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Role of Hog-1 mitogen activated protein kinase in the *Saccharomyces cerevisiae* UV response

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The UV stress response in mammalian cells is characterized by transcriptional changes aimed at regulating cell growth, repair and survival. Previous studies have suggested that highly conserved mitogen-activated protein kinase (MAPK) pathways mediate UV-induced stress signalling. Here, we examined the role of the Hog-1 and Mpk-1 MAPKs in the *Saccharomyces cerevisiae* UV response. Deletion of both Hog-1 and Mpk-1 enhanced sensitivity to UVC-induced cell death. However, activation of these pathways by osmotic stress prior to irradiation failed to cross-protect wild type cells from death. A dose-dependent upregulation of active Hog-1 was observed in response to UVC irradiation, but elevated expression caused by pre-treatment with hyper-osmotic medium correlated with a decrease in viability. We speculate that Hog-1, like its mammalian homologue, may also activate pro-apoptotic pathways when highly expressed.

Ultraviolet (UV) radiation from sunlight is a major contributing factor in the etiology of human skin cancer and immunosuppression (9). While damage to DNA causes mutations which induce carcinogenesis, UV-dependent changes in gene expression also occur without DNA damage (25). The cellular UV response is characterized by dramatic changes in the transcriptome, including increased expression of DNA repair enzymes and proteins involved in regulating cell growth (15). In mammals, UV-activated signal transduction pathways are comprised largely of mitogen activated protein kinases (MAPKs). These enzymes are activated by phosphorylation on tyrosine and threonine residues of a conserved Thr-Gly-Tyr (TXY) motif (2). At least four major MAPK cascades exist in mammals: extracellular signal-regulated kinase (ERK) 1/2, ERK4, p38, and Janus N-terminal linked kinase (JNK). UVC irradiation induces signalling via ERK1/2, p38, and JNK (13) leading to activation of c-Jun, a transcription factor of the activating protein 1 (AP-1) family (8). Also induced in response to mitogenic signals, AP-1 appears to play two distinct roles: its activation in some cell types promotes apoptosis, while in others it is protective (15).

The budding yeast *Saccharomyces cerevisiae* is a model eukaryote that has been widely used for studying stress signalling pathways in both mammalian and fungal cells. At least five signalling pathways which include a MAPK cascade have been identified in *S. cerevisiae*, but the UV response in yeast has been poorly characterized to date (13). Bryan *et al.* (4) have reported that yeast cells lacking the Mpk-1 MAPK exhibit impaired survival following UV irradiation. Other studies have also suggested a role for the high osmolarity glycerol (Hog) pathway, the ERK pathway, and the mitogen-activated protein kinase homolog (Mpk-1) pathway (13). The yeast Hog-1 kinase is homologous to mammalian p38, while Mpk-1 belongs to the ERK1/2 family. Like p38, Hog-1 mediates the hyperosmotic and oxidative stress response in mammalian cells (11,14). Mpk-1 plays a key role in maintaining cell wall integrity and is induced by hypo-osmotic shock, cell wall damage, and oxidative stress (7). It is unclear whether this functional homology between yeast and mammalian MAPK cascades extends to other types of stress signalling.

To investigate the role of Hog-1 and Mpk-1 in the *S. cerevisiae* UV response, we first evaluated the effect of *hog-1* and *mpk-1* deletions on cell survival following irradiation at 254 nm. To determine whether pre-activation of these signalling pathways could protect from UVC-induced cell death, we treated cells with hyper- and hypo-osmotic media before exposure to UVC. The effect of radiation on the expression of phosphorylated Hog-1 was then evaluated by western blotting. While deletion of either kinase impaired cell survival following exposure to UVC, induction of kinase activity prior to exposure did not correlate with increased viability.

MATERIALS AND METHODS

Yeast strains and culture conditions. The *S. cerevisiae* strains used in this study were haploid *MATα* wild-type, *Δhog-1*, or *Δmpk-1* on the BY4742 background (*Δhis3 Δleu2 Δlys2 Δura3*). Strains were produced by the Stanford *S. cerevisiae* Genome Deletion Project and obtained from Dr. Vivien Measday (UBC, Wine Research Centre). The cultures were grown at 30°C with gentle aeration in liquid yeast peptone dextrose (YPD) medium (1% yeast extract, 2% peptone, 2% D-glucose) prior to experimental conditions.

