Molecular Cell, Vol. 9, 1307–1317, June, 2002, Copyright ©2002 by Cell Press

Hog1 Kinase Converts the Sko1-Cyc8-Tup1 Repressor Complex into an Activator that Recruits SAGA and SWI/SNF in Response to Osmotic Stress

Markus Proft and Kevin Struhl[1]
Department of Biological Chemistry
and Molecular Pharmacology
Harvard Medical School
Boston, Massachusetts 02115

Summary

The yeast ATF/CREB repressor Sko1(Acr1) regulates genes that are induced upon hyperosmotic stress by recruiting the Cyc8(Ssn6)-Tup1 corepressor complex to target promoters. During hyperosmotic stress, Hog1 MAP kinase associates with target promoters, phosphorylates Sko1, and converts Sko1 into a transcriptional activator. Unexpectedly, Tup1 remains bound to target promoters during osmotic stress. Sko1, Hog1, and Tup1 are all important for recruitment of SAGA histone acetylase and SWI/SNF nucleosome-remodeling complexes to osmotic-inducible promoters, and both complexes are important for activation upon osmotic stress. Thus, osmotic induction involves a switch of Sko1-Cyc8-Tup1 from a repressing to an activating state in a process that is triggered by Hog1 phosphorylation. Cyc8-Tup1 is not simply a corepressor but is also involved in recruiting SWI/SNF and SAGA during the transcriptional induction process.

Introduction

In yeast cells, diverse classes of genes are actively repressed during normal growth conditions by targeted recruitment of the Cyc8-Tup1 corepressor complex (Smith and Johnson, 2000). In many cases, Cyc8-Tup1 represses classes of genes that are expressed under specific but distinct conditions of environmental stress such as poor carbon source, hypoxia, DNA damage, mitochondrial dysfunction, and osmotic stress (DeRisi et al., 1997). Cyc8-Tup1 is recruited to promoters by a variety of DNA binding proteins, each of which represses genes in a specific pathway. The DNA binding repressors utilize different surfaces of Cyc8 and Tup1 for recruitment of the corepressor complex to target promoters (Komachi et al., 1994; Tzamarias and Struhl, 1994, 1995). Transcriptional repression by the corepressor complex is mediated by a distinct region of Tup1 that is presumed to function through protein-protein interactions (Tzamarias and Struhl, 1994).

The mechanism by which Cyc8-Tup1 inhibits transcription has been heavily investigated but only partially understood. There is considerable genetic and molecular evidence suggesting that Cyc8-Tup1 repression involves direct contacts to subunits of the mediator subcomplex of RNA polymerase II holoenzyme (Balciunas and Ronne, 1995; Kuchin et al., 1995; Wahi and Johnson, 1995; Song et al., 1996; Kuchin and Carlson, 1998; Conlan et al., 1999; Gromoller and Lehming, 2000; Papamichos-Chronakis et al., 2000; Han et al., 2001; Zaman et al., 2001). However, a number of mediator subunits have been implicated, and a coherent picture of how Tup1-mediator interactions lead to repression has yet to be elucidated. There is also considerable evidence that Cyc8-Tup1 represses transcription by altering chromatin structure. Tup1 interacts with underacetylated H3 and H4 histone tails (Edmondson et al., 1996) and several histone deacetylases (Watson et al., 2000; Wu et al., 2001) in vitro, and repressed promoters show localized deacetylation of histone H3 (Bone and Roth, 2001; Deckert and Struhl, 2001; Wu et al., 2001). It is likely that Cyc8-Tup1 repression involves effects on both chromatin structure and the transcription machinery.

As Cyc8-Tup1-repressed genes play crucial roles in cell survival under various stress conditions, it is important to understand how repression of these genes is quickly and efficiently relieved upon environmental stimuli. It is generally assumed that, upon environmental stress, recruitment of Cyc8-Tup1 is inhibited by inactivating the relevant DNA binding repressor (Smith and Johnson, 2000). As a consequence, transcriptional activators (whose function might or might not be regulated by the environmental stress) acting at the promoter stimulate expression of the gene. DNA binding repressors can be rapidly inactivated by proteolysis (Johnson et al., 1998), nuclear export (De Vit et al., 1997), loss of DNA binding (Huang et al., 1998), or blocking the interaction with Cyc8-Tup1 (Proft et al., 2001). Inactivation of the DNA binding repressor often involves rapid phosphorylation by a specific protein kinase in response to environmental stress. However, the fate of the Cyc8-Tup1 corepressor at target promoters under conditions of environmental stress has never been examined.

