Regulation of Snf1 Protein Kinase in Response to Environmental Stress*

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The Saccharomyces cerevisiae Snf1 protein kinase, a member of the Snf1/AMPK (AMP-activated protein kinase) family, has important roles in metabolic control, particularly in response to nutrient stress. Here we have addressed the role of Snf1 in responses to other environmental stresses. Exposure of cells to sodium ion stress, alkaline pH, or oxidative stress caused an increase in Snf1 catalytic activity and phosphorylation of Thr-210 in the activation loop, whereas treatment with sorbitol or heat shock did not. Inhibition of respiratory metabolism by addition of antimycin A to cells also increased Snf1 activity. Analysis of mutants indicated that the kinases Sak1, Tos3, and Elm1, which activate Snf1 in response to glucose limitation, are also required under other stress conditions. Each kinase sufficed for activation in response to stress, but Sak1 had the major role. In sak1 Δtos3 Δelm1 Δ cells expressing mammalian Ca2+/calmodulin-dependent protein kinase α, Snf1 was activated by both sodium ion and alkaline stress, suggesting that stress signals regulate Snf1 activity by a mechanism that is independent of the upstream kinase. Finally, we showed that Snf1 protein kinase is regulated differently during adaptation of cells to NaCl and alkaline pH with respect to both temporal regulation of activation and subcellular localization. Snf1 protein kinase becomes enriched in the nucleus in response to alkaline pH but not salt stress. Such differences could contribute to specificity of the stress responses.

The Snf1/AMP-activated protein kinase (AMPK)2 family is highly conserved among eukaryotes and plays a central role in responses to metabolic stress (1, 2). In mammals, AMPK regulates glucose and lipid metabolism and is involved in regulating the energy balance both at the cellular and whole body levels. AMPK is activated by hormones, including leptin and adiponectin, and by stresses that cause depletion of cellular ATP and hence elevation of the AMP:ATP ratio, including glucose deprivation, exercise, hypoxia, ischemia, heat shock, oxidative stress, and metabolic poisons such as inhibitors of the tricarboxylic acid cycle and the respiratory chain (2–4). Hyperosmotic stress also activates AMPK, although exposure of cells to sorbitol does not appear to increase the cellular AMP:ATP ratio (5).

In the yeast Saccharomyces cerevisiae, Snf1 protein kinase similarly has important functions in metabolic control. Snf1 protein kinase is required for the adaptation of yeast cells to glucose limitation and for growth on carbon sources that are less preferred than glucose, such as sucrose (hence the name Snf, for sucrose-nonfermenting) and nonfermentable carbon sources (6). The kinase is activated by glucose limitation (7–10), and although the glucose signal(s) regulating Snf1 protein kinase is not known, AMP does not activate Snf1 in vitro (7, 8, 11, 12). Snf1 protein kinase has also been implicated in responses to starvation for other nutrients besides carbon, notably nitrogen (13–15). Snf1 regulates the transcription of a large set of genes (16), controls the activity of metabolic enzymes involved in fatty acid metabolism and carbohydrate storage (7, 12, 17), and has roles in such nutrient-responsive cellular processes as meiosis (18), aging (19, 20), autophagy (21), and filamentous invasive growth (14, 22, 23).

Genetic evidence indicates that Snf1, like AMPK, also has roles in responses to a variety of other environmental stresses. The snf1 Δ mutant exhibits reduced resistance to sodium and lithium ions (24–26), other toxic cations such as hygromycin B (24), alkaline pH (27), and genotoxic stress caused by hydroxyurea, methyl methane sulfonate, or cadmium (28). In addition, snf1 Δ mutant cultures show reduced thermotolerance in the stationary phase (13). Snf1 has been shown to affect transcription of stress-responsive genes such as ENA1, encoding Na+-ATPase; the snf1 Δ mutant is defective in induction of ENA1 in response to alkaline or sodium ion stress (25, 27). Interestingly, when cells are starved for glucose, Snf1 also promotes induction of ENA1 (26), activates heat shock transcription factor Hsf1 (29, 30), and phosphorylates the stress-responsive transcription factor Msn2, thereby inhibiting its nuclear accumulation (31, 32). These findings suggest a role for Snf1 in the regulation of protective mechanisms against various stresses in response to glucose depletion.

