Molecular Biology of the Cell
Vol. 19, 308–317, January 2008

Multiple Pathways Differentially Regulate Global Oxidative Stress Responses in Fission Yeast

Dongrong Chen,*†‡ Caroline R.M. Wilkinson,†‡ Stephen Watt,* Christopher J. Penkett,*§ W. Mark Toone,†‖ Nic Jones,† and Jürg Bähler*

*Cancer Research UK Fission Yeast Functional Genomics Group, Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1HH, United Kingdom; and †Paterson Institute for Cancer Research, University of Manchester, Manchester M20 4BX, United Kingdom

Submitted August 2, 2007; Revised October 24, 2007; Accepted November 1, 2007
Monitoring Editor: Jonathan Weissman

Cellular protection against oxidative damage is relevant to ageing and numerous diseases. We analyzed the diversity of genome-wide gene expression programs and their regulation in response to various types and doses of oxidants in *Schizosaccharomyces pombe*. A small core gene set, regulated by the AP-1–like factor Pap1p and the two-component regulator Prr1p, was universally induced irrespective of oxidant and dose. Strong oxidative stresses led to a much larger transcriptional response. The mitogen-activated protein kinase (MAPK) Sty1p and the bZIP factor Atf1p were critical for the response to hydrogen peroxide. A newly identified zinc-finger protein, Hsr1p, is uniquely regulated by all three major regulatory systems (Sty1p-Atf1p, Pap1p, and Prr1p) and in turn globally supports gene expression in response to hydrogen peroxide. Although the overall transcriptional responses to hydrogen peroxide and *t*-butylhydroperoxide were similar, to our surprise, Sty1p and Atf1p were less critical for the response to the latter. Instead, another MAPK, Pmk1p, was involved in surviving this stress, although Pmk1p played only a minor role in regulating the transcriptional response. These data reveal a considerable plasticity and differential control of regulatory pathways in distinct oxidative stress conditions, providing both specificity and backup for protection from oxidative damage.

INTRODUCTION

Reactive oxygen species (ROS) are generated as metabolic by-products of aerobically growing cells and after exposure to environmental agents such as UV and ionizing radiation. An excess of ROS leads to oxidative stress by directly or indirectly damaging DNA, proteins, and lipids. ROS are implicated in aging and apoptosis and in numerous diseases, including cancer (Halliwell and Gutteridge, 1999; Finkel and Holbrook, 2000). In contrast, evidence is accumulating that ROS also provide vital signaling functions for diverse cellular processes (Rhee, 2006; Veal *et al*., 2007). Therefore, cells need to precisely tune ROS homeostasis and oxidative stress defense mechanisms to maintain healthy ROS levels and, accordingly, they have evolved sophisticated ways to sense and respond to ROS (Temple *et al*., 2005). In the laboratory, various oxidants such as hydrogen peroxide (H_2O_2, [HP]) are used to trigger and analyze responses to oxidative stress.

This article was published online ahead of print in *MBC in Press* (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E07–08–0735) on November 14, 2007.

‡ These authors contributed equally to this work.

Present addresses: § EMBL Outstation-Hinxton, European Bioinformatics Institute, Cambridge CB10 1SD, United Kingdom; ‖ Samuel Lunenfeld Research Institute, Toronto, Ontario, M5G 1X5, Canada.

Address correspondence to: Jürg Bähler (jurg@sanger.ac.uk).

Abbreviations used: CESR, core environmental stress response; HP, hydrogen peroxide; Md, menadione (Md); TBH, *t*-butylhydroperoxide; MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species; TF, transcription factor.

The fission yeast *Schizosaccharomyes pombe* is a popular model organism to study oxidative stress response pathways, most of which show remarkable conservation in multicellular eukaryotes (Toone *et al*., 2001; Ikner and Shiozaki, 2005). At least three signaling pathways are involved in directing the transcriptional response to oxidative stress in fission yeast. 1) A mitogen-activated protein kinase (MAPK) cascade, similar to the mammalian c-Jun NH_2-terminal kinase and p38 pathways (Torres, 2003), activates the Spc1p/Sty1p MAPK (Millar *et al*., 1995; Shiozaki and Russell, 1995; Degols *et al*., 1996; Degols and Russell, 1997; Buck *et al*., 2001). Sty1p in turn phosphorylates and regulates the stability of the bZIP transcription factor (TF) Atf1p, which is related to mammalian ATF-2 (Takeda *et al*., 1995; Shiozaki and Russell, 1996; Wilkinson *et al*., 1996; Gaits *et al*., 1998; Lawrence *et al*., 2007). The Sty1p–Atf1p pathway is activated in response to multiple environmental stresses, and mutants defective in the pathway are hypersensitive to ROS and several other stresses (Degols *et al*., 1996; Nguyen *et al*., 2000; Quinn *et al*., 2002). Genome-wide analyses have uncovered hundreds of genes whose expression is modulated by Sty1p and Atf1p in response to HP and other stressors (Smith *et al*., 2002; Chen *et al*., 2003; Rodriguez-Gabriel *et al*., 2003; Watson *et al*., 2004; Wilhelm and Bähler, 2006). The MAPK-controlled response to oxidative stress also involves posttranscriptional and posttranslational levels of regulation (Sanchez-Piris *et al*., 2002; Rodriguez-Gabriel *et al*., 2003, 2006; Dunand-Sauthier *et al*., 2005; Martin *et al*., 2006). 2) Pap1p is an AP-1-like TF similar to mammalian Jun; it is required for survival during oxidative stress by activating genes functioning in oxidant protection after stress-induced nuclear accumulation (Toda *et al*., 1991; Toone *et al*., 1998; Kudo *et al*., 1999). Similar to the budding yeast orthologue Yap1p

(Toone *et al*., 2001), Pap1p is a redox sensor that is directly activated by increased ROS levels (Castillo *et al*., 2002; Veal *et al*., 2004; Vivancos *et al*., 2004). However, Pap1p is inactivated when HP levels are too high; under such conditions, it requires the Sty1p-dependent induction of the sulfiredoxin Srx1p to become reactivated (Bozonet *et al*., 2005; Vivancos *et al*., 2005). Pap1p and Sty1p-Atf1p seem to have both overlapping and specialized roles in oxidative stress, with Pap1 dominating the response to low ROS levels and Sty1p-Atf1p dominating the response to high ROS levels (Quinn *et al*., 2002; Madrid *et al*., 2004; Vivancos *et al*., 2006). 3) Finally, a multistep phosphorelay system seems to be specialized for oxidative stress signaling in *S. pombe* (Ikner and Shiozaki, 2005). The two-component response regulator Prr1p functions in ROS defense, probably as a direct transcriptional regulator for some oxidative stress response genes independently of the Sty1p and Pap1 pathways (Ohmiya *et al*., 1999; Nguyen *et al*., 2000; Ohmiya *et al*., 2000; Buck *et al*., 2001).

Little is known about the relationships between and the relative importance of the three known oxidative stress response pathways in *S. pombe* described above. It is also not clear to what extent the genome-wide responses vary as a function of the specific oxidative stress encountered and whether additional key regulators are involved in these responses. Here, we present global analyses of fission yeast gene expression in response to different types and doses of oxidants and we identify additional regulators with functions in the oxidative stress response. This study highlights a substantial sophistication and plasticity in the stress response genes and in the pathways involved in regulating these genes as a function of the type and dose of oxidative stress.

MATERIALS AND METHODS

Strains and Stress Experiments

We used the wild-type strain 972 h^- (Leupold, 1970), and the following isogenic deletion mutant strains were obtained by crossing out auxotrophic markers: *pap1Δ::ura4* h^- (Toone *et al*., 1998), *sty1Δ::ura4* h^- and *atf1Δ::ura4* h^- (Chen *et al*., 2003), *prr1Δ::his7* h^- (Buck *et al*., 2001), and *pmk1Δ::ura4* h^- (Bimbo *et al*., 2005). The *atf1Δ pap1Δ* h^- double mutant strain was obtained by crossing the above-mentioned single mutants and using polymerase chain reaction (PCR) to confirm the mutant genotype. The *sty1Δ pmk1Δ* h^- double mutant strain was made by deleting *sty1* in the *pmk1Δ::ura4* mutant by using the hygromycin B marker (Sato *et al*., 2005). The *hsr1* (SPAC3H1.11) gene was deleted using the hygromycin B marker to obtain strain *hsr1Δ::hph* h^-; correct integration was checked by PCR and sequencing. Standard media and *S. pombe* methods were used (Moreno *et al*., 1991).

For stress experiments, strains were grown in yeast extract (YE) medium at 30°C to a titer of $\sim 4 \times 10^6$ cells/ml (Chen *et al*., 2003). Cells were harvested immediately before and at different times (5–60 min) after oxidant addition to the same culture. Harvesting was by gentle centrifugation (2000 rpm for 2 min), and pellets were immediately frozen in liquid nitrogen. Hydrogen peroxide (H1009; Sigma Chemical, Poole, Dorset, United Kingdom) was added to a final concentration of 0.07, 0.5, or 6 mM; menadione sodium bisulfite (M-5750; Sigma Chemical) was added to a final concentration of 5 mM, and *tert*-butylhydroperoxide (B2633; Sigma Chemical) was added to a final concentration of 2 mM. The same oxidant concentrations were used for the Northern and Western blot experiments.

