A Cooperative Role for Atf1 and Pap1 in the Detoxification of the Oxidative Stress Induced by Glucose Deprivation in Schizosaccharomyces pombe

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In Schizosaccharomyces pombe, glucose concentrations below a certain threshold trigger the stress-activated protein kinase (SAPK) signal transduction pathway and promote increased transcription of Atf1-dependent genes coding for the general stress response. Removal of glucose specifically induces the nuclear accumulation of green fluorescent protein-labeled Pap1 (GFP-Pap1) and the expression of genes dependent on this transcription factor. In contrast, depletion of the nitrogen source triggers the SAPK pathway but does not activate Pap1-dependent gene transcription, indicating that carbon stress rather than growth arrest leads to an endogenous oxidative condition that favors nuclear accumulation of Pap1. The reductant agents glutathione or N-acetylcysteine suppress the nuclear accumulation of GFP-Pap1 induced by glucose deprivation without inhibiting the activation of the MAPK Sty1. In addition, cells expressing a mutant GFP-Pap1 unable to accumulate into the nucleus upon hydrogen peroxide-mediated oxidative stress failed to show this protein into the nucleus in the absence of glucose. These results support the concept of a concerted action between the SAPK pathway and the Pap1 transcription factor during glucose exhaustion by which glucose limitation induces activation of the SAPK pathway prior to the oxidative stress caused by glucose deprivation. The ensuing induction of Atf1-dependent genes (catalase) decreases the level of hydroperoxides allowing Pap1 nuclear accumulation and function. Congruent with this interpretation, glucose-depleted cells show higher adaptive response to exogenous oxidative stress than those maintained in the presence of glucose.

Glucose is a powerful signaling molecule that promotes major metabolic changes in cells (1). Glucose metabolism produces compounds directly related to the detoxification of intracellular hydroperoxides formed as byproducts by ongoing metabolic processes (2). In human tumor cells, which typically show strong glycolysis and a reduced rate of respiration, deprivation of glucose causes a strong metabolic oxidative stress characterized by increased steady state levels of intracellular hydroperoxides and glutathione disulfide (3, 4). Strong evidence indicates that the absence of glucose also triggers signaling cascades that activate transcription factors and the expression of stress-related genes attempting to redirect cellular functions (2). Remarkably, glucose metabolism in some fermenting yeasts is quite similar to that of tumor cells. In particular, the Crabtree-positive fission yeast S. pombe ferments glucose under aerobic conditions. Unlike Saccharomyces cerevisiae, this yeast lacks enzymes of the glyoxylate cycle that maintain di- auxic growth after glucose depletion and utilizes very few non-sugar carbon sources (5, 6). Thus, as soon as glucose disappears and respiration of the fermentation products is impaired, cultures of S. pombe may suffer nutritional stress.

The mitogen-activated protein kinase (MAPK)1 signaling pathways are critical for the sensing and response of eukaryotic cells to changes in the external environment (7, 8). These MAPK cascades are highly conserved through evolution and serve to transduce signals to the nucleus, resulting in new patterns of gene expression (9, 10). The identification of a highly conserved stress-activated protein kinase (SAPK) pathway in S. pombe allows us to analyze the mechanisms by which SAPKs are activated in a system more amenable than higher eukaryotic cells (11–15). In this yeast, the central element of the SAPK cascade is the MAPK Sty1 (also known as Spc1 or Ph11), which is highly homologous to mammalian p38 kinase and becomes activated by a similar series of stresses (12, 13, 15–17). MAPK Sty1 is directly phosphorylated by MAPK kinase Wis1; however, the transmission pathway of the stress signal to Wis1 is dual, and either MAPK kinase Wis1 (also known as Wis4 or Wk1) or MAPK kinase Win1 is responsible for Wis1 phosphorylation (18). A response regulator protein, Mcs4, associates with Wk1, and probably with Win1, to regulate MAPK kinase kinase activity in response to several stimuli (17, 19). In S. pombe different transcription factors function downstream of the MAPK Sty1 cascade, among which Atf1 and Pap1 have been studied extensively. Atf1 (also known as Gad7 or Mts1) is a mammalian ATF-2 homologue b-ZIP protein that associates to and is phosphorylated by Sty1 following different stresses (20–22). In fact, Sty1 is the only

1 The abbreviations used are: MAPK, mitogen-activated protein kinase; SAPK, stress-activated protein kinase; DEM, diethyl maleate; Ha, hemagglutinin; Ha6H, epitope comprising hemagglutinin antigen plus six histidine residues; GFP, green fluorescent protein; EMM, Edinburgh minimal medium; GSH, glutathione; NAC, N-acetyl-L-cysteine; kbp, kilobase pair; ASK1, apoptosis signal-regulating kinase 1.

