

The Hog1 MAP kinase pathway and the Mec1 DNA damage checkpoint pathway independently control the cellular responses to hydrogen peroxide

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Abstract

The DNA damage checkpoint pathway and the MAP kinase pathway respond to various forms of environmental as well as endogenous stresses through signal transduction mechanisms involving protein kinases. Both pathways are intertwined in mammalian cells, but potential crosstalk between these two pathways in budding yeast has not been examined yet. We show that the Rad53 checkpoint kinase and the Hog1 MAP kinase of *Saccharomyces cerevisiae* become phosphorylated upon exposure to hydrogen peroxide, indicative of activation of the DNA damage checkpoint and MAP kinase pathways in response to oxidative stress. Rad53 kinase is equally activated in wild type and in *hog1-Δ* cells. Likewise, the activation of Hog1 MAP kinase is not dependent on Mec1 kinase, the central checkpoint kinase in budding yeast. Mutants in either pathway are sensitive to hydrogen peroxide and the double mutants exhibit a near perfectly additive phenotype. These data demonstrate that the DNA damage checkpoint pathway and the MAP kinase pathway respond to oxidative stress independently of each other and suggest that these two stress signaling pathways are activated by different types of insults induced by hydrogen peroxide.

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1. Introduction

The DNA damage checkpoint system is an evolutionarily conserved surveillance mechanism that coordinates the cellular responses to DNA lesions and stalled replication forks [1]. Some of the cellular events regulated by the checkpoint response include cell cycle arrest, transcriptional induction of DNA metabolism genes, suppression of late-firing origins, and DNA repair. The DNA damage checkpoint signal transduction pathway consists of sensors, adaptor proteins, and effector kinases [2]. The *Saccharomyces cerevisiae* PIK-like kinases, Mec1 (ATR in mammals) and Tel1 (ATM in mammals), perform sensor and signaling functions. Unlike in mammals, where ATR and ATM are activated by different forms of DNA damage, Mec1 in budding yeast is activated by all forms of genotoxic stress,

whereas Tel1 functions in telomere metabolism with only a minor role in the DNA damage checkpoint in wild type cells. Mec1 is the primary signaling kinase in budding yeast and the DNA damage checkpoints are virtually eliminated in *mec1* cells. Mec1 kinase is also critical for the signal sensing stage, and Mec1 was proposed to be activated by binding to RPA-coated single-stranded DNA through its interacting partner, Ddc2 [3]. Once activated, Mec1 phosphorylates and activates the downstream effector kinases, Rad53 (Chk2 in mammals and Cds1 in *S. pombe*) and Chk1, through the adaptor proteins Rad9 (in case of DNA damage) and Mrc1 (in case of replication block) [2]. Budding yeast also contains Dun1, another checkpoint kinase with similarity to Chk2/Cds1, which is phosphorylated by Rad53 [4] and Mec1 [5]. Activation of the DNA damage checkpoint in *S. cerevisiae* can be conveniently monitored as an electrophoretic mobility shift of Rad53 kinase, which is caused by *trans*-phosphorylation by the Mec1 and Tel1 kinases and subsequent Rad53 auto-phosphorylation [6,7].

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The mitogen activated protein kinase (MAPK) pathway is a signal transduction cascade that involves sequential phosphorylation events and responds to environmental stresses including high osmolarity, oxidative stress, and heat shock [8,9]. The MAPK pathway is composed of three protein kinases: a MAPK kinase kinase (MAPKKK), which activates a MAPK kinase (MAPKK), which in turn phosphorylates and activates a MAPK. An activated MAPK is then capable of entering the nucleus and regulating the expression of target genes by phosphorylating relevant transcription factors. The HOG pathway of *S. cerevisiae* responds to high osmolarity in the growth media [10]. The hallmark of the activation of the HOG pathway is the immediate, within less than one minute, tyrosine phosphorylation of Hog1 MAPK, which is detected in immunoblots using an anti-phospho-tyrosine antibody [11]. Hog1 activation occurs in media containing 200–500 mM NaCl, and expectedly, *hog1-Δ* cells are extremely sensitive to high salt concentrations in the growth media [10]. Besides the osmoregulatory HOG pathway, budding yeast has other MAPK pathways that respond to different environmental stimuli including the Fus3 MAPK pathway that is involved in the mating pheromone response pathway; the Kss1 pathway controlling the invasive growth response; Slt2 MAPK regulating the cell cycle and cell wall construction in response to high temperatures; and the Smk1 pathway controlling spore wall assembly [8,9]. The *S. pombe* Hog1 homolog, Spc1, is activated not only in response to high osmolarity but also to oxidative and other forms of stress [12].