Hyper- and hypo-osmotic shock treatment. Overnight cultures of wild type cells were diluted in isotonic medium (0.5 M NaCl, 20% YPD) to an OD_{600} of 0.4 and grown to an OD_{600} of 1.0

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prior to centrifugation at 2000 × g for 10 minutes. Cell pellets were resuspended in 50 ml iso-osmotic, hyper-osmotic (1.0 M NaCl, 20% YPD) or hypo-osmotic (20% YPD) medium and incubated at 30°C with gentle aeration for 0, 1, 5, 15, and 30 minutes.

Cross protection against UVC irradiation by osmotic shock pretreatment. Osmotic shock pretreatment was carried out as described above with a 2 minute incubation period. Ten ml aliquots of each culture were irradiated with UVC (254 nm) in 100 mm × 15 mm plastic Petri dishes using a Stratalinker 1800 UV crosslinker (Stratagene, La Jolla, CA) at 0, 50, 100, and 150 J/m^2. 100 l of 10^{-4} and 10^{-5} dilutions were plated on solid YPD (2% agar) in triplicates to assess cell viability. Plates were incubated at 30°C for 90 hours before enumeration of colonies.

Preparation of yeast lysate. Five to ten ml cell cultures were centrifuged at 2000 × g for 3 minutes at 4°C, then resuspended in 200 l RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethanesulphonyl floride, 1 mM Na_3VO_4, 1 mM NaF, 1 g/ml aprotinin, 1 g/ml leupeptin, 1 g/ml pepstatin). The cell suspension was transferred to a screw-cap microfuge tube containing 200 g of 0.2 mm diameter glass beads (Sigma, St. Louis, MO). Cells were mechanically disrupted with FastPrep FP120 (Thermo Electron Corp., Waltham, MA) at a speed setting 6 for 3 successive 45-second cycles. The lysates were centrifuged at 13,000 × g for 5 minutes at 4°C and the supernatant collected for storage at -80°C. Protein concentration was determined by Bradford assay (Bio-Rad Laboratories, Hercules, CA) using chicken egg albumin as a standard.

Western blot analysis. Samples were thawed on ice, diluted in 6× loading buffer (300 mM Tris-HCl pH 6.8, 600 mM DTT, 12% SDS, 0.6% bromophenol blue, 60% glycerol), and heated for 5 minutes at 95°C. Ten to twenty five g protein was loaded per lane of a 10% acrylamide gel (4ml H2O, 3.34ml 30% acrylamide/bis, 2.5ml Tris pH 8.8, 100ul 10%w/v SDS, 100ul 10%w/v ammonium persulphate, 7ul TEMED) and subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to nitrocellulose membranes (cat. 1703956, Bio-Rad Laboratories, Hercules, CA) and blocked overnight at 4°C with 5% BSA in tris-buffered saline (TBS) with 0.1% Tween-20. Blots for phosphorylated Hog-1 and Mpk-1 were probed with rabbit anti-phospho-p38 MAPK (Thr180/Tyr182, cat. 9211; Cell Signaling, Beverly, MA) and mouse anti-phospho-p44/42 MAPK (Thr202/Tyr204, cat. 9106; Cell Signaling), respectively. Primary antibodies were diluted 1:1000 in 5% BSA with 0.1% Tween-20 for 2h at room temperature or overnight at 4°C. Blots were incubated with HRP-conjugated goat anti-rabbit IgG or rabbit anti-mouse IgG secondary antibodies (1:2000 or 1:4000, cat. 7074 and 7076; Cell Signaling) for 2 h at room temperature. Blots were treated with ECL reagent (cat. RPN2135, Amersham Pharmaceuticals, Amersham, UK) according to the manufacturer's instructions then exposed to x-ray film. Membranes were incubated at 50°C for 30 minutes with stripping buffer (2% sodium dodecyl sulphate, 100 mM 2-mercaptoethanol, 50mM Tris pH 6.8) to remove antibodies, then re-probed for nonphosphorylated Hog-1 and Mpk-1 with rabbit anti-p38 MAPK (cat. 9212; Cell Signaling) and anti-p44/42 MAPK (cat. 4695; Cell Signaling) as described above for phospho-proteins. After processing, images of films were captured digitally and band densities were quantified by spot densitometry using the Alpha Imager analysis system (Alpha Innotech Corp., San Leandro, CA).

