transduction · MAPK pathways · SAPK pathways · Fission yeast · Stress response

Introduction

Yeasts know how to make good bread, beer, and even better wine, as well as other valuable and tasteful foodstuffs. This reason alone largely justifies the particular appeal of yeasts within the microbial universe. Moreover, in addition to obvious anthropological interests, these organisms are model systems suitable for use in solving fundamental biological questions far removed from direct industrial concern. In this context, the budding yeast *Saccharomyces cerevisiae* is often taken as the paradigm of yeast cells, likely because it is the best known eukaryote at the molecular level. However, *S. cerevisiae* may not always be the preferred single-celled model eukaryote. The fission yeast *Schizosaccharomyces pombe* exhibits many differences with the budding yeast *S. cerevisiae* and the two species have diverged as much from each other as much as from humans [34]. *S. pombe* is probably more closely related to higher eukaryotes [10, 41] and thus offers a more significant approach to solving biological issues relevant to mammalian cells. The identification in *S. pombe* of a stress-activated pathway homologous to that present in higher metazoans allows the mechanisms underlying intracellular sensing to be analyzed by amenable genetic manipulation. Unlike *S. cerevisiae*, which has a variety of distinct signal transduction pathways to perceive environmental changes such that, quite often, researchers cannot see the wood for the trees, a distinctive feature of *S. pombe* is that the response to multiple stresses is funneled through a common integrative pathway. Because of its relative simplicity, we shall focus on this pathway to highlight some aspects of intracellular sensing.

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Basic MAPK signaling module

Signal transduction pathways are important elements of the cellular framework that sense extracellular stimuli and communicate with the nucleus to initiate adaptive changes in gene expression. This kind of sensing can be methodologically studied under standard stress conditions thereby providing invaluable clues as to how these pathways operate. The sensing cascades transduce signals to enable cells to adapt and grow under potentially lethal conditions, so that there is an obvious relationship between stress tolerance and growth control. Studies

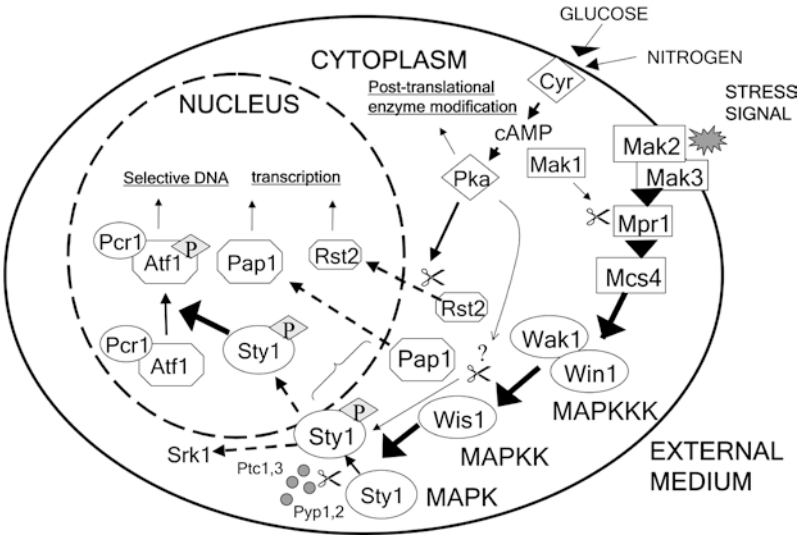

Fig. 1 The regulatory network involving the SAPK signaling pathway in *Schizosaccharomyces pombe*. Not all the elements shown are active in all kinds of responses (see text) and other components are likely missing. Inhibitory action is indicated by the *scissors* symbol

carried out during the past few years have revealed a role for a family of evolutionarily conserved protein kinases, mitogen-activated-protein kinases (MAPKs), that are involved in the cellular response to changes in environmental conditions [8, 11, 12]. In mammalian cells, the JNK and p38 protein kinases have been characterized as MAPKs whose activity is triggered by stress [14, 19, 33]. These kinases are also known as stress-activated-protein-kinases (SAPKs) and they display the ability to either phosphorylate transcription factors or translocate them from the cytoplasm to the nucleus resulting in selective activation of transcription. In the fission yeast *S. pombe*, the MAPK Sty1 is the effector kinase (Fig. 1) required for cellular response to a wide range of stimuli including nutritional stress, temperature changes, oxidative status, osmotic conditions, heavy-metal toxicity, and the presence of DNA-damaging agents such as UV light [6, 7, 17, 25, 38].