Here, we analyze the association of transcriptional regulatory proteins with Cyc8-Tup1-repressed promoters in vivo in cells undergoing transcriptional induction in response to environmental stress. Specifically, we examine regulation by Sko1(Acr1), an ATF/CREB repressor (Nehlin et al., 1992; Vincent and Struhl, 1992) that inhibits transcription of several genes that are inducible by hyperosmotic stress (Proft and Serrano, 1999; Garcia-Gimeno and Struhl, 2000; Pascual-Ahuir et al., 2001b; Rep et al., 2001). Sko1-mediated repression requires the Cyc8-Tup1 corepressor (Proft and Serrano, 1999; Garcia-Gimeno and Struhl, 2000), and Sko1 interacts with Tup1 (Pascual-Ahuir et al., 2001a; Proft et al., 2001). Release from Cyc8-Tup1 repression upon osmotic shock requires the Hog1 MAP kinase, which phosphorylates Sko1 at multiple sites (Proft et al., 2001).

Our results demonstrate that the role of Sko1 goes far beyond recruiting Cyc8-Tup1 to repressed promoters. Hog1-dependent phosphorylation converts Sko1 from a repressor into an activator that recruits Hog1 itself and both the SAGA histone acetylase and SWI/SNF nucleosome-remodeling complexes to the promoter. Unexpectedly, Tup1 remains at the promoter even under inducing conditions and is important for recruitment of SAGA and SWI/SNF. Thus, the Cyc8-Tup1 corepressor complex is important both for transcriptional repression

[1]Correspondence: kevin@hms.harvard.edu

of osmotically regulated promoters under normal conditions and for the rapid transcriptional induction that occurs upon hyperosmotic stress.

Results

Sko1 and Tup1 Are Bound to Stress-Regulated Promoters Both in the Absence and Presence of Osmotic Stress

Sko1 represses a subset of defense genes that are highly inducible by hyperosmotic stress (Proft and Serrano, 1999; Garcia-Gimeno and Struhl, 2000; Pascual-Ahuir et al., 2001b; Rep et al., 2001). In this work, we studied three Sko1-regulated genes: *GRE2*, encoding a protein similar to plant isoflavonoid reductases; *AHP1*, encoding alkylhydroperoxide reductase; and *HAL1*, encoding an ion homeostasis determinant. When yeast cells are challenged with moderate salt concentrations (0.4 M NaCl), these genes are highly, though transiently, induced within 5 min.

We utilized chromatin immunoprecipitation to follow Sko1 binding and recruitment of the Tup1 corepressor in the living cell during this switch from repressed to induced transcription. Chromatin from a yeast strain expressing a functional HA-Sko1 fusion protein from its natural locus was immunoprecipitated with antibodies against the HA-epitope or natural Tup1, and the resulting material was analyzed by quantitative PCR in real time using primers spanning the *GRE2*, *AHP1*, and *HAL1* promoter regions. As expected, Sko1 and Tup1 bind strongly to all promoters tested in the absence of stress (Figures 1A and 1B). Remarkably, however, Sko1 and Tup1 are bound to these promoters 5 min after salt treatment. Sko1 binding at *AHP1* during salt induction is as strong as during nonstress conditions, while Sko1 binding at *HAL1* and *GRE2* is less efficient. Tup1 recruitment both under stress and nonstress conditions completely depends on Sko1, because Tup1 binding is not observed in a *sko1* deletion strain (Figure 1B).

To monitor directly when *GRE2*, *AHP1*, and *HAL1* transcription was activated under the stress conditions applied, we analyzed the association of TATA binding protein (TBP) using the same chromatin samples described above. TBP occupancy is low (*AHP1*) or not detectable (*GRE2*, *HAL1*) under normal growth conditions, whereas salt stress stimulated the recruitment of TBP within 3 min (data not shown) with maximal occupancy at 5 min (Figure 1C). TBP occupancy is not further increased at later time points in all three cases (data not shown). Thus, Sko1 binds and recruits the Cyc8-Tup1 corepressor to both repressed and fully activated stress promoters.