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known kinase involved in Atf1 phosphorylation during stress. Transcription of a wide array of stress-response genes like gpx1 (coding for glutathione peroxidase), ntp1 (neutral trehalase), ctt1 (cytoplasmic catalase), fbp1 (fructose-1,6-bisphosphatase), or ste11 (a high mobility group protein involved in the regulation of sexual differentiation) is controlled by Sty1 through Atf1 (21, 22). Another transcription factor, Pap1, encoded by the pap1 gene, has been isolated as required for survival to oxidative stress and, like its S. cerevisiae homologue YAP1, shows high homology to mammalian c-Jun (23). S. pombe cells deleted in pap1 show high sensitivity to oxidative stress but not to osmotic stress or nutrient deprivation (24). Moreover, Pap1 activity is regulated at the level of cellular localization. In glucose-growing cells Pap1 localizes to the cytoplasm but accumulates in the cell nucleus upon oxidative stress with hydrogen peroxide or the glutathione-depleting agent diethyl maleate (DEEM). Hydrogen peroxide reversibly oxidizes two cysteine residues in Pap1 (at positions 278 and 501), whereas DEEM induces a non-reversible modification (25). As a result, modified Pap1 is unable to interact in both cases with the exportin Crm1 through the nuclear export signal of yeast (25, 501), whereas DEM induces a non-reversible modification (25). 

In particular genes for defense against oxidative stress, like ctt1 (cat-

alase), ttr1 (thioredoxin reductase), or sod1 (superoxide dismutase) (24, 26). In contrast to Atf1, Pap1 is neither phosphorylated nor a substrate for Sty1 upon stress conditions; however, Sty1 presence/function is needed for nuclear accumulation of Pap1 in response to high concentrations of hydrogen peroxide, but not at low concentrations of the proxidant (27, 28). Several results have been reported previously that carbon starvation is an environmental stress able to activate the MAPK Sty1 in S. pombe (29, 30). However, the contribution of the SAPK pathway to the cellular responses under glucose deprivation after fermentative growth has not been investigated in detail. Transfer of cells from a glucose-rich to a glucose-free culture medium without an alternative carbon source may help to reveal characteristic responses normally masked under conditions of sugar availability or cell growth. Following such an approach we have analyzed the stress signal induced by glucose deprivation in cultures maintained under similar osmotic conditions to avoid the incidence of conditions unrelated to the absence of glucose. In this work we demonstrate that glucose limitation in S. pombe not only promotes activation of the SAPK signaling pathway that results in increased expression of Atf1-dependent stress-related genes but also induces an oxidative stress that favors the concerted expression of additional genes depending on the transcription factor Pap1. These results may help us to understand the mechanisms underlying the comparative resistance of glucose-depleted cells against external oxidative conditions.