MAPK Sty1 is activated by dual phosphorylation at neighboring Thr-171 and Tyr-173 residues by another kinase, MAPKK Wis1, which is activated in turn through phosphorylation at Ser-469 and Thr-473 residues by either of two MAPKKKs, Wak1 or Win1 (Fig. 1). The upstream regulator protein Mcs4 apparently binds to MAPKKKs, modulating their activity after receiving the signal from a control system that senses the triggering stimulus and initiates the phosphorylation cascade [3, 28]. The components of this MAPK phosphorylation pathway are homologous to those forming the Hog1 osmosensing MAPK pathway in *S. cerevisiae*, which in baker's yeast is only operative in response to changes in the osmotic pressure of the extracellular medium. Most importantly, the *S. pombe* Sty1 MAPK pathway is homologue to the mammalian JNK and p38 stress-activated protein kinase cascades [14, 19, 33] that regulate the activity of the transcription factors c-Jun and ATF2 [8, 13, 19, 20] (Fig. 2). In the fission yeast, several transcription factors that have been similarly implicated in the response to various stresses are under the regulation of the MAPK Sty1 pathway

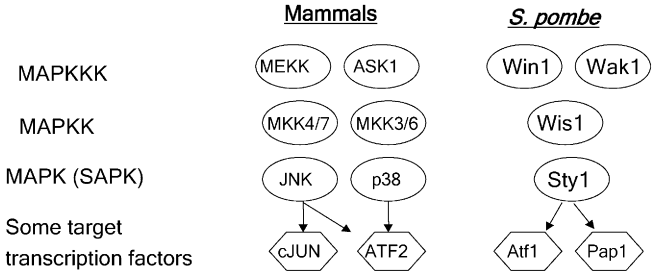

Fig. 2 Homology between the SAPK-pathways in mammals and *S. pombe*. These cascades are similarly regulated and control the activity of transcription factors that determine biological responses to various extracellular stress-inducing conditions. In mammals they are involved in proliferation, differentiation, DNA repair and apoptosis. In *S. pombe* they may control response to oxidative stress, high osmolarity, heat shock, nutritional limitation and UV stress, as well as meiosis and resistance to drugs and heavy metals

(Fig. 2). Not all the elements of this basic MAPK pathway module are operative in all cases. For instance, it has yet to be determined whether Wis1 is activated in the same fashion by all the stress conditions that are known to activate Sty1, or whether components like Mak2 and Mak3 participate in the response to all types of stress (Fig. 1).

Sensing and response to oxidative stress conditions

Aerobic organisms produce reactive oxygen intermediates as a result of incomplete reduction of oxygen during respiration, β-oxidation of fatty acids, exposure to radiation, and the action of some drugs [48]. These harmful molecules interfere with cellular processes due to their ability to damage DNA, lipid membranes, and proteins. The survival of cells under oxidative stress conditions depends on a chain of protective reactions to eliminate these toxic oxygen intermediates. For example, the expression of an appropriate set of proteins, namely superoxide dismutases, catalases, and peroxidases, is

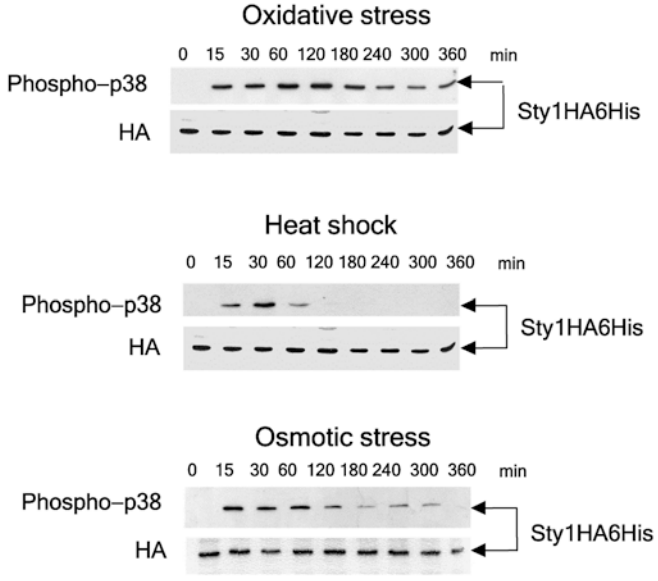

Fig. 3 Kinetics of stress-induced activation of Sty1p in *S. pombe*. A wild-type strain of the fission yeast carrying a Ha6H-tagged chromosomal version of *sty1* was grown in YES medium to mid-exponential phase and subjected to oxidative stress (1 mM H_2O_2), heat shock (40 °C) or osmotic shock (0.5 M NaCl) for the times indicated. Aliquots were harvested and Sty1 was purified by affinity chromatography. Activated Sty1p was detected by immunoblotting with anti-(phospho-p38) antibodies. Total Sty1p was determined by immunoblotting with anti-Ha antibody as loading control

often regulated by transcription factors that react to intracellular signals and bind to specific sequences contained in the promoter region of stress-responsive genes [23]. The response of yeasts to H_2O_2-induced oxidative stress is probably the most thoroughly studied among the stressful conditions imposed on cells of *S. pombe* [9, 32]. One interesting property of this response is that it is dose-dependent and involves at least two closely related mechanisms. These are discussed in the following sections.

