Lipid Hydroperoxides Activate the Mitogen-activated Protein Kinase Mpk1p in *Saccharomyces cerevisiae*[*]

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*S. cerevisiae* is capable of responding to oxidants, including lipid peroxidation products. We investigate here the role of the mitogen-activated protein kinase Mpk1p in protection against linoleic acid hydroperoxide (LoaOOH), a product of radical attack on an unsaturated lipid. *MPK1* was found to be required for resistance to LoaOOH. Furthermore, Mpk1p was rapidly and transiently phosphorylated in response to LoaOOH. This phosphorylation was dose-dependent and stimulated by sublethal concentrations as low as 1 μM in the external medium. Such low doses have been shown to result in resistance to subsequent challenge with a higher dose through the process of adaptation. However *MPK1* was not essential for this adaptive response. *MPK1* was also not involved in cell cycle modulation and acted independently of the cell cycle-regulating Oca1p.

Transcriptional profiling of the *mpk1Δ* cells during LoaOOH stress indicated that Mpk1p may be important in effecting changes to the cell surface and metabolism during LoaOOH exposure. Furthermore, it revealed that Mpk1p is required for the regulation of 97 LoaOOH-responsive transcripts. Evidence is presented that the activation of Mpk1p may be caused by the activation of protein kinase C by LoaOOH.

Damage caused by reactive oxygen species to cellular constituents includes peroxidation of lipids. Peroxidation of membrane lipids is thought to directly interfere with membrane integrity and membrane-associated functions. It also leads to production of further toxic metabolites, such as lipid hydroperoxides and reactive aldehydes, including malondialdehyde and 4-hydroxynonenal (1–3). We have studied the effects of lipid peroxidation on yeast by exposing cells to linoleic acid hydroperoxide (13-hydroperoxylinoleic acid; LoaOOH). This compound is one of the most toxic peroxides to yeast cells (4, 5).

*S. cerevisiae*, like other organisms, responds to oxidative stress by adapting to become resistant (6), by undergoing cell division-cycle delay (7), and by altering its gene expression (8, 9). A number of transcription factors are known to be involved in the cellular responses to oxidants, including Yap1p, Skn7p (10–12), and the general stress-responsive Msn2p and Msn4p (13, 14). However, the complex signal transduction pathways that govern these responses are now only being elucidated (15–17).

Mitogen-activated protein kinases (MAPKs) are serine/threonine kinases that form the final step in a signaling cascade comprised of three sequentially activating kinases (18). MAPK pathways are present and well conserved in eukaryotes from yeast to humans. In multicellular organisms at least two families of MAPK (JNK and p38) are involved in stress responses (19). In *Schizosaccharomyces pombe*, the p38 homologue, Sty1, is also activated by a variety of stress conditions, including oxidative stress (20). The *S. cerevisiae* p38 homologue, Hog1p, is activated in response to hyperosmotic stress and heat shock (21–23). However it does not appear to be activated by H₂O₂ (Ref. 22; however, see Ref. 24). In *S. cerevisiae* at least one more MAPK, Mrp1p (Stlp2), is stress responsive.

The Mpk1p pathway (the cell-integrity pathway) is thought to be important in maintaining cellular integrity during cell wall remodeling, heat, and hypo-osmotic shock (25–27). The pathway is under the control of protein kinase C (PKC) and is comprised of a MAPK kinase kinase, Bck1p, two redundant MAPK kinases, Mkk1p and Mkk2p, and the Mpk1p MAPK (reviewed in Ref. 28).

Here we report on the involvement of the cell-integrity pathway in an oxidative stress response of *S. cerevisiae*. Mpk1p is phosphorylated in a time- and dose-dependent manner in response to LoaOOH. *MPK1* is required for proper resistance to the peroxide, but not for adaptation or cell cycle modulation. We also show that Mpk1p plays a role in the transcriptional response to the peroxide.