As the HOG MAP kinase pathway is essential for the osmotic induction of Sko1-dependent genes (Proft and Serrano, 1999; Garcia-Gimeno and Struhl, 2000; Pascual-Ahuir et al., 2001b; Rep et al., 2001), we addressed whether Hog1 kinase affects Sko1 binding and Tup1 recruitment in vivo. In the absence of stress, Sko1 binding to the target promoters is comparable in wild-type and *hog1* deletion strains (Figure 1A). However, in the absence of Hog1, osmotic stress significantly reduces Sko1 binding to the *GRE2* and *AHP1* promoters, although it does not affect Sko1 binding to *HAL1* promoter. Tup1 recruitment is enhanced in the absence of a functional HOG pathway, with the effect being modest in unstressed cells and more pronounced during osmotic induction considering that salt-stressed *hog1* cells have less Sko1 bound at *GRE2* and *AHP1* promoters. As expected, stress-induced TBP recruitment is impaired in a *hog1* mutant (Figure 1C). Taken together, our results indicate that osmotic-inducible transcription is not simply the result of a loss of Sko1 binding and/or Tup1 recruitment, but rather occurs in the presence of both proteins.

Sko1 Recruits the Hog1 MAP Kinase to Stress-Regulated Promoters

As Hog1 kinase interacts with and phosphorylates Sko1 (Proft et al., 2001), we used a functional HA-tagged Hog1 derivative to address whether Hog1 physically associates with Sko1-bound promoters. As assayed by chromatin immunoprecipitation, Hog1 is associated with the *GRE2* and *AHP1* promoter regions exclusively under stress conditions (Figure 1D). The presence of Hog1 at the tested promoters coincides well with the association of TBP and therefore is a feature of transcriptionally active promoters. Moreover, in a *sko1* deletion strain, Hog1 does not associate with these promoters, suggesting that Sko1 recruits Hog1 kinase to CRE-sites of stress-activated promoters.

Hog1 phosphorylates three residues within the N terminus of Sko1 (Proft et al., 2001). In cell-free extracts from osmotically stressed cells, Hog1 coimmunoprecipitates the Sko1 N-terminal region (1–315) but not Sko1 variants that lack this domain (Figures 2A and 2B). Therefore, the Sko1 N terminus is responsible and sufficient to interact with Hog1. Furthermore, the Sko1-Hog1 interaction is not altered when the three Hog1-phosphorylation sites in Sko1 are mutated (Figure 2C), indicating that this interaction does not depend on phosphorylation. These results strongly suggest that, upon stimulation by salt stress, the N-terminal domain of Sko1 directly recruits Hog1 to CRE-sites in vivo.

Sko1 Is a Stress- and Hog1-Dependent Activator

Our results that Sko1 recruitment of Hog1 kinase to promoters upon osmotic stress coincides with transcriptional activation suggest that Sko1 can activate transcription. We therefore fused intact Sko1 or the N-terminal 315 residues with the Gal4 DNA binding domain and assayed transcription on a genomic *GAL1-LacZ* reporter gene (Figure 3). Strikingly, Gal4-Sko1 activates transcription exclusively under salt stress conditions, with the Sko1 N-terminal region activating more strongly than intact Sko1. Sko1-mediated activation does not occur in a *hog1* mutant strain, indicating that it depends on Hog1 kinase. Analysis of comparable Gal4-Sko1 derivatives in which the three Hog1-phosphorylation sites are mutated (Proft et al., 2001) reveals that salt-induced activation is largely abolished by preventing Hog1 phosphorylation of Sko1. Thus, osmotic induction and Hog1 phosphorylation converts Sko1 repressor into a transcriptional activator.