Signal transmission through the SAPK pathway

When cells are exposed to low levels of H_2O_2, phosphorylation of the final effector MAPK Sty1 (Fig. 3) is triggered through a signaling pathway that initiates with a two-component system regulated by histidine kinases Mak2 and Mak3 and which includes either of the two redundant MAPKKKs, Wak1 or Win1 (Fig. 1) [3, 28]. However, following exposure to a higher level of H_2O_2, the signal transduction pathway regulates Sty1 phosphorylation by means of a mechanism that is independent of the initial two-component system and which requires the joint action of both MAPKKKs, i.e. Wak1 and Win1 [32]. Interestingly, the individual transcription factors that operate in this gene response also vary as a function of the range of H_2O_2 concentrations, which establishes a molecular basis to allow a graded response by the cell. Thus, a low-level signal stress determines that Pap1 is the transcription factor activating gene targets, whereas in the presence of high concentrations of H_2O_2 Atf1 (likely together with Pcr1) is the main factor controlling the transcriptional response [32]. On the whole, this might be a strategy for fine tuning and adaptation to external conditions. Indeed, in nature stresses are variable in intensity and therefore the cell's response must be appropriate to the particular level of stress. Exposure of *S. pombe* to low levels of H_2O_2 promotes an adaptive response that protects cells from further potential exposures to the same or related agents. Under such conditions, expression of $tpx1^+$, which codes for thioredoxin peroxidase, is quickly induced in a Pap1-dependent manner. However, in response to a stronger and potentially lethal exposure an acute survival response is mounted that includes increased expression of $gpx1^+$, which codes for glutathione peroxidase under the control of Atf1. Because both types of response are dependent on Sty1 but rely on different transcription factors, an $atf1^-$ mutant strain is insensitive to oxidative stress when grown on media containing low concentrations of H_2O_2 but is sensitive to higher doses of the stimulus. By contrast, a $pap1^-$ mutant is quite sensitive to low concentrations of H_2O_2 but surprisingly insensitive to higher concentrations of H_2O_2, in particular when compared to an $atf1^-$ mutant. Finally, a double mutant ($atf1^-pap1^-$) behaves identically to a $sty1^-$ strain and is unable to respond to an oxidative challenge under any condition. Another gene, ctt^+, which encodes catalase, is expressed over a wide range of concentrations of the oxidative stimulus in spite of the fact that transcription factors change depending on its concentration. Congruent with this, the *ctt* promoter contains both Pap1 and Atf1 binding sites [26].

Regulatory aspects of oxidative signal transduction

Although many pieces have still to be found before solving the entire puzzle, major questions are being answered while trying to understand the regulation of distinct responses by Sty1. It has been proposed that the intensity of MAPK Sty1 activation is a mechanism whereby signaling through a single pathway can result in distinct outcomes. In systems other than *S. pombe* it is known that the activation of certain pathways can lead even to opposite results, such as proliferation or differentiation, depending on the level of activation [24, 35]. In *S. pombe*, it has proven that, as the level of oxidative stress increases, there is a corresponding increase in the levels of active, phosphorylated Sty1. On the other hand, Atf1 is a nuclear target that is phosphorylated by MAPK Sty1 only after activated MAPK is itself translocated into the nucleus by triggering of the signaling pathway (Fig. 1). Atf1 contains eleven potential phosphorylation sites, and a cumulative level of this type of covalent modification might be critical for its transcription-activating activity, a property that in turn would be related to the level of Sty1 activation. By

contrast, the subcellular localization of the transcription factor Pap1 is mainly cytoplasmic in the absence of stressing factors [51]. Moreover, Pap1 is not a substrate directly phosphorylated by Sty1, although its activation and nuclear translocation are Sty1-dependent [51]. In response to low levels of H_2O_2, Pap1 accumulates quickly in the nucleus, but the cytoplasm-to-nucleus shift rapidly slows down when the level of the inducer increases. In short, the action of Pap1 appears topographically regulated by its nuclear location. It seems reasonable that stronger oxidative conditions induce disulfide bonds within the Pap1 molecule, which thereby assumes an inactive conformation not compatible with effective nuclear residence and action (Fig. 1).