Although *hog1* cells were found to be sensitive to oxidative stress [13], Hog1 activation, evidenced by tyrosine phosphorylation, could not be demonstrated and a novel mechanism of Hog1 activation, independent of tyrosine phosphorylation, was suggested [10,13]. We decided to further investigate Hog1 MAPK activation in response to oxidative stress. Since the DNA damage checkpoint is activated in response to oxidative stress [14], we wanted to determine any potential crosstalk between the DNA damage checkpoint response and the HOG MAPK response to oxidative stress. Such crosstalk had been identified in mammalian cells [15,16]. Here we report that Hog1 MAPK is tyrosine-phosphorylated and activated in response to H₂O₂ treatment and that this activation is independent of an active DNA damage checkpoint. We also show that the Mec1-dependent DNA damage checkpoint response invoked by H₂O₂ is fully intact in *hog1-Δ* cells. Moreover, the results of epistasis analysis of *hog1-Δ*, *mec1-kd* and *rad53-Δ* mutants suggest that the HOG and the DNA damage checkpoint pathways operate independently in response to oxidative stress in budding yeast.

2. Materials and methods

2.1. Strains

All strains used in this work are of the W303 background and contain the corrected *RAD5* allele, which was

Table 1
S. cerevisiae strains used in this study^a

| Strain | Relevant genotype | Reference |
|-----------|----------------------------------|--------------|
| W303 RAD5 | <i>SML1</i> | R. Rothstein |
| WDHY 1741 | <i>MEC1</i> | This study |
| WDHY 1742 | <i>mec1-kd</i> | This study |
| WDHY 1838 | <i>hog1-Δ::KAN</i> | This study |
| WDHY 1839 | <i>mec1-kd hog1-Δ::KAN</i> | This study |
| WDHY 1901 | <i>rad53-Δ::HIS3</i> | This study |
| WDHY 1916 | <i>rad53-Δ::HIS3 hog1-Δ::KAN</i> | This study |

^a All strains are of the W303 background with the common genotype of *MATa ade2-1 can1-100 leu2-3,112 his3-11 ura3-1 trp1-1 RAD5*. The WDHY strains contain in addition *sml1::HIS3* to suppress *mec1* and *rad53* lethality.

kindly provided by Dr. R. Rothstein (Columbia University) (see Table 1). WDHY 1741 (*MEC1*) and 1742 (*mec1-kd*; D2224A, N2229K) are *RAD5* variants of strains kindly provided by Dr. T. Petes (University of North Carolina). Both *MEC1* and *mec1-kd* alleles are tagged with an HA epitope, which does not compromise the function of the protein [17]. The remaining WDHY strains contain the untagged *MEC1* wild type locus. WDHY 1838 (*hog1-Δ::KAN*) was constructed by PCR-mediated gene replacement using primers HOG1F (forward primer) 5'-CTATCGTATATAATAATGACCACTAACGAGGAATTCATTACGTACGCTGCAGGTTCGA-3' and HOG1R (reverse primer) 5'-GAC-ATTAAAAAACACGTTTACTGTTGGAACCTATTAGCGATCGATGAATTCGAGCT-3'. The chromosomal *HOG1* gene was replaced with the *KAN* cassette from pKANMX6. Correct genomic integration of *KAN* cassette was confirmed by PCR and Western blots using anti-Hog1 antibodies (Santa Cruz Biotechnology). All experiments were performed at 30 °C.

2.2. Immunoblotting experiments

Logarithmically growing YPD cultures were left untreated or treated with the indicated concentrations of either H₂O₂ or NaCl for 10 min. Total protein extracts were prepared using trichloroacetic acid precipitation [18]. Approximately 15 μg of total protein extract from each sample were electrophoresed on a 9% polyacrylamide gel and transferred onto nitrocellulose membrane. Membranes were either immuno-blotted with anti-Rad53 (Santa Cruz Biotechnology), anti-phospho-tyrosine 4G10 (Upstate Biotechnology), or anti-phospho-p38 (Cell Signaling) antibodies. Some membranes were re-blotted with the mouse monoclonal anti-*S. cerevisiae* Xrn1 antibody H8 [19] as a loading control.

2.3. Survival experiments

For the spot assay, overnight cultures (OD₆₀₀ 8–12) were serially diluted and spotted on YPD plates containing the indicated concentrations of H₂O₂ or NaCl. Plates were incubated at 30 °C for 3 days. For the acute exposure ex-

periment, exponentially growing cultures were treated with 10 mM H_2O_2 and aliquots were removed at the indicated time points, diluted, and plated on YPD plates. Surviving colonies were counted after 3 days of incubation at 30 °C. The experiment was performed three times and the means with standard deviations are given.