Three upstream kinases, Sak1, Tos3, and Elm1, phosphorylate Thr-210 in the activation segment of the Snf1 catalytic subunit (33–35). Mutants lacking all three kinases do not activate Snf1 in response to glucose limitation. Evidence suggests that the three kinases make different contributions to cellular regulation under conditions of different carbon source availability; however, in all cases tested, Sak1 was the major player (36–38). It remains possible that the other two kinases are more prominently involved in responses to other types of stress besides carbon stress.

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2 The abbreviations used are: AMPK, AMP-activated protein kinase; YEP, yeast extract-peptone;YPD, yeast extract-peptone-dextrose; AICA, S-aminomimidazole-4-carboxamidine; GFP, green fluorescent protein; CaMKKα, Ca2+/calmodulin-dependent protein kinase kinase α.
In this paper, we have addressed the role of Snf1 protein kinase in the response to environmental stresses, including ionic, hyperosmotic, and oxidative stress, alkaline pH, and heat shock. To determine whether Snf1 is activated by stress, we assayed Snf1 activity and phosphorylation of Thr-210 after exposure of cells to various stress conditions, and we examined the subcellular localization of Snf1 protein kinase. We have also addressed the roles of the Snf1-activating kinases Sak1, Tos3, and Elm1 in stress responses.

**EXPERIMENTAL PROCEDURES**

**Strains and Media**—*S. cerevisiae* strains were derivatives of W303-1A (MATα trp1 leu2 his3 ura3 ade2 can1) and W303-1B (MATα trp1 leu2 his3 ura3 ade2 can1). Relevant mutant alleles were sak1Δ::KanMX4, tos3Δ::KanMX4, elm1Δ::KanMX4, elm1Δ::ADE2, and elm1Δ::URA3. The hog1::LEU2 allele (39) was a gift from F. Posas. Yeast cultures were grown in yeast extract-peptone (YPE) containing 2% dextrose (YPD) at 30 °C unless otherwise specified. For cells carrying a plasmid, cultures were grown in synthetic complete medium lacking uracil and containing 2% dextrose. To prepare liquid medium at alkaline pH, medium containing 0.1 M HEPES was adjusted to the desired pH with 5 M NaOH. To prepare solid medium at alkaline pH, 4× YPD containing 0.4 M HEPES was adjusted to the desired pH at room temperature and was then mixed with agar precooled to 50 °C.

**Assay of Snf1 Catalytic Activity by Phosphorylation of SAMS Peptide**—Cultures were grown to exponential phase (A$_{600}$ of 1.0) in YPD, and cells (100 ml) were harvested by filtration. The filter was immediately transferred to the appropriate medium, and cells were resuspended, incubated for the indicated time, and harvested again by filtration. Cells were then scraped from the filter and frozen immediately in liquid nitrogen. In the case of oxidative stress, hydrogen peroxide (H$_2$O$_2$; 30% v/v) was added to the YPD culture to the indicated final concentration, and the culture was incubated for 5 min before harvesting. Cell extracts were prepared as described (36), and protein concentrations were determined by Bio-Rad assay. Assays for phosphorylation of a synthetic peptide (HMRSAMSGLHLVKRR; SAMS peptide) (40) were performed as described (7, 36) using different protein concentrations to confirm linearity. Kinase activity is expressed as nmol of phosphate incorporated into the peptide per min per mg of protein (40).