Statistical analysis. Mean cell concentrations and standard deviations were determined from three or six plate replicates in a single viability assay. Statistical significance of the differences among the mean % viability in each group were determined using a 1-way ANOVA with Tukey-Kramer post test (intra-group comparisons) and 2-way ANOVA with Bonferroni post test (inter-group comparisons). For all statistical analyses, GraphPad Prism 4 (GraphPad, San Diego, CA) was used. P values less than 0.05 were considered to be significant.

RESULTS

Deletion of *hog-1* or *mpk-1* enhances sensitivity to UVC. Irradiation of *S. cerevisiae* BY4742 with 50 J/m^2 UVC significantly decreased the viability of the *mpk-1* mutant but not the *hog-1* mutant or the wild type strain (Fig. 1, $p<0.001$). Approximately 60% of *mpk-1* cells were no longer able to form colonies after plating on solid YPD. A similar reduction in viability was observed in the *hog-1* mutant but not the wild type strain when the UVC dosage was increased to 100 J/m^2. The difference between the wild type and mutant strains was less pronounced at 150 J/m^2 UVC, although no more than 25% of wild type cells were rendered non-culturable. While an increase in mean survival of the mutant strains was noted at this high dosage, this increase was not significant and the *mpk-1* mutant strain remained significantly less viable than the wild type ($p<0.05$). Thus, both mutations were associated with increased susceptibility to UVC-induced cell death, with a more pronounced effect at lower dosages.

Hypo-osmotic shock induces expression of phosphorylated Mpk-1. A putative Mpk-1 protein was detected using an antibody against the phosphorylated form of the human homologue, p42/44 (ERK1/2). The western blot in Fig. 2a shows a protein with an apparent molecular mass of approximately 40 kDa, the only strong band detected with a size close to that expected for Mpk-1. Table 1 provides a semi-quantitative analysis of the western blot, suggesting that a 3-fold induction of this protein occurs one minute following hypo-osmotic shock and then declines toward the steady-state level over the next 30 minutes. No readily detectable variation in phosphorylated Mpk-1 (p-Mpk-1) expression was observed in response to hyper-osmotic shock (Fig. 2a), but the hyper-osmotic samples cannot be directly compared to the untreated cells as only half the amount of protein is present in these lanes.

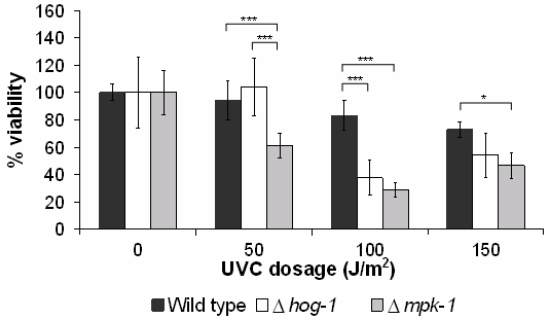

FIG. 1. Effect of *hog-1* and *mpk-1* deletions on the viability of *S. cerevisiae* BY4742 following UVC irradiation. Strains were exposed to UVC at the indicated dosage. Viability was estimated by colony counts and expressed as a relative percentage of the non-irradiated control. Values represent mean ± standard deviation of three or six plate counts. Statistical significance at $p<0.001$ (***) or $p<0.05$ (*) was determined by a two-way ANOVA with Bonferroni post-test.