3. Results

3.1. Hog1 MAP kinase is activated in response to H_2O_2 treatment

Previous studies had shown that the MAP kinase Hog1 is only phosphorylated, and thus activated, in response to

osmotic stress [8–10]. However, *hog1* cells are not only sensitive to high osmolarity but also to oxidative stress induced by H_2O_2 [13]. Yet a 10-min exposure of wild type cells to either 0.4 mM [10] or 10 mM [13] H_2O_2 did not result in detectable Hog1 tyrosine phosphorylation, and thus it was concluded that the HOG pathway responds to oxidative stress by a mechanism not involving tyrosine phosphorylation. We decided to analyze the H_2O_2 response in greater detail. We treated wild type cells with varying concentrations of H_2O_2 for 10 min and analyzed Hog1 phosphorylation using anti-phospho-tyrosine and anti-phospho-p38 antibodies. The latter antibody specifically detects the dually phosphorylated form of MAPK from a variety of eukaryotic species [20]. As shown in Fig. 1A, we could detect Hog1

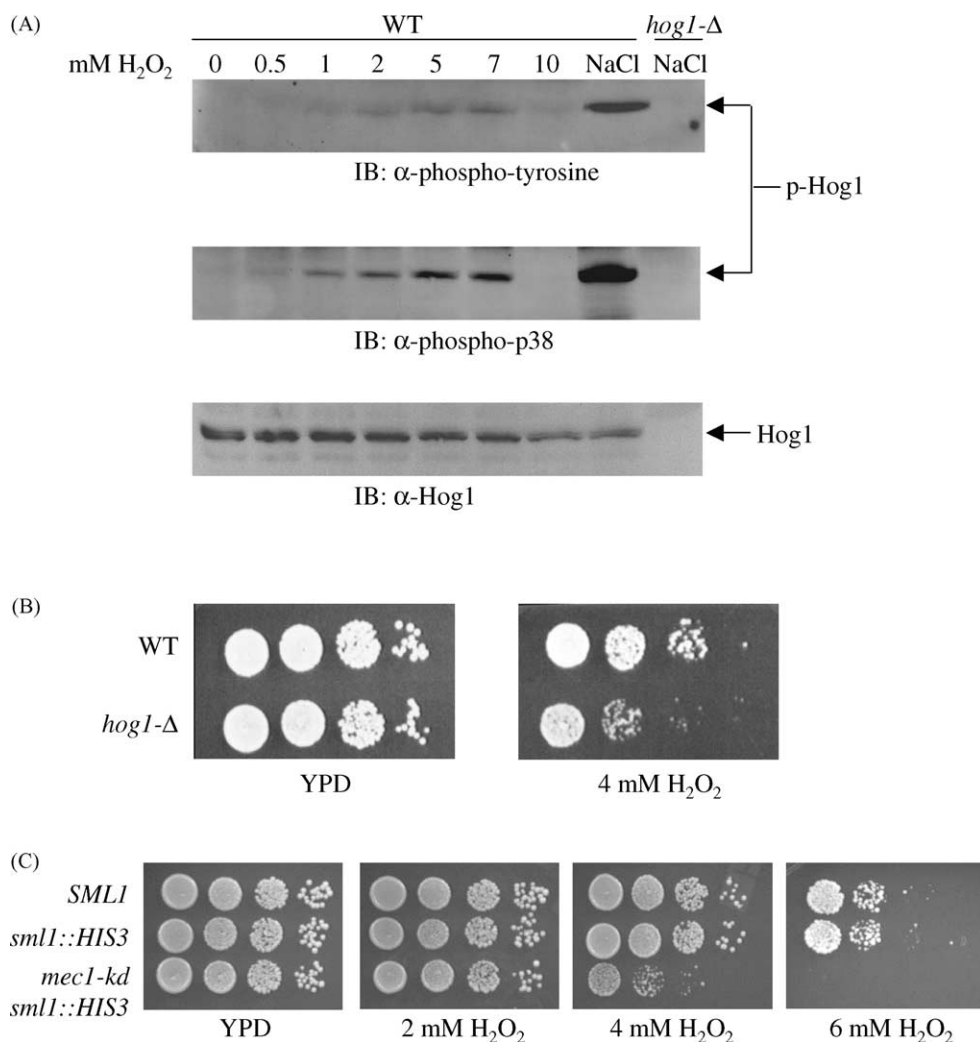


Fig. 1. Hog1 MAPK is activated in response to H_2O_2 stress. (A) Logarithmically growing cultures ($OD_{600} = 1$) were left untreated or treated with varying concentrations of H_2O_2 for 10 min. Total protein extracts were analyzed on SDS-PAGE and subsequently blotted with anti-phospho-tyrosine antibody (upper panel) or anti-phospho-p38 antibody (middle panel). Wild type (WDHY 1741) and *hog1*-Δ (WDHY 1838) cells were also treated with 0.5 M NaCl for 10 min, and extracts were analyzed on the same gels as positive and negative controls, respectively. The membrane was re-blotted with anti-Hog1 antibody to determine protein level during H_2O_2 and NaCl treatments (lower panel) (IB, immunoblot; pHog1, phosphorylated Hog1). (B) Wild type (WDHY 1741) and *hog1*-Δ (WDHY 1838) cells were serially diluted and spotted onto YPD plates either containing no H_2O_2 or containing 4 mM H_2O_2 . Plates were incubated at 30 °C and photographed 3 days after spotting. (C) *SML1* (W303a), *sml1::HIS3* (WDHY 1741), and *mec1-kd sml1::HIS3* (WDHY 1742) cells were serially diluted and spotted onto YPD plates containing the indicated concentrations of H_2O_2 . Plates were photographed 3 days after spotting.