For the dilution assays, exponentially growing cells at concentrations of $\leq 2 \times 10^6$ cells/ml were serially diluted fivefold from a starting dilution of 1×10^6 cells/ml, and 7.5 μl of each dilution was spotted onto YE plates containing no drug, 0.8 mM *t*-butylhydroperoxide (TBH), or 1 mM HP. The plates were incubated at 30°C for 3 d.

Flow cytometry was carried out with ethanol-fixed cells stained with propidium iodide by using a FACScan (BD Biosciences, San Jose, CA) as described previously (Sazer and Sherwood, 1990). DNA and septa were detected with 1 μg/ml 4,6-diamidine-2-phenyl-indole and 50 μg/ml calcofluor, respectively, using a BX51 microscope (Olympus, Tokyo, Japan) (using cells that had been fixed as for fluorescence-activated cell sorting analysis).

Microarray Experiments and Data Evaluation

Isolation of total RNA, labeling, and microarray hybridization was as described previously (Lyne *et al*., 2003). The experimental design was as described by Chen *et al*. (2003). In short, we used pools of samples from stress experiments with wild-type cells as a reference for microarray hybridizations, and after data acquisition the ratios were divided by the ratios of untreated wild-type cells (0 min). Thus, the reported ratios represent the expression levels at each time point relative to the expression levels of untreated wild-type cells from the same type of stress experiment. For repeated experiments, the cyanine (Cy) dyes were swapped between the experimental and reference samples. An overview of the experiments performed for the different oxidants, time points, and strains is provided in Supplemental Table 1. In total, we used data from 158 microarrays for this study. The complete processed data set is available from our website (http://www.sanger.ac.uk/PostGenomics/S_pombe/), and all raw data are available from the ArrayExpress repository (http://www.ebi.ac.uk/arrayexpress/), accession number E-MEXP-1083.

Genes with statistically significant changes in expression levels as a function of oxidant treatment were determined using the analysis of variance parametric test in GeneSpring (Agilent Technologies, Palo Alto, CA) with a Benjamini and Hochberg false discovery rate correction at 0.01. In most cases, genes that showed only minor changes at all time points were filtered out from the lists of differentially expressed genes as specified in the figure legends. Hierarchical clustering was performed in GeneSpring using standard or Pearson correlations with genes containing no data in ≥50% of the conditions being discarded; for Figure 4, genes were first grouped using the classic clustering with standard correlation in ArrayMiner 5 (Optimal Design, Brussels, Belgium). The significance of overlaps between different gene lists was calculated in GeneSpring by using a standard Fisher's exact test, and the p values were adjusted with a Bonferroni multiple testing correction. GeneSpring was also used to search for enriched sequence motifs within gene lists.

Northern and Western Blots

Northern assays were carried out as described previously (Quinn *et al*., 2002). For protein extract preparation, cells were grown to $\sim 2 \times 10^6$ cells/ml and treated with either HP or TBH as described above. Cells were harvested by filtration before and after treatment and frozen on liquid nitrogen. The filters were thawed in STOP buffer (150 mM NaCl, 50 mM NaF, 10 mM EDTA, and 1 mM NaN_3, pH 8), and cells were washed off and harvested by centrifugation. The cells were resuspended in lysis buffer with phosphatase inhibitor cocktail 2 (Sigma Chemical; catalog no. P5726). The composition of the lysis buffer was 50 mM HEPES, pH 7.5, 40 mM β-glycerophosphate, 1 mM sodium vanadate, 50 mM NaF, 0.5% NP-40, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 1 complete mini protease inhibitor tablet. After glass bead lysis, the supernatant was boiled in 2X SDS loading buffer. Extracts (50 μg) were separated by SDS-polyacrylamide gel electrophoresis on a 10% gel and analyzed by Western blotting with anti-phospho p38 (GenWay, San Diego, CA) or anti-hemagglutinin (HA) (Roche Diagnostics, Indianapolis, IN) diluted 1:1000.

RESULTS AND DISCUSSION

Gene Expression Responses to Different Doses of Hydrogen Peroxide

We studied the dose-dependent responses of fission yeast by using three doses of HP: 0.07 mM (low), 0.5 mM (medium), and 6 mM (high). These doses were chosen based on experience from previous studies (Quinn *et al*., 2002) and effects on cell mortality (see below). All stress experiments were performed with cells exponentially growing in rich medium. Figure 1A shows an overview of genes with changes in transcript levels in the three experiments. HP led to a dramatic and global reprogramming of gene expression. The expression of >3000 genes significantly changed in at least one dose of HP, and ~1800 genes changed >2-fold in at least one time point. The cellular response to the low dose of HP markedly differed from the response to the medium and high doses. Relatively few genes were regulated at this dose compared with the higher doses. Only ~150 genes were strongly induced at all three doses (Figure 1A, cluster 1).

The overall gene expression responses to medium and high doses of HP were similar, but the changes in transcript levels were strongest at the medium dose. For example, >99% of the previously identified core environmental stress response (CESR) genes (Chen *et al*., 2003; Gasch, 2007) were significantly regulated at the medium dose, but only 53% of these genes were regulated at the high dose. However, some genes, were induced in the high dose but not in the lower

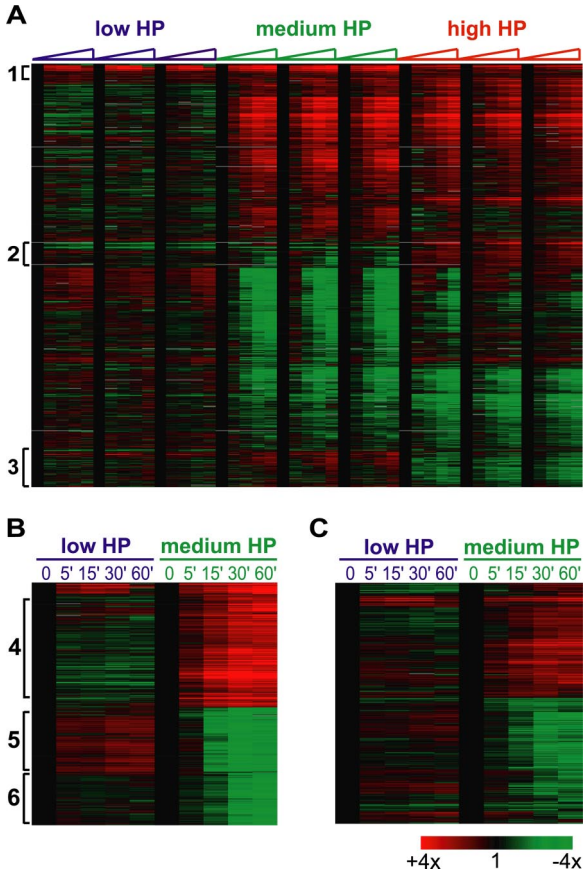

Figure 1. Changes in gene expression in response to different doses of HP. Hierarchical cluster analyses with columns representing experimental time points and rows representing genes. The mRNA levels at each time point relative to the levels in the same cells before HP treatments are color coded as indicated at the bottom, with missing data in gray. (A) Three independent biological repeats each of wild-type cells exposed to low, medium, and high doses of HP as indicated on top. Cells were collected before and at 5, 15, 30, and 60 min after exposure to HP. Data for 3466 genes with significant differential expression in at least one of the three doses are shown. Brackets on the left indicate clusters of genes described in the text. Annotated gene lists of these clusters are provided in Supplemental Table 3. (B) Expression profiles of CESR genes in wild-type cells exposed to low and medium doses of HP (with expression ratios from three repeats averaged). Data shown are for 888 genes that are included in the large lists of induced and repressed CESR genes from Chen *et al*. (2003), and they are also significantly regulated in the dose experiments. Brackets on the left indicate clusters of genes described in the text. Annotated gene lists of these clusters are provided in Supplemental Table 3. (C) Expression profiles of non-CESR genes in wild-type cells exposed to low and medium doses of HP as in B. Data shown are for 2578 genes that are not included in the CESR genes from Chen *et al*. (2003) but are significantly regulated in the dose experiments.

doses (Figure 1A, cluster 2); these genes were also induced in the other oxidants tested (see below). Notably, a cluster of genes that was specifically repressed at the high dose (Figure 1A, cluster 3) showed a significant overlap with a list containing the 20% of genes with the longest open reading frames ($p \sim 5 \times 10^{-26}$). This could reflect that long transcripts become preferentially degraded upon exposure to high doses of HP. It is likely that the gene expression differences between the medium and high concentration of HP mainly reflect that cells exposed to the high dose have been hit so hard that the regulatory response is partly compromised; accordingly, the cell mortality was 67% under these conditions, whereas only 22% of cells were killed by the medium dose, and no decrease in survival was evident at the low dose. For the rest of this article, we therefore focus on the responses to low and medium doses of HP.