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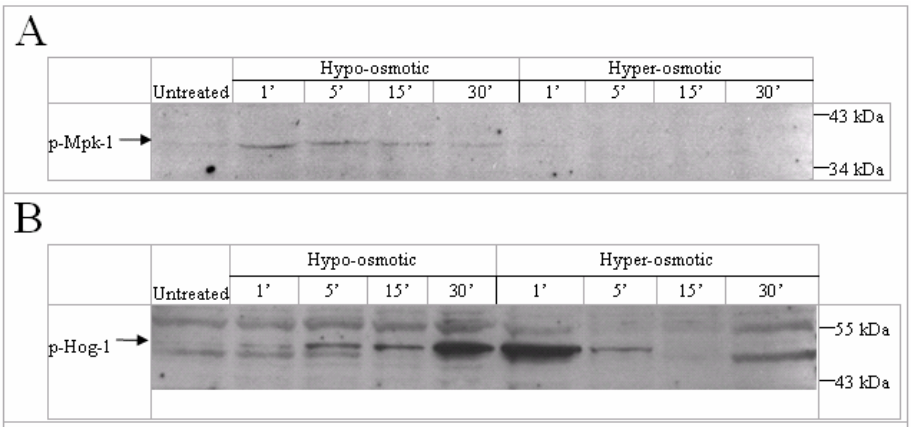

FIG. 2. Activation of yeast MAPKs in response to osmotic stress. Cells exposed to hypo- or hyper-osmotic medium were lysed at the indicated time points. (a) Western blot probed with anti-phospho p42/44 MAPK antibody. 20 g or 10 g protein was loaded for hypo-osmotic and hyper-osmotic samples, respectively. Location of molecular mass standards is shown at right. (b) Western blot probed with anti-phospho p38 MAPK antibody. 20 g protein was loaded in each lane.

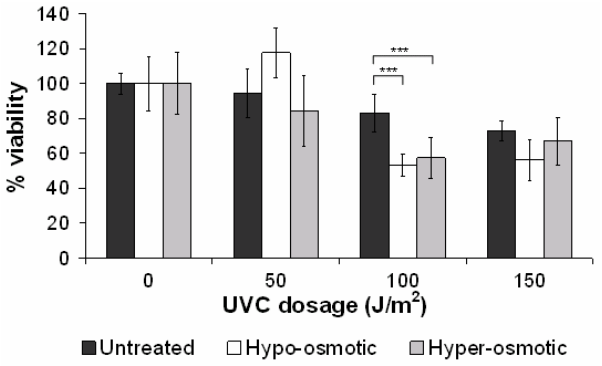

FIG. 3. Effect of hypo- and hyper-osmotic shock on *S. cerevisiae* BY4742 viability following UVC irradiation. Wild type cells were exposed to hypo- or hyper-osmotic medium for 2 minutes prior to UVC irradiation at the indicated dosage. Viability was estimated by colony counts and expressed as a relative percentage of the non-irradiated control. Values represent mean ± standard deviation of three or six plate counts. Statistical significance at $p<0.001$ (***) was determined by a two-way ANOVA with Bonferroni post-test.

Hyper-osmotic shock induces expression of phosphorylated Hog-1. A putative Hog-1 protein was detected using an antibody against the phosphorylated form of the human homologue, p38 MAPK. Fig. 2b shows a protein with an apparent molecular mass of approximately 50 kDa; other bands of similar size were detected with weaker banding patterns and minimal variation among samples. A coomassie blue membrane stain indicated that all wells were loaded with similar amounts of protein (data not shown). The densitometry data in Table 1 suggest that more than a 3-fold induction of the phosphorylated form of this protein occurs one minute following hypo-osmotic shock, followed by a return to steady-state levels by fifteen minutes. Comparison of the expression profiles in Fig. 2a and 2b suggests that phosphorylated Hog-1 (p-Hog-1) returns to steady-state levels more rapidly than Mpk-1. Fig. 2b also indicates that levels of p-Hog-1 increase gradually following hypo-osmotic shock, with the highest expression level observed at 30 minutes.

Table 1. Western blot densitometry for semi-quantitative analysis of p-Mpk-1 and p-Hog-1 induction following hypo- or hyper-osmotic shock. Fold induction is relative to the untreated (0 min) sample.