Another control checkpoint in MAPK-directed transcriptional regulation may be found in the upper part of the SAPK pathway. A third histidine kinase, Mak1, related to Mak2 and Mak3, has an inhibitory role on Sty1 activation at least under some conditions. This has been revealed by the observation that under low oxidative stimulus deletion of *mak1* increases Sty1 phosphorylation [32]. Such inhibition may hinder the signal coming from Mak2 and Mak3, resulting in depressed Sty1 activation and a concomitant decreased level of Atf1 phosphorylation, thus maintaining Pap1 as the critical transcription factor. At higher levels of oxidative stress, the inhibitory filter-effect of Mak1 on Sty1 activation is lost and Atf1 becomes increasingly important in the transcriptional response.

Activation of the SAPK pathway by other stimuli

Heat shock

MAPK Sty1 is strongly activated by phosphorylation when cells are exposed to heat shock (45–48 °C) (Fig. 3). Also, mutant cells of *S. pombe* lacking Sty1 lose viability more quickly than do wild-type cells at high temperature, indicating that Sty1 activation is essential to establish cellular thermotolerance [7]. Activated Sty1 phosphorylates downstream transcription factors to induce stress response genes. Among the strongly induced genes is *tps1*$^+$, responsible for the synthesis of the non reducing disaccharide trehalose, which is known to be an important stress metabolite related to thermal resistance [40, 46].

As outlined above, in *S. pombe* Wis1 is the MAPKK that phosphorylates MAPK Sty1 at Thr-171 and Tyr-173 residues. The phosphorylating activity of Wis1 on Sty1 may in part be counteracted by two tyrosine-specific phosphatases, Pyp1 and Pyp2 [25] (see Fig. 1). Also, dephosphorylation at threonine residues is achieved by another set of specific serine/threonine phosphatases, Ptc1 and Ptc3 [27]. Since activation of MAPK Sty1 requires both threonine and tyrosine phosphorylation, dephosphorylation of Tyr-171 alone is sufficient to inactivate Sty1. Simultaneous deletion of *pyp1*$^+$ and *pyp2*$^+$, encoding the two tyrosine-phosphatases, brings about a lethal hyperactivation of Sty1, which is similar to that achieved by *wis1*$^+$ overexpression [25, 38]. These observations suggest that, in general terms, the function of MAPK Sty1 might be regulated either by the dual phosphorylation of Sty1 protein, achieved by Wis1, or by the Tyr-173 dephosphorylation carried out by Pyp1/Pyp2. During osmostress and oxidative stress, the corresponding signals are mainly mediated through phosphorylation of Wis1 at its Ser-469 and Thr-473 residues, and the substitution of these sites on Wis1 with unphosphorylatable residues abolishes signaling to Sty1 in both cases [27]. In other words, this indicates that these two stresses are channeled to Sty1 via the first mentioned strategy of control. However, several studies have concluded that the signal elicited by heat shock can be transmitted to Sty1 independently of MAPKK Wis1 by blocking the negative regulatory control by Pyp1/Pyp2 [25, 38]. In contrast with the strong activation of Wis1 induced by osmostress and oxidative stress, Wis1 activation is relatively weak upon heat shock [27]. More importantly, the tyrosine phosphatase activity that dephosphorylates Sty1 at Tyr-173 is significantly inhibited during heat shock [27], which is consistent with the idea that inhibition of the tyrosine phosphatases for Sty1 is the crucial factor contributing to the maintained activation of Sty1 in response to heat shock. Because Tyr-173 dephosphorylation by Pyp1/Pyp2 is a major mechanism for Sty1 inactivation, inhibition of these enzymes is expected to play a key role in activation of Sty1 upon heat shock. The relatively weak activation of Wis1 at high temperature implies that under these conditions the upstream regulators of the MAPK pathway are not fully functional and that a sensor dedicated to heat shock stimuli might never have evolved upstream of the SAPK cascade in *S. pombe*. This makes sense since the Pyp1/Pyp2 phosphatases are sensitive to heat and might themselves serve as stress sensors. Interestingly, in cells unstressed by heat these enzymes are soluble cytoplasmic proteins but the bulk of Pyp1/Pyp2 becomes insoluble within minutes of exposure to heat shock. One possible explanation for these results is that, upon raising temperature, changes in conformation, modification, or the interaction with other proteins lead to the inhibition of Pyp1/Pyp2. Likely, subsequent attenuation of Sty1 in heat-shocked cells is brought about by phosphatases Ptc1 and Ptc3, which dephosphorylate Thr-171 [27].