We also separately clustered the CESR (Figure 1B) and non-CESR genes (Figure 1C) that were significantly regulated in the low doses, medium doses, or both. Notably, CESR genes showed an inverse correlation between induced and repressed genes in low and medium doses of HP: in low HP, CESR genes that are normally induced during stress tended to be repressed (Figure 1B, cluster 4), whereas most CESR genes that are normally repressed during stress were weakly induced (Figure 1B, cluster 5). Cluster 5 contains many genes involved in ribosome biogenesis ($p \sim 5 \times 10^{-61}$). Genes encoding ribosomal proteins, in contrast, are highly enriched in cluster 6 ($p \sim 2 \times 10^{-138}$), and these genes were not induced at the low dose (Figure 1B, cluster 6). Genes of these two clusters also showed differences in expression profiles at the medium dose of HP: although cluster 5 genes were maximally repressed at 30 min, repression of cluster 6 genes was delayed and showed lowest transcript levels at 60 min (Figure 1B). The regulation of CESR genes is discussed in more detail below. Although the distinction into CESR and non-CESR genes is somewhat arbitrary, the non-CESR genes showed an overall weaker regulation and less inverse correlation in expression levels between low and medium doses of HP compared with CESR genes (Figure 1, B and C).

Gene Expression Responses to Different Types of Oxidants

We next analyzed the global responses of fission yeast to two additional oxidants: menadione (Md) and TBH. Md is a quinine that causes the intracellular accumulation of oxidative species; it produces superoxide radicals that can be further oxidized in the cell to HP. TBH is a large organic peroxide ($C_4H_{10}0_2$) and, like HP, it acts directly as a ROS. Both TBH and the medium dose of HP led to a cell-cycle arrest in G2-phase, whereas Md showed only marginal effects on cell-cycle progression (Supplemental Figure S1). Figure 2 compares the Md and TBH experiments with the HP data. Overall, the gene expression response to TBH was similar to the medium-HP dose response. In comparison, the response to Md was much weaker and looked like a muted version of the response to TBH. Md triggered similar responses at 0.07, 0.5, and 5 mM, and the data shown are for the highest dose tested, which is 5 times higher than what has been used for budding yeast (Gasch *et al*., 2000). The number of genes significantly regulated in TBH was >5-fold higher than in Md. Unlike HP at low dose, Md did not lead to a "reverse" regulation of CESR genes: most genes were regulated in the same direction, albeit much weaker, as in the stronger stresses. One exception was the cluster enriched for ribosomal protein genes ($p \sim 6 \times 10^{-170}$; Figure 2, cluster 9). These are the very genes that did not show any reverse regulation at the low dose of HP (see also Figure 1B, cluster 6). The differences in expression levels of mRNAs for ribosomal proteins may reflect oxidant-specific effects on growth rate.

A cluster of <100 genes was induced in response to Md and TBH, but it was repressed in response to the medium and low doses of HP (Figure 2, cluster 7). However, many of these genes were also induced in the high dose of HP (Figure 1A, cluster 2; $p \sim 3 \times 10^{-32}$). The following gene lists showed significant overlaps with this cluster: 1) genes induced in *mcs6* and *pmh1* mutants, which are defective in a cyclin-dependent kinase-activating kinase complex required

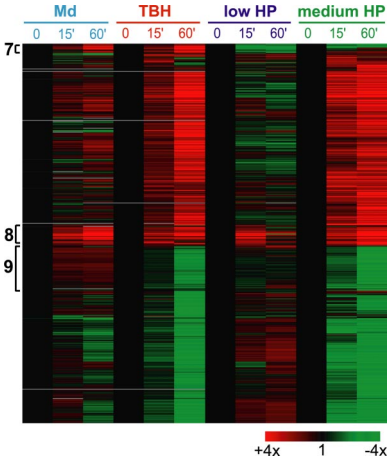

Figure 2. Changes in gene expression in response to four different oxidative stress conditions. Hierarchical cluster analysis with columns representing experimental time points, and rows representing the 1324 genes that show significant differential expression and at least twofold changes in one or more of the four stress conditions (with expression ratios from two or three repeats averaged). For each stress condition, data are shown from 0, 15, and 60 min after stress exposure as indicated on top. The mRNA levels at each time point relative to levels in the same cells before oxidative stress are color coded as indicated at the bottom, with missing data in gray. Brackets on the left indicate clusters of genes described in the text. Annotated gene lists of these clusters are provided in Supplemental Table 3.

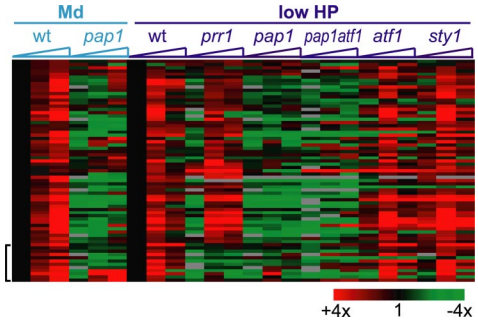

Figure 3. Regulation of gene expression in response to low dose of HP and Md. Oxidative stress experiments performed in wild type (wt) and in different deletion mutant backgrounds as indicated on top; columns for each experiment represent data from 0, 15, and 60 min after stress exposure. The mRNA levels at each time point relative to levels in wild-type cells before oxidative stress are color-coded as indicated at the bottom with missing data in gray. The 69 genes that were significantly regulated in both stresses and showed >1.5-fold change in at least one stress in wild-type cells were used for clustering. The bracket indicates the 13 genes that require both Pap1p and Prr1p for induction in HP (Table 1).

for cell cycle progression ($p \sim 4 \times 10^{-17}$; Lee *et al*., 2005); 2) genes for heat-shock proteins (Chen *et al*., 2003) and with the gene ontology (GO) association "protein folding" ($p \sim 5 \times 10^{-15}$); and 3) genes induced in response to cisplatin ($p \sim 2 \times 10^{-13}$; Gatti *et al*., 2004). This distinct response, characterized by specific gene groups, may reflect cellular defects and/or defense triggered by Md and TBH but not by the lower doses of HP. Another cluster of 127 genes was induced in all three oxidants and also in all doses of HP (Figure 2, cluster 8); these "core oxidative stress genes" are discussed below.

Together, TBH and the medium dose of HP lead to strong and similar stress responses, including the CESR genes. Md and the low dose of HP, in contrast, trigger much weaker responses, but many of the same genes are subtly regulated in the same or reverse direction, respectively, compared with the stronger stresses. Three regulatory pathways for the transcriptional response to oxidative stress are known, with major players represented by the Sty1p MAPK and the Atf1p TF, the Pap1p TF, and the Prr1p response regulator (see *Introduction*). Below, we analyze the gene expression signatures in deletion mutants of these key regulators to obtain a global perspective on the relative contribution of different pathways in the oxidative stress responses.

Pap1p and Prr1p Regulate Gene Expression in Response to Weak Oxidative Stress

Figure 3 shows a cluster analysis of the two conditions that led to a relatively weak gene expression response (low dose of HP and Md; see Figure 2), focusing on genes that were induced in both stresses. These genes tended to be induced in all four oxidative stress conditions and accordingly showed a strong overlap with genes of cluster 8 in Figure 2. Almost all of these genes were repressed rather than induced in *pap1*Δ mutants and thus require Pap1p for induction in both stresses. The Pap1p-dependent genes were not significantly enriched for reported Pap1p-binding sites (Fujii *et al*., 2000), but they were enriched for a related yet distinct sequence motif present in 28 of the 69 genes (GCTTAC; $p \sim 0.03$). Some differences between the two stresses were evident: ~10 genes that required Pap1p for induction in HP were less or not dependent on Pap1p in Md (Figure 3, bottom). In contrast to *pap1*Δ mutants, the gene expression response in *atf1*Δ and *sty1*Δ mutants was similar to wild-type cells, and the *pap*Δ *atf1*Δ double mutant showed defects similar to the *pap1*Δ single mutant (Figure 3). Thus, Atf1p and Sty1p are not or only marginally involved in regulating these genes.

Prr1p is known to be involved in regulating at least some Pap1p-dependent genes (Ikner and Shiozaki, 2005). We therefore also analyzed *prr1*Δ mutants in low HP. Before adding HP, most of the Pap1p-dependent genes were repressed in both *pap1*Δ and *prr1*Δ mutants (Figure 3). Thus, for basal expression levels in the absence of stress, Pap1p and Prr1p seem to be of similar importance. However, the majority of Pap1p-dependent genes were largely independent of Prr1p for stress induction (Figure 3). We identified only 13 genes that require both Pap1p and Prr1p for induction in HP (Figure 3, bracket). This latter group includes well-known oxidative stress defense genes, besides several uncharacterized genes (Table 1). This gene list was too small to reveal any significantly enriched sequence motifs. We found no genes that only required Prr1p without also requiring Pap1p for induction after HP treatment. We conclude that the response to weak oxidative stress mainly requires Pap1p, whereas Prr1p, along with Pap1p, controls the basal expression of these genes and the induction of a small subset of them. The Sty1p–Atf1p pathway is not critical under these conditions, and the CESR is not launched.

