J. Biochem. **139**, 797–803 (2006)
doi:10.1093/jb/mvj089

Activation of the HOG Pathway upon Cold Stress in *Saccharomyces cerevisiae*

Michio Hayashi and Tatsuya Maeda*
Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032

Received February 4, 2006; accepted March 3, 2006

When *Saccharomyces cerevisiae* cells are exposed to hyper-osmotic stress, the high-osmolarity glycerol response (HOG) pathway is activated to induce osmotic responses. The HOG pathway consists of two upstream osmosensing branches, the SLN1 and SHO1 branches, and a downstream MAP kinase cascade. Although the mechanisms by which these upstream branches transmit signals to the MAP kinase cascade are well understood, the mechanisms by which they sense and respond to osmotic changes are elusive. Here we show that the HOG pathway is activated in an SLN1 branch–dependent manner when cells are exposed to cold stress (0°C treatment). Dimethyl sulfoxide (DMSO) treatment, which rigidifies the cell membrane, also activates the HOG pathway in both SLN1 branch– and SHO1 branch–dependent manners. Moreover, cold stress, as well as hyper-osmotic stress, exhibits a synergistic effect with DMSO treatment on HOG pathway activation. On the other hand, ethanol treatment, which fluidizes the cell membrane, partially represses the cold stress–induced HOG pathway activation. Our results suggest that both osmosensing branches respond to the rigidification of the cell membrane to activate the HOG pathway.

Key words: cold-stress, HOG pathway, membrane fluidity, *Saccharomyces cerevisiae*, stress-activated MAP kinase pathway.

Abbreviations: HOG pathway, high-osmolarity glycerol response pathway; MAP kinase, mitogen-activated protein kinase; DMSO, dimethyl sulfoxide; HPt domain, histidine-containing phosphotransfer domain.

Cells show adaptive responses to various extracellular changes. In *Saccharomyces cerevisiae*, increases in extracellular osmolarity induce the activation of the high-osmolarity glycerol response (HOG) pathway, which in turn provokes osmotic responses (*1*, Fig. 1). The HOG pathway consists of two upstream osmosensing branches, SLN1 and SHO1 branches, and a downstream MAP kinase cascade. The SLN1 branch, consisting of Sln1p, Ypd1p, and Ssk1p, constitutes a multi-step His-Asp phosphorelay (*2–4*). Under normal osmotic conditions, the transmembrane protein Sln1p phosphorylates its own histidine kinase domain (*3, 4*). The phosphate group is transferred to the receiver domain of Sln1p, then to the HPt domain of Ypd1p, and finally to the receiver domain of Ssk1p (*3, 4*). Upon hyper-osmotic stress, Sln1p phosphorylation is repressed, and thus phosphotransfer to Ssk1p via Ypd1p is also inhibited. As a result, unphosphorylated Ssk1 activates Ssk2p and Ssk22p MAP kinase kinase kinases (MAPKKKs) to phosphorylate Pbs2p MAP kinase kinase (MAPKK) (*5, 6*). Phosphorylated Pbs2p phosphorylates Hog1p MAP kinase (MAPK) on threonine 174 and tyrosine 176 residues, the phosphorylation of both of which is required for Hog1p activity (*7, 8*). In the case of the SHO1 branch, Pbs2p MAPKK is recruited to the transmembrane anchor Sho1p upon hyper-osmotic stress (*5, 9*), and is activated by Ste11p MAPKKK, which also participates in the pheromone signal transduction pathway (*10*). Despite considerable information about the machinery by which these two osmosensing branches activate the downstream MAP kinase pathway, the actual mechanism by which they sense and respond to osmotic changes is not well understood. In particular, it is not clear whether hyper-osmolarity is sensed directly or indirectly through other physical parameters reflecting hyper-osmolarity, such as decreased turgor pressure, which also activates the HOG pathway through the SLN1 branch (*11*). Other stimuli that activate the HOG pathway are also known: heat stress activates the pathway through the SHO1 branch (*12*) and some oxidative stress does so through both the SLN1 and SHO1 branches (*13*), although increased Hog1p phosphorylation is not detected in the latter case. The homologous pathway in *Schizosaccharomyces pombe* is also activated by various stresses, including hyper-osmotic, heat, oxidative, UV, starvation, and cold stresses (*14–19*).

The cell membrane largely consists of a lipid bilayer. Membrane fluidity is determined by free movement of the acyl group of membrane phospholipids, lateral diffusion of phospholipids, wobbling of phospholipids, and the interaction among various domains (rafts) of the cell membrane. Various factors influence membrane fluidity. For example, increases in temperature cause increases in membrane fluidity (*20*), and decreases in temperature cause decreases in membrane fluidity in various organisms including yeast (*21–24*). Dimethyl sulfoxide (DMSO) added *in vitro* causes decreases in fluidity in an artificial membrane (*25*), as well as the cellular membrane

*To whom correspondence should be addressed. Tel: +81-3-5841-7820, Fax: +81-3-5841-7899, E-mail: maeda@iam.u-tokyo.ac.jp