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Conditions**—The strains used in the study are described in Table I. The strains were generated using standard yeast molecular biology methods. To delete *OCA1*, the *HIS3* gene was amplified by polymerase chain reaction using the primers: GAGAGTCACATC-TACCATCACTTTTCAACAGAAAGGAGTGTTCCACCCTGGCGAGATTGTACATGAGTGTCG and GCGAATGGGCCGTGCATCCTGAAACATTCAAACTTTAGGTAAATGGTCCTGTGCGGTATT (region of homology to the genomic *OCA1* sequence is underlined). The product was used to replace the entire open reading frame of *OCA1* (YNL099C) with *HIS3* in the wild-type strain BY4741 (Brachmann et al., Ref. 29). After integration was confirmed by polymerase chain reaction. The *oca2Δ* (ynl056wΔ) and *siw14Δ* (ynl032wΔ) strains were obtained by transduction from the respective homozygous-deletion diploid mutants (Y2221 and Y23559 from Euroscarb) and the deletions confirmed. The triple deletion mutant was obtained by deleting the *OCA1* gene, using the method described above, in the double *siw14Δoca2Δ* mutant, which...
was generated by mating, sporulation, and spore dissection. Synthetic complete medium (5) was used in all experiments. Yeast was grown at 30°C with shaking. Liquid cultures were always 1.5 of the flask volume.

LoaOOH Synthesis, Tests of Sensitivity, Adaptation, Cell Cycle Delay, and Actin Visualization—LoaOOH synthesis, spot tests for sensitivity, and the microtitre plate-based method for assessing adaptation and colony-forming ability have been described (4, 5). For each spot-test at least three concentrations in the range of 0.05–0.3 mM LoaOOH were used. For caffeine sensitivity 12 mM caffeine was used and growth examined after 3 days. To examine the ability of cells to delay in the G1 phase of the cell cycle, strains were grown to OD600 = 0.4 and treated with 40 mM LoaOOH. After 90 min, samples were fixed in 75% ethanol. Unbudded cells were scored based on cell and nuclear morphology after staining with 4,6-diamidino-2-phenylindole dihydrochloride. To visualize actin, one volume of 150 μM EGTA and protease inhibitors were added to four volumes of cells. The cells were incubated for 5 min, followed by fixation in 1% PIPES, pH 6.9, 5 mM EGTA, 5 mM MgCl2, and 3.7% formaldehyde. Cells were permeabilized in the same buffer without formaldehyde, with 0.1% Triton X-100, and stained with TRITC-phalloidin (4 μg/ml; Sigma). Means and standard deviations of cell counts were calculated based on a binomial distribution as previously described (5).

LoaOOH Treatment, Protein Extraction, and Western Blotting—Cells were grown to OD600 of 0.4, except for the 20 μM LoaOOH treatment where the starting OD600 was 0.2. Either the whole culture or an aliquot of the appropriate strain was treated for the indicated times with the indicated doses. Cells were harvested by centrifugation at 4°C after mixing with crushed ice prechilled at −30°C (10 g of ice per 40 ml of cells). The cell pellet was washed with 1 ml of ice-cold 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and frozen in an ethanol bath at −80°C. Proteins were extracted in 80 μl of ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% (v/v) glycerol, 1% (v/v) Triton X-100, 0.1% sodium dodecyl sulfate, 5 mM EDTA, 1 mM sodium orthovanadate, 50 mM β-glycerophosphate with 10 μl of yeast-specific and 25 μl of general protease inhibitors (Sigma) per ml) by agitation in a minibead-beater for 5 bursts of 30 s with acid-washed glass beads. Protein concentration was determined using the DC Protein Assay (BioRad) and bovine serum albumin as a standard. 50 μg of protein per lane was separated by SDS-PAGE on a gradient gel (Gels; Gradipore, NSW, Australia). The proteins were transferred to a polyvinylidene difluoride membrane using a Trans-Blot electrophoretic cell (BioRad) according to the manufacturer’s instructions. After transfer, the residual proteins in the gel were stained with Coomassie Brilliant Blue (Sigma) to confirm even loading. Membranes were probed with either the phospho-p44/42 MAP Kinase (Th-202/Tyr-204) antibody (Cell Signaling Technology) or the anti-Mpk1 antibody (yN-19; Santa Cruz Biotechnology) essentially as described. The antibody reacts with a band of ~43 kDa that was absent in the mpk1Δ strain.