In brief, Sty1 is kept inactive in unstressed cells mainly through dephosphorylation of Tyr-173 by Pyp1/Pyp2 phosphatases, but, when cells are exposed to heat shock, inhibition of these phosphatases combined with a basal, moderate activation of Wis1 results in a strong activation of Sty1. This is clearly distinct from osmostress and oxidative stress, which induce activation of Sty1 solely in a Wis1-dependent manner [39]. MAPK regulation by specific phosphatases has also been reported in budding yeast and mammalian cells; therefore, this mechanism could be widespread in other MAPK pathways of lower and higher eukaryotes.

Cold stress

Low temperature is an important environmental signal for all living organisms, and the adaptive response to cold stress involves the synthesis of several types of proteins to avoid its damaging effects. In bacteria, thermal downshifts induce the synthesis of Csp proteins, a class of cold-shock proteins that function as RNA chaperones favoring efficient translation of mRNA at low temperature [30]. Eukaryotes also display a cold-shock response that triggers the synthesis of specific proteins, although no proteins homologous to bacterial Csps have been isolated so far.

Whereas in *S. pombe* cells a marked rise in temperature impairs the interaction of Pyp1/Pyp2 tyrosine-phosphatases with their substrate Sty1, a downshift in temperature (cold stress) induces a very different mechanism, in which Sty1 activation is mediated by the complete Wak1/Wis1-Win1-Sty1 cascade in a way more closely related to an osmostress or oxidative stress than to a heat shock (Fig. 4). A possible rationale for this is that low temperature triggers a marked production of oxygen-reactive species or a lower water activity (a_w) [29, 53]. Essentially, when cultures of the fission yeast are subjected to a thermal downshift from 28 °C to 10–15 °C MAPK Sty1 is transiently activated by phosphorylation and the kinetics of this phosphorylation are clearly delayed compared to those of other stresses such as heat shock and osmotic or oxidative stresses [47] (Fig. 3). Similar to its role in other stresses, Wis1 is responsible for Sty1 activation during cold shock. In turn, Wis1 activation under these conditions is only partially dependent on MAPKKK Wak1, suggesting that signal transmission follows a branched pathway, with the redundant MAPKKK Win1 acting as an alternative transducer to Wis1 which subsequently activates the effector MAPK Sty1 [47]. Induction of this response is completely abolished in cells disrupted in the upstream response regulator Mcs4 (Fig. 4), thereby suggesting a role for elements from the upper part of the phosphorylation cascade. Also, the bZIP transcription factor Atf1 becomes phosphorylated in a Sty1-dependent way during cold shock and this phosphorylation was found to be responsible for the increased expression of several genes, including *gdp1*$^+$ (related to the synthesis of the cryoprotectant glycerol), *ctt1*$^+$ (encoding catalase) and those involved in trehalose metabolism (Fig. 4). Strains of *S. pombe* in which the transcription factor Atf1 or Pcr1 is deleted were unable to grow at low temperature whereas strains disrupted in any member of the SAPK pathway were able to do so [47]. This result is striking because it suggests that although *S. pombe* responds to low temperatures by inducing the SAPK pathway its function is dispensable during growth under these conditions. Instead, the presence of the transcription factors per se appears critical to ensure growth in the cold by an as yet undefined mechanism. This is a role independent of their normal function as SAPK-driven multifunctional switches that activate specific responses against stress-inducing extracellular conditions.