Differential Requirements for Sty1p and Pmk1p MAPKs in Strong Oxidative Stresses

Figure 4 shows a cluster analysis of the two conditions that led to a strong gene expression response (TBH and medium dose of HP; Figure 2). The genes modulated in these conditions consisted to a large part of the less conservative sets of CESR genes as defined previously (Chen *et al*., 2003). Four major clusters were evident. Genes were either induced

Table 1. Genes that require both Pap1p and Prr1p for induction in HP

Gene name	Annotation
ctt1	Catalase
gly1	Predicted threonine aldolase
gpa2	Heterotrimeric G protein α-2 subunit
meu8	Member of aldehyde dehydrogenase family
SPAC11D3.05	Membrane transporter
SPAC11D3.13	Member of DJ-1 or PfpI family
SPAC1F8.04c	Protein with amidohydrolase family domain
SPAC2E1P3.01	Member of zinc-binding dehydrogenase family
hsr1	Zinc finger protein, low similarity to *S. cerevisiae* Msn2p
SPAC869.02c	Predicted nitric oxide oxidoreductase
SPBC409.13	Predicted dimethyl-ribityllumazine synthase (riboflavin synthesis)
trr1	Thioredoxin reductase
trx1	Thioredoxin

Annotations are based on *S. pombe* GeneDB at http://www.genedb.org/genedb/pombe/index.jsp.

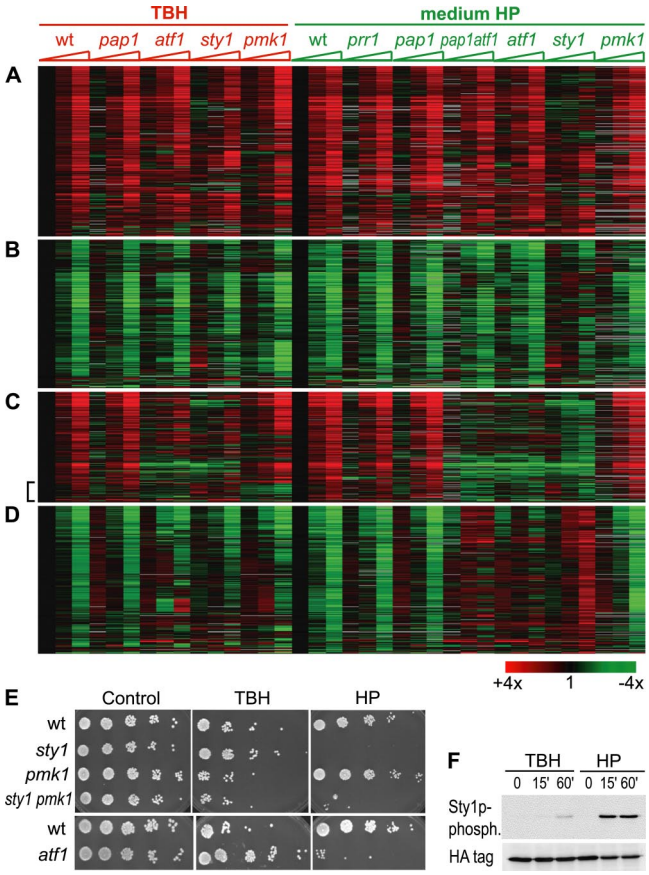

Figure 4. Regulation of gene expression in response to TBH and medium dose of HP and differential requirements for the MAP kinases Sty1p and Pmk1p. Oxidative stress experiments performed in wt and in different mutant backgrounds as indicated on top; columns for each experiment represent data from 0, 15, and 60 min after stress exposure. The mRNA levels at each time point relative to levels in wild-type cells before oxidative stress are color coded as indicated at the bottom, with missing data in gray. The 2095 genes that were significantly regulated in either stress and showed >1.5-fold change in at least one stress in wild-type cells were used for clustering. These genes were first clustered into five main groups, and genes within each group were then clustered hierarchically. (A) Cluster of 620 genes that are induced in both stresses and in most regulatory mutants. (B) Cluster of 499 genes that are repressed in both stresses and in most regulatory mutants. (C) Cluster of 386 genes that are induced in both stresses and dependent on Atf1p and Sty1p. The bracket indicates genes that require Pmk1p for induction in TBH. (D) Cluster of 525 genes that are repressed in both stresses and dependent on Atf1p and Sty1p. (E) Serial dilutions of exponentially growing cultures of wt cells, *sty1*Δ and *pmk1*Δ single and double mutants, and *atf1*Δ cells were spotted on plates without oxidant (control) and with addition of TBH or HP. (F) Western analyses of protein extracts from TBH and HP stress-time course experiments. Top, antibody probing Sty1p phosphorylation; bottom: anti-HA antibody as input control of HA-tagged Sty1p.

(Figure 4A) or repressed (Figure 4B) in both stresses and in all tested regulatory mutants, although changes in gene expression tended to be lower in *sty1*Δ mutants, most notably in HP stress. Other clusters of genes were also induced (Figure 4C) or repressed (Figure 4D) in both stresses, but in *sty1*Δ and *atf1*Δ mutants treated with TBH, these gene expression changes were less pronounced, and when treated with HP, they even showed a reverse regulation compared with wild-type cells. The *pap1*Δ and *prr1*Δ mutants, in contrast, had no effect on regulation of the genes in Figure 4, A–D, and the *pap1*Δ *atf1*Δ double mutant in HP showed a similar effect to the *atf1*Δ single mutant.

The regulatory patterns of the large number of genes in Figure 4 suggest that Sty1p and Atf1p are less critical for gene expression control in response to TBH compared with HP. This raised the possibility that additional regulators are involved in controlling the TBH response. Pmk1p/Spm1p is another *S. pombe* MAPK similar to metazoan extracellular signal-regulated kinase extracellular signal-regulated kinase (p42/p44) (Toda *et al.*, 1996; Roux and Blenis, 2004). Recent data indicate that Pmk1p can be activated in response to a variety of stresses (Madrid *et al.*, 2006). We therefore also analyzed *pmk1*Δ mutants under our conditions. Notably, *pmk1*Δ but not *sty1*Δ mutants were hypersensitive to TBH, whereas *sty1*Δ but not *pmk1*Δ mutants died in HP (Figure 4E). Moreover, Sty1p became strongly phosphorylated in HP but less so in TBH (Figure 4F), reflecting differential activation in response to the two oxidants. Pmk1p, however, has been shown to be activated by TBH but not by low or medium doses of HP (Madrid *et al.*, 2006). Note that *sty1*Δ and especially *atf1*Δ mutants were actually more resistant to TBH compared with both wild-type and the *sty1*Δ *pmk1*Δ double mutant (Figure 4E). This may reflect cross-talk between the Sty1p and Pmk1p MAPK pathways. Consistent with these findings, it has been shown that Pmk1p is hyperactivated in *atf1*Δ mutants (Madrid *et al.*, 2006).

Unlike for cell survival, however, Pmk1p seemed to play only a minor role for gene expression control in response to TBH (Figure 4, A–D). An exception was a small cluster of ~40 genes, which were strongly dependent on Pmk1p for induction in TBH (Figure 4C, bracket). These genes also depended on Atf1p but not on Sty1p in TBH, whereas in HP they depended on Atf1p and Sty1p but not on Pmk1p for induction. Thus, a small set of genes is regulated by Pmk1p instead of Sty1p in TBH stress. These genes showed no strong enrichment for any particular GO terms or functional groups. They included *gcn2*, which encodes a kinase that plays an important and conserved role in stress resistance by regulating the translation factor eIF 2α (Zhan *et al.*, 2004; Dunand-Sauthier *et al.*, 2005). It is possible that *gcn2* and/or other Pmk1p-dependent genes are critical targets that promote survival in TBH, although this seems unlikely given

that *atf1*Δ mutants were actually more resistant to TBH (Figure 4E). Alternatively, Pmk1p may exert its key functions at the posttranslational level. We conclude that Sty1p is the main MAPK regulating the response to HP, whereas Pmk1p is more critical for cell survival in TBH, although it plays only a minor role in the regulation of the large number of genes modulated in this condition.