EXPERIMENTAL PROCEDURES

Strains, Plasmids, and Growth Conditions—The S. pombe strains employed in this study were the wild type strain TK003 (h- leu1–32) (14) and the mutants TK107 (h- leu1–32 ura4-D18 sat4::ura4) (14), NT146 (h- leu1–32 ura4-D18 sat4::ura4) (20), TP108-3c (h- leu1–32 ura4-D18 sat4::ura4) (23), and GA334 (h- leu1–32 ura4-D18 sat4::ura4) (23). Strains JM1521 (h- ade6-M210 his3–666 leu1–32 ura4-D18 sty1::Ha6H(ura4)) and JM1821 (h- ade6-M216 leu1–32 ura4-D18 atf1::Ha6H(ura4)) harbor a genomic copy of sty1 or atf1 tagged with two copies of the Ha epitope and six histidine residues, respectively (23). To visualize the localization of a GFP-Pap1 fusion, we used strain EHH14, which harbors an integrated copy of the wild type GFP-Pap1 chimeric gene under the control of the thiamine-repressible promoter nmt1 (25). Strain EHH14.2C78A encodes a mutated version of GFP-Pap1 in the cysteine residue 278 of Pap1, which is critical for protein oxidation mediated by hydrogen peroxide (25). Plasmid p41GFP-Pap1 expresses an amino-terminal GFP-fused variant of Pap1 under the control of an attenuated version (41X) of the nmt1 promoter (24). S. pombe strains were routinely grown with shaking at 28 °C in EMM2 (32) with 7% glucose (repressing conditions) to a final A600 of 0.5, recovered by filtration, and resuspended in the same medium without glucose but with glyceral, sorbitol, glucose plus glyceral, or glucose plus sorbitol, depending on the particular experiment (see below). When indicated, reduced glutathione (GSH, 0.16 mM) or N-acetyl-l-cysteine (NAC, 30 mM) was added (2, 33). Culture media were supplemented with adenine, leucine, histidine, or uracil (100 mg/liter, all obtained from Sigma) depending on the requirements for each particular strain. Transformation of yeast strains was performed by the lithium acetate method as described elsewhere (32).

Protein Extraction and Detection of Activated Sty1-Ha6H and Atf1-Ha6H Proteins following Glucose Deprivation—Yeast cells grown in EMM2 with 7% glucose to an A600 of 0.5 (actual glucose concentration ~6%, determined by the glucose oxidase method) were recovered by filtration and resuspended in the same medium devoid of glucose. EMM2 without glucose was osmotically equilibrated with 3% glyceral, 2.8% glyceral plus 0.5% glucose, 2.5% glyceral plus 1% glucose, 6% sorbitol, 5% sorbitol plus 0.5% glucose, or 5% sorbitol plus 1% glucose. At different times, the cells from 30 ml of culture were harvested by centrifugation at 4 °C, and yeast pellets were immediately frozen in liquid nitrogen. Under these conditions, the previously described Sty1 phosphorylation resulting from centrifugation (30, 32) was not observed in unstrengthened cultures. To analyze Sty1, total yeast homogenates prepared under different conditions employing chilled acid-washed glass beads and lysis buffer (10% glyceral, 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Nonidet P-40) supplemented with specific protease and phosphatase inhibitor cocktails (Sigma). The lysates were removed and cleared by centrifugation at 13,900 × g for 15 min. Ha6H-tagged Sty1 was purified by using nickel-nitrilotriacetic acid-agarose beads (Qiagen Inc.) as reported previously (32). The purified proteins were resolved in 10% SDS-polyacrylamide gels, transferred to nitrocellulose filters (Amer- sham Biosciences), and incubated with either mouse anti-Ha (Roche Applied Science, clone 12C5A) or mouse antiphospho-pS8 (New Eng- land Biolabs) antibodies. The immunoreactive bands were revealed with anti-mouse horseradish peroxidase-conjugated anti-

body (Sigma) and the ECL system (Amer-sham Biosciences). For Atf1- Ha6H purification, the pelleted cells were lysed into denaturing lysis buffer (6 M guanidine HCl, 0.1 M sodium phosphate, 50 mM Tris HCl, pH 8.0), and the Atf1 protein was isolated by affinity precipitation on nickel-nitrilotriacetic acid-agarose beads as described previously (34). The purified proteins were resolved in 6% SDS-polyacrylamide gels, transferred to nitrocellulose filters (Amer-sham Biosciences), and incubated with a mouse anti-Ha antibody (12C5A).

RNA Isolation and Hybridization—Yeast cells grown in EMM2 with 7% glucose to an A600 of 0.5 were recovered by filtration and resuspended in the same medium with 3% glyceral. Volumes of 50 ml of the yeast cultures were recovered at different times, and total RNA preparations were obtained as described by Franco et al. (35) and resolved through 1.5% agarose-formaldehyde gels. Northern (RNA)-hybridization analy- ses were performed as described by Sambrook et al. (36). The probes employed were amplified by PCR and included the following: a 0.7-kbp fragment of the apt1 gene (23) that was amplified with the 5'-oligonucleotide CCCACTATGCTCTACC and the 3'-oligonucleotide AAGCTT- TACCTGGCG, a 0.4-kbp fragment of the gpx1 gene (37) amplified with the 5'-oligonucleotide TTCTAGACTGGGCT and the 3'-oligonucleotide ACACTTCGATAGT, a 0.9-kbp fragment of the trt1 gene (38) amplified with the 5'-oligonucleotide GTGACTCAACAAAAG and the 3'-oligonucleotide TAACTCGGTATCTCC, a 2.1-kbp fragment of the gpy2 gene (39) amplified with the 5'-oligonucleotide TAAAGGCGCTTCT- TGGA and the 3'-oligonucleotide AAAGGGCTTGAAACCCCTG, a 1-kbp fragment of the fbp1 gene (39) amplified with the 5'-oligonucleotide CTCTTACCCAGCCGTAAG TCC and the 3'-oligonucleotide GATCTGGACAGGAATCCGAC. Probes for ttr1 and leu1 were prepared as reported previously (32). To establish quantitative conclusions, the relative mRNA levels of the RNAs were determined by using the comparative CT method (2) and compared with the internal control (leu1 mRNA).