Time (min)	Fold induction of p-Mpk-1 following hypo-osmotic shock	Fold induction of p-Hog-1 following hyper-osmotic shock
0	1.0	1.0
1	2.7	3.3
5	2.4	1.3
15	1.7	0.7
30	0.5	0.8

Pre-treatment with hyper- or hypo-osmotic medium does not protect from UVC-induced cell death. Since deletion of *hog-1* or *mpk-1* appeared to promote UVC-induced cell death (Fig. 1), we sought to determine whether activation of these signalling pathways prior to irradiation would have a protective effect. Fig. 3 shows that no significant protection against UVC-induced death was observed when cells were pre-treated with hypo- or hyper-osmotic medium to activate Mpk-1 or Hog-1, respectively. In fact, both pre-treatments significantly decreased cell viability compared to the untreated control following irradiation with 100 J/m^2

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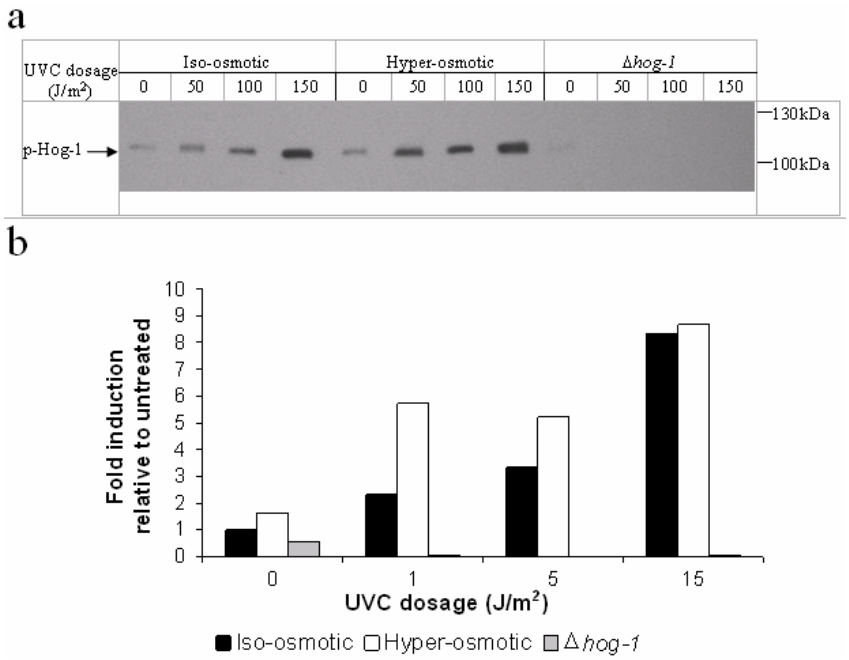

FIG. 4. Induction of phosphorylated Hog-1 by UVC in *S. cerevisiae* BY4742. Wild type cells were exposed to iso- or hyper-osmotic medium prior to UVC irradiation at the indicated dosage. The □*hog-1* strain was used as a negative control. (a) Western blot probed with anti-phospho p38 MAPK antibody. 25 g protein was loaded in each lane. Location of molecular mass standards is shown at right. (b) Western blot densitometry for semi-quantitative analysis of kinase induction.

UVC ($p<0.001$). No significant differences were observed at other dosages.

UVC irradiation causes upregulation of phosphorylated Hog-1 in a dosage-dependent manner. A putative p-Hog-1 protein was detected with an apparent molecular mass near 100 kDa (Fig. 4a), approximately twice the size of that shown at 50 kDa in Fig. 2b. A very weak signal was detected at this lower molecular mass. Neither the 50 kDa nor the 100 kDa form was expressed in the □*hog-1* mutant (Fig. 4a) with the exception of a faint band at 100 kDa in the non-irradiated sample. The 100 kDa protein was constitutively expressed in the wild-type strain following 4 hours of growth in 20% YPD, and its up-regulation correlated with increased
UVC dosage. A coomassie blue membrane stain indicated that all wells were loaded with similar amounts of protein (data not shown). Fig. 4b shows that hyper-osmotic shock prior to irradiation increased levels of this putative p-Hog-1 isoform by up to 50%, although this varied significantly between samples exposed to different dosages. Cells exposed to both iso- and hyper-tonic medium demonstrated increased p-Hog-1 expression in a UVC dosage-dependent manner, with an 8-fold induction observed at 150 J/m^2.

Attempts to detect p-Mpk-1, as well as the non-phosphorylated forms of Mpk-1 and Hog-1, were unsuccessful. The anti-p-Mpk-1, anti-p38 and anti-p42/44 antibodies failed to produce a detectable signal by western blot. Blots for -actin, intended for use as a loading control, revealed numerous non-specific bands; none of these appeared to be the protein of interest.