Regulation of Core Oxidative Stress Response Genes

We then focused on the core oxidative stress response genes that were induced in all four stresses (Figure 5). These genes are a subset of gene clusters described above (Figures 2, cluster 8; and 3). They include several well-known genes involved in oxidative stress response such as *ctt1* (catalase), *trx1* (thioredoxin), and *trr1* (thioredoxin reductase) and dehydrogenases and glutathione *S*-transferases (Supplemental Table 2). The core list also contains numerous uncharacterized genes and three pseudogenes. The following gene lists showed significant overlaps with the core gene list: genes induced after exposure to ionizing radiation ($p \sim 7 \times 10^{-50}$; Watson *et al.*, 2004), genes specifically induced in HP among five stresses ($p \sim 2 \times 10^{-24}$; Chen *et al.*, 2003), and the GO category oxidoreductase activity ($p \sim 1 \times 10^{-9}$). These genes therefore seem to be generally induced in response to conditions causing oxidative damage.

Induction of most of these core oxidative stress response genes was Pap1p-dependent in all four conditions, most notably in low HP (Figure 5A), as also highlighted by the average expression profiles (Figure 5B). Note that almost all of these genes required Pap1p and Prr1p also for basal levels of expression in the absence of stress. In medium HP only, Atf1p and Sty1p also played some role in regulating these genes, whereas Pap1p became somewhat less important, although it remained a critical regulator for more than half of the genes (Figure 5, A and B). The function of Prr1p for HP-dependent gene induction was relatively minor compared with Pap1p in low HP, but it was almost as critical as Pap1p in medium HP. As reported previously (Quinn *et al.*,

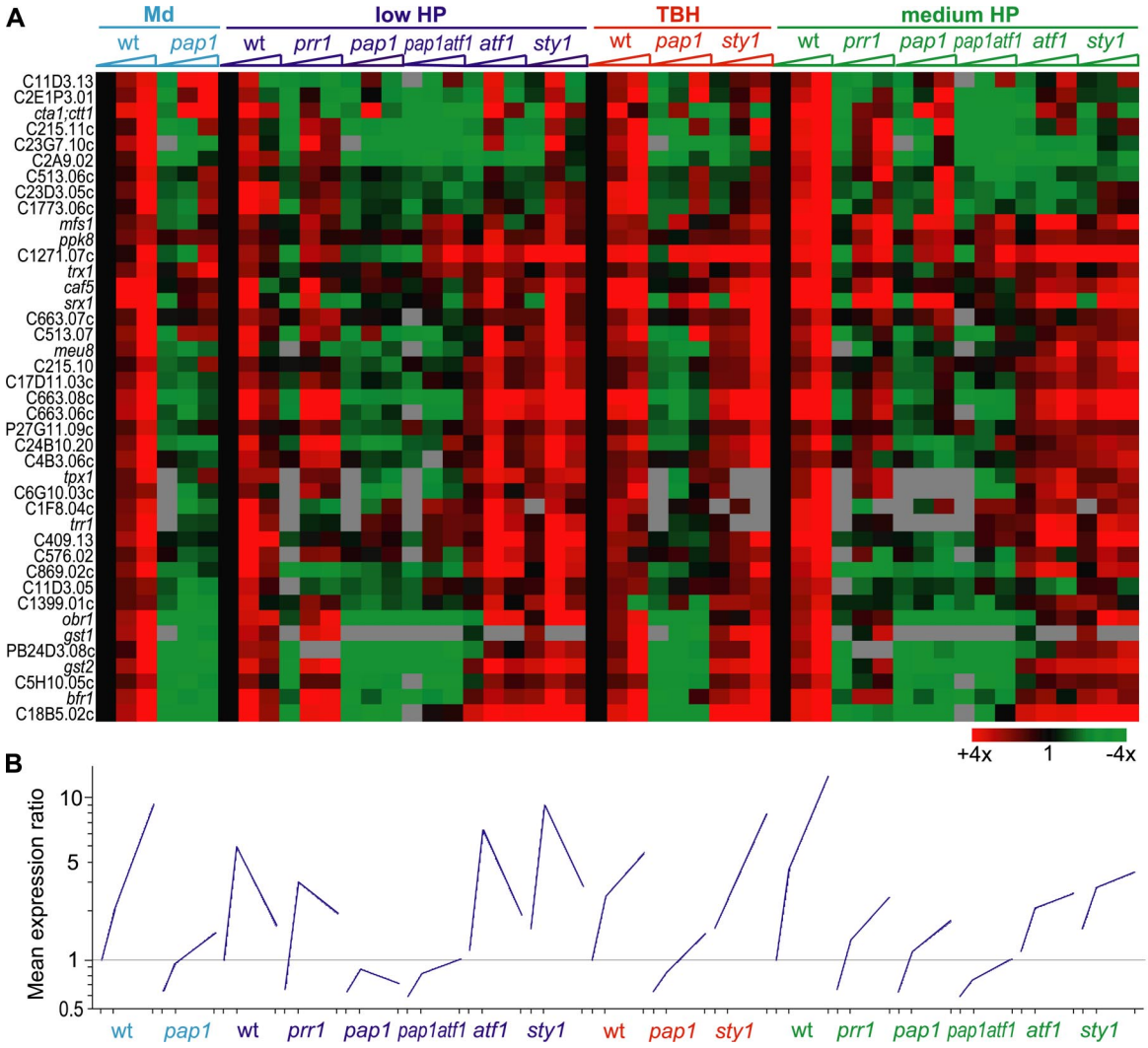

Figure 5. Regulation of core genes induced in all four oxidative stress conditions. (A) Oxidative stress experiments performed in wt and in different deletion mutant backgrounds as indicated on top; columns for each experiment represent data before and at 15 and 60 min after stress exposure. The mRNA levels at each time point relative to levels in wild-type cells before oxidative stress are color coded as indicated at the bottom, with missing data in gray. The 41 genes that were significantly regulated and showed >1.5-fold change in all four stress experiments in wild-type cells were used for clustering. The gene names are indicated at left, and annotations are provided in Supplemental Table 2. (B) Average gene expression profiles for the 41 genes shown in A in the different stress conditions.

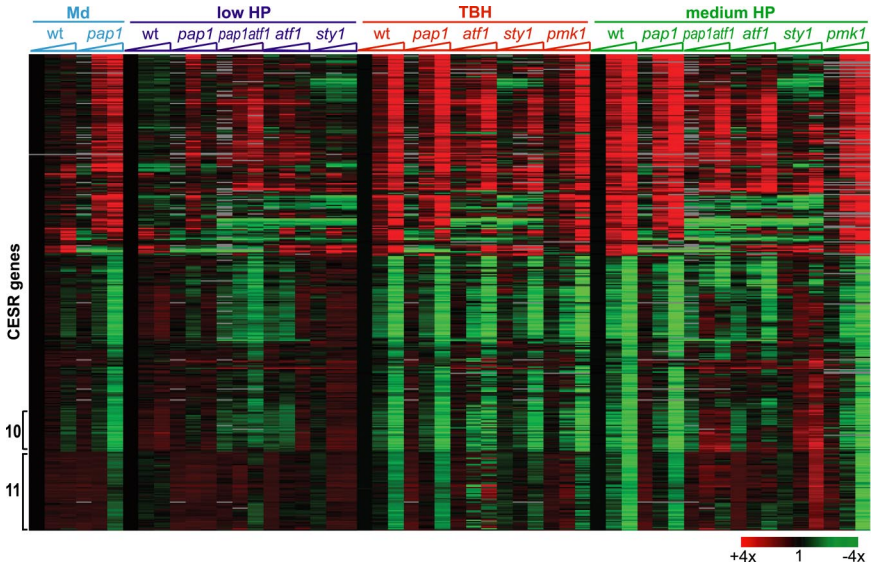

Figure 6. Regulation of CESR genes in oxidative stress. Expression profiles of CESR genes in the different stress conditions. Data shown are for 694 genes that are included in the large lists of induced and repressed CESR genes from Chen *et al*. (2003) and are also significantly regulated in the dose experiments. Oxidative stress experiments performed in wt and in different deletion mutant backgrounds as indicated on top; columns for each experiment represent data before, and at 15 and 60 min after stress exposure. The mRNA levels at each time point relative to levels in wild-type cells before oxidative stress are color coded as indicated in B, with missing data in gray. Brackets on the left indicate clusters enriched for genes encoding ribosomal biogenesis proteins (cluster 10) and ribosomal proteins (cluster 11). Annotated gene lists of these clusters are provided in Supplemental Table 3.

2002), some genes such as *ctt1* were dependent on both Pap1p and Atf1p/Sty1p for induction, with Pap1p being the dominant regulator in low HP, and Atf1p/Sty1p becoming more important in medium HP; accordingly, these genes were more affected in the *pap1*Δ *atf1*Δ double mutant than in either single mutant, being strongly repressed rather than induced in the double mutant (Figure 5A, top rows). However, this combined regulation seems to be the exception rather than the rule, and most of the core oxidative stress response genes mainly rely on Pap1p and on Prr1p for regulation.