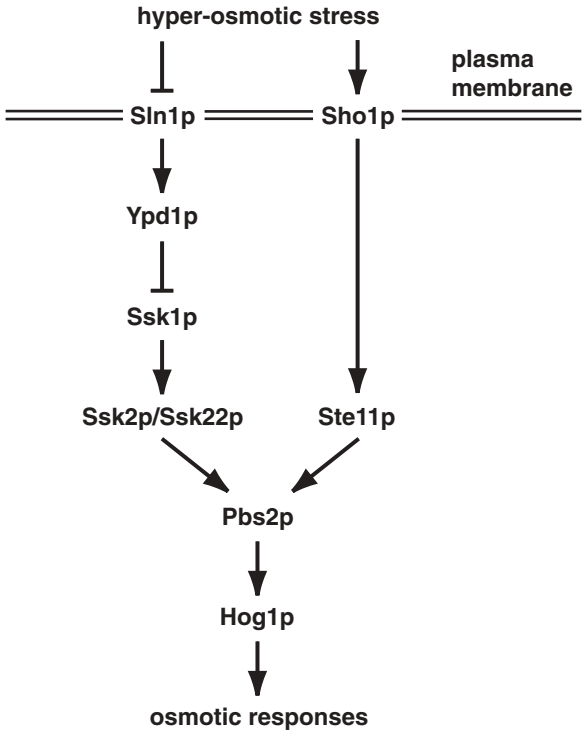

Fig. 1. **High-osmolarity glycerol response (HOG) pathway.**

(*26, 27*). Conversely, ethanol added *in vitro* causes increased fluidity of the cellular membrane (*28–30*).

A number of reports have shown that membrane fluidity functions in sensing temperature. For example, low temperature or cold stress is proposed to be sensed as decreases in membrane fluidity in cyanobacteria (*31*), in *Bacillus subtilis* (*32*), and in plants (*27*). Membrane fluidity altered by temperature or a membrane fluidizer, benzyl alcohol, also affects the induction temperature of heat shock genes in cyanobacteria (*24*). Similarly, in yeast, alcohols that increase membrane fluidity lower the maximum induction temperature of heat shock–induced genes (*33*), and an increase of unsaturated fatty acid induced by heat acclimation increases the induction temperature of heat shock–induced genes (*34*). Membrane fluidity also affects osmotic responses. It was reported that membrane fluidity is reduced with hyper-osmotic stress (*35–37*). In fact, hyper-osmotic stress-specific genes are induced by mutations that change the phospholipid composition of the cellular membrane in *Escherichia coli* (*38*). Furthermore, the Slt2p/Mpk1p pathway in yeast, or the cell integrity MAPK pathway, is activated by decreases in extracellular osmolarity (*39*), up-shift of temperature (*40, 41*), and chlorpromazine treatment, all of which induce membrane fluidization (*40*). Interestingly, a cold sensor in cyanobacterium *Synechocystis*, the Hik33 histidine kinase, also acts as a sensor for hyper-osmotic stress, raising the possibility that Hik33 senses decreases in membrane fluidity as a cue for hyper-osmotic stress, in addition to cold (*42*).

In this study, we found that the HOG pathway is activated by cold stress. Moreover, DMSO treatment also caused HOG pathway activation. Conversely, ethanol treatment partially repressed HOG pathway activation. These results suggest that the fluidity of the plasma membrane is sensed by upstream branches of the HOG pathway.

MATERIALS AND METHODS

Yeast Strains and Media—All *S. cerevisiae* strains used in this study are isogenic to TM101 (*MAT*α *ura3-52 leu2Δ1 his3Δ200*), a *GAL2*$^+$ derivative of S288C (*3, 5, 43*). Their relevant genotypes are as follows: TM101, wild-type; TM193, *ssk1::URA3*; TM231, *pbs2::URA3*; TM253, *ssk2::LEU2 ssk22::LEU2*; and TM282, *MAT***a** *trp1Δ63 sho1::URA3*. YPD medium contains 1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) glucose.

Stress Treatments—For cold stress, cells were grown to the logarithmic phase at 30°C in YPD medium, then 2-ml aliquots of culture were transferred to 2-ml sampling tubes and incubated at 30, 10, or 0°C. DMSO and ethanol were obtained from Wako Pure Chemical. For DMSO treatment, DMSO was added to the cell culture at a concentration of 1, 3, or 10% (v/v) and cultures were incubated at 30°C. For ethanol treatment, ethanol was added to the cell culture at a concentration of 0.3, 1, or 3% (v/v) and cultures were incubated for another 1 h at 30°C. Cells treated with or without 0.5 M sorbitol stimulation for 5 min at 30°C were used for control samples.

Western Blotting—Cells were harvested by spin-down for 20 s, then suspended in sample buffer (20 μl each) and immediately boiled for 4 min. After centrifugation for 4 min, aliquots of cell lysate corresponding to 0.5 A_{600} equivalents of cells were subjected to immunoblotting. Phospho-Hog1p was detected with anti–phospho-p38 antibody (Cell Signaling Technology) and anti-rabbit antibody labeled with peroxidase (Amersham Biosciences). Hog1p was detected with anti-Hog1p polyclonal antibody yC-20 (Santa Cruz Biotechnology) and anti-goat antibody labeled with peroxidase (Santa Cruz Biotechnology).

RESULTS

Activation of the HOG Pathway upon Cold Stress—Using immunoblot analysis, we found that phosphorylation occurred when exponentially growing culture at 30°C was transferred to an ice-water bath (0°C) (Fig. 2A, top). Phosphorylation was not observed in cells kept at 30°C or transferred to 10°C (Fig. 2A, top). Upon transferring cells to 4°C, weaker Hog1p phosphorylation than at 0°C was observed (data not shown). An immunoblot of the same cell extracts with an anti-Hog1p antibody revealed that an equal amount of Hog1p was loaded on each lane (Fig. 2A, bottom). These results indicate that the HOG pathway is activated in response to decreased extracellular temperature besides increased extracellular osmolarity or heat shock. Compared with sorbitol stimulation, phosphorylation at 0°C was weaker and slower in kinetics. It should be noted that decreased temperature itself is not sufficient, but that a certain magnitude of temperature decrease is required for Hog1p phosphorylation. The temperature shift from 30°C to 0°C is referred to as cold stress hereafter.