**Immunoblot Analysis**—Proteins (2 μg) were separated on 7.5% SDS-PAGE and analyzed by immunoblotting using anti-AMPK-Thr-172-AMPK antibody (Cell Signaling Technologies). Before the membrane was reprobed with anti-Snf1 antibody (6), it was incubated in 0.2 M glycine, pH 2, for 5 min. ECL-Plus or ECL-Advance (Amersham Biosciences) was used for visualization.

**RESULTS**

**Snf1 Protein Kinase Is Activated in Response to Some Environmental Stresses**—We first examined Snf1 protein kinase activity in cells exposed to salt stress. For this and other experiments, wild-type cells of the W303 genetic background were grown to exponential phase in rich medium containing 2% glucose (YPD). Cells were collected by rapid filtration, resuspended in YPD medium containing 1 M NaCl and harvested at time points from 5 to 60 min. Extracts were prepared. Snf1 was partially purified, and Snf1 catalytic activity was assayed by phosphorylation of the synthetic SAMS peptide substrate. Snf1 activity increased about 3-fold within 5 min, and the activation persisted for more than 1 h (Fig. 1A). In cultures grown to mid-log phase in YPD containing 2% dextrose, to prepare liquid medium at alkaline pH, 4× YPD containing 0.4 M HEPES was adjusted to the desired pH at room temperature and was then mixed with agar precooled to 50 °C.

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containing 1 mM NaCl, Snf1 catalytic activity was elevated (0.87 ± 0.14 nmol/min/mg). To determine the effects of NaCl concentration, cells were shifted to medium containing 0.5, 1.0, or 1.5 mM NaCl for 5 min, and Snf1 activity was assayed; activation was maximal in 1 mM NaCl (Fig. 1B). To determine whether ionoc an hyperosmotic stress was responsible, we exposed cells to sorbitol at concentrations up to 1.5 M; Snf1 activity did not increase substantially (Fig. 1C).

We then tested other stress conditions, including alkaline pH, heat shock, and oxidative stress. Yeast cells prefer an acidic environment, and a shift to alkaline pH causes stress. To assay the effects of alkaline pH on Snf1 activity, cells were grown to exponential phase in YPD (pH of the culture ~6.2), collected by filtration, and resuspended in YPD medium buffered to pH 6–8. Exposure to alkaline pH elevated Snf1 activity within 5 min (Fig. 1D), with a 5-fold increase in Snf1 activity at pH 8. However, after 1 h at pH 8, Snf1 activity was reduced to near basal levels (0.66 ± 0.11 nmol/min/mg); we confirmed that the pH of the medium remained alkaline.

To test the effects of heat shock, we resuspended the cells in prewarmed YPD at 42 and 50 °C and incubated them for 5 min; no activation of Snf1 was detected in response to heat shock (Fig. 1E). In similar experiments, no increase in Snf1 activity was observed when cells were incubated at 40 or 45 °C for 20 and 40 min (data not shown). Immunoblot analysis showed that levels of Snf1 protein did not decrease (data not shown).

To examine the response to oxidative stress, we added H2O2 to the culture at concentrations up to 1 mM for 5 min. Treatment with 0.3 mM H2O2 caused a modest (2-fold) increase in Snf1 activity (Fig. 1F), which was evident by 1 min (data not shown); results were similar for 0.3–1 mM H2O2 after 10 min (data not shown).

We next examined the effect of sudden inhibition of respiratory metabolism. Cells were grown in YEP containing 2% glycerol, a nonfermentable carbon source; utilization of glycerol requires Snf1 function, and the kinase was active in these cells (Fig. 1G). Respiratory was then blocked by the addition of antimycin A, an inhibitor of the cytochrome bc1 complex (41, 42). Snf1 activity increased within 5 min (Fig. 1G). The addition of antimycin A to cells growing by fermentation of glucose had little effect on Snf1 activity (data not shown). In mammalian cells, antimycin A causes depletion of ATP and activates AMPK (2).