phosphorylation in a concentration-dependent manner from 0.5 to 7 mM H_2O_2 , but curiously not at 10 mM. Note that the Hog1 protein level is essentially the same at 10 mM H_2O_2 and 0.5 M NaCl treatments. Therefore lack of phosphorylated Hog1 at 10 mM H_2O_2 is not a consequence of diminished Hog1 protein levels. Extract from *hog1-Δ* cells was analyzed to demonstrate the specificity of the anti-Hog1 antibody (Fig. 1A, last lane, lower panel). Likewise, the specificity of the antibodies against the phosphorylated form of Hog1 is demonstrated by the strong signals in response to osmotic stress (NaCl, Fig. 1A second to last lane, upper and middle panel) and the extremely attenuated signal in the absence of Hog1 activation (Fig. 1A, lanes 1 upper and middle panel). In the experiment described here, we utilized two antibodies and observed a stronger signal with the anti-phospho-p38 antibody. Therefore, all the subsequent experiments were performed with this antibody only.

3.2. *hog1-Δ* cells are sensitive to H_2O_2

To confirm the previous observation that *hog1* mutants are sensitive to H_2O_2 [10], we constructed a *hog1-Δ* strain in our W303 strain background (see Section 2) and analyzed its phenotype. As shown in Fig. 1B, *hog1-Δ* cells are more sensitive to 4 mM H_2O_2 present in the growth media than wild type cells. Chronic exposure to 10 mM H_2O_2 concentration in plates did not allow growth of either wild type or *hog1-Δ* strains (data not shown). Since our strains contain an *sml1* mutation to suppress the lethality of the *mec1* null allele [21], we verified that the *sml1* mutation did not sensitize cells to H_2O_2 , which is confirmed by the data shown in Fig. 1C. Together the results from Fig. 1 show that the Hog1 MAPK pathway is activated in response to H_2O_2 treatment and survival in the presence of oxidative stress by H_2O_2 requires functional Hog1 kinase.

3.3. H_2O_2 -induced Hog1 activation is independent of Mec1 kinase

H_2O_2 is a potent activator of the DNA damage checkpoint response [14]. We decided to examine whether a functional DNA damage checkpoint response was necessary for the H_2O_2 -induced activation of Hog1. We utilized a kinase defective *mec1* strain (*mec1-kd*), which has two amino acid substitutions in the catalytic domain and is as checkpoint defective as a *MEC1* deletion strain [17]. As shown in Fig. 2, neither the NaCl- nor the H_2O_2 -induced activation of Hog1 require a functional Mec1 kinase. These results show that the Mec1 pathway is not required for activation of the HOG pathway.

3.4. H_2O_2 -induced Rad53 activation is independent of Hog1 kinase

Oxidative stress induced by H_2O_2 activates the DNA damage checkpoint pathway, as inferred by the electrophoretic mobility shift of Rad53 kinase (Fig. 3A). This result is con-