DISCUSSION

The recognition of stressful conditions by cellular sensors is followed by activation of signal transduction pathways leading to modification of gene expression to promote survival. Mitogen-activated protein kinase (MAPK) cascades play an essential role in many stress signalling pathways and are highly conserved among eukaryotes (2,20). Previous studies have demonstrated functional homology between mammalian p38 MAPK and the yeast Hog-1 kinase (10). The present study suggests that the UVC-induced stress response is also conserved.

The *S. cerevisiae* Hog-1 MAPK is activated in response to high osmolarity and is required for cell survival under these conditions (7,18); we predicted that this correlation between activation and survival might be extended to UVC-induced stress. Most studies report that UVC stimulates all three MAPKs and activates the transcription factor AP-1 in mammals (8). Multiple sensing mechanisms are involved in MAPK-mediated AP-1 activation, including the epidermal growth factor receptor (EGFR) and protein kinase C (13). Indeed, inhibition of EGFR or ERK activation may lead to an increase in UV-induced

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apoptosis, suggesting that activation of MAPKs is an important survival mechanism in response to UVC (5). We sought to determine the function of Mpk-1 and Hog-1 MAPK activation in the *S. cerevisiae* UV response. In particular, we asked whether pre-activation of these stress-signalling pathways cross-protects against UVC-induced cell death, and whether UVC irradiation can activate Hog-1.

To establish a functional role for Mpk-1 and Hog-1 in the UVC-induced stress response of *S. cerevisiae*, wild type and □*mpk-1* or □*hog-1* deletion mutants were exposed to UVC. The dosage-dependent killing of the wild type strain was also observed in the □*mpk-1* and □*hog-1* deletion mutants, but with significantly more cell death (Fig. 1). Given that Hog-1 and Mpk-1 appear to be required for optimal survival following UVC irradiation, it is tempting to speculate that they mediate expression of repair or resistance genes. Bryan *et al.* reported that at UV doses of 150 J/m^2, Mat-*mpk-1* cells are close to three times more sensitive than wild type cells (4). A similar effect was observed in this study. The authors also reported a minor decrease in viability of Hog-1 mutants but did not examine activation in wild type cells. While we have chosen to focus on Hog-1 due to availability of effective antibodies, we have confirmed a role for both kinases in UVC-induced signalling.

As demonstrated in Fig. 2, the phosphorylated forms of Mpk-1 and Hog-1 were strongly up-regulated one minute following resuspension in hypo- or hyper-osmotic medium, respectively. Upon phosphorylation by their respective MAPK kinases, Hog-1 and Mpk-1 translocate to the nucleus where they target multiple transcription factors (2). Activated Hog-1 may also remain in the cytoplasm where it mediates post-transcriptional and translational effects, or it may act directly on cytoplasmic targets such as membrane transporters (8). The RIPA buffer used in cell lysis extracts cytoplasmic, membrane, and nuclear proteins. Thus, our blots likely reflect total cellular levels of the phosphorylated kinases. However, it is important to note that the identity of these proteins has not been confirmed with certainty; it is possible that the proteins observed in Fig.2 are not in fact p-Hog-1 or p-Mpk-1. While the TXY motif is well-conserved between yeast and mammals (21), it is unknown which residues around the TXY motif were included in the antigenic peptide used to make the antibodies employed in this study. Indeed, no bands were observed on a subsequent p-Mpk-1 when a more dilute secondary antibody was employed.

Nevertheless, the putative Mpk-1 and Hog-1 kinases exhibited rapid and transient activation in response to hyper- or hypo-osmotic stress, as reported previously (7). This suggests that control of these pathways is tightly regulated by negative feedback mechanisms that activate phosphatases or inhibit upstream sensors. Dephosphorylation of Hog-1 may occur more quickly than Mpk-1 due to higher affinity interactions with specific tyrosine phosphatases. For example, Ptp3 is a cytoplasmic tyrosine phosphatase that preferentially acts on Hog-1 and is transcriptionally induced following Hog-1 activation (17). While other phosphatases such as Msg5 act only on Mpk-1, it is uncertain whether this kinase can participate in a negative feedback loop to induce expression of phosphatase-encoding genes (18). In any case, it is likely that phosphatase localization, specificity, and expression levels – in concert with inhibition of upstream activators by the phosphorylated kinases – regulate the kinetics of Hog-1 and Mpk-1 signalling.