Fluorescence Microscopy—To localize the GFP-Pap1 fusion, yeast cultures were grown in EMM2 with 7% glucose (with or without GSH and NAC) to an A600 of 0.5, recovered by filtration, and resuspended in the same medium with 6% glucose but lacking the nitrogen source (ammonium) at pH 7 (EMM2-N). S. pombe cells were grown in 0.01% lithium acetate method as described elsewhere (32).
clear staining, 3 μl of Hoechst in 50% glycerol was added. Fluorescence microscopy was performed on a Leica DM 4000B microscope with a 100 objective. Images were captured with a cooled Leica DC 300F camera and IM50 software and then imported into Adobe PhotoShop 6.0 (Adobe Systems, Mountain View, CA).

Cell Viability Assays and Analytical Determinations—Yeast strains grown in YES medium (2% glucose and 0.6% yeast extract) with 7% glucose to an A$_{600}$ of 0.5 were recovered by filtration, resuspended for 1 h at 28 °C in the same medium with either 6% glucose or 3% glycerol, and treated for 1 h with 80 μM H$_2$O$_2$. The samples were diluted and spread in triplicate onto plates containing YES solid medium, and cell viability was measured by their ability to form colonies on this medium after incubation at 28 °C for 5 days. Results represent the mean values ± S.D. from three different experiments. Glucose concentration in the growth media was assayed by the glucose oxidase method (40). Protein determination was performed according to Lowry et al. (41).

RESULTS

SAPK Activation during Glucose Depletion—We first analyzed the kinetics of Sty1 activation due to glucose exhaustion. An exponentially glucose-growing culture of strain JM1521, which harbors a genomic copy of sty1$^+$ tagged with two copies of the Ha epitope and six histidine residues (32), was shifted to a similar medium containing a non-assimilated carbon source (glycerol or sorbitol) instead of glucose. It should be noted that S. pombe does not feed on glycerol alone unless glucose is present for initial growth (42) and that sorbitol is not a carbon source for this yeast (43) either. These compounds were used to prevent drastic osmotic changes in the non-glucose-containing medium and, hence, to avoid any potential disturbance of the SAPK pathway unrelated to glucose deprivation (44). Samples were collected at different times from the new medium. Sty1 and Atf1 were purified by affinity chromatography. Activated Sty1 was detected by immunoblotting with antiphospho-p38 antibodies. Total Sty1 and Atf1 (in both phosphorylated and unphosphorylated forms) were determined by immunoblotting with anti-Ha antibody.

Fig. 1. SAPK pathway activation in S. pombe under glucose limitation. Strains JM1521 and JM1821 carrying Ha6H-tagged chromosomal versions of the sty1$^+$ or the atf1$^+$ gene, respectively, were grown in EMM2 plus 7% glucose to early log phase and transferred to the same medium with 3% glycerol (A), 2.5% glycerol plus 1% glucose (B), 2.8% glycerol plus 0.5% glucose (C), 6% sorbitol (D), 5% sorbitol plus 1% glucose (E), and 5.5% sorbitol plus 0.5% glucose (F). Aliquots were harvested at different times, and Sty1 or Atf1 was purified by affinity chromatography. Activated Sty1 was detected by immunoblotting with antiphospho-p38 antibodies. Total Sty1 and Atf1 (in both phosphorylated and unphosphorylated forms) were determined by immunoblotting with anti-Ha antibody.