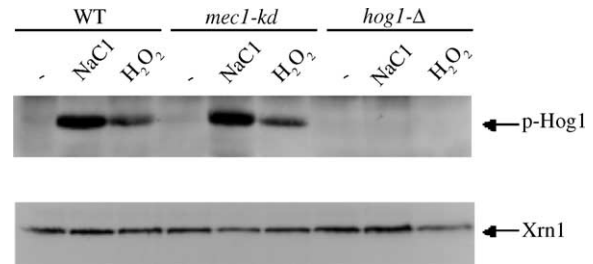


Fig. 2. Hog1 activity in response to H_2O_2 is independent of the DNA damage checkpoint pathway. Exponentially growing cultures of wild type (WDHY 1741), *mec1-kd* (WDHY 1742), and *hog1-Δ* (WDHY 1838) cells were left untreated, treated with 0.5 M NaCl, or 5 mM H_2O_2 for 10 min. Total protein extracts were analyzed by SDS-PAGE and blotting with anti-phospho-p38 antibody. The membrane was then re-blotting with anti-Xrn1 antibody as a loading control (pHog1, phosphorylated Hog1).

sistent with a previous study, which, in addition, demonstrated DNA damage checkpoint-dependent cell cycle arrest in response to H_2O_2 [14]. We wanted to determine if Hog1 was required for checkpoint activation in response to H_2O_2 . For this experiment we used 1 mM H_2O_2 for 30 min, as we observed a very sharp optimum around 0.5–1 mM (data not shown) for the activation of Rad53 kinase, consistent with previous studies [14]. Note that Hog1 is activated by 1 mM H_2O_2 (Fig. 1A). A 10-min treatment with 5 mM H_2O_2 , which does activate Hog1 kinase (Figs. 1 and 2), did not activate Rad53 kinase (data not shown). As shown in Fig. 3A, Rad53 kinase is equally activated in response to H_2O_2 in both wild type and *hog1-Δ* cells, suggesting that the H_2O_2 -induced checkpoint activation occurs independent of the HOG pathway. Expectedly, H_2O_2 -induced Rad53 phosphorylation is dependent on the Mec1 DNA damage checkpoint kinase, as no Rad53 phosphorylation was observed after H_2O_2 treatment in *mec1-kd* cells (Fig. 3A).

3.5. Checkpoint mutants are not sensitive to high salt

We were interested to see if Rad53 kinase is activated in response to 0.5 M NaCl treatment, a potent activator of the HOG pathway. Results from Fig. 3B show that Rad53 kinase is not activated in either wild type or *hog1-Δ* cells, treated with 0.5 M NaCl for 10 and 30 min. Note that Hog1 MAPK is activated under these conditions (Fig. 1 and [10]). Rad53 phosphorylation in NaCl-containing media was monitored up to 28 h and no activation was detected (data not shown). We also show in Fig. 3C that neither *mec1-kd* nor *rad53-Δ* cells are sensitive to high NaCl concentrations. These results indicate that 0.5 M NaCl treatment does not activate the checkpoint pathway and that the high salt sensitivity phenotype of *hog1-Δ* cells is independent of Mec1 kinase.

3.6. *hog1-Δ* checkpoint-deficient double mutant cells display an additive sensitivity to H_2O_2

To further examine the interdependency of the two stress signaling pathways, we compared the H_2O_2 sensitivity pro-