Finally, we tested whether metformin or 5-aminoimidazole-4-carboxamide (AICA) riboside, which activate AMPK in mammalian cells, would similarly activate Snf1 protein kinase in yeast cells. The biguanide metformin is widely used in the treatment of type 2 diabetes and causes activation of AMPK (43), apparently as an indirect effect of inhibition of complex 1 of the respiratory chain (44). In mammalian cells, AICA-riboside is phosphorylated to yield an AMP mimetic, which activates AMPK (45). The addition of metformin (up to 10 mM) to yeast cells grown in YPD or in YEP containing 2% glycerol did not affect Snf1 activity after 5 or 30 min (data not shown). In a similar experiment, the addition of AICA-riboside (2 mM) to yeast cells had no effect.

Phosphorylation of Thr-210 of Snf1 in Response to Stress—Snf1 is phosphorylated on Thr-210 in the activation loop in response to glucose limitation (9). To examine the phosphorylation of Thr-210 in cells exposed to NaCl, alkaline pH, or oxidative stress, we carried out immunoblot analysis of the protein samples assayed above using antibody against phospho-Thr-210 of AMPK, which also specifically recognizes phospho-Thr-210 of Snf1 (15, 34, 46). In each case, Thr-210 was phosphorylated, whereas Snf1 protein levels did not significantly change (Figs. 2 and 3D). The extent of phosphorylation in cells exposed to different salt concentrations or pH roughly paralleled the activation of Snf1 catalytic activity (compare with Fig. 1). These results are in agreement with a previous report that Thr-210 is phosphorylated in cells exposed to 0.8 M NaCl for 1 h (9). We did not detect increased phosphorylation after heat shock (5 min at 42 or 50 °C and 20 or 40 min at 45 °C; data not shown).

Protein Kinases Sak1, Tos3, and Elm1 Activate Snf1 in Response to Environmental Stress—Sak1, Tos3, and Elm1 are responsible for activation of Snf1 when cells are subjected to centriofugation or carbon stress (33, 34). To determine whether these kinases also activate Snf1 in response to other stresses, we exposed sak1Δ tos3Δ elm1Δ triple mutant cells to 1 mM NaCl, pH 8, or 0.3 mM H2O2 for 5 min as described above. Snf1 protein kinase was not activated, as determined by assaying phosphorylation of the SAMS peptide (<0.1 nmol/mg/min; Fig. 3A and data not shown), and no phosphorylation of Thr-210 was detected by immunoblot analysis (Fig. 3C). These findings indicate that activation of Snf1 protein kinase in response to all of these different stress conditions requires Sak1, Tos3, or Elm1.

We next addressed the possibility that the three upstream kinases exhibit specificity for activation of Snf1 in response to different stresses. To examine the requirement for individual upstream kinases in the response to salt stress, we treated the
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Activation of Snf1 protein kinase in cells lacking upstream kinases—These findings indicate that Sak1 is the major, although not the sole, upstream kinase that activates Snf1 in response to these different stresses.

The basal Snf1 catalytic activity was low in cells expressing only Tos3 or Elm1, activity increased upon exposure to 1 M NaCl for 5 min (Fig. 3A). Sak1 was sufficient for nearly normal activity in glucose-grown tos3Δ cells and for activation to wild-type levels upon salt stress. Thus, activation of Snf1 in response to salt stress signals can be mediated by any one of these upstream kinases, with Sak1 being the most effective.

We further assessed the possibility that the different upstream kinases have different relative importance after adaptation to high salt concentration than during acute stress. Cultures of single mutant cells were grown to mid-log phase in YPD containing 1 M NaCl, and Snf1 catalytic activity was assayed; Sak1 remained the primary upstream kinase under these conditions (Fig. 3D).

Finally, we considered the possibility that the high osmolarity glycerol (HOG) mitogen-activated protein kinase (MAPK) pathway (47) has a role in regulating the activation of Snf1 in response to salt stress. Wild-type and hog1Δ mutant cells were grown in YPD and assayed after no treatment or after exposure to 1 M NaCl for 5 min. The mutation had no detectable effect on Snf1 activity (data not shown).