Alonso-Monge *et al.* (1) have observed that pre-treatment of cells with sublethal amounts of oxidant enhances survival when cells are challenged with higher doses. This cross-protection was Hog-1-dependent. We did not observe a similar protective effect when Hog-1 and Mpk-1 were activated by osmotic stress prior to UVC irradiation. First, it should be noted that pre-activation of Hog-1 or Mpk-1 may not confer any additional protection because of the very rapid phosphorylation of these kinases in response to stimuli.

Second, while multiple stimuli may activate the same MAPK pathway, they likely have specific effects on the kinetics of phosphorylation, cellular localization, and activation of gene expression (17). For example, Marques *et al.* have demonstrated that LPS-induced Hog-1 phosphorylation peaks after 10 minutes, while maximal phosphorylation in response to hyperosmotic stress occurs within five minutes (16). The sensor kinases that detect the stress likely recruit MAPK-interacting molecules which contribute to this signalling specificity. For this reason, activation of Hog-1 via one pathway may not contribute to the increased activity of another pathway if the appropriate adaptor molecules are not present.

The duration and magnitude of kinase activation also determines the outcome of signalling (12). Prolonged hyperactivation of Hog-1 may produce lethal effects (24). While Fig. 4 suggests that hyperactivation did not occur at 100 J/m^2 when hyper- and hypo-osmotic pre-treatment appeared to increase UV sensitivity, it is possible that Hog-1 mediated activation of apoptotic pathways can occur in the presence of high levels of p-Hog-1. A role for p38 in cell-cycle progression has been reported in several organisms; Xue *et al.* have demonstrated that high levels of p38/Hog-1 signalling results in a biochemical cascade that feeds into apoptotic pathways (23). Mammalian cells regularly exposed to osmotic imbalances respond by arresting in G_1-S, G_2, and mitosis; mammalian p53

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inhibits transition from G_1 to S and is phosphorylated by p38 (22). Hog-1 activation by UVC may work by similar mechanisms to inhibit cell cycle progression and induce apoptosis. Although *S. cerevisiae* does not have a p53 homologue, it does possess a closely related serine/threonine protein kinase, YGR262c (6). Another possibility is that cellular damage caused by osmotic stress was not repaired before UVC exposure, which may have made the cells more sensitive.

It is also conceivable that negative feedback inhibition as a result of osmotic stress suppresses rather than enhances the UVC-induced stress response. As noted above, expression of negative regulators such as phosphatases can be transcriptionally induced by the same stimuli that activate MAPKs (17). Thus, pre-activation of these pathways may also activate the phosphatases which inhibit protective signalling. While this hypothesis is not supported by the presence of p-Hog-1 in Fig. 4, phosphatases not only remove phosphate groups but also regulate the nuclear translocation of MAPKs and cross-talk between different pathways. Indeed, it has been estimated that 7% of the yeast transcriptome is altered in response to mild osmotic shock; post-transcriptional regulation of gene expression and control of protein synthesis also occur (10). It is possible that among these diverse responses, some may inhibit cell survival following exposure to UVC. Finally, we cannot rule out the possibility of cross-talk between MAPK pathways and other signalling events (23). It is possible that the activation of Mpk-1 and Hog-1 pathways can confer cross-protection against UVC irradiation only when the signal input does not surpass a threshold level. At input levels that override this threshold level, a cross talk event may occur between these stress signal pathways and other pro-apoptotic or inhibitory pathways.