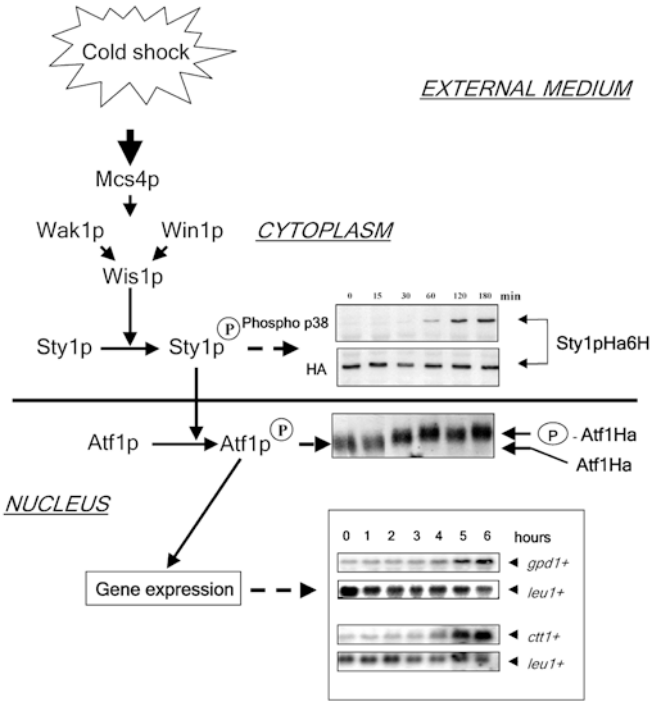

Fig. 4 Cold-induced activation of the SAPK pathway in *S. pombe*. Activation of Sty1 in cells subjected to cold stress (15 °C) was determined as indicated in Fig. 3. This activation is dependent on Wis1, Wak1/Win1 and Mcs4 since the increased level of Sty1 phosphorylation was blocked in the corresponding mutants. There is also a typical Atf1 shift due to subsequent Atf1 phosphorylation in cells carrying a chromosomal copy of Ha-tagged *atf1* after transfer from 28 to 15 °C for the times indicated. Phosphorylation of nuclear Atf is, in turn, Sty1-dependent because it does not occur in *sty1*-disrupted cells. The Atf1-tagged protein was purified with Ni^{2+}-nitrilotriacetic acid beads and analyzed by SDS/PAGE followed by immunoblotting with anti-Ha antibodies. To demonstrate that SAPK regulates the induction of various genes during cold through transcription factor Atf1p, total RNA was extracted from cells at the indicated intervals and samples were resolved in agarose-formaldehyde gels. After transfer to nylon membranes, they were hybridized with ^{32}P-labeled probes for *gdp1*$^+$ and *ctt1*$^+$ using *leu1*$^+$ as loading control. This increase in gene expression was not achieved by *atf1*-disrupted cells

Other physical stresses

Yeast cells encounter a variety of other physical stresses in nature or laboratory conditions in addition to oxidative attack or temperature changes. The fine mechanisms by which *S. pombe* cells sense and regulate Sty1 activation in response to other stresses, such as osmotic, high-pressure stress, nutrient limitation, UV irradiation, and DNA damage, are largely unknown. Many studies involving cells of *S. pombe* under osmostress conditions have concluded that Sty1 is an effector in signal transmission [7] (see Fig. 3) and that the upstream regulator Mcs4 mediates activation of the signaling pathway [36]. Wak1/Win1 and Wis1 are also required for the cellular

response to osmostress in addition to Sty1 [25, 36, 37]. However, characterization of primary sensors for this and other stimuli remains elusive. This is in contrast to the *S. cerevisiae* Hog1 MAPK cascade, which has been studied in more detail [2]. The Hog1 pathway is activated by the action of two distinct receptors. One is a phosphorelay receptor that acts through the MAPKKKs Ssk2 and Ssk22 whereas the other receptor acts through the MAPKKK Ste11 [21, 31]. Both converge on activation of the MAPKK Pbs2, which is the kinase that phosphorylates MAPK Hog1 (homologous to *S. pombe* Sty1). This activation leads, in turn, to the induction of a set of protective genes.

Finally, exposure of *S. pombe* cells to UV light results in increased expression of several genes and this response is also dependent on the Sty1 protein kinase pathway [6, 18]. Apparently, the stress-activated MAPK cascade mediates UV-induced gene expression by increasing mRNA half-life [18].

Novel targets for Sty1 stress-activated MAPK

As expected, analysis of mutants impaired in the function of the transcription factors Atf1/Pcr1 and Pap1 reveals a considerable overlap with the phenotype of Sty1 mutants. This is consistent with the structural framework of the basic module of the SAPK pathway (Fig. 1). However, several phenotypic traits appear to be Sty1-specific, suggesting that there are other downstream targets for Sty1 besides the well characterized transcription factors considered so far. For example, the sensitivity and survival response of cells to certain DNA damaging agents is dependent upon Sty1-stimulated gene transcription but is not necessarily mediated by transcription factors Atf1/Pcr1 or Pap1 [42]. Recently, the identification of other targets for Sty1 has been facilitated by microarray analysis. This has allowed the characterization of a novel regulatory protein that acts downstream of the Sty1 MAPK and whose expression is up-regulated in a Sty1-dependent manner following exposure to stress [42]. Notably, this target is not a transcription factor but a protein kinase, named Srk1p (from Sty1 regulated kinase 1). In addition to being induced by the Sty1 MAPK cascade, Srk1p is also post-translationally phosphorylated by Sty1 kinase and then translocates to the nucleus, where substrates for this kinase might amplify the stress response (Fig. 1). The identification of further intranuclear targets for Srk1 will improve our understanding of how stress signals are relayed out of the MAPK module. Protein kinase Srk1 shows a high degree of homology to the Rck2 protein kinase found in *S. cerevisiae*, which is a substrate for the omostress sensing Hog1 MAPK [1, 49]. It is likely that other substrates for Sty1 may exist since deletion of *srk1*$^+$ does not result in any obvious stress sensitivity. Rather, this protein kinase appears to be involved in the regulation of sexual development as an inhibitor of meiosis [42]. To date, however, it is not clear which transcription factors are involved in the expression of *srk1*$^+$ itself.