Mutation of the Hog1 phosphorylation sites increases the interaction between Sko1 and Tup1 (Proft et al., 2001) and also between Sko1(1–315) and Tup1 (data not

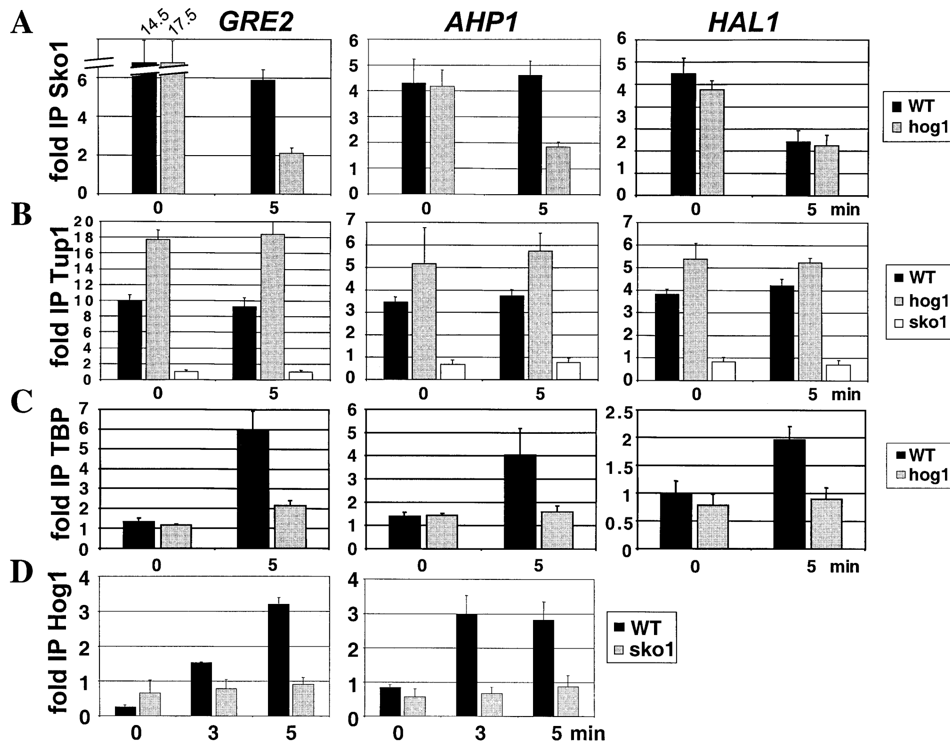

Figure 1. Sko1 and Tup1 Are Bound to CRE Sites under Repressing and Activating Conditions, while Sko1 Recruits Hog1 Exclusively Upon Osmotic Stress

(A) Sko1 association with the indicated promoters in wild-type (MAP37; black bars) or *hog1* mutant (MAP36; gray bars) cells expressing $(HA)_3$-Sko1 that were osmotically stressed by 0.4 M NaCl for the indicated time. The fold-Sko1 occupancy in control untagged cells = 1 (data not shown).

(B) Tup1 association in the same samples. Tup1 occupancy was also analyzed in *sko1* mutant cells (MAP19; white bars).

(C) TBP occupancy in the same samples.

(D) Hog1 recruitment to the indicated promoters in wild-type (MAP51; black bars) or *sko1* mutant (MAP54; gray bars) cells expressing $(HA)_3$-Hog1. Immunoprecipitation efficiencies are presented as the fold over the *POL1* coding sequence control.

shown). To examine whether the lack of activation by unphosphorylatable Gal4-Sko1 is (at least partially) due to favoring corepressor recruitment, we analyzed Gal4-Sko1-mediated activation in a *cyc8* deletion strain. In the absence of Cyc8, activation by Sko1 and Sko1(1–315) is strictly regulated by osmotic stress but is slightly more efficient (Figure 3). More importantly, the loss of activation caused by mutating the Hog1 phosphorylation sites in Sko1 is partially suppressed by deletion of *CYC8*. Taken together, our results show that Sko1 can activate transcription strictly dependent on phosphorylation by the Hog1 MAP kinase. However, in the absence of Cyc8-Tup1, phosphorylation of Sko1 by Hog1 is no longer the essential step for activation, and at least some Sko1-mediated transcription occurs in the absence of Hog1 phosphorylation.