The HOG pathway has two upstream sensing branches, the SLN1 and SHO1 branches (Fig. 1). We examined whether the phosphorylation of Hog1p upon cold stress is SLN1 branch- or SHO1 branch-dependent. As shown

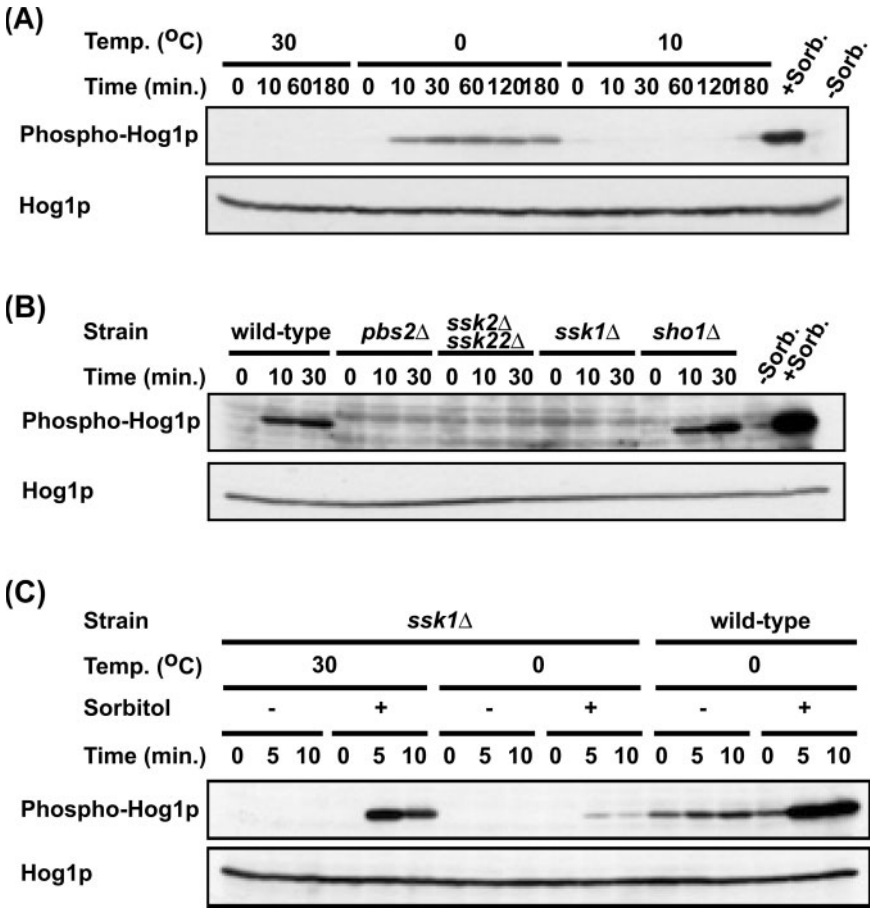

Fig. 2. **The HOG pathway is activated upon cold stress.** (A) Phosphorylation of Hog1p upon cold stress. Wild-type TM101 cells were grown to the logarithmic phase (A_{600} = 0.5–0.9) at 30°C in YPD medium, then aliquots of cell culture were transferred to 30, 0, or 10°C. At the indicated times, cell lysates were prepared for immunoblotting. Phospho-Hog1p and Hog1p were detected using appropriate antibodies. Cells stimulated with or without 0.5 M sorbitol (+ Sorb. and – Sorb., respectively) were used as a control. (B) Hog1p is phosphorylated through the SLN1 branch upon cold stress. TM101 (wild-type), TM231 (*pbs2Δ*), TM253 (*ssk2Δ ssk22Δ*), TM193 (*ssk1Δ*), and TM282 (*sho1Δ*) cells were used. Hog1p phosphorylation was examined as in (A). (C) The SHO1 branch hardly functions at 0°C. TM193 (*ssk1Δ*) and TM101 (wild-type) cells were grown to the logarithmic phase (A_{600} = 0.5) at 30°C in YPD medium. Aliquots of cell culture were transferred to 30 or 0°C and incubated for 5 min, then distilled water or 5 M sorbitol was added to give a sorbitol concentration of 0 or 0.5 M, respectively. At the indicated times, cell lysates were prepared for immunoblotting.

in Fig. 2B, Hog1p phosphorylation upon cold stress was observed in wild-type and *sho1Δ* cells, but not observed in *pbs2Δ*, *ssk2Δ ssk22Δ*, or *ssk1Δ* cells. This result indicates that the phosphorylation of Hog1p upon cold stress depends only on the SLN1 branch.

Activation of the HOG Pathway by DMSO Treatment—Decreases in extracellular temperature lead to decreases in membrane fluidity. We hypothesized that the SLN1 branch senses the rigidification of the plasma membrane. To test this hypothesis, we examined Hog1p phosphorylation upon membrane rigidification. DMSO rigidifies an artificial membrane (*44*) and was used as a membrane rigidifier in a rat synaptic membrane (*26*) and plant alfalfa cells (*27*). On adding 10% (v/v) DMSO to the cell culture, Hog1p was phosphorylated (Fig. 3A): however, under this condition, cell growth was also hampered. To minimize the toxic effect of DMSO treatment we employed a milder condition of 3% DMSO, under which the growth curve of cells was not perturbed (data not shown). Hog1p was not phosphorylated on adding 3% DMSO or shifting to 10°C, whereas it was observed on adding 3% DMSO in combination with shifting to 10°C. These results indicate that DMSO treatment activates the HOG pathway, and that cold stress and DMSO treatment have synergistic effects on the phosphorylation of Hog1p.