Fig. 2. The SAPK pathway regulates the induction of different stress-responsive genes under glucose limitation. Strains TK003 (wild type (WT)) and NT146 (Δatf1) were grown in EMM2 plus 7% glucose to early log phase and transferred to the same medium with 3% glycerol for the times indicated. Total RNA was extracted from each sample, and 20 μg was resolved in 1.5% agarose-formaldehyde gels. Denatured RNAs were transferred to nylon membranes and hybridized with 32P-labeled probes of fbp1, pyp2, gpx1, ctt1, and leu1 (loading control).
stated that Atf1 of unstressed cells migrates in gel as a single protein of ~85 kDa that undergoes a phosphorylation-dependent band shift under different stresses (32). As shown in Fig. 1A, glucose deprivation induced a Sty1-dependent band shift in the migration of Atf1 because of in vivo phosphorylation, whose initial kinetics matched closely that observed for Sty1 phosphorylation. Essentially identical results were obtained when sorbitol was used to balance osmolarity (Fig. 1D), confirming that the activation of the SAPK pathway is exclusively due to glucose limitation.

To clarify whether SAPK activation was triggered only after a complete exhaustion of glucose, we further analyzed the glucose deprivation-induced phosphorylation of Sty1/Atf1. As shown in Fig. 1, B and E, a switch from high glucose-containing medium to osmotically equilibrated medium (containing glycerol or sorbitol) with glucose concentrations of 1% (w/v) or higher (not shown) did not induce Sty1/Atf1 phosphorylation. However, lower glucose concentrations (0.5% or less) prompted a rapid and transient increase in both Sty1 and Atf1 phosphorylation. Essentially identical results were obtained when sorbitol was used to balance osmolarity (Fig. 1D), confirming that the activation of the SAPK pathway is exclusively due to glucose limitation.

Different Stress-responsive Genes Are Induced through the SAPK Pathway by Glucose Depletion—Because a decreased glucose concentration fully activates the SAPK pathway in S. pombe, we determined the expression of a set of genes previously described as totally or partially dependent on Sty1 through its main downstream effector Atf1. Cells from glucose-growing cultures were transferred to the same medium but containing glycerol or sorbitol as the sole carbon source. Samples were taken at different times, and total RNAs were hybridized with probes corresponding to several stress-responsive genes. The expression of the glucose-repressible fbp1 gene, coding for fructose-1,6-bisphosphatase, which is required to assimilate non-fermentable carbon sources (39), was absent in glucose-growing wild type cells but was sharply induced upon transfer to non-glucose-containing medium (Fig. 2). Similar results were obtained when yeast cultures reached the early stationary phase of growth.3 Hence, these data demonstrate that the activation of the SAPK pathway in S. pombe by a downshift in glucose concentration takes place even in the presence of a certain amount of this carbon source.

![Fig. 3. Glucose deprivation induces nuclear accumulation of Pap1.](image)

FIG. 3. Glucose deprivation induces nuclear accumulation of Pap1. Strain EHH14 (pap1 with an integrated version of the GFP-Pap1 fusion under the control of the nmt1 promoter) was grown in EMM2 with 7% glucose to an A_{540} of 0.5 and recovered by filtration. The cells then either were resuspended in (A) the same medium with either 3% glycerol or 6% sorbitol for 30 min and then shifted back to the same medium plus 6% glucose or were resuspended in (B) EMM2 plus 6% glucose and then shifted to the same medium without a nitrogen source (EMM2-N). Samples were taken at different times, and the cellular distribution of the GFP-Pap1 protein was determined by fluorescence microscopy.

3 E. Hidalgo, unpublished results.
Glucose removal—Nuclear accumulation of Pap1 mediated by hydrogen peroxide is due to the reversible oxidation of two cysteine residues in the Pap1 amino acid sequence, which prevents interaction with exportin Crm1 (25). To gain insight into the nature of the stress triggered by the absence of glucose, we investigated the nuclear accumulation of GFP-Pap1 during the transition to medium without glucose in strain EHH14.C278A, which expresses a version of GFP-Pap1 with a substitution in cysteine 278 to alanine. This variant GFP-Pap1 cannot accumulate into the nucleus following an oxidative stress induced by external addition of hydrogen peroxide (25). Fig. 5A shows that contrary to control strain EHH14, the mutated version of GFP-Pap1 failed to accumulate into the nucleus in the absence of glucose even at longer incubation times. However, treatment of the same cells with DEM, which induces a non-reversible modification at cysteines 523 and 532 in the Pap1 primary structure (25), provoked a clear nuclear retention of the fusion protein (Fig. 5A). These results strongly suggest that the mechanism responsible for the nuclear accumulation of GFP-Pap1 observed upon removal of glucose is an oxidative stress mediated by hydrogen peroxide.