Nutritional sensing

The level of intracellular cAMP plays a role in regulating the adaptation to external conditions and the induction of cellular differentiation [22]. This nucleotide is produced by adenylate cyclase, which in *S. pombe* is encoded by *cyr1*$^+$. Since cAMP is made using ATP as substrate, cells under conditions of active energy-yielding metabolism are more likely to contain high level of cAMP. In *S. pombe*, the main target of cAMP is cAMP-dependent protein kinase A (Pka1) and, unlike in *S. cerevisiae*, only one *pka*$^+$ gene is present in the fission yeast [22]. At low cAMP levels, Pka1 activity is maintained silent in a Pka-Cgs complex. In contrast, at high cAMP levels, the regulatory subunit Cgs is sequestered from Pka1, which acquires full catalytic phosphorylating activity upon dissociation from the blocking component. This is a critical step for sensing and response because it links the status of the cell, as determined by external nutritional factors, to intracellular biochemical reactions. For instance, conditions unfavorable to cell growth decrease cAMP levels, resulting in decreased Pka1 activity. Down-regulation of Pka1 during starvation allows both the adaptation to adverse conditions and the onset of sexual reproduction through gross changes in gene expression. A considerable body of evidence has emerged from these, and related, observations to indicate that the response of *S. pombe* to nutrient deprivation triggers a sensing pathway by which many transcription factors are negatively regulated through Pka1.

The Pka1-Rst2 connection

The above suggestion has received strong support by the finding that Pka1 activity suppresses the transcription of *ste11*$^+$ (which is in turn responsible for the expression of genes required for the initiation of sexual reproduction) as well as the transcription of *fbp1*$^+$ (which encodes the key enzyme for gluconeogenesis, fructose-1,6-bis-phosphatase) [15]. It is likely that many other genes are under the regulation of the Pka1-Rst2 pathway. Induction of *ste11*$^+$ expression occurs in response to nitrogen starvation, whereas expression of *fbp1*$^+$ is induced in response to glucose consumption. Cells lacking *fbp1*$^+$ fail to proliferate on non-fermentable carbon sources because they cannot generate glucose by gluconeogenesis. Pka1 regulates both sexual development and gluconeogenesis through this recently uncovered pathway by means of differential phosphorylation of the transcription factor Rst2, which is similar to the *S. cerevisiae* transcription factors Adr1, Msn2, and Mig1 involved in glucose repression. Fission yeast Rst2 is a zinc-finger protein that in the unphosphorylated state binds to an

upstream STRE-like motif in the promoter region of *ste11*$^+$ and *fbp1*$^+$ thereby mediating the transcriptional control of these genes by Pka1 [15]. Direct phosphorylation of Rst2 by Pka1 suppresses its transcription-activating activity (Fig. 1). There is evidence for a correlation between the subcellular localization of Rst2 and the activity of Pka1 in the cell since this transcription factor shuttles between the nucleus and cytoplasm, which explains why the transcription-activating function of Rst2 is Pka1-dependent. Rst2 is mostly located in the cytoplasm when Pka1 activity is high and tends to be nuclear when Pka1 activity is low. Although the Pka1-Rst2 pathway (Fig. 1) clearly underlies modulation of *ste11*$^+$ and *fbp1*$^+$ transcription in response to nutritional conditions, there must be additional pathways connecting the presence of nitrogen to repression of *ste11*$^+$ and the presence of glucose to repression of *fbp1*$^+$. In fact, several factors other than Rst2 have been shown to participate in the activation and repression of these genes [15]. Interestingly, the Pka1-Rst2 pathway and the MAPK pathway outlined earlier regulate transcription of *ste11*$^+$ and *fbp1*$^+$ in an opposite manner. Activation of the Pka1-Rst2 pathway results in the inhibition of expression of both genes, whereas activation of Sty1 MAPK stimulates their transcription. This situation exemplifies the existence of cross-talk between regulatory pathways.