We monitored the time course of Hog1p phosphorylation after 10% DMSO was added. At 5 min after the addition of DMSO, Hog1p phosphorylation was already observed (Fig. 3B). A similar time course was observed for phosphorylation induced by adding 3% DMSO in combination with shifting to 10°C (data not shown).

SLN1 branch– or SHO1 branch–dependency of Hog1p phosphorylation induced by 10% DMSO was examined. As shown in Fig. 3C, Hog1p phosphorylation was observed in all cells examined except *pbs2Δ* cells. In detail, *sho1Δ* cells showed a somewhat stronger and more persistent signal than *ssk2Δ ssk22Δ* or *ssk1Δ* cells. These results indicate that Hog1p phosphorylation induced by 10% DMSO treatment depends more on the SLN1 branch than on the SHO1 branch. A similar result was obtained for phosphorylation on adding 3% DMSO in combination with shifting to 10°C (data not shown).

Effects of DMSO Addition on Hyper-Osmotic Stress–Induced Hog1p Phosphorylation—To determine whether membrane rigidification affects the hyper-osmotic stress sensing of the pathway, we examined the effects of DMSO treatment on Hog1p phosphorylation induced by hyper-osmotic stress. Phosphorylation of Hog1p was observed following 3% DMSO treatment in combination with 0.5 M (and to a lesser extent, 0.2 M) sorbitol stimulation, whereas it was not induced by either treatment alone as shown in Fig. 3D. This result indicates that DMSO treatment and hyper-osmotic stress have synergistic effects on Hog1p phosphorylation.

The SHO1 Branch Is Defective at Low Temperature—In response to DMSO treatment, Hog1p phosphorylation is