Snf1 is Regulated by Sodium Ion and Alkaline Stress When Activated by a Heterologous Upstream Kinase—These findings show that each of the three upstream kinases activates Snf1 in response to salt stress suggest that each kinase mediates salt stress signaling or that signaling is independent of the upstream kinase. To distinguish between these possibilities, we took advantage of the ability of a heterologous kinase, mammalian Ca2+/calmodulin-dependent protein kinase kinase α (CaMKKα). We took advantage of the ability of a heterologous kinase, mammalian Ca2+/calmodulin-dependent protein kinase kinase α

sak1Δ, tos3Δ, and elm1Δ single mutants with 1 M NaCl for 5 min and assayed Snf1 protein kinase activity. The sak1Δ mutant showed a marked decrease in Snf1 activity relative to wild type, whereas the elm1Δ mutant showed a modest decrease and the tos3Δ mutant was indistinguishable from wild type (Fig. 3A). Similar results were observed previously for the response of these mutants to abrupt glucose depletion (36). The sak1Δ mutant still showed a 3-fold increase in Snf1 activity in response to salt stress, because the basal Snf1 activity was also lower in the mutant than in the wild type. Mutation of SAK1 also impaired activation of Snf1 in response to alkaline pH and oxidative stress (Fig. 3B; compare Fig. 1). Immunoblot analysis showed that phosphorylation of Thr-210 was reduced in the sak1Δ mutant relative to wild type (Fig. 3C). These findings indicate that Sak1 is the major, although not the sole, upstream kinase that activates Snf1 in response to these different stresses.

To determine whether each of the upstream kinases alone suffices for activation of Snf1 by salt stress, we examined double mutants expressing only Tos3 (sak1Δ elm1Δ), Elm1 (sak1Δ tos3Δ), or Sak1 (tos3Δ elm1Δ). Although basal Snf1 catalytic activity was low in cells expressing only Tos3 or Elm1, activity increased upon exposure to 1 M NaCl for 5 min (Fig. 3A). Sak1 was sufficient for nearly normal activity in glucose-grown tos3Δ elm1Δ cells and for activation to wild-type levels upon salt stress. Thus, activation of Snf1 in response to salt stress signals can be mediated by any one of these upstream kinases, with Sak1 being the most effective.
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(CaMKKα), to function as a Snf1-activating kinase. Expression of CaMKKα suffices for activation of Snf1 protein kinase in glucose-deprived $sak1\Delta tos3\Delta elm1\Delta$ yeast cells (48).

To assess the requirement for native yeast upstream kinases to mediate salt and alkaline pH stress signals, we examined $sak1\Delta tos3\Delta elm1\Delta$ cells expressing CaMKKα from a plasmid. Cells were grown to mid-log phase in synthetic medium with selection for the plasmid and were shifted to medium containing 1 M NaCl or buffered to pH 8 for 5 min. In both cases, stress caused elevation of Snf1 catalytic activity, as judged by phosphorylation of the SAMS peptide; in control cells carrying the vector, virtually no activity was detected (Fig. 4). Thus, mammalian CaMKKα conferred activation of Snf1 in response to two different stress signals. As it is unlikely that CaMKKα is positively regulated by both sodium ion and alkaline stress signals in yeast cells, these findings provide strong evidence that both of these stress signals regulate Snf1 protein kinase activity by a mechanism that is independent of the upstream kinases.