Consistent with the finding that the core oxidative stress response genes were mainly regulated by Pap1p, they showed an overlap with genes induced upon overexpression of Int6p, which has been shown to increase the transcriptional activity of Pap1p ($p \sim 2 \times 10^{-33}$; Jenkins *et al*., 2005). Interestingly, we also observed an overlap with genes in the amino acid metabolism module ($p \sim 1 \times 10^{-14}$; Tanay *et al*., 2005). This raises the possibility that Pap1p controls the amino acid metabolism genes in fission yeast, whose regulation has been well studied in budding yeast and involves another bZIP TF, Gcn4p (Hinnebusch, 2005). Consistent with this idea, the fission yeast orthologue of the translation initiation factor Gcn2p, which regulates Gcn4p translation (Hinnebusch, 2005), is up-regulated by both oxidative stress and amino acid starvation (Zhan *et al*., 2004).

In low HP, the induction of the core oxidative stress response genes was more rapid and more transient than in the other conditions (Figure 5, A and B; also see Figure 1), consistent with the previous finding that Pap1p goes to the nucleus faster at a low dose of HP than at a high dose (Quinn *et al*., 2002). Recent data provide insight into the mechanistic basis of this differential regulation of Pap1p to different doses of HP (Bozonet *et al*., 2005; Vivancos *et al*., 2005): Pap1p activity depends on the peroxiredoxin Tpx1p; in high doses of HP, Tpx1p is inhibited until it becomes reactivated in a Sty1p-dependent manner by the sulfiredoxin Srx1p. The increased dependency of the core genes on Sty1p-Atf1p at the higher dose of HP is therefore probably an indirect effect reflecting the requirement for the MAPK pathway for Pap1p reactivation. Notably, *tpx1* and *srx1* were themselves among the core oxidative stress response genes (Figure 5A and Supplemental Table 2); in medium HP, their induction was most severely compromised in the *pap1*Δ *atf1*Δ double mutant, whereas expression levels were much less affected in either single mutant or in the *sty1*Δ mutant. This result points to a positive feedback mechanism for the regulation of Pap1p activity involving both Pap1p and the Sty1p–Atf1p pathway. We conclude that Pap1p and, to a lesser extent, Prr1 are the direct regulators of the core oxidative stress response genes in all conditions tested.

Regulation of CESR Genes during Different Oxidative Stresses

We also separately analyzed the regulation of the CESR genes (Chen *et al*., 2003) under the four different conditions (Figure 6). As also shown in Figure 1B, the CESR genes were regulated "the wrong way round" in the low dose of HP, with CESR induced genes tending to be slightly repressed and CESR repressed genes tending to be slightly induced. This is consistent with the idea that Pap1p can directly or indirectly inhibit the Sty1p pathway, as suggested by Vivancos *et al*. (2005). In Md, in contrast, where Pap1p-dependent transcription was delayed (Figure 5, A and B), the CESR genes tended to be weakly regulated in the expected direction, suggesting that the Sty1p pathway is not inhibited under these conditions.

As described above (Figures 1B and 2), the cluster enriched for ribosomal protein genes (Figure 6, cluster 11) showed different gene expression patterns compared with the ribosomal biogenesis gene cluster (Figure 6, cluster 10) and the other CESR-repressed genes. This finding suggests differences in regulation for the ribosomal protein genes: whereas all CESR-repressed genes require Sty1p for repression, Atf1p seems to be mainly required for repression of ribosomal protein genes. At least some of these differences in regulation could also be realized at the posttranscriptional level, e.g., by regulating mRNA turnover. Consistent with this possibility, the mRNAs for ribosomal proteins have longer poly(A) tails than those for ribosomal biogenesis proteins (Lackner *et al*., 2007). This resembles the situation in budding yeast where genes for ribosomal protein and biogenesis show similar yet distinct regulatory patterns (Jorgensen *et al*., 2004; Wade *et al*., 2006).

In both weak stresses (Md and low HP), the CESR was activated in the *pap1*Δ mutant, which was even more pronounced in the *pap1*Δ *atf1*Δ double mutant in low HP (Figure 6). These data may reflect that in the absence of Pap1p the

cells cannot adequately protect themselves against low levels of ROS, and they compensate by activating the CESR that is normally only launched in response to strong stress. The two strong stresses (TBH and medium HP) led to a pronounced CESR that was largely independent of Pap1p. Part of the CESR could be triggered in the absence of Atf1p (also see Chen *et al*., 2003). Sty1p was required for both induction and repression of most CESR genes, and in its absence the regulation of many of these genes was reversed (Figure 6). However, as also seen in Figure 4, the Sty1p pathway played a more prominent role in regulating the response to HP, and it was less critical for the response to TBH. The CESR genes were largely independent of Pmk1p, although in TBH the ribosomal protein genes were less repressed in *pmk1*Δ mutants (Figure 6, cluster 11). In conclusion, the Sty1p pathway is the major regulator of the CESR genes in response to HP and, to a much lesser degree, to TBH.

Hsr1, a Novel Regulator for the Response to Hydrogen Peroxide

A predicted zinc-finger TF (encoded by SPAC3H1.11) raised our interest, because its mRNA was among the few transcripts that required both Prr1p and Pap1p for induction in the low dose of HP (Table 1). We named this gene hydrogen peroxide stress regulator (*hsr1*) for reasons described below. The levels of *hsr1* mRNA changed <1.5-fold in Md and TBH, but they were induced >2-fold in the medium dose of HP, and this induction required Prr1p and Pap1p as well as Atf1p and Sty1p (Figure 7A); the dependency on Atf1p and Sty1p was strongest, and even the basal expression levels in unstressed cells were lower in the *atf1*Δ and *sty1*Δ mutants and further decreased during stress. This requirement for all four regulators was unique. We therefore deleted this gene to analyze its role in the global response to the medium dose of HP. On average, the induction of the core oxidative stress response genes as well as the CESR-induced genes was approximately twofold lower than in wild-type cells (Figure 7B). Thus, Hsr1p is involved in regulating both of these distinct gene sets that are otherwise differentially controlled by either Pap1p/Prr1p or by Sty1p-Atf1p, although it plays a relatively minor role compared with the respective specialized regulators. The induction of at least two genes with potential alcohol dehydrogenase functions, however, was strongly affected in the *hsr1*Δ mutants (Figure 7C). The *hsr1*Δ mutants were slightly more sensitive to HP than wild-type cells but not nearly as sensitive as *sty1*Δ mutants (Figure 7D), in accordance with the relatively minor global effect on gene expression.

Hsr1p shows a weak similarity to Msn2p of budding yeast, which is involved in the response to multiple stresses, including oxidants (Hasan *et al*., 2002, and references cited therein), but Hsr1p lacks the protein kinase A sites. Hsr1p contains a nuclear localization signal but no detectable nuclear export signal, and, unlike Pap1p, it may therefore localize constitutively in the nucleus. Consistent with this view, Hsr1p shows nuclear localization in unstressed cells according to the large-scale study of Matsuyama *et al*. (2006).

We conclude that Hsr1p is heavily controlled by all known regulators involved in the response to HP and, in turn, it is required for the full transcriptional induction of genes induced in HP stress regulated by the different factors. Hsr1p is thus part of a positive feedback for the response to HP stress.

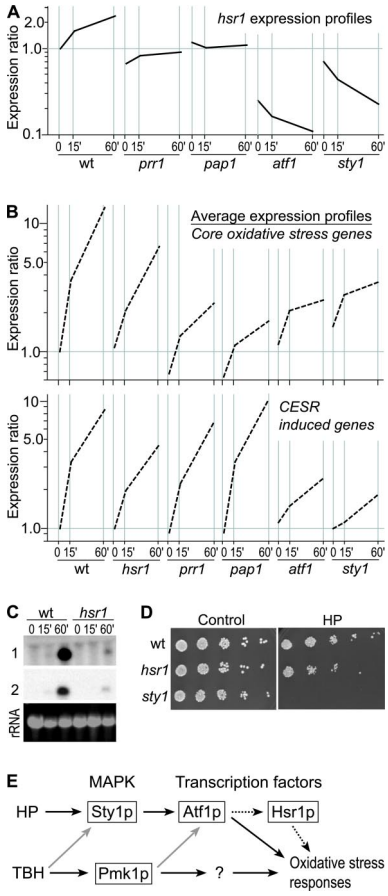

Figure 7. Hsr1p is involved in the response to HP stress. (A) Gene expression profiles of *hsr1* in response to the medium dose of HP in wild type and different mutant strains and time points as indicated at the bottom. The mRNA levels at each time point relative to levels in the wild-type cells before HP treatments are indicated as expression ratios. (B) Average gene expression profiles of the 41 core oxidative stress genes (top; as in Figure 5) and 324 induced CESR genes (bottom; Chen *et al*., 2003) in response to the medium dose of HP in wild-type and different mutant strains and time points as indicated at the bottom. (C) Northern analyses of two genes whose induction in response to the medium dose of HP is much lower in *hsr1*Δ mutants compared with wt cells. Row 1, SPAC23D3.05c, an alcohol dehydrogenase pseudogene; row 2, SPBC1773.06c, predicted to encode an alcohol dehydrogenase. The rRNA amounts are shown as a loading control. (D) Serial dilutions of exponentially growing cultures of wt cells and *hsr1*Δ and *sty1*Δ mutants were spotted on plates without oxidant (control) or with addition of HP. (E) Scheme of the regulatory events in response to stress caused by HP or TBH. The gray arrows indicate weak links, and dashed arrows indicate a pathway that seems to be only activated in HP. See main text for details.