RNA Extraction and Microarray Analysis—Cells were treated, harvested, and frozen as above. The RNA was obtained by breaking cells in TRIzol reagent (Invitrogen) in a mini-bead beater in the presence of acid-washed glass beads at 4°C, and extracted according to the manufacturer’s instructions. The RNA was further purified using the RNeasy kit (Qiagen, Dusseldorf, Germany), including DNase I treatment. RNA quality was checked by agarose gel electrophoresis and ethidium bromide staining, as well as by A260/280 determination.

SDS-PAGE and Western Blotting—SDS-PAGE and Western blots were obtained from the Clive and Vera Ramaciotti Centre for Gene Function Analysis (Sydney, Australia). Oligonucleotide probes (MWG Biotech, Ebersberg, Germany) addressing 6250 yeast open reading frames were printed in duplicate on epoxy-coated glass substrates (Eppeendorf, Hamburg, Germany) and were blocked immediately prior to use with ethanediol according to the manufacturer’s instructions.

RNA from the wild type, isolated before and after treatment was compared with a reference sample and the values were zero-transformed during analysis, while the RNA from the oca1Δ before and after treatment was compared with the equivalent RNA from the wild type. The RNA samples from mpk1Δ before and after treatment, were directly compared. Each comparison was performed in duplicate. Labeling and hybridization was carried out according to a modification of the protocol described by Hughes et al. (31). 20 μg of total RNA was reverse-transcribed incorporating 5'-3-aminomethyl)-dUTP (Sigma). The cDNA was labeled by coupling the aminoallyl-dUTP to N-hydroxy succinimide esters of either Cy3 or Cy5 (Amersham Biosciences). The labeled cDNA of the two samples to be compared was mixed and hybridized to the array overnight at 37°C in DIG Easy Hyb (Roche Applied Science) containing 0.5 mg/ml Escherichia coli RNA and 0.5 mg/ml denatured herring sperm DNA. The slides were then washed three times for 20 min in SSC (3 mM sodium chloride, 0.3 mM trisodium citrate, pH 7.5), 0.1% (v/v) SDS at 50°C, rinsed several times in SSC, dried by centrifugation, and scanned.

Image analysis was performed in GenePix Pro 3.0 (Axon Instruments). The signal for a gene was deemed “present” if: (a) no artifacts were associated with the spot, and (b) the program could identify the spot intensity above the background intensity. The data were imported into GeneSpring 5.0 (Silicon Genetics), where all further analysis was performed. Data were normalized by the LOWESS normalization method. Only the genes deemed “present” on all six slides were further considered. Data from the experiments comparing oca1Δ to the wild type were processed in a similar manner.

Functional enrichment in the obtained sets was determined using FunSpec (32) addressing the MIPS data base. For information on individual gene products MIPS was consulted (www.mips.biochem.mpg.de). Novel putative regulatory elements, yeast RSA regulatory tools was used (rsat.ulb.ac.be/rsat/). The frequency of the occurrence of the elements in the set was compared with the frequency in the genome determined using PatMatch (seq.yeastgenome.org/cgi-bin/SGD/PAT-MATCH/nph-patmatch) and the probability of accidental enrichment calculated based on a hypergeometric distribution.

Additional Material—The normalized microarray data from the experiments analyzing the expression changes in the mpk1Δ and wild-type strains, and the derived gene lists are available as supplementary material.

RESULTS

Mpk1p Is Required for Resistance to LoaOOH and Is Phosphorylated in Response to Peroxide—The investigation of the activation of the cell-integrity pathway in response to LoaOOH stems from our interest in the OCA1 gene. We have previously shown that this gene is required for resistance to LoaOOH, and for a G1 cell cycle delay in response to this peroxide (5). Other studies have pointed out that it encodes a putative tyrosine phosphatase and that it has two homologues in yeast (33): SIW14 and the open reading frame of unknown function (YNLO56W) that we named OCA2. The growth of yeast cells with deletions of either of the three genes on solid medium was inhibited by the presence of LoaOOH or caffeine (Fig. 1), and the caffeine sensitivity was especially pronounced. Caffeine sensitivity is one of the phenotypes of the mutants of the cell-integrity pathway (28), and previous studies have shown that SIW14 interacts genetically with MPK1 (34). Thus it appeared possible that Oca1p may act in the cell-integrity path-
way, and, in turn, that this pathway may be involved in mediating responses to LoaOOH.