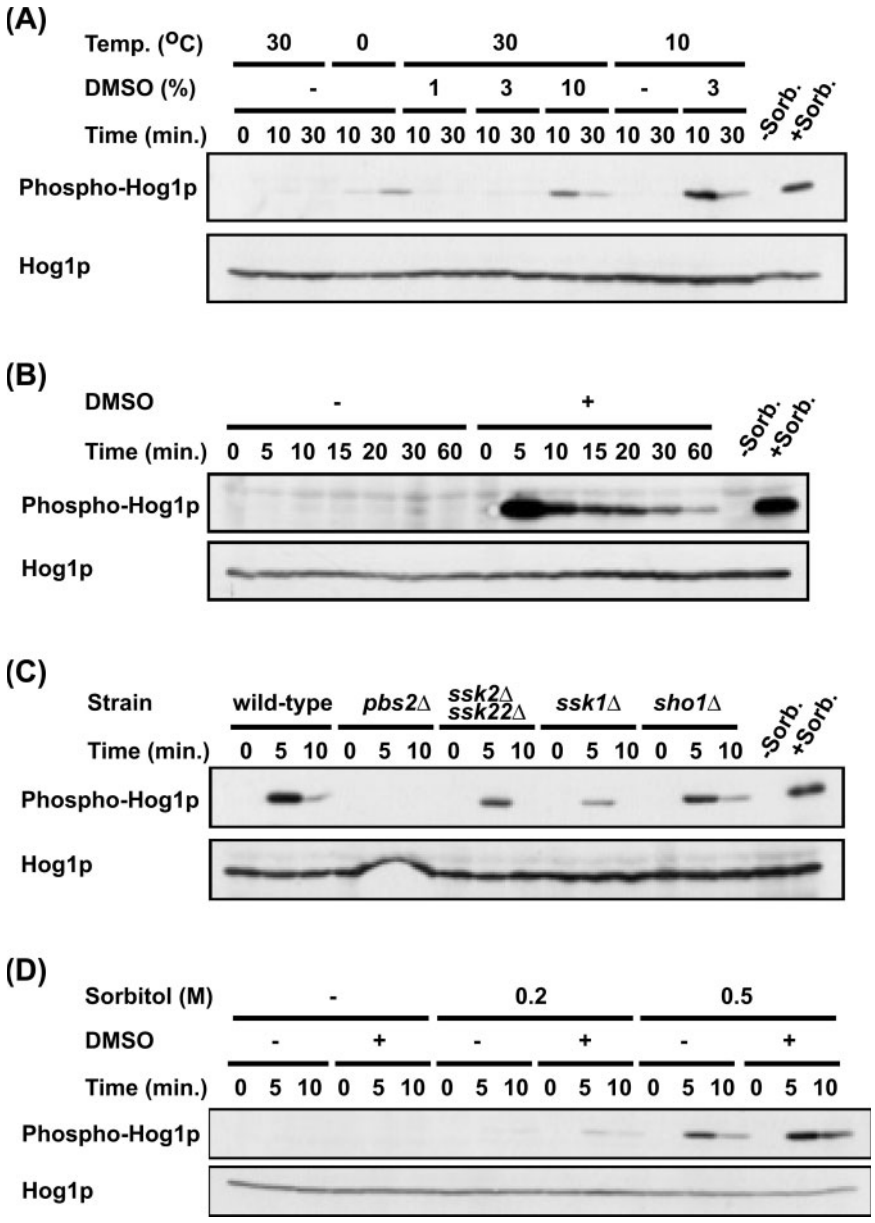

Fig. 3. **Phosphorylation of Hog1p by DMSO treatment.** (A) Phosphorylation of Hog1p by the addition of DMSO. Wild-type TM101 cells were grown to the logarithmic phase (A_{600} = 0.5–0.7) at 30°C in YPD medium. Aliquots of cell culture were treated by addition of DMSO to a concentration of 0, 1, 3, or 10% (v/v) (0, 0.141, 0.423, or 1.41 mM, respectively), then incubated at 30, 10, or 0°C. At the indicated times, cell lysates were prepared for immunoblotting. (B) Time course of Hog1p phosphorylation after the addition of DMSO. Wild-type TM101 cells were grown to the logarithmic phase (A_{600} = 0.8–1.0) at 30°C in YPD medium. Aliquots of cell culture were treated by addition of distilled water or DMSO to give a DMSO concentration of 0 or 10% (v/v), then incubated at 30°C. At the indicated times, cell lysates were prepared for immunoblotting. (C) Hog1p is phosphorylated through both the SLN1 and SHO1 branches by the addition of DMSO. TM101 (wild-type), TM231 (*pbs2Δ*), TM253 (*ssk2Δ ssk22Δ*), TM193 (*ssk1Δ*), and TM282 (*sho1Δ*) cells were used. Hog1p phosphorylation was examined as in (B). (D) Hyper-osmotic stress and the DMSO treatment have a synergistic effect on Hog1p phosphorylation. Wild-type TM101 cells were grown to the logarithmic phase (A_{600} = 0.8) at 30°C in YPD medium. Aliquots of cell culture were treated by addition of distilled water, DMSO (at 3%), and/or sorbitol (at indicated concentrations) in the combinations indicated, then incubated at 30°C. At the indicated times, cell lysates were prepared for immunoblotting.

observed in both SLN1 branch– and SHO1 branch–dependent manners, as in Hog1p phosphorylation induced by hyper-osmotic stress. However, upon cold stress, only the SLN1 branch was responsible for Hog1p phosphorylation, for which there are two possible explanations: the SHO1 branch is not able to sense the membrane rigidification induced by cold stress, or the SHO1 branch is not functional at low temperature irrespectively of the rigidity of the membrane. To test the latter possibility, Hog1p phosphorylation in response to hyper-osmotic stress was examined in *ssk1Δ* cells at 0°C. In *ssk1Δ* cells, Hog1p phosphorylation upon sorbitol stimulation was observed at 30°C, but was drastically decreased at 0°C (Fig. 2C). Similar results were obtained in experiments using *ssk2Δ ssk22Δ* cells (data not shown). These results indicate that the SHO1 branch is hardly functional at low temperature, even for sensing hyper-osmotic stress.

Repression of HOG Pathway Activation by Ethanol Treatment—As shown above, Hog1p was phosphorylated upon cold stress or by DMSO treatment, both of which cause membrane rigidification. It can therefore be asked whether membrane fluidization represses Hog1p phosphorylation. Ethanol affects the plasma membrane in yeast (*45*), and also increases membrane fluidity in the rat synaptic membrane (*28*) and in yeast (*29, 30*). Adding 3% (v/v) ethanol alone to the cell culture did not cause Hog1p phosphorylation for at least 1 h (data not shown). Hog1p phosphorylation induced by cold stress was partially repressed by pretreatment with 3% ethanol for 1 h (Fig. 4A), and this effect was dose-dependent. Similar results were obtained with 3% ethanol treatment for 15 or 30 min instead of 60 min (data not shown). These results suggest that Hog1p phosphorylation in response to cold stress was repressed by fluidization of the cell membrane

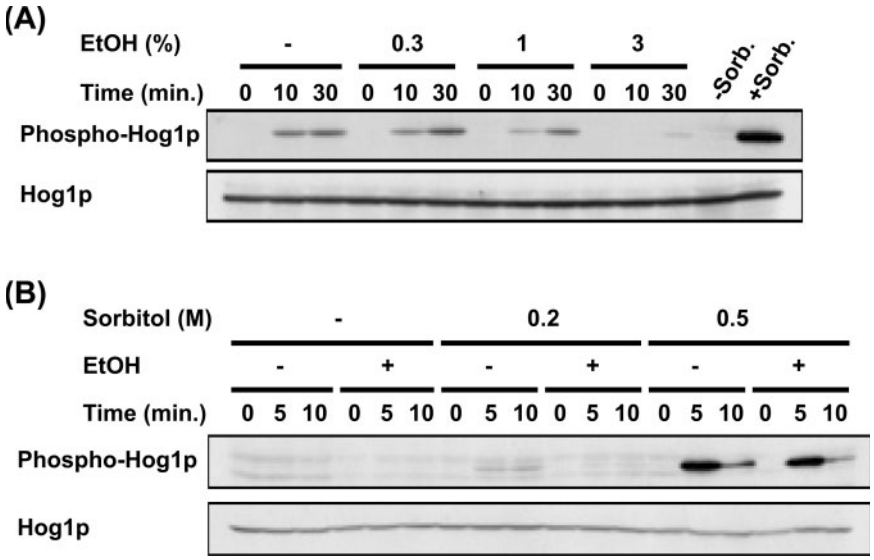

Fig. 4. **Repression of Hog1p phosphorylation by ethanol treatment.** (A) Ethanol treatment represses Hog1p phosphorylation induced by cold stress. Wild-type TM101 cells were grown to the logarithmic phase (A_{600} = 0.6) at 30°C in YPD medium. Aliquots of cell culture were pretreated with ethanol at a concentration of 0, 0.3, 1, or 3% (v/v) (0, 51, 171, or 514 mM, respectively) for 1 h at 30°C, then transferred to 0°C and incubated further. At the indicated times, cell lysates were prepared for immunoblotting. (B) Ethanol treatment represses Hog1p phosphorylation induced by weak hyper-osmotic stress. Wild-type TM101 cells were grown to the logarithmic phase (A_{600} = 0.4) at 30°C in YPD medium. Aliquots of cell culture were pretreated with ethanol at a concentration of 0 or 3% (v/v) for 1 h at 30°C, then distilled water or sorbitol was added to each cell culture to give a sorbitol concentration of 0, 0.2, or 0.5 M. At the indicated times, cell lysates were prepared for immunoblotting.

induced by ethanol treatment. Treatment with 3% ethanol also partially repressed Hog1p phosphorylation induced by hyper-osmotic stress (Fig. 4B).