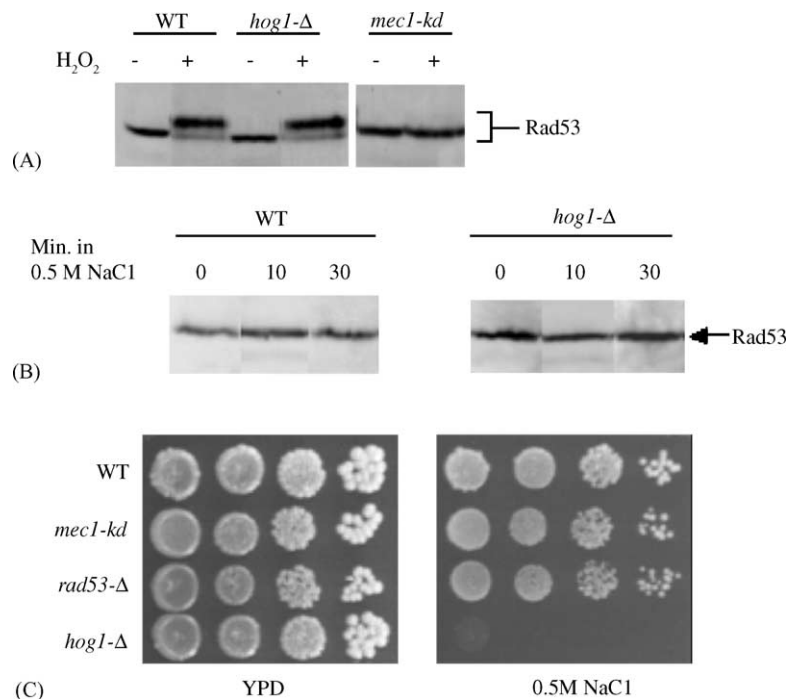


Fig. 3. Rad53 activation in response to H₂O₂ is independent of HOG pathway. (A) Logarithmically growing wild type (WDHY 1741), *hog1-Δ* (WDHY 1838), and *mec1-kd* (WDHY 1742) cells were left untreated or treated with 1 mM H₂O₂ for 30 min. Total protein extracts were analyzed by SDS-PAGE and blotted with anti-Rad53 antibody. (B) Logarithmically growing wild type (WDHY 1741) and *hog1-Δ* (WDHY 1838) cells were treated with 0.5 M NaCl. The Rad53 phosphorylation status was monitored as in (A). (C) Wild type (WDHY 1741), *mec1-kd* (WDHY 1742), *rad53-Δ* (WDHY 1901), and *hog1-Δ* (WDHY 1838) cultures were serially diluted and plated on YPD plates containing no NaCl or 0.5 M NaCl. The plates were photographed 3 days after spotting.

file of *mec1-kd*, *rad53-Δ*, *hog1-Δ* single mutants to the corresponding double mutants. We examined the survival of these cells in two ways: growth in the presence of H₂O₂ (chronic exposure, Fig. 4A), and during acute exposure (Fig. 4B). As evident from both panels of Fig. 4, the most sensitive single mutant is the *mec1-kd* strain. *hog1-Δ* cells are slightly more sensitive than *rad53-Δ* cells. The more pronounced sensitivity of *mec1* cells compared to *rad53* cells is consistent with the upstream role of Mec1 kinase in the DNA damage checkpoint pathway [1,2] and suggests that the DNA damage response pathways controls multiple effectors that contribute to the resistance against hydrogen peroxide.

Easily quantifiable phenotypes, like survival after H₂O₂ exposure, allow epistasis analysis, which permits conclusions about pathway organization. Epistasis between two mutations suggests that both gene products function in the same pathway. Epistasis is indicated by a double mutant phenotype that is identical to one of the single mutants, usually the more sensitive one. Additivity is found, when the double mutant displays a survival that is the product of the survival frequencies of the single mutants. In this case, both pathways do not compete for the same substrate, and one pathway cannot compensate for the absence of another pathway. Synergism is suggested by a double mutant phenotype that greatly exceeds additive sensitivity of the respective single mutant phenotypes. Synergism suggests that two pathways

can compete for the same substrate, and that the absence of one pathway can be compensated for by another pathway.

The double mutants, in which the HOG and the DNA damage checkpoint signaling pathways were disabled, exhibited perfect additivity in their H₂O₂ sensitivity compared to the respective single mutant strains. The predicted additive phenotype of *mec1-kd hog1-Δ* cells would be a survival of 1.3% at 30 min (product of the individual survivals). The observed survival for that strain is 3.6%, which, given the standard deviation, falls in the expected range for additivity. The same analysis also reveals additivity for the *rad53-Δ hog1-Δ* strain (predicted: 5.1%; found: 6.8%). The results from the chronic and the acute exposure are fully congruent. These results show that the Mec1 DNA damage checkpoint pathway and the HOG pathway are required for survival during the continued growth in the presence of oxidative stress (2–3 days) as well as during acute exposure (up to 30 min). These results suggest that the Mec1 DNA damage checkpoint pathway and the Hog1 MAPK pathway respond independently to oxidative stress.

4. Discussion

We have shown that the Hog1 MAPK is tyrosine-phosphorylated and hence activated in response to H₂O₂ treatment. Hog1 tyrosine phosphorylation appears to be very de-