Hog1 and Tup1 Are Important for Recruitment of SAGA and SWI/SNF to Sko1-Bound Promoters

The SAGA histone acetylase and SWI/SNF nucleosome-remodeling complexes can be recruited to promoters in vivo by transcriptional activators (Cosma et al., 1999; Bhaumik and Green, 2001; Larschan and Winston, 2001; Deckert and Struhl, 2002). We addressed whether the conversion of Sko1 repressor into an activator during osmotic stress resulted in recruitment of SAGA and SWI/SNF to promoters using strains with Myc-tagged versions of Ada2, Spt20, and Swi2, respectively. At the *GRE2* and *AHP1* promoters, both coactivator complexes are recruited in response to osmotic stress with comparable kinetics to that observed for TBP (Figures 4A–4D). Analysis of the same samples with antibodies against acetylated histone H3 and H4 tails reveals that histone H3 acetylation at both promoters increases during osmotic stress (Figure 4E), whereas histone H4 acetylation is unaffected (data not shown). Increased SAGA and SWI/SNF association at both promoters under salt stress is abolished in *sko1* and *hog1* deletion mutants. Thus, the osmotic-inducible and Hog1-dependent conversion of Sko1 repressor into an activator results in recruitment of SWI/SNF and SAGA to target promoters in a manner that correlates well with transcriptional induction of target genes.

Surprisingly, Tup1 is important for SAGA and SWI/SNF recruitment upon stress (Figure 4). In *tup1* mutant cells, the levels of SAGA and SWI/SNF association at *AHP1* is similar to that observed in *sko1* or *hog1* mutants, while the levels at *GRE2* are reduced in comparison to the wild-type strain. It is important to note that the Sko1- and Tup1-dependence of SAGA- and SWI/SNF-recruitment are specific for osmotic stress at the *GRE2* and

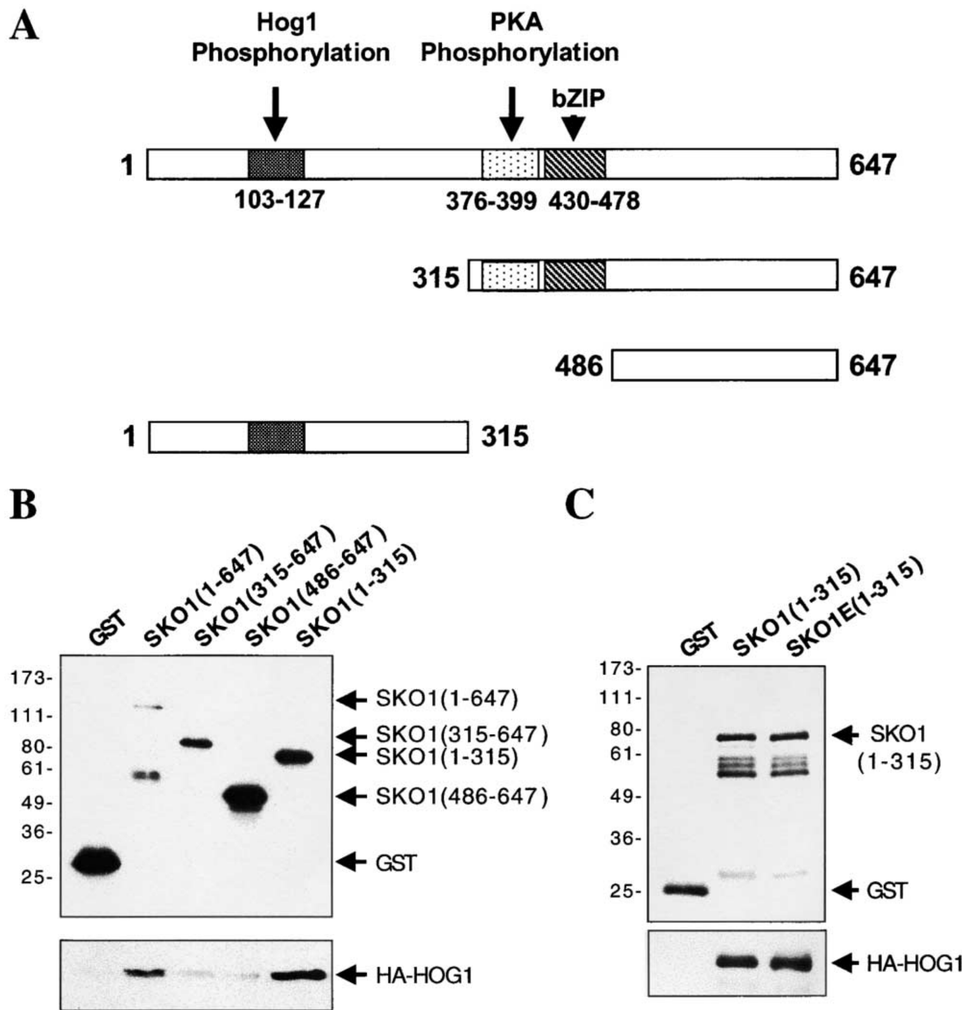

Figure 2. The Sko1 NH_2-Terminal Domain (1–315) Interacts with Hog1 Independently of Phosphorylation

(A) Diagram of Sko1 functional domains in the wild-type and mutant proteins used in this study.