DISCUSSION

In this study, we have shown that the HOG pathway, which is the osmosensing MAP kinase pathway in *S. cerevisiae*, is activated upon cold stress, *i.e.*, a rapid and substantial decrease in temperature. The HOG pathway is known to be activated by several stimuli, including extracellular hyper-osmolarity, heat stress, oxidative stress, and changes in turgor pressure, and now cold stress can be added to the list (*7, 11–13*). Low temperature induces decreases in membrane fluidity (*21–24*). DMSO treatment [10% (v/v)], which also rigidifies the membrane, also activates the HOG pathway. Moreover, a combination of mild cold stress (10°C) and mild DMSO treatment [3% (v/v)] activates the HOG pathway, whereas these treatments alone do not. On the other hand, ethanol treatment, which induces membrane fluidization, represses cold stress–induced HOG pathway activation. These results suggest that cold stress induces activation of the HOG pathway through rigidification of the cell membrane. The yeast Slt2p/Mpk1p MAPK pathway, or the cell integrity pathway, is activated by decreases in extracellular osmolarity (*39*), up-shifts of temperature (*40, 41*) and chlorpromazine treatment for membrane stretching (*40*). All these stress conditions increase membrane fluidity. Therefore, the HOG pathway and the Slt2p/Mpk1p pathway may sense opposite changes in cell membrane fluidity.

Each stimulus that activates the HOG pathway may do so through a mechanism specific to the stimulus. Alternatively, there may be a common consequence of some of these stimuli that is sensed by a common sensing mechanism. Consistent with the latter possibility, it was proposed that hyper-osmotic stress activates the HOG pathway partly through decreased turgor pressure (*11*). Hyper-osmotic stress, as well as low temperature, reduces membrane fluidity (*35–37*). It therefore is possible that both hyper-osmotic and cold stresses activate the HOG pathway through decreased membrane fluidity. In fact, mild hyper-osmotic stress (0.2 M) in combination with mild DMSO treatment also activated the HOG pathway (Fig. 3D). Consistently, the cyanobacteria sensor Hik33 is also activated by both hyper-osmotic stress and low temperature, and is proposed to do so through sensing decreases in membrane fluidity as the primary signal (*42*). More studies are necessary to confirm this hypothesis, however.

Decreases in temperature induce the rigidification of any cell membrane. However, the localization of Sln1p and Sho1p at the plasma membrane suggests that it is fluidity of the plasma membrane that affects HOG pathway activation (*9, 11*). A synergistic effect between cold stress and exogenously applied DMSO, as well as an antagonistic effect between cold stress and exogenously applied ethanol, is consistent with this view.

Different stimuli show different dependences on two upstream sensing branches. Hyper-osmolarity activates the HOG pathway by both SLN1 branch– and SHO1 branch–dependent mechanisms (*5*), heat stress by the SHO1 branch–dependent mechanism (*12*), oxidative stress by both SLN1 branch– and SHO1 branch–dependent mechanisms (*13*), and changes in turgor pressure by the SLN1 branch–dependent mechanism (*11*). Although both cold stress and DMSO treatment induce membrane rigidification, they show different branch dependences: cold stress activates the HOG pathway only by the SLN1 branch–dependent mechanism (Fig. 2B), but DMSO treatment does so by both SLN1 branch– and SHO1 branch–dependent mechanisms (Fig. 3C). This discrepancy, however, is most likely because the SHO1 branch is hardly functional at 0°C, not even in response to hyper-osmolarity (Fig. 2C). Furthermore, mild cold stress in combination with mild DMSO treatment activates the HOG pathway through both mechanisms. These results suggest that

the HOG pathway is activated upon decreases in membrane fluidity through both the SLN1 and the SHO1 branches, but the latter is defective at 0°C.

Although any decrease in temperature leads to some rigidification of the membrane, it appeared that a certain amount of decrease is required for activation of the HOG pathway. The phosphorylation of Hog1p is observed when cells are shifted from 30°C to 0°C (Fig. 2A), but is not when they are shifted to 10°C. Shifting from 30°C to 4°C induces more phosphorylation of Hog1p than to 10°C but less than to 0°C (data not shown). This may be due to phase transition rather than mere rigidification of the membrane being required to activate the HOG pathway. In line with this suggestion, phase transition of the plasma membrane in *Aspergillus nidulans* occurs upon shifting from 28°C to 5°C, while shifting from 30°C to 10°C only causes changes in membrane fluidity (*31*).

The requirement of a gross temperature decrease for activation of the HOG pathway raises the possibility that this activation may not be a physiological response. In fact, we were not able to find a significant difference in growth or survival rates after cold stress between wild-type and *hog1Δ* strains (data not shown). In contrast, the homologous pathway in *S. pombe* is activated by milder and more physiologically relevant temperature change, from 28°C to 15°C (*19*). Further investigation is required to appraise the physiological significance of the activation of the HOG pathway upon cold stress.

During the last stages of preparing this manuscript, a report describing cold stress–induced activation of the HOG pathway was published online in JBC Papers in Press (*46*). The observations described, however, differ from ours at several points. Most noticeably, DMSO treatment was reported to selectively stimulate the SLN1 branch, whereas we found that it stimulates both the SLN1 and SHO1 branches. The reason for the discrepancy is not currently clear, although the different DMSO concentrations used might be a cause. Based on their observations, the authors of the report hypothesize that change in membrane fluidity specifically activates the SLN1 branch, and that the specificity accounts for the SLN1 branch–dependence of the cold stress–induced HOG pathway activation. In contrast, we propose that the SLN1 branch–dependence is caused because the SHO1 branch is hardly functional at low temperature, even though change in membrane fluidity can activate both branches.