Requirement for Snf1 and Snf1-activating Kinases for Cellular Resistance to Stress—Previous studies indicated that phosphorylation of Snf1 on Thr-210 is not essential for resistance to hydroxyurea (28) or for resistance to toxic cations such as hygromycin B, mediated by the Trk high-affinity potassium transporter (24). To determine whether phosphorylation of Snf1 protein kinase by the upstream kinases is required for tolerance of cells to the stress conditions examined here, we compared wild-type, snf1Δ, sak1Δ, and sak1Δ tos3Δ elm1Δ cells. Both snf1Δ and triple mutant cells showed a marked reduction in growth in the presence of 1 or 1.5 M NaCl but were resistant to 1.5 M sorbitol (Fig. 5 and data not shown); similar results were reported for snf1Δ cells of the S288C genetic background (26). The sak1Δ single mutant, in which activation of Snf1 is reduced but not abolished (Fig. 3), exhibited little or no impairment of growth (Fig. 5); tos3Δ and elm1Δ also did not markedly impair growth (data not shown). Both wild-type and sak1Δ cells grew on medium buffered to pH 8, whereas snf1Δ and sak1Δ tos3Δ elm1Δ mutant cells did not (Fig. 5). Taken together, these findings suggest that phosphorylation of Thr-210 by upstream kinases is required for tolerance of salt and alkaline stress.

We did not detect sensitivity of the snf1Δ mutant to $H_2O_2$ as judged by an assay for growth inhibition (data not shown), in accord with a previous report (28). The modest activation of the kinase observed upon treatment of cells with $H_2O_2$ (Fig. 1F) is apparently not critical for cell survival.

We also tested both overnight and mid-log phase cultures for heat sensitivity by exposing cells to 50°C for up to 90 min, because reduced viability at 50°C was reported previously for snf1Δ cells (different genetic background) in stationary phase cultures (13). The $snf1\Delta$ and $sak1\Delta tos3\Delta elm1\Delta$ cultures showed reduced viability at 50°C (Fig. 5 and data not shown). Together with evidence that Snf1 activity and phosphorylation of Thr-210 did not increase significantly in wild-type cells in response to heat shock (Fig. 1E and data not shown), these results suggest that phosphorylation of Snf1 is required for thermotolerance but that low level activation of Snf1 suffices.
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We showed previously that nuclear localization of Snf1 requires activation (36). To exclude the possibility that activation by sodium ion stress is restricted to kinase complexes that do not contain Gal83, we assayed β-subunit mutants. The sip1Δ and sip2Δ mutants showed increased Snf1 catalytic activity after exposure to 1 mM NaCl for 5 min, and the gal83Δ mutant showed much reduced activation (data not shown), consistent with evidence that Gal83 is the major β subunit (50). These findings indicate that activation of Snf1 protein kinase by salt stress is not accompanied by nuclear localization.

DISCUSSION

We addressed the role of Snf1 protein kinase in the response to various environmental stresses. Exposure of cells to sodium ion stress, alkaline pH, and oxidative stress resulted in phosphorylation of Thr-210 of Snf1 and elevation of Snf1 catalytic activity, whereas treatment with sorbitol and heat shock did not. Inhibition of respiratory metabolism by addition of antimycin A to cells growing on nonfermentable carbon sources also caused an increase in Snf1 activity. In mammalian cells, treatment with antimycin A is thought to elevate AMPK activity by depleting ATP; other stress conditions that activate AMPK are also associated with elevated AMP:ATP ratios (2, 4), with the notable exception of sorbitol treatment (5). It is not yet clear whether changes in the AMP:ATP ratio affect Snf1 activity, although published evidence does not support this idea (7, 8, 11, 12). Nonetheless, these findings indicate that Snf1, like AMPK, is activated in response to a variety of environmental stresses.

We determined the roles of the Snf1-activating kinases Sak1, Tos3, and Elm1 in stress responses. The sak1Δ tos3Δ elm1Δ triple mutant cells were defective in activation of Snf1 catalytic activity and phosphorylation of Thr-210 in response to NaCl stress, alkaline pH, and oxidative stress. Analysis of single and double mutants indicated that Sak1 is primarily responsible for activation, as is also the case for carbon stress (36–38). Although Tos3 and Elm1 each sufficed for some activation of Snf1 in response to salt stress, Sak1 alone conferred activation to nearly wild-type levels. These results indicate that Sak1 is the principal Snf1-activating kinase in the cascade, which is consistent with evidence that Sak1 is associated with Snf1 in a stable complex (51).