(B) Coprecipitation of $(HA)_3$-Hog1 by the indicated GST-Sko1 fusion proteins. GST-containing proteins (upper panel) and $(HA)_3$-Hog1 (lower panel) were detected by Western blotting using antibodies against GST and the HA epitope. Protein standards are given at the left in kDa.

(C) Coprecipitation of $(HA)_3$-Hog1 by GST-Sko1(1–315) and GST-Sko1E(1–315), a derivative in which all three Hog1-phosphorylation sites are mutated (Proft et al., 2001).

AHP1 promoters. High SAGA and to some extent higher SWI/SNF occupancy is observed in *sko1* and *tup1* mutants under non-stress conditions (Figure 4), but this corresponds to increased transcription dependent on other activators that bind the *GRE2* promoter such as Yap1, Aca1, and Aca2 (Garcia-Gimeno and Struhl, 2000; Rep et al., 2001). As expected from the ability of these other activators to function at *GRE2* in the absence of Tup1 under nonstress conditions, TBP occupancy is elevated in *sko1* and *tup1* mutant strains (Figure 1C). Importantly, these other activators do not appear to function under conditions of osmotic stress, because *sko1* mutant strains have background levels of TBP occupancy. It is very likely that the high mRNA levels observed in salt-stressed *sko1* cells (Proft et al., 2001; Rep et al., 2001) reflect RNA that is synthesized prior to osmotic stress but is not significantly degraded during the short induction time.

Tup1 Co-Occupies Osmotically Induced Promoters with SAGA and SWI/SNF

The observation that Tup1 is important for SAGA and SWI/SNF recruitment upon stress strongly suggests that Tup1 should co-occupy stress-induced promoters with these chromatin-modifying complexes. To directly show that Tup1 is bound together with SAGA or SWI/SNF at the same stress-activated promoters, we performed sequential chromatin immunoprecipitation. Specifically, Myc-tagged Ada2 or Swi2 were first immunoprecipitated from unstressed and stressed cells, and the resulting material was subjected to a second immunoprecipitation using Tup1 antibodies (Figure 5).

Strikingly, the fold-enrichments at the *GRE2* promoter observed in the sequential immunoprecipitations (15 to 25-fold) are roughly equal to the product of the fold-enrichments of the individual precipitations (3 to 5-fold). The high enrichment of the *GRE2* promoter in the se-