To test this hypothesis, the sensitivity to LoaOOH of the mpk1Δ mutant was examined by assessing its capacity to grow in the presence of LoaOOH. Indeed, the deletion of this gene resulted in pronounced sensitivity to LoaOOH (Fig. 2A).

The sensitivity of the deletion strain may result from the involvement of Mpk1p in signaling the presence of LoaOOH. If that was the case, then the activity of Mpk1p should be modulated in response to the peroxide. MAPKs are known to be activated by dual phosphorylation of a threonine and a tyrosine residue (18). We assessed the levels of this phosphorylated form of Mpk1p (Mpk1pTpYp) during treatment with LoaOOH using the antibody against dually phosphorylated human p42/44, which has been shown to recognize the doubly phosphorylated yeast homologue, Mpk1p (30, 35). Treatment of the wild-type strain with 40 μM LoaOOH in exponential growth phase resulted in a rapid (within 5 min) increase in levels of Mpk1pTpYp. This response was transient and the level of Mpk1pTpYp returned to below the basal level within 60 min (Fig. 2B). The time taken for the level of Mpk1pTpYp to return to basal was longer in response to a lower dose (20 μM) indicating that this decline may be due to a feedback mechanism (Fig. 2C). The levels of total Mpk1p were also determined and were found not to change during treatment with 40 μM LoaOOH (Fig. 2B), confirming that the changes in the levels of the dually phosphorylated Mpk1p were due to post-translational modification.

MPK1 Acts Independently of OCA1 and Is Not Required for the G1 Delay—For the reasons noted above, it seemed possible for Oca1p to be involved in the cell-integrity pathway. If it was acting upstream of Mpk1p, then the activation of this MAPK in the oca1Δ strain should be altered. However, OCA1 was not required for the phosphorylation of Mpk1p, nor were either of its two homologues (Fig. 3A). This effect was not the result of the possible functional redundancy of the three genes, since Mpk1p phosphorylation still occurred even in the triple deletion mutant (Fig. 3B). Furthermore, deletion of OCA1 exacerbated the sensitivity of a mpk1Δ strain (Fig. 3C; note that mpk1Δ is more sensitive than the oca1Δ). This allowed us to conclude that OCA1 and MPK1 act in two separate LoaOOH-resistance pathways.

The cell-integrity pathway is known to interact with the cell cycle machinery (36, 37), and thus MPK1 may provide another level of regulation of the LoaOOH-induced G1 delay. To examine this, wild-type and mpk1Δ cells were treated with 40 μM LoaOOH in the exponential growth phase. The G1 delay was assessed by scoring the percentage of unbudded cells present after 90 min of treatment when the most pronounced change was observed in the wild type. The accumulation of unbudded G1 cells occurred both in the mpk1Δ and the wild-type strain (Fig. 4). This was different from oca1Δ, which was included as a control in this experiment, and indicated that MPK1 was not required for this effect of LoaOOH. Furthermore, the cell cycle delay appeared even more pronounced in the mpk1Δ, which may be related to its increased sensitivity to LoaOOH.

*MPK1 Phosphorylation Is Stimulated by Sublethal Doses of LoaOOH but MPK1 Is Not Essential for Adaptation—*Cells respond differently to different doses of LoaOOH. Cell cycle delay only occurs in response to medium to high doses (5) while low doses of LoaOOH induce resistance to subsequent treatment with an otherwise lethal dose, an adaptive response (4). To further understand the physiological role of Mpk1p, the dose...
dependence of its phosphorylation was examined. It was found that this process was stimulated within 5 min of exposure to doses as low as 1 μM in external medium (Fig. 5A).