To our knowledge, this is the first report of a dosage-dependent effect of UVC irradiation on Hog-1 activation. UVC exposure induced p-Hog-1 expression in wild type *S. cerevisiae,* suggesting that the kinase activity increased with UVC dosage even in the absence of pre-activation by hyper-osmotic stress. While cells treated with hyper-osmotic medium did exhibit higher p-Hog-1 levels compared to cells pre-incubated in iso-osmotic medium (Fig. 4b), this effect did not correlate with increased cell survival, likely for reasons described above. While Fig. 4 suggests that low levels of p-Hog1 were present in the non-irradiated mutant strain, this weak signal may result from spill-over between wells. However, the discrepancy in protein size between the two anti-phospho-p38 blots presented in this paper is disconcerting. The single strong band depicted in Fig. 4 ran at approximately twice the apparent molecular mass as that in Fig 2a. This doubling in size suggests that the protein may be dimerized, perhaps due to inadequate reduction by the dithiothreitol present in the sample buffer. Phosphorylated ERK is known to dimerize in vitro and in vivo to form a high molecular mass complex; the monomers bind via a hydrophobic zipper with ion pairs on either side (19). It has been suggested that p38 also forms homodimers as it shares a similar domain; however, sufficient crystal structure data for Hog-1 is unavailable. As the ERK homodimeric complex is typically dissociated by treatment with -mercaptoethanol, it would also be unusual to detect it in the presence of sufficient dithiothreitol concentrations. Nevertheless, one freeze-thawing cycle may have rendered the reducing agent less active than expected. No evidence for extensive covalent modification of Hog-1 exists in the literature.

Taken together, our data provide support for previous reports of rapid and transient up-regulation of p-Hog-1 and p-Mpk-1 in response to osmotic stress. We have also demonstrated impaired survival of *hog-1* and *mpk-1* mutants following UVC irradiation between 50 and 150 J/m^2. While UVC induces up-regulation of wild type p-Hog-1 in a dosage-dependent manner, further increases in expression resulting from osmotic shock pre-treatment may increase sensitivity to UVC-induced cell death. Additional studies are necessary to confirm these preliminary findings.

S. cerevisiae provides a useful model for studying stress signalling because of the wide range of genetic and biochemical tools available for this organism. The present study adds to increasing evidence that MAPK pathways are well conserved between mammals and yeast. The latter may prove useful in elucidating the signal transduction mechanisms involved in UV-induced carcinogenesis and in screening potential drug candidates. Furthermore, understanding the mechanisms of cellular defence in yeast may aid in the development of therapeutic strategies which target stress responses in pathogenic fungi.

FUTURE EXPERIMENTS

Whether p-Hog-1 levels increase as a result of phosphorylation or due to changes in Hog-1 transcription or translation remains to be examined. However, the rapid rate at which p-Hog-1 levels are induced suggests that a post-transcriptional mechanism is involved. Antibodies to Hog-1 are commercially available and could be used to evaluate total kinase levels. A positive control for these western blots would be useful. Further optimization of the western blotting methods described here is also necessary to confirm the identity of the MAPKs of interest.

While we have suggested that total cellular p-Hog-1 levels increase in response to UVC, we have not demonstrated upregulation of gene products activated

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by this kinase, which requires nuclear translocation. Immunocytochemistry to examine Hog-1 translocation to the nucleus would prove useful in this regard. Evaluation of downstream products of Hog-1 activation, particularly *GDP1*, would also determine the extent to which p-Hog-1 levels reflect changes in gene expression. Another approach to studying these pathways is the use of recently-developed hyper-active MAPK mutants to eliminate the possibility of cross-talk between non-specific pathways (3). To begin to determine whether pro-apoptotic signalling contributes to UVC-induced cell death in the presence of Hog-1 activation, it would also be informative to blot for apoptotic markers or assay for caspase activity in wild type, deletion mutant, and hyper-activated Hog-1 mutants exposed to UVC.

Much work remains to be done to elucidate the mechanisms by which UVC exposure activates Hog-1. Whether membrane receptors similar to mammalian EGFR act as sensors or whether distinct cell membrane receptors are present in yeast are questions that remain to be answered. The mutants now available through the *S. cerevisiae* Genome Deletion Project will undoubtedly prove useful in answering these questions; screening mutants for impaired survival and signalling in response to UVC may help determine which genes are involved in these signalling cascades.

Finally, it also would be prudent to consider the wavelength-dependence of the UV response in future studies. Most UVC radiation is blocked by the ozone layer; hence, UVA and UVB are more physiologically relevant to human skin cancer. However, UVC is the best-studied form of UV radiation and will likely contribute significantly to our understanding of the many pathways which can lead to UV-induced carcinogenesis.

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