We thank Dr. Fred Winston for yeast strains. Thanks are also due to all the members of the Maeda laboratory for help, advice, and discussion. This work was supported in part by a Grant-in-Aid for Exploratory Research (KAKENHI 15657032) from the Japan Society for the Promotion of Science (JSPS) and a Grant-in-Aid for Scientific Research on Priority Areas (KAKENHI 14086203) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) (both to T. M.).

REFERENCES

1. Gustin, M.C., Albertyn, J., Alexander, M., and Davenport, K. (1998) MAP kinase pathways in the yeast *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **62**, 1264–1300
2. Ota, I.M. and Varshavsky, A. (1993) A yeast protein similar to bacterial two-component regulators. *Science* **262**, 566–569
3. Maeda, T., Wurgler-Murphy, S.M., and Saito, H. (1994) A two-component system that regulates an osmosensing MAP kinase cascade in yeast. *Nature* **369**, 242–245
4. Posas, F., Wurgler-Murphy, S.M., Maeda, T., Witten, E.A., Thai, T.C., and Saito, H. (1996) Yeast HOG1 MAP kinase cascade is regulated by a multistep phosphorelay mechanism in the SLN1-YPD1-SSK1 "two-component" osmosensor. *Cell* **86**, 865–875
5. Maeda, T., Takekawa, M., and Saito, H. (1995) Activation of yeast PBS2 MAPKK by MAPKKKs or by binding of an SH3-containing osmosensor. *Science* **269**, 554–558
6. Posas, F. and Saito, H. (1998) Activation of the yeast SSK2 MAP kinase kinase kinase by the SSK1 two-component response regulator. *EMBO J.* **17**, 1385–1394
7. Brewster, J.L., de Valoir, T., Dwyer, N.D., Winter, E., and Gustin, M.C. (1993) An osmosensing signal transduction pathway in yeast. *Science* **259**, 1760–1763
8. Schüller, C., Brewster, J.L., Alexander, M.R., Gustin, M.C., and Ruis, H. (1994) The HOG pathway controls osmotic regulation of transcription via the stress response element (STRE) of the *Saccharomyces cerevisiae CTT1* gene. *EMBO J.* **13**, 4382–4389
9. Reiser, V., Salah, S.M., and Ammerer, G. (2000) Polarized localization of yeast Pbs2 depends on osmostress, the membrane protein Sho1 and Cdc42. *Nat. Cell Biol.* **2**, 620–627
10. Posas, F. and Saito, H. (1997) Osmotic activation of the HOG MAPK pathway via Ste11p MAPKKK: scaffold role of Pbs2p MAPKK. *Science* **276**, 1702–1705
11. Reiser, V., Raitt, D.C., and Saito, H. (2003) Yeast osmosensor Sln1 and plant cytokinin receptor Cre1 respond to changes in turgor pressure. *J. Cell Biol.* **161**, 1035–1040
12. Winkler, A., Arkind, C., Mattison, C.P., Burkholder, A., Knoche, K., and Ota, I. (2002) Heat stress activates the yeast high-osmolarity glycerol mitogen-activated protein kinase pathway, and protein tyrosine phosphatases are essential under heat stress. *Eukaryot. Cell* **1**, 163–173
13. Singh, K.K. (2000) The *Saccharomyces cerevisiae* Sln1p-Ssk1p two-component system mediates response to oxidative stress and in an oxidant-specific fashion. *Free Radic. Biol. Med.* **29**, 1043–1050
14. 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
15. Shiozaki, K. and Russell, P. (1995) Counteractive roles of protein phosphatase 2C (PP2C) and a MAP kinase kinase homolog in the osmoregulation of fission yeast. *EMBO J.* **14**, 492–502
16. Kato, T., Okazaki, K., Murakami, H., Stettler, S., Fantes, P.A., and Okayama, H. (1996) Stress signal, mediated by a Hog1-like MAP kinase, controls sexual development in fission yeast. *FEBS Lett.* **378**, 207–212
17. 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
18. Shieh, J.C., Wilkinson, M.G., Buck, V., Morgan, B.A., Makino, K., and Millar, J.B. (1997) The Mcs4 response regulator coordinately controls the stress-activated Wak1-Wis1-Sty1 MAP kinase pathway and fission yeast cell cycle. *Genes Dev.* **11**, 1008–1022
19. Soto, T., Beltrán, F.F., Paredes, V., Madrid, M., Millar, J.B., Vicente-Soler, J., Cansado, J., and Gacto, M. (2002) Cold induces stress-activated protein kinase-mediated response in the fission yeast *Schizosaccharomyces pombe*. *Eur. J. Biochem.* **269**, 5056–5065