Since Mpk1p phosphorylation was stimulated by such low concentrations of the peroxide, we examined the involvement of Mpk1p signaling in the adaptive response by determining the ability of the mpk1Δ strain to adapt. Exponentially growing wild-type and mpk1Δ strains were either exposed to a low dose (1 μM) of LoaOOH for 1 h, or left untreated, after which their ability to grow in the presence of higher concentrations of the peroxide was determined. Surprisingly, the cells could still mount an adaptive response even in the absence of Mpk1p (Fig. 5B), indicating that even though the protein was phosphorylated in response to very low doses of LoaOOH it was not essential for the adaptive response.

MPK1 Is Involved in the Transcriptional Response to LoaOOH—Mpk1p has been shown to regulate at least two transcription factors, and the examination of genome-wide transcriptional changes upon artificial activation of Mpk1p revealed it to be involved in the regulation of cell-wall genes (36, 38, 39). We wanted to examine if MPK1 was also required for a proper transcriptional response to LoaOOH. We have thoroughly characterized the genome-wide response of the wild-type strain to LoaOOH and the results of this investigation will be given elsewhere. To identify any alterations in this transcriptional response that may result from the absence of MPK1, exponentially growing wild-type or mpk1Δ cells were exposed to 30 μM LoaOOH in the exponentially growing wild-type or mpk1Δ cells were determined as described under “Experimental Procedures.” The normalized ratio (treated/untreated) in the wild type is given on the y-axis, while for the mutant it is shown on the x-axis. The genes whose transcripts were deemed to be present on all slides are shown. The 2-fold difference boundary of induction in the mutant versus the wild type is indicated by the outer diagonal lines. The 2-fold change boundary for the expression in the wild type alone is given by the two outer vertical lines. The position of genes belonging to subsets a or b is indicated. B, numbers of genes whose transcriptional regulation depends on OCA1 or MPK1 from the ~500 genes that are 2-fold up-regulated or ~700 that are 2-fold down-regulated in the wild-type strain. The genes in the sets were found to be 2-fold misregulated in the oca1Δ or mpk1Δ strains. Note that the sets dependent on MPK1 correspond to sets a and b. C, the ratios (treated/untreated) of mRNA for MSN4, ENO1, and TRX2 in the wild-type, mpk1Δ, and oca1Δ strains are given. The results presented in both B and C are derived from microarray data.

Fig. 4. The mpk1Δ strain undergoes G1-delay in response to LoaOOH. The accumulation of unbudded cells in response to 40 μM LoaOOH during asynchronous-exponential growth was analyzed after treatment for 90 min in the wild-type, mpk1Δ, and oca1Δ strains. Cells were scored based on cell and nuclear morphologies, and the means and S.D. presented were calculated based on a binomial distribution.

Fig. 5. Mpk1p phosphorylation is stimulated by low doses of LoaOOH but MPK1 is not required for adaptation. A, aliquots of an exponentially growing culture were treated with a range of LoaOOH concentrations for 5 min and analyzed for the levels of Mpk1pTpYp as described in the legend to Fig. 2B. B, mpk1Δ and the wild-type strain were grown to exponential phase, diluted to OD600 of 0.1 in pre-warmed SC medium, and incubated in a microtiter plate for 1 h with (round symbols) or without (square symbols) 1 μM LoaOOH pretreatment. The cells were then treated with 30 μM LoaOOH (filled-in symbols) or left untreated (open symbols) and the culture growth monitored by measuring OD600. The lowest of three different pretreatment concentrations examined, all giving similar results, is shown.