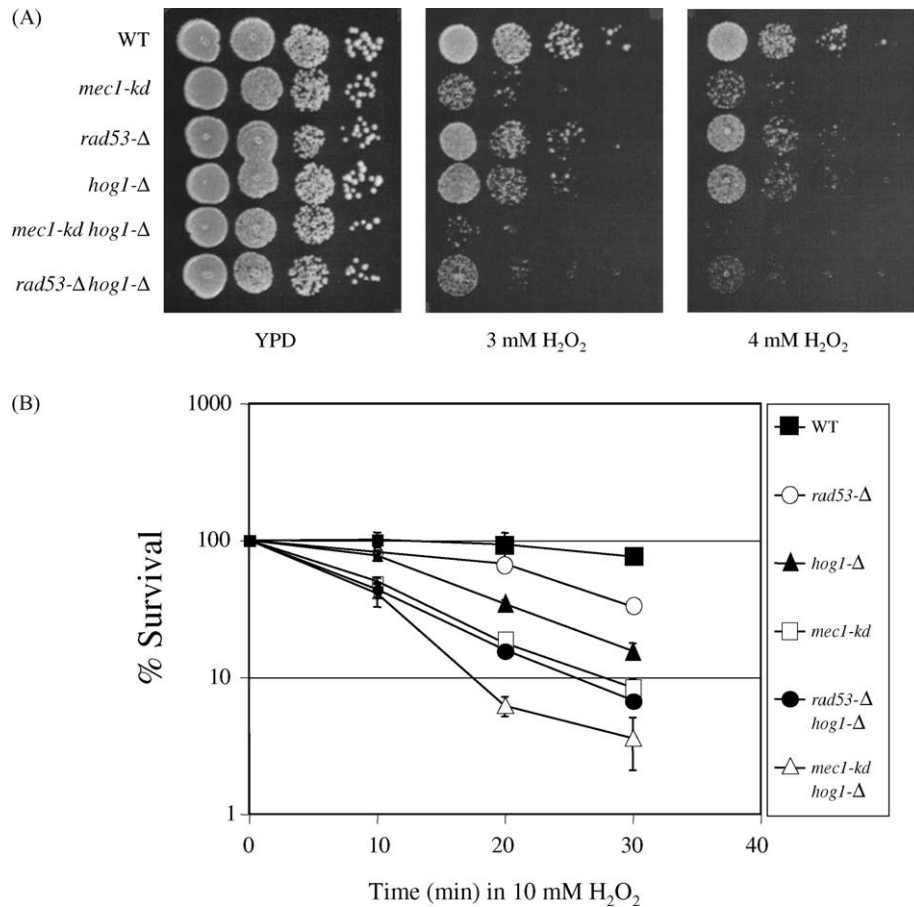


Fig. 4. *mec1-kd hog1-Δ* cells display additive sensitivity to H₂O₂. (A) Cultures of wild type (WDHY 1741), *mec1-kd* (WDHY 1742), *rad53-Δ* (WDHY1901), *hog1-Δ* (WDHY 1838), *mec1-kd hog1-Δ* (WDHY 1839), and *rad53-Δ hog1-Δ* (WDHY 1916) cells were serially diluted and spotted onto YPD plates containing the indicated concentrations of H₂O₂. Plates were incubated at 30 °C and photographed 3 days after spotting. (B) Logarithmically growing cultures of the strains described in (A) were incubated in the presence of 10 mM H₂O₂ and aliquots were removed at 10-min intervals, diluted, and plated on YPD plates to determine survival. The means of three different experiments are presented. The error bars indicate one standard deviation and where not visible errors are smaller than the symbol used.

pendent on the concentration of H₂O₂, starting at 0.5–1 mM. Maximum phosphorylation was observed at 5–7 mM, whereas after treatment with 10 mM H₂O₂ no Hog1 tyrosine phosphorylation could be detected. These results explain the previous failure to detect Hog1 tyrosine phosphorylation in response to H₂O₂, as in those studies either too low (0.4 mM [10]) or too high a concentration (10 mM [13]) of H₂O₂ was used. It is intriguing that there is no detectable Hog1 tyrosine phosphorylation with 10 mM H₂O₂. This failure is not a consequence of low Hog1 protein levels, as the same Hog1 level responded well to osmstress (Fig. 1A). Moreover, under the exact conditions (10 mM H₂O₂ for 10 min) that fail to elicit detectable Hog1 tyrosine phosphorylation (Fig. 1A), *hog1* mutant cells begin to show sensitivity to H₂O₂ exposure that is accentuated at longer exposure times (Fig. 4B). These data suggest that Hog1 is required also for survival at high H₂O₂ concentrations but the mechanism of its action under these conditions remains unclear. It is possible that under these conditions Hog1 acquires additional post-translational modifications that do not allow detection by the anti-

bodies used to visualize Hog1 activation through Tyr phosphorylation.

A similar observation was made with the DNA damage checkpoint pathway. Rad53 kinase is activated in cells exposed to 1 mM H₂O₂ but not to 5 mM H₂O₂ (Fig. 3A and data not shown). However, *rad53-Δ* cells are more sensitive to high doses of H₂O₂ than wild type cells (Fig. 4), even in the absence of detectable Rad53 phosphorylation. Thus, also the DNA damage checkpoint is required for survival at high H₂O₂ concentrations. While Rad53 auto-phosphorylation, evidenced by a pronounced electrophoretic mobility shift and indicative of activation, is readily detectable at low hydrogen peroxide concentration, high concentrations (>5 mM) do not elicit a detectable shift. The mechanism of how Rad53 and the DNA damage checkpoint contribute to survival under these conditions is unclear. It is possible that such high concentrations of hydrogen peroxide interfere with the cellular physiology that the checkpoint cascade no longer functions properly. It is interesting to note that the two pathways (HOG, DNA damage checkpoints) cease

to respond at different concentrations of H₂O₂, suggesting that both pathways have evolved to respond to different physiological situations.