Post-transcriptional modification of enzymes induced by stress

Although many genes activatable by stress code for enzymes that participate directly in coping with adverse extracellular conditions (e.g. *ctt*$^+$ in oxidative stress, *gpd*$^+$ in osmostress, and *tps*$^+$ in heat shock), others code for enzymes whose role in the stress response is less clear. However, irrespective of the nature of the triggering extracellular stimulus, many genes are markedly induced as part of a general stress response. As a result of this so-called cross-adaptation cells adapted to one type of stress can survive under other stress conditions. While stress-response genes can be used as probes for the stress status of the cell, in some cases, their increased transcription alone does not determine the actual degree of function of the gene products.

Another important role of Pka1 in the sensing systems of *S. pombe* includes post-translational modification of enzymes that play a pivotal role in cellular metabolism. In this context, the modulation of neutral trehalase has been studied in some detail. This enzyme is responsible for mobilizing the pool of trehalose, a disaccharide that is both reserve and stress metabolite in yeast cells [4, 52]. Discrepancies observed during stress between the expression of the structural gene *ntp1*$^+$ and the specific activity of the corresponding enzyme Ntp1 [45] suggest that post-transcriptional control of Ntp1 occurs besides transcriptional regulation. In analogy to the Ras-adenylate cyclase-cAMP signaling pathway of *S. cerevisiae* [50], we have proposed the existence in the fission yeast of a cAMP-Pka1 signal transduction pathway related to the activation of this hydrolytic enzyme [5]. Ras proteins, however, are not involved in the *S. pombe* transduction pathway. Coexistence in the cytoplasm of the trehalase enzyme and the substrate trehalose requires some regulatory system to control short-term breakage of the disaccharide in response to external conditions. Addition of glucose to derepressed cells triggers trehalase activation, which is preceded by a sharp peak in cAMP levels. Addition to the fission yeast cells of the nucleotide itself promotes an even quicker activation effect, suggesting the involvement of a cAMP-dependent mechanism in the activation of the enzyme. Since deactivation of trehalase activated in vivo can be achieved by treatment with phosphatase in vitro, the results are compatible with the interpretation that trehalase activation results from phosphorylation of the enzyme protein by cAMP-dependent Pka1 [5]. Nevertheless, Pka1 is not the only actor. Instead, cells devoid of Pka1 are unable to activate trehalase by addition of cAMP but, surprisingly, they still activate trehalase after addition of glucose, thus revealing the existence of a cAMP-independent pathway[43, 44]. This alternative pathway for Ntp1 activation relies on a redundant protein kinase, Sck1, which was first characterized in the fission yeast as a cAMP-independent suppressor of Pka1 deficiency [16]. Recent evidence indicates that Sty1 is needed not only for proper *ntp1*$^+$ induction in response to stress but also for modulating the function of Pka1 and Sck1 kinases in the stress-induced activation of neutral trehalase. This illustrates the subtle interaction between different signaling pathways to modulate the activity of stress-responsive enzymes.

Final remarks

As in mammalian cells, the SAPK pathway is activated in *S. pombe* by a wide range of different stresses. This raises the issue of how a single multifunctional pathway can respond selectively, at least in part, in such diverse circumstances. Although the common core of the stress signaling pathways is becoming apparent in *S. pombe*, many details still need to be delineated upstream and downstream of the key molecule Sty1, particularly with respect to the nature of specific sensors. The time course of Sty1 activation is different for various stresses and could be the result of either differential phosphorylation or different primary triggering receptors. The cAMP-Pka1 pathway somehow counterbalances various functions of the SAPK pathway, but the exact cross-talk between these two signal transduction systems is still unclear. This latter pathway is central to many regulatory circuits and may also control the post-translational modification of certain key enzymes. Thus, we are just at the threshold of elucidating systems of signal transduction for diverse stimuli.

The present review was prepared in beloved memory of Herman Jan Phaff, in whose labs at Cruess Hall, Davis, California, one of the authors (TGV) spent several unforgettable years back in the 1970s. Very often, those dedicated to contemplating the activity of the unseen but hinted at microbes are thought of as dull people that lack any feeling for visible, everyday life. But quite to the contrary, Prof. Phaff was a vital scientist who loved many aspects of human existence, especially his mastering of the cello, as all who knew him or of were well aware of. Herman's fine sense and enjoyment of life were apparent whether he was studying yeasts or playing this rather difficult instrument at the Davis Opera House or with the Bay Area Symphony Orchestra. He clearly realized that scientific research and playing music have much in common, both pursuits mobilizing the deepest in the human soul to bring out the best and leave the unnecessary behind. One might wonder whether yeasts themselves somehow sense music. This and other questions remain unresolved for us but perhaps Prof. Phaff has discovered their answers by now.

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