20. Mejía, R., Gómez-Eichelmann, M.C., and Fernández, M.S. (1995) Membrane fluidity of *Escherichia coli* during heat-shock. *Biochim. Biophys. Acta* **1239**, 195–200
21. Henry, S.A., and Keith, A.D. (1971) Membrane properties of saturated fatty acid mutants of yeast revealed by spin labels. *Chem. Phys. Lipids* **7**, 245–265
22. Alonso, A., Queiroz, C.S., and Magalhães, A.C. (1997) Chilling stress leads to increased cell membrane rigidity in roots of coffee (*Coffea arabica* L.) seedlings. *Biochim. Biophys. Acta* **1323**, 75–84
23. Swan, T.M., and Watson, K. (1997) Membrane fatty acid composition and membrane fluidity as parameters of stress tolerance in yeast. *Can. J. Microbiol.* **43**, 70–77
24. Horváth, I., Glatz, A., Varvasovszki, V., Török, Z., Páli, T., Balogh, G., Kovács, E., Nádasdi, L., Benkö, S., Joó, F., and Vígh, L. (1998) Membrane physical state controls the signaling mechanism of the heat shock response in *Synechocystis* PCC 6803: identification of *hsp17* as a "fluidity gene". *Proc. Natl. Acad. Sci. USA* **95**, 3513–3518
25. Lyman, G.H. and Preisler, H.D. (1976) Membrane action of DMSO and other chemical inducers of Friend leukaemic cell differentation. *Nature* **262**, 360–363
26. Mrak, R.E. (1992) Opposite effects of dimethyl sulfoxide and ethanol on synaptic membrane fluidity. *Alcohol* **9**, 513–517
27. Örvar, B.L., Sangwan, V., Omann, F., and Dhindsa, R.S. (2000) Early steps in cold sensing by plant cells: the role of actin cytoskeleton and membrane fluidity. *Plant J.* **23**, 785–794
28. Chin, J.H. and Goldstein, D.B. (1977) Effects of low concentrations of ethanol on the fluidity of spin-labeled erythrocyte and brain membranes. *Mol. Pharmacol.* **13**, 435–441
29. Lloyd, D., Morrell, S., Carlsen, H.N., Degn, H., James, P.E., and Rowlands, C.C. (1993) Effects of growth with ethanol on fermentation and membrane fluidity of *Saccharomyces cerevisiae*. *Yeast* **9**, 825–833
30. Alexandre, H., Berlot, J.P., and Charpentier, C. (1994) Effect of ethanol on membrane fluidity of protoplasts from *Saccharomyces cerevisiae* and *Kloeckera apiculata* grown with or without ethanol, measured by fluorescence anisotropy. *Biotechnol. Tech.* **8**, 295–300
31. Murata, N. and Los, D.A. (1997) Membrane fluidity and temperature perception. *Plant Physiol.* **115**, 875–879
32. Aguilar, P.S., Hernandez-Arriaga, A.M., Cybulski, L.E., Erazo, A.C., and de Mendoza, D. (2001) Molecular basis of thermosensing: a two-component signal transduction thermometer in *Bacillus subtilis*. *EMBO J.* **20**, 1681–1691
33. Curran, B.P.G. and Khalawan, S.A. (1994) Alcohols lower the threshold temperature for the maximal activation of a heat shock expression vector in the yeast *Saccharomyces cerevisiae*. *Microbiology* **140**, 2225–2228
34. Chatterjee, M.T., Khalawan, S.A., and Curran, B.P.G. (1997) Alterations in cellular lipids may be responsible for the transient nature of the yeast heat shock response. *Microbiology* **143**, 3063–3068
35. Yamazaki, M., Ohnishi, S., and Ito, T. (1989) Osmoelastic coupling in biological structures: decrease in membrane fluidity and osmophobic association of phospholipid vesicles in response to osmotic stress. *Biochemistry* **28**, 3710–3715
36. Laroche, C., Beney, L., Marechal, P.A., and Gervais, P. (2001) The effect of osmotic pressure on the membrane fluidity of *Saccharomyces cerevisiae* at different physiological temperatures. *Appl. Microbiol. Biotechnol.* **56**, 249–254
37. Beney, L., Mille, Y., and Gervais, P. (2004) Death of *Escherichia coli* during rapid and severe dehydration is related to lipid phase transition. *Appl. Microbiol. Biotechnol.* **65**, 457–464
38. Inoue, K., Matsuzaki, H., Matsumoto, K., and Shibuya, I. (1997) Unbalanced membrane phospholipid compositions affect transcriptional expression of certain regulatory genes in *Escherichia coli*. *J. Bacteriol.* **179**, 2872–2878
39. Davenport, K.R., Sohaskey, M., Kamada, Y., Levin, D.E., and Gustin, M.C. (1995) A second osmosensing signal transduction pathway in yeast: Hypotonic shock activates the PKC1 protein kinase-regulated cell integrity pathway. *J. Biol. Chem.* **270**, 30157–30161
40. Kamada, Y., Jung, U.S., Piotrowski, J., and Levin, D.E. (1995) The protein kinase C-activated MAP kinase pathway of *Saccharomyces cerevisiae* mediates a novel aspect of the heat shock response. *Genes Dev.* **9**, 1559–1571
41. Zarzov, P., Mazzoni, C., and Mann, C. (1996) The SLT2(MPK1) MAP kinase is activated during periods of polarized cell growth in yeast. *EMBO J.* **15**, 83–91
42. Mikami, K., Kanesaki, Y., Suzuki, I., and Murata, N. (2002) The histidine kinase Hik33 perceives osmotic stress and cold stress in *Synechocystis* sp. PCC 6803. *Mol. Microbiol.* **46**, 905–915
43. Winston, F., Dollard, C., and Ricupero-Hovasse, S.L. (1995) Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. *Yeast* **11**, 53–55
44. Lyons, J.M. (1973) Chilling injury in plants. *Annu. Rev. Plant Physiol.* **24**, 445–466
45. Inoue, Y., Tsujimoto, Y., and Kimura, A. (1998) Expression of the glyoxalase I gene of *Saccharomyces cerevisiae* is regulated by high osmolarity glycerol mitogen-activated protein kinase pathway in osmotic stress response. *J. Biol. Chem.* **273**, 2977–2983
46. Panadero, J., Pallotti, C., Rodríguez-Vargas, S., Randez-Gil, F., and Prieto, J.A. (2006) A downshift in temperature activates the HOG pathway, which determines freeze tolerance in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **281**, 4638–4645