Fig. 6. Genome-wide transcriptional response to LoaOOH in the mpk1Δ strain compared with the wild type. The genome-wide transcriptional changes resulting from 1 h of treatment with 30 μM LoaOOH in the exponentially growing wild-type or mpk1Δ cells were determined as described under “Experimental Procedures.” The normalized ratio (treated/untreated) in the wild type is given on the y-axis, while for the mutant it is shown on the x-axis. The genes whose transcripts were deemed to be present on all slides are shown. The 2-fold difference boundary of induction in the mutant versus the wild type is indicated by the outer diagonal lines. The 2-fold change boundary for the expression in the wild type alone is given by the two outer vertical lines. The position of genes belonging to subsets a or b is indicated. B, numbers of genes whose transcriptional regulation depends on OCA1 or MPK1 from the ~500 genes that are 2-fold up-regulated or ~700 that are 2-fold down-regulated in the wild-type strain. The genes in the sets were found to be 2-fold misregulated in the oca1Δ or mpk1Δ strains. Note that the sets dependent on MPK1 correspond to sets a and b. C, the ratios (treated/untreated) of mRNA for MSN4, ENO1, and TRX2 in the wild-type, mpk1Δ, and oca1Δ strains are given. The results presented in both B and C are derived from microarray data.
Determining the functions associated with the transcripts whose treated/untreated ratio was altered in the mpk1Δ relative to the wild-type strain after treatment could provide insight into the cellular functions affected by the absence of MPK1. The sets of genes with either higher or lower untreated/treated ratio in the deletion mutant relative to the wild-type strain were examined for statistically significant overlaps with genes sets classified based on the available information regarding the function or the localization of the gene product. Classifications available from the MIPS data base were addressed using the FunSpec program (32). The results are given in Table II. The set of genes with lowered treated/untreated ratio in the mpk1Δ strain was enriched for those encoding proteins localizing to the cell wall, confirming the importance of MPK1 in the regulation of cell wall-associated functions. Interestingly, the set also included genes coding for proteins involved in homeostasis of protons, as well as other membrane proteins (Table II), indicating that MPK1 may also be important for the maintenance of plasma membrane-associated functions during LoaOOH stress. The deletion of MPK1 resulted in reduced expression of genes encoding proteins involved in metabolism and energy production. The set of genes whose transcripts were up-regulated in the absence of MPK1 were not enriched for any of the MIPS functional categories.

The sets of transcripts misregulated in the mpk1Δ relative to the wild-type strain included those whose ratio changed less than 2-fold in the wild-type strain. This implied that some of the changes observed on deletion of MPK1 occurred to compensate for its absence. To differentiate between these compensatory changes, and those genes for whose induction or repression MPK1 may be required, we identified the subsets that were induced or repressed by LoaOOH at least 2-fold in the wild type within the set of genes that were misregulated in the mpk1Δ strain. These subsets (a and b) are indicated in Fig. 6A and the genes they contain are listed in Table III. In total, Mpk1p appeared to be required for the regulation of 97 transcripts in response to LoaOOH. The subset of genes whose up-regulation was impaired in the mpk1Δ strain (subset a) contained 61 genes. It was enriched for functions associated with energy production, and in particular glycolysis and gluconeogenesis (Table III), implicating MPK1 in directly controlling metabolic changes that occur in cells in response to stress (8, 9). MPK1 also appeared required for regulation of several genes involved in stress responses, including the transcription factor Msn4p, as well as those encoding plasma membrane-associated proteins. Furthermore, MPK1 may also be important for proper down-regulation of 36 genes (set b, Fig. 6A and Table III).

Putative regulatory elements, over-represented in the promoters of the genes in each of the sets a and b could be found. Within set a, 52 of 61 genes (85%) contained a motif (CAGCAG; allowing one-base substitution) in their promoter, compared with 56% of the genes in the genome (p = 3.1 × 10^{-8}). In set b, a similar enrichment was observed for the sequence ACCATCAA (64% in the set versus 40% in the genome, p = 1.1 × 10^{-5}). This further indicated that the genes in these sets could be part of an Mpk1p-dependent regulon.

A significant proportion of the transcriptional changes that occur in the wild-type strain in response to LoaOOH are independent of MPK1 (Fig. 6A). It was interesting to examine how many of these genes may require OCA1 for proper regulation and if the putative MPK1 and OCA1-dependent regulons are independent. We have examined the difference in gene expression between isogenic oca1Δ and wild-type strains using microarrays, and while the results of this study will be described elsewhere in depth, the relevant comparison is illustrated in Fig. 6B. When the data from this experiment were analyzed in the same way as described for the mpk1Δ strain, a much smaller OCA1-dependent regulon was uncovered. Importantly,
sets requiring MPK1 for proper induction or repression in response to LoaOOH

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**TABLE III**

**Sets requiring MPK1 for proper induction or repression in response to LoaOOH**

Set a, genes with reduced induction in mpk1Δ, b, genes with reduced repression in mpk1Δ strain. Functional enrichment was determined using Funspec. Categories from MIPS database with p (probability of accidental enrichment) < 0.001 are given.