A role for the HOG pathway in controlling the oxidative stress response has been proposed previously [13]. *hog1* cells are sensitive to H₂O₂ ([13]; this study). The two independent osmotic sensors, Sho1 and Sln1, are also required for the oxidative response, as the corresponding mutants show sensitivity to H₂O₂ [13]. Also mutants in the MAPKK of the HOG pathway, *PBS2*, which activates Hog1 in response to osmotic stress, exhibit H₂O₂ sensitivity [13]. Consistent with a biologically relevant role of Hog1 in oxidative stress response, is the Hog1-dependent transcriptional induction of *CTT1* and *TSA2* genes, which code for anti-oxidants [10,22]. Moreover, the Hog1-activated Sko1 transcription factor is required for the regulation of a set of genes implicated in protection from oxidative damage [23]. Our results, showing activating Hog1 phosphorylation in response to oxidative stress, suggest that oxidative stress by H₂O₂ is another input signal to activate the HOG MAPK pathway in addition to osmotic stress.

The DNA damage checkpoints specifically respond to DNA damage and replication stress. Oxidative damage leads to base modifications and DNA strand breaks that are either directly detected by checkpoint sensors or give rise to stalled replication forks that elicit checkpoint activation during S-phase [1]. In *S. cerevisiae*, this signal is nuclear-limited and does not diffuse to the cytoplasm [24]. The oxidative damage signal that activates the MAP kinase pathway is unknown. The activation of the Jun N-terminal kinase (JNK) and p38 stress kinase pathways in mammalian cells is nuclear-independent. Activation of the MAP kinase pathway in response to alkylating agents as well as to UV radiation is independent of the DNA damage induced by these agents and equally efficient in enucleated cells, demonstrating that the MAP kinase signal is cytoplasmic [25,26]. Consistent with these observations, we propose that in *S. cerevisiae* the HOG pathway responds to cytoplasmically-contained oxidative damage to proteins and/or lipids, whereas the Mec1 DNA damage checkpoint pathway is triggered by DNA damage signals initiating in the nucleus.

The functional differentiation between the HOG and DNA damage checkpoint pathways in response to stress is also apparent at the signaling output level. Hog1 regulates the cell cycle in response to osmotic stress, and presumably in response to oxidative stress, by modulating the enzymatic activity of the central p34^{Cdc28} kinase through controlling the inhibitory tyrosine phosphorylation of p34 [27]. The DNA damage checkpoint-mediated cell cycle arrest was found to be independent of Cdc28 tyrosine phosphorylation in *S. cerevisiae* [28]. This suggests that the two pathways respond not only to different input signals but also employ different effectors in their signaling output in budding yeast. The perfect additivity of the H₂O₂ sensitivity in double mutants disabling both pathways supports this conclusion.

In mammalian cells, several studies have shown an interdependency between the MAPK and the DNA damage checkpoint pathways. JNK is activated in response to ionizing radiation and this activation is defective in Ataxia Telangiectasia (A–T) cells, which contain a mutation in ATM, a central PIK-like kinase in the mammalian DNA damage checkpoints [15]. This checkpoint dependency was specific for ionizing radiation, as UV-induced activation of stress-activated protein kinase was fully intact in A–T cells. Furthermore, mutants in the JNK kinase pathway, unlike *hog1* mutants, are sensitive to both ionizing and UV radiation. This suggests an upstream role of the DNA damage checkpoint in activating JNK in response to ionizing radiation. Moreover, p38 MAPK, the mammalian Hog1 homolog, is required for the initiation of the G2/M arrest after UV exposure [16]. Inhibition of p38 eliminates the UV-induced cell cycle arrest in both human and mouse cells. Unlike its mammalian homolog p38, Hog1 kinase does not appear to have a role in mediating responses to ionizing and UV radiation. Hog1 is not phosphorylated when cells are treated with these agents, and *hog1* cells are no more sensitive to ionizing or UV radiation than wild type cells [13]. A possible explanation for this apparent discrepancy is that, unlike in higher eukaryotes, in budding yeast, the nuclear membrane does not break down during mitosis [29]. This deprives Hog1, which is localized in the cytoplasm in its non-activated state, of the possibility to be activated in response to a nuclear signal. Likewise, the DNA damage checkpoint proteins and the checkpoint signal are contained in the nucleus [24] and unavailable to function in activating the MAP kinase pathway.

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