CONCLUSIONS

The dose and type of oxidant strongly influences the gene expression response. A relatively small core oxidative stress response is launched irrespective of dose and oxidant, and this response is universally regulated by Pap1p and, to some extent, Prr1p. Pap1p and Prr1p are also critical to maintain basal expression levels of these genes. These core genes make up the majority of the response to weak oxidative stresses. Pap1p can be regarded as a rheostat for the cell that regulates ROS homeostasis, and it is in charge of an adaptive response to mild ROS imbalance. In strong oxidative

stresses, in contrast, additional genes including the CESR are also modulated, leading to a much larger response. Although the overall responses to TBH and the medium-dose HP are similar, two MAPK pathways are differentially required for the acute responses to the strong oxidative stress conditions. The Sty1p pathway plays a more prominent role in regulating genes in response to HP, and it is not required for survival in TBH. Instead, Pmk1p promotes survival in TBH, although it plays a relatively minor role in gene expression control, and it may mainly function at the post-translational level. This differential requirement for Sty1p and Pmk1p in two oxidative stress conditions is surprising given that Sty1p is otherwise activated by and required for survival in a wide range of different stresses. TBH and HP are usually applied interchangeably to study oxidative stress in various organisms, but our data indicate that they can lead to distinct regulatory responses. Hsr1p, a newly identified predicted TF, is unique in that it is regulated by all three major regulatory systems (Sty1p-Atf1p, Pap1p, and Prr1p) and, in turn, it globally promotes gene expression in response to HP. These findings are summarized in Figure 7E. Our data highlight the considerable richness, specialization, and plasticity in regulatory mechanisms used by the cell to effectively respond to different types and doses of oxidants. The multiple pathways interact with each other and may provide both specificity and redundancy for cellular protection from oxidative damage.

ACKNOWLEDGMENTS

We thank S. Marguerat for comments on the manuscript, the Sanger Institute Microarray facility for array printing, and M. Balasubramanian (Temasek Life Sciences Laboratory, Singapore) and J. Millar (University of Warwick, United Kingdom) for strains. We apologize to colleagues in the field for not citing all relevant papers due to space limitations. D.C. and W.M.T. have been supported by the EMF Biological Research Trust. Research in the Bähler and Jones laboratories is funded by Cancer Research UK.

REFERENCES

Bimbo, A. *et al*. (2005). Systematic deletion analysis of fission yeast protein kinases. Eukaryot. Cell *4*, 799–813.

Bozonet, S. M., Findlay, V. J., Day, A. M., Cameron, J., Veal, E. A., and Morgan, B. A. (2005). Oxidation of a eukaryotic 2-Cys peroxiredoxin is a molecular switch controlling the transcriptional response to increasing levels of hydrogen peroxide. J. Biol. Chem. *280*, 23319–23327.

Buck, V., Quinn, J., Soto Pino, T., Martin, H., Saldanha, J., Makino, K., Morgan, B. A., and Millar, J. B. (2001). Peroxide sensors for the fission yeast stress-activated mitogen-activated protein kinase pathway. Mol. Biol. Cell *12*, 407–419.

Castillo, E. A., Ayte, J., Chiva, C., Moldon, A., Carrascal, M., Abian, J., Jones, N., and Hidalgo, E. (2002). Diethylmaleate activates the transcription factor Pap1 by covalent modification of critical cysteine residues. Mol. Microbiol. *45*, 243–254.

Chen, D., Toone, W. M., Mata, J., Lyne, R., Burns, G., Kivinen, K., Brazma, A., Jones, N., and Bähler, J. (2003). Global transcriptional responses of fission yeast to environmental stress. Mol. Biol. Cell *14*, 214–229.

Degols, G., and Russell, P. (1997). Discrete roles of the Spc1 kinase and the Atf1 transcription factor in the UV response of *Schizosaccharomyces pombe*. Mol. Cell. Biol. *17*, 3356–3363.

Degols, G., Shiozaki, K., and Russell, P. (1996). Activation and regulation of the Spc1 stress-activated protein kinase in *Schizosaccharomyces pombe*. Mol. Cell. Biol. *16*, 2870–2877.

Dunand-Sauthier, I., Walker, C. A., Narasimhan, J., Pearce, A. K., Wek, R. C., and Humphrey, T. C. (2005). Stress-activated protein kinase pathway functions to support protein synthesis and translational adaptation in response to environmental stress in fission yeast. Eukaryot. Cell *4*, 1785–1793.

Finkel, T., and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. Nature *408*, 239–247.

Fujii, Y., Shimizu, T., Toda, T., Yanagida, M., and Hakoshima, T. (2000). Structural basis for the diversity of DNA recognition by bZIP transcription factors. Nat. Struct. Biol. *7*, 889–893.

Gaits, F., Degols, G., Shiozaki, K., and Russell, P. (1998). Phosphorylation and association with the transcription factor Atf1 regulate localization of Spc1/Sty1 stress-activated kinase in fission yeast. Genes Dev. *12*, 1464–1473.

Gasch, A. P. (2007). Comparative genomics of the environmental stress response in ascomycete fungi. Yeast *24*, 961–976.

Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B., Storz, G., Botstein, D., and Brown, P. O. (2000). Genomic expression programs in the response of yeast cells to environmental changes. Mol. Biol. Cell *11*, 4241–4257.

Gatti, L. *et al*. (2004). Global gene expression of fission yeast in response to cisplatin. Cell. Mol. Life Sci. *61*, 2253–2263.

Halliwell, B., and Gutteridge, J. M. C. (1999). Free Radicals in Biology and Medicine. Oxford, United Kingdom: Oxford University Press.

Hasan, R., Leroy, C., Isnard, A.-D., Labarre, J., Boy-Marcotte, E., and Toledano, M. B. (2002). The control of the yeast H_2O_2 response by the Msn2/4 transcription factors. Mol. Microbiol. *45*, 233–241.

Hinnebusch, A. G. (2005). Translational regulation of GCN4 and the general amino acid control of yeast. Annu. Rev. Microbiol. *59*, 407–450.

Ikner, A., and Shiozaki, K. (2005). Yeast signaling pathways in the oxidative stress response. Mutat. Res. *569*, 13–27.

Jenkins, C.C.L., Mata, J., Crane, R. F., Thomas, B., Akoulitchev, A., Bähler, J., and Norbury, C. J. (2005). Activation of AP-1-dependent transcription by a truncated translation initiation factor. Eukaryot. Cell *4*, 1840–1850.

Jorgensen, P., Tyers, M., and Warner, J. R. (2004). Forging the factory: ribosome synthesis and growth control in budding yeast. In: Cell Growth: Control of Cell Size, ed. M.N. Hall, M. Raff, and G. Thomas, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 329–370.

Kudo, N., Taoka, H., Toda, T., Yoshida, M., and Horinouchi, S. (1999). A novel nuclear export signal sensitive to oxidative stress in the fission yeast transcription factor Pap1. J. Biol. Chem. *274*, 15151–15158.

Lackner, D. H., Beilharz, T. H., Marguerat, S., Mata, J., Watt, S., Schubert, F., Preiss, T., and Bähler, J. (2007). A network of multiple regulatory layers shapes gene expression in fission yeast. Mol. Cell *26*, 145–155.

Lawrence, C. L., Maekawa, H., Worthington, J. L., Reiter, W., Wilkinson, C. R., and Jones, N. (2007). Regulation of *S. pombe* Atf1 protein levels by Sty1-mediated phosphorylation and heterodimerisation with Pcr1. J. Biol. Chem. *282*, 5160–5170.

Lee, K. M. *et al*. (2005). Impairment of the TFIIH-associated CDK-activating kinase selectively affects cell cycle-regulated gene expression in fission yeast. Mol. Biol. Cell *16*, 2734–2745.

Leupold, U. (1970). Genetical methods for *Schizosaccharomyes pombe*. Methods Cell Physiol. *4*, 169–177.

Lyne, R., Burns, G., Mata, J., Penkett, C. J., Rustici, G., Chen, D., Langford, C., Vetrie, D., and Bähler, J. (2003). Whole-genome microarrays of fission yeast: characteristics, accuracy, reproducibility, and processing of array data. BMC Genomics *4*, 27.

Madrid, M., Soto, T., Franco, A., Paredes, V., Vicente, J., Hidalgo, E., Gacto, M., and Cansado, J. (2004). A cooperative role for Atf1 and Pap1 in the detoxification of the oxidative stress induced by glucose deprivation in *Schizosaccharomyces pombe*. J. Biol. Chem. *279*, 41594–41602.