We also showed that Snf1 protein kinase is regulated differently during adaptation of cells to NaCl and alkaline pH with respect to both temporal regulation of activation and subcellular localization. First, the time course of Snf1 activation was specific to the environmental stress. Upon exposure of cells to 1 mM NaCl, activation was rapid and persistent. In contrast, upon exposure to alkaline pH, Snf1 was again rapidly activated, but activity returned to basal levels within 1 h. These findings are in accord with studies showing rapid alterations in genomic expression patterns in response to many stresses and, in particular, with findings that the changes in response to alkaline pH are generally more transient than those occurring as a result of exposure to increased salt concentration (52).

Second, Snf1 protein kinase was differently regulated in response to high salt and alkaline pH with respect to its subcel-
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lular localization. Snf1 and the β subunit Gal83, which are cyto-
solic in unstarved cells, became enriched in the nucleus when
cells were subjected to alkaline pH, as is also the case for carbon
stress. In contrast, they did not become nuclear in response to
salt stress but rather remained largely nuclear excluded. Such
differential localization confers differential access to substrates,
notably nuclear proteins, and most likely contributes to the
specificity of stress responses. These findings could account for
the phosphorylation of the nuclear repressor Mig1 by
Snf1 in response to carbon stress but not salt stress (9, 49).

We found that sak1Δ tos3Δ elm1Δ triple mutant cells, like
snf1Δ cells, exhibited defects in growth in 1 M NaCl or alkaline
pH and sensitivity to heat shock, suggesting that phosphoryla-
tion of Thr-210 by upstream kinases is required for resistance of
yeast cells to these stress conditions. The sensitivity to heat
shock, which did not detectably activate Snf1 in wild-type cells,
further suggests that very low levels of Snf1 activity suffice for
thermotolerance. Previous studies showed that phosphoryla-
tion of Thr-210 is not essential for resistance to hygromycin B
or hydroxyurea, and it has been suggested that weak Snf1 activity
is sufficient (24, 28). We also note that the sak1Δ mutation
significantly impaired activation of Snf1 protein kinase in response to salt stress, alkaline pH, glucose depletion, and
growth on nonfermentable carbon sources, but we and others
(this study and Refs. 33 and 35–38) have not observed marked
growth defects under any of these conditions. Taken together,
these findings suggest that the Snf1 protein kinase pathway is
robust and that low levels of Snf1 activity suffice for growth
under many different stress conditions.

The mechanism(s) that mediates regulation of Snf1 protein
kinase activity by stress signals is not yet clear. We have pre-
presented evidence that the yeast Snf1-activating kinases are not
required to mediate stress signaling. In sak1Δ tos3Δ elm1Δ cells
expressing mammalian CaMKKa, Snf1 was activated by both
high salt and alkaline pH, strongly suggesting that regulation
is independent of the particular upstream kinase. Studies of
sak1Δ tos3Δ elm1Δ cells in which mammalian LKB1 or
CaMKKa served as the Snf1-activating kinase similarly indi-
cate that a mechanism independent of the upstream kinases
mediates glucose signaling (48). Stress signals could act
directly on Snf1 protein kinase, perhaps controlling its
accessibility for phosphorylation or dephosphorylation, or
such signals could act on the Reg1-Glc7 form of protein
phosphatase 1, which dephosphorylates Thr-210 (9). It
remains possible, however, that the yeast upstream kinases
are also directly regulated by stress signals and contribute in
part to the regulatory response.

It has long been recognized that Snf1 protein kinase and
AMPK have similar roles in metabolic control in response to
nutrient signals. These studies extend the parallels between
Snf1 and AMPK and suggest that yeast will serve as a useful
model system for understanding the roles of Snf1/AMPK path-
ways in responses to environmental stress.

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