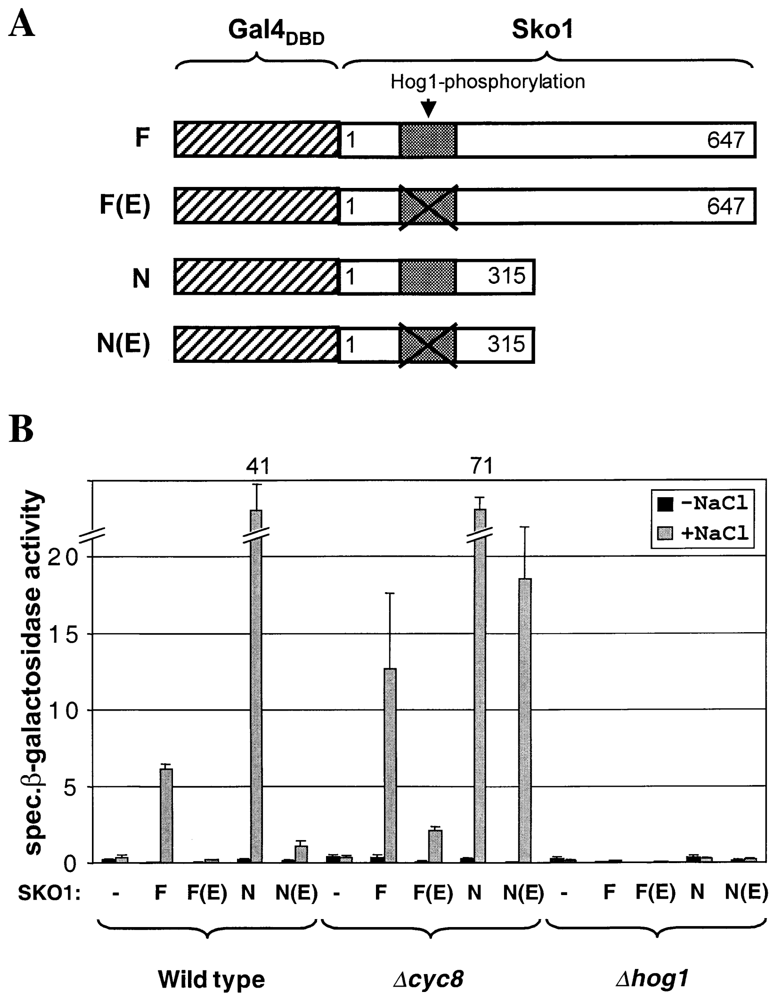

Figure 3. Sko1 Is a Stress- and Hog1-Dependent Activator

(A) Diagram of Sko1 regions fused to the Gal4 DNA binding domain ($Gal4_{DBD}$). Protein variants marked (E) contain mutations of all three Hog1 phosphorylation sites (Proft et al., 2001).

(B) Expression of the integrated *GAL1-lacZ* reporter gene in wild-type (SFY526), *cyc8* (MAP34), or *hog1* mutant (MAP57) cells containing the indicated Gal4-Sko1 fusions or the empty pGBT9 vector (−) that were (+NaCl) or were not (−NaCl) subjected to hyperosmotic stress (0.4 M NaCl for 45 min). β-galactosidase values are given in $nmol \cdot min^{-1} \cdot mg^{-1}$.

quential immunoprecipitations occurs exclusively in the salt-treated sample (Figure 5), even though the levels of Tup1, Myc-Ada2, and Myc-Swi2 proteins are comparable under normal and inducing conditions (data not shown). As such, the samples from normal cells represent an important control for the sequential chromatin immunoprecipitation procedure. These results demonstrate that Tup1 co-occupies the *GRE2* promoter together with SAGA or SWI/SNF complexes in response to osmotic stress.

SAGA and SWI/SNF Are Important for Osmotic Induction in Wild-Type but Not Tup1-Deficient Cells

Although *tup1* mutant cells show reduced or nondetectable levels of SAGA and SWI/SNF association upon stress, TBP association with stress-induced promoters is equally or even more efficient than in wild-type cells (Figure 4C). These results suggest that SAGA and SWI/SNF are important for transcriptional activation in the presence of Cyc8-Tup1 but are dispensable in the absence of the corepressor. We directly examined the role of SAGA and SWI/SNF in stress-activated transcription of *GRE2* and *AHP1* using *gcn5* and *swi2* deletion mutants. As shown in Figure 6, *gcn5* mutants show reduced activation of *GRE2* and *AHP1* transcription, while *swi2* mutants show reduced transcription only for the *AHP1* gene. In all cases, additional deletion of *TUP1* compensates for the loss of SAGA or SWI/SNF activity (Figure 6). We conclude that SAGA and SWI/SNF recruitment is an important feature for osmotic stress-induced transcription of Cyc8-Tup1 repressed genes. However, recruitment of these coactivator complexes is not important for salt-induced transcription in mutant strains lacking the Cyc8-Tup1 corepressor.

Discussion

The MAP Kinase Hog1 Switches Sko1 from a Repressor to an Activator in Response to Osmotic Stress

Our chromatin immunoprecipitation experiments confirm previous work indicating that Sko1 inhibits transcription by recruiting the Cyc8-Tup1 corepressor (Proft and Serrano, 1999; Garcia-Gimeno and Struhl, 2000) and that the interaction of Sko1 with Cyc8-Tup1 decreases upon osmotic shock due to phosphorylation of Sko1 by the Hog1 kinase (Proft et al., 2001). Under normal growth conditions in which osmotically inducible genes are repressed, Sko1 and Tup1 are bound to stress-regulated, CRE-containing promoters, and Tup1 association re-