**DISCUSSION**

This is the first report of the activation of the cell-integrity pathway in a condition of oxidative stress in yeast. Whether this response is common to all types of oxidants remains to be determined.

The sensitivity of the mpk1Δ mutant may be due to alterations in its cell wall (28). Indeed, deletion of some cell wall genes has been noted to result in sensitivity to LoaOOH (42). However, Mpk1p must also play an active role in the protection against lipid hydroperoxides, since it is activated in the wild-type strain upon exposure to LoaOOH and is required for proper regulation of at least a subset of LoaOOH-responsive genes. Furthermore, the activation of Mpk1p appears to be very sensitive to the peroxide and occurs at low doses that do not affect the cell cycle, nor the growth or viability of the cultures (5).

The cell-integrity pathway may provide immediate protection from the peroxide, since it is activated rapidly. The cellular functions associated with transcripts that are misregulated in the mpk1Δ strain indicate that Mpk1p may be important in effecting changes to the plasma membrane, cell wall and metabolism during LoaOOH stress. Mpk1p may, in concert with observed reorganization of the actin cytoskeleton, aid the repair or replacement of cell surface structures damaged by LoaOOH. Modulation of plasma membrane properties, which has not been investigated previously in connection with the
PKC such as those encoded by the Msn4 family of genes (28). The rhythmic nature of LoaOOH makes it most likely to affect membrane lipids and proteins. Such damage to the plasma membrane may cause the activation of the pathway. Interestingly, the rapid activation of the pathway is also observed in hypo-osmotic conditions (26), while it is delayed during heat shock (27). It is possible that LoaOOH stress and hypo-osmolarity may be linked to the halt/reduction in membrane phosphatase is an interesting candidate for effecting this Mpk1p dephosphorylation (30).

How is Mpk1p activated? The cell-integrity pathway is thought to sense damage to the cell surface (28). The hydrophobic nature of LoaOOH makes it most likely to affect membrane lipids and proteins. Such damage to the plasma membrane may cause the activation of the pathway. Interestingly, the rapid activation of the pathway is also observed in hypo-osmotic conditions (26), while it is delayed during heat shock (27). It is possible that LoaOOH stress and hypo-osmolarity causes a common and immediate disturbance to the cell surface (28). The hydrophobic nature of LoaOOH makes it most likely to affect membrane lipids and proteins. Such damage to the plasma membrane may cause the activation of the pathway. Interestingly, the rapid activation of the pathway is also observed in hypo-osmotic conditions (26), while it is delayed during heat shock (27). It is possible that LoaOOH stress and hypo-osmolarity cause a common and immediate disturbance to the cell surface such as a depolarization of the plasma membrane. This disturbance could be sensed by any of the sensors upstream of PKC such as those encoded by the WSC family of genes (28). The nature of the signal and the mechanism of activation of these sensors are unclear. The examination of their functioning during LoaOOH stress may shed further light on this question.

Exposure to oxidants results in an alteration to the intracellular redox balance. The way this change is sensed by the cell is beginning to be understood, and appears to be based on the redox properties of Cys residues in specific proteins (43). In the case of the activation of the cell-integrity pathway, Pck1p itself is a possible sensor since the activity of its mammalian homologues is altered by redox changes. Oxidation of the Cys residues forming the Cys finger in the regulatory (C1) domain acts to activate the kinase (reviewed in Ref. 44). Oxidant activation of mammalian Pck1p has been observed with several oxidants, including hydrogen peroxide and lipid hydroperoxides (45–47). The equivalent residues are present in yeast Pck1p, and may be directly oxidized by LoaOOH. This property of PKCs may make them a very suitable redox sensor. The activation may be only transient if the catalytic domain Cys residues are oxidized subsequent to the regulatory domain ones, since this oxidation event has been shown to deactivate the kinase (44).

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