Madrid, M., Soto, T., Khong, H. K., Franco, A., Vicente, J., Perez, P., Gacto, M., and Cansado, J. (2006). Stress-induced response, localization, and regulation of the Pmk1 cell integrity pathway in *Schizosaccharomyces pombe*. J. Biol. Chem. *281*, 2033–2043.

Martin, V., Rodriguez-Gabriel, M. A., McDonald, W. H., Watt, S., Yates, J. R., 3rd, Bähler, J., and Russell, P. (2006). Cip1 and Cip2 are novel RNA-recognition-motif proteins that counteract Csx1 function during oxidative stress. Mol. Biol. Cell *17*, 1176–1183.

Matsuyama, A. *et al*. (2006). ORFeome cloning and global analysis of protein localization in the fission yeast *Schizosaccharomyces Pombe*. Nat. Biotechnol. *24*, 841–847.

Millar, J. B., Buck, V., and Wilkinson, M. G. (1995). Pyp1 and Pyp2 PTPases dephosphorylate an osmosensing MAP kinase controlling cell size at division in fission yeast. Genes Dev. *9*, 2117–2130.

Moreno, S., Klar, A., and Nurse, P. (1991). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol. *194*, 795–823.

Nguyen, A. N., Lee, A., Place, W., and Shiozaki, K. (2000). Multistep phosphorelay proteins transmit oxidative stress signals to the fission yeast stress-activated protein kinase. Mol. Biol. Cell *11*, 1169–1181.

Ohmiya, R., Kato, C., Yamada, H., Aiba, H., and Mizuno, T. (1999). A fission yeast gene *prr1* that encodes a response regulator implicated in oxidative stress response. J. Biochem. *125*, 1061–1066.

Ohmiya, R., Yamada, H., Kato, C., Aiba, H., and Mizuno, T. (2000). The Prr1 response regulator is essential for transcription of ste11+ and for sexual development in fission yeast. Mol. Gen. Genet. *264*, 441–451.

Quinn, J., Findlay, V. J., Dawson, K., Millar, J. B., Jones, N., Morgan, B. A., and Toone, W. M. (2002). Distinct regulatory proteins control the graded transcriptional response to increasing H_2O_2 levels in fission yeast *Schizosaccharomyces pombe*. Mol. Biol. Cell *13*, 805–816.

Rhee, S. G. (2006). Cell signaling. H2O2, a necessary evil for cell signaling. Science *312*, 1882–1883.

Rodriguez-Gabriel, M. A., Burns, G., McDonald, W. H., Martin, V., Yates, J. R., 3rd, Bähler, J., and Russell, P. (2003). RNA-binding protein Csx1 mediates global control of gene expression in response to oxidative stress. EMBO J. *22*, 6256–6266.

Rodriguez-Gabriel, M. A., Watt, S., Bähler, J., and Russell, P. (2006). Upf1, an RNA helicase required for nonsense-mediated mRNA decay, modulates the transcriptional response to oxidative stress in fission yeast. Mol. Cell. Biol. *26*, 6347–6356.

Roux, P. P., and Blenis, J. (2004). ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol. Mol. Biol. Rev. *68*, 320–344.

Sanchez-Piris, M., Posas, F., Alemany, V., Winge, I., Hidalgo, E., Bachs, O., and Aligue, R. (2002). The serine/threonine kinase Cmk2 is required for oxidative stress response in fission yeast. J. Biol. Chem. *277*, 17722–17727.

Sato, M., Dhut, S., and Toda, T. (2005). New drug-resistant cassettes for gene disruption and epitope tagging in *Schizosaccharomyces pombe*. Yeast *22*, 583–591.

Sazer, S., and Sherwood, S. W. (1990). Mitochondrial growth and DNA synthesis occur in the absence of nuclear DNA replication in fission yeast. J. Cell Sci. *97*, 509–516.

Shiozaki, K., and Russell, P. (1995). Cell-cycle control linked to extracellular environment by MAP kinase pathway in fission yeast. Nature *378*, 739–743.

Shiozaki, K., and Russell, P. (1996). Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast. Genes Dev. *10*, 2276–2288.

Smith, D. A., Toone, W. M., Chen, D., Bähler, J., Jones, N., Morgan, B. A., and Quinn, J. (2002). The Srk1 protein kinase is a target for the Sty1 stress-activated MAPK in fission yeast. J. Biol. Chem. *277*, 33411–33421.

Takeda, T., Toda, T., Kominami, K., Kohnosu, A., Yanagida, M., and Jones, N. (1995). Schizosaccharomyces pombe atf1 encodes a transcription factor required for sexual development and entry into stationary phase. EMBO J. *14*, 6193–6208.

Tanay, A., Regev, A., and Shamir, R. (2005). Conservation and evolvability in regulatory networks: the evolution of ribosomal regulation in yeast. Proc. Natl. Acad. Sci. USA *102*, 7203–7208.

Temple, M. D., Perrone, G. G., and Dawes, I. W. (2005). Complex cellular responses to reactive oxygen species. Trends Cell Biol. *15*, 319–326.

Toda, T., Dhut, S., Superti-Furga, G., Gotoh, Y., Nishida, E., Sugiura, R., and Kuno, T. (1996). The fission yeast $pmk1^+$ gene encodes a novel mitogen-activated protein kinase homolog which regulates cell integrity and functions coordinately with the protein kinase C pathway. Mol. Cell. Biol. *16*, 6752–6764.

Toda, T., Shimanuki, M., and Yanagida, M. (1991). Fission yeast genes that confer resistance to staurosporine encode an AP-1-like transcription factor and a protein kinase related to the mammalian ERK1/MAP2 and budding yeast FUS3 and KSS1 kinases. Genes Dev. *5*, 60–73.

Toone, W. M., Kuge, S., Samuels, M., Morgan, B. A., Toda, T., and Jones, N. (1998). Regulation of the fission yeast transcription factor Pap1 by oxidative stress: requirement for the nuclear export factor Crm1 (Exportin) and the stress-activated MAP kinase Sty1/Spc1. Genes Dev. *12*, 1453–1463.

Toone, W. M., Morgan, B. A., and Jones, N. (2001). Redox control of AP-1-like factors in yeast and beyond. Oncogene *20*, 2336–2346.

Torres, M. (2003). Mitogen-activated protein kinase pathways in redox signaling. Front. Biosci. *8*, d369–d391.

Veal, E. A., Day, A. M., and Morgan, B. A. (2007). Hydrogen peroxide sensing and signaling. Mol. Cell *26*, 1–13.

Veal, E. A., Findlay, V. J., Day, A. M., Bozonet, S. M., Evans, J. M., Quinn, J., and Morgan, B. A. (2004). A 2-Cys peroxiredoxin regulates peroxide-induced oxidation and activation of a stress-activated MAP kinase. Mol. Cell *15*, 129–139.

Vivancos, A. P., Castillo, E. A., Biteau, B., Nicot, C., Ayte, J., Toledano, M. B., and Hidalgo, E. (2005). A cysteine-sulfinic acid in peroxiredoxin regulates H2O2-sensing by the antioxidant Pap1 pathway. Proc. Natl. Acad. Sci. USA *102*, 8875–8880.

Vivancos, A. P., Castillo, E. A., Jones, N., Ayte, J., and Hidalgo, E. (2004). Activation of the redox sensor Pap1 by hydrogen peroxide requires modulation of the intracellular oxidant concentration. Mol. Microbiol. *52*, 1427–1435.

Vivancos, A. P., Jara, M., Zuin, A., Sanso, M., and Hidalgo, E. (2006). Oxidative stress in *Schizosaccharomyces pombe*: different H_2O_2 levels, different response pathways. Mol. Genet. Genomics *276*, 495–502.

Wade, C. H., Umbarger, M. A., and McAlear, M. A. (2006). The budding yeast rRNA and ribosome biosynthesis (RRB) regulon contains over 200 genes. Yeast *23*, 293–306.

Watson, A., Mata, J., Bähler, J., Carr, A., and Humphrey, T. (2004). Global gene expression responses of fission yeast to ionizing radiation. Mol. Biol. Cell *15*, 851–860.

Wilhelm, B. T., and Bähler, J. (2006). The genomics of stress response in fission yeast. In: The Mycota, vol. XIII, Fungal Genomics, ed. A.J.P. Brown, Berlin, Germany: Springer, 97–111.

Wilkinson, M. G., Samuels, M., Takeda, T., Toone, W. M., Shieh, J. C., Toda, T., Millar, J. B., and Jones, N. (1996). The Atf1 transcription factor is a target for the Sty1 stress-activated MAP kinase pathway in fission yeast. Genes Dev. *10*, 2289–2301.

Zhan, K., Narasimhan, J., and Wek, R. C. (2004). Differential activation of eIF2 kinases in response to cellular stresses in *Schizosaccharomyces pombe*. Genetics *168*, 1867–1875.