Glucose deprivation in tumor cells causes an intracellular oxidative stress that is responsible for the activation of the SAPK pathway, and this effect can be avoided when the cells are preincubated in the presence of scavengers for peroxide radicals such as GSH or NAC (2). With these precedents, cells from strain EHH14 were pregrown in minimal medium plus glucose in the presence of 0.16 mM GSH or 30 mM NAC and then transferred to isotonic medium without glucose plus the reducing agents. Under these conditions, GFP-Pap1 fusion protein failed to accumulate in the nucleus (Fig. 5B), indicating that both GSH and NAC are able to effectively inactivate the intracellular peroxide radicals arising from the shift to medium lacking glucose. However, Sty1 displayed a typical pattern of phosphorylation independently in the presence of GSH (Fig. 5C) or NAC (data not shown). This result indicates that in S. pombe the original cause of the SAPK activation observed during the transition from glucose to non-glucose conditions is the absence of glucose itself and not an internal oxidative stress.

Sty1 Regulates Pap1 Nuclear Accumulation in Glucose-deprived Cells—Several studies have shown that the nuclear accumulation of Pap1 induced by hydrogen peroxide is depend-
ent on MAPK Sty1 function at high oxidative doses, whereas at low doses (0.07–0.2 mM H₂O₂) it occurs in the absence of Sty1 (27, 28). We studied the cellular localization of a GFP-Pap1 fusion protein expressed in the Δsty1 strain TK107. As shown in Fig. 6, contrary to wild type cells, deletion of sty1Δ inhibited GFP-Pap1 nuclear accumulation after a shift to osmotically stabilized medium without glucose, even in cells maintained for longer incubation times. In contrast, GFP-Pap1 was found in the nucleus when the cells were treated by DEM. This result, together with those shown in Fig. 5, strongly suggest that during the transition to glucose deprivation S. pombe cells undergo a high peroxide stress. Congruent with this interpretation, ctt1Δ expression was completely absent in Δsty1 cells depleted from glucose (not shown).

Pap1-regulated Gene Expression after Glucose Depletion—In S. pombe cells subjected to mild oxidative conditions, the nuclear accumulation of Pap1 enhances the expression of a number of genes whose products are involved in the oxidative stress response (27). Because the above results indicated that glucose deprivation causes an intracellular stress by peroxide, we determined whether the response included a Pap1-dependent up-regulation of genes coding for enzymes involved in peroxide detoxification. Fig. 7 shows that glucose deprivation was accompanied by a moderate but reproducible increase in the expression of two genes, apt1Δ (p25) and trr1Δ (thioredoxin reductase), whose expression under oxidative stress specifically relies upon the transcription factor Pap1 (24). Maximal expression for both genes was observed 60 min after glucose starvation and, as expected, was not observed in the pap1Δ mutant strain TP108-3c (Fig. 7), indicating that nuclear accumulation of Pap1 correlates with its transcriptional activity. As mentioned earlier, the absence of Atf1 transcription factor under glucose deprivation prompted a partial attenuation in ctt1Δ expression (Fig. 2). We observed that the increase in ctt1Δ expression induced by the absence of glucose was also partially dependent on Pap1 (Fig. 7). Moreover, deletion of both transcription factors prevented ctt1Δ induction under glucose starvation (Fig. 7). Thus, in S. pombe both Atf1 and Pap1 transcription factors regulate the expression of genes involved in the response against the endogenous oxidative stress caused by glucose starvation.

Stress Adaptation Induced by Glucose Deprivation—The above results support that in S. pombe glucose deprivation induces an endogenous oxidative stress alleviated by the activ-
FIG. 7. Pap1 modulates the expression of different genes involved in the oxidative stress response under glucose limitation. Strains TK003 (wild type (WT)) and TP108-3c (Δpap1) were grown in EMM2 plus 7% glucose to early log phase and transferred to the same medium without glucose (3% glycerol) for the times indicated. Total RNA was extracted from each sample, and 20 μg was resolved in 1.5% agarose-formaldehyde gels. The denatured RNAs were transferred to nylon membranes and hybridized with 32P-labeled probes of atf1, trr1, ctt1, and leu1 (loading control).

FIG. 8. Both Atf1 and Pap1 are responsible for the increase in the protection of S. pombe cells against an exogenous acute oxidative stress after glucose deprivation. Strains TK003 (wild type (WT)), TK107 (Δsty1), TP108-3c (Δpap1), NT146 (Δatf1), and CA334 (Δatf1Δpap1) were grown in YES medium with 7% glucose, resuspended for 1 h at 28 °C in the same medium with 6% glucose (filled bars) or 3% glycerol as a non-metabolizable carbon source (open bars), and treated for 1 h with 80 mM H2O2. Cell viability was measured by their ability to form colonies on YES plates after incubation at 28 °C for 5 days. Results represent mean values ± S.D. from three different experiments.

DISCUSSION

One major finding of this work is the demonstration that the signal transduction pathway that responds to hypoglycemia in S. pombe becomes activated at low levels of glucose prior to the subsequent oxidative stress produced in the absence of this sugar. We have shown that Sty1 phosphorylation takes place with decreased concentrations of glucose even in the presence of thiol antioxidants, which rescue cells from the oxidative stress caused by glucose depletion. In response to glucose exhaustion there is also an increased induction of both Atf1-dependent (ctt1+, gpx1+, bpf1+, and pyp2+) and Pap1-dependent (ctt1+, apt1+, and trr1+) genes, indicating that some change in the induced oxidative stress must occur to explain the functional activity of these two transcription factors. In evaluating our results, we have taken into account that Atf1 primarily controls the transcriptional response to high concentrations of hydrogen peroxide, whereas Pap1 activates target genes in response to low levels of hydrogen peroxide (27). We also have taken into consideration that the expression of ctt1+ is induced in an Atf1/Pap1-dependent manner under glucose depletion. Therefore, our observations support the interpretation that the accumulation of Pap1 by increasing concentrations of oxidant
Atf1- and Pap1-mediated Responses during Glucose Depletion

Two critical issues need to be addressed to gain a better understanding of the regulation of the response of S. pombe to glucose deprivation. Firstly, the identity of the upstream elements in the glucose signaling pathway has to be determined, and secondly, the origin of the oxidative effect by glucose deprivation must be outlined. Yeasts appear endowed with fine-tuning to extracellular conditions by multiple glucose sensor systems. In S. cerevisiae, for example, the changes induced by glucose limitation in natural diauxic shifts begin well before complete glucose exhaustion, allowing a differential gene response to different thresholds of glucose limitation (50). However, glucose detection systems and associated signal transduction pathways are still under active examination in both budding and fission yeasts (51, 52), and the complex responsible for sensing glucose limitation in S. pombe is as yet unknown. In any case, the activation signal for Sty1 phosphorylation following glucose depletion appears to be transduced through the main elements of the SAPK pathway, as Sty1 activation was completely abolished in cells disrupted in the upstream response regulator Mscs4 or MAPK kinase Wis1 (30).2

The causal factors behind the self-inflicted oxidation in glucose-deprived cells also remain elusive. S. pombe is able to ferment glucose in aerobic conditions, and the major pathways for glucose metabolism include glycolysis, which results in the formation of pyruvate, and the pentose phosphate cycle, which yields NADPH as cellular reducing power. Pyruvate, in addition to its role in energy metabolism, has been shown to scavenge hydrogen peroxide and other hydroperoxides (3). Also, NADPH has been demonstrated to participate in the metabolic decomposition of reactive oxygen species, as this cofactor is a source of reducing equivalents for the glutathione/glutathione peroxidase/glutathione reductase system. Exposure of yeast cells to hydrogen peroxide alters glucose metabolism to favor generation of NADPH, and mutant cells lacking key enzymes of the pentose phosphate pathway show increased sensitivity to hydroperoxides by NAC eliminates SAPK activation, apparently by suppressing the redox-sensitive activation of MAPK kinase ASK1 and its downstream targets in the signal transduction pathway (34).
Atf1- and Pap1-mediated Responses during Glucose Depletion

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