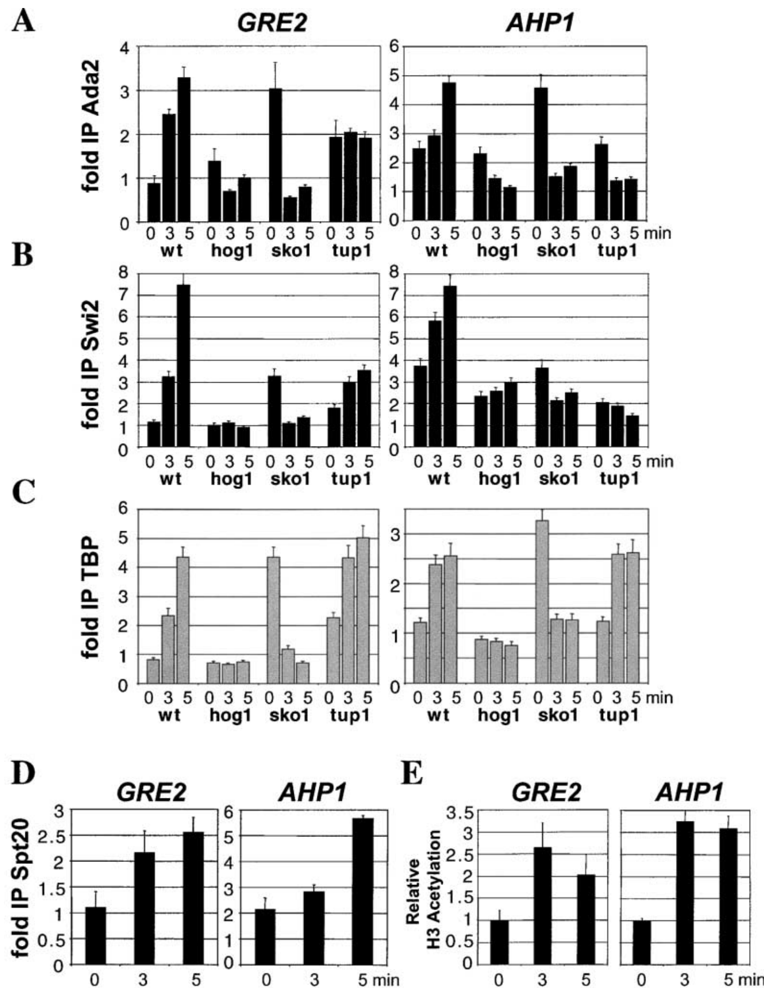

Figure 4. Stress-Stimulated SAGA and SWI/SNF Recruitment Depends on Sko1, Hog1, and Tup1

(A) Association of 18Myc-Ada2, a SAGA subunit, with the indicated promoters in wild-type (K8135), *hog1* (MAP62), *sko1* (MAP63), and *tup1* (MAP66) cells that were osmotically stressed by 0.4 M NaCl for the indicated times.

(B) Association of 18Myc-Swi2, a SWI/SNF subunit, with the indicated promoters in wild-type (K8126), *hog1* (MAP64), *sko1* (MAP65), and *tup1* (MAP67) cells that were osmotically stressed by 0.4 M NaCl for the indicated times.

(C) TBP occupancy in the same samples as in (B).

(D) Association of 3Myc-Spt20, a SAGA-specific subunit, with the indicated promoters in wild-type (YDH254) cells that were osmotically stressed by 0.4 M NaCl for the indicated times.

(E) Activation of Sko1-regulated genes by osmotic stress is associated with increased histone H3 acetylation. Crosslinked chromatin from wild-type (MAP37) cells that were stressed with 0.4 M NaCl for the indicated times was immunoprecipitated with antibodies against acetylated H3 histone tails, and the amounts of immunoprecipitated and input material for the indicated promoter regions were determined by quantitative PCR. The level of histone H3 acetylation before stress is arbitrarily set to 1 for each promoter. Immunoprecipitation efficiencies for (A)–(D) are presented as the fold over the *POL1* coding sequence control.

quires Sko1. The Tup1:Sko1 binding ratio at target promoters is increased in the absence of Hog1, presumably reflecting higher affinity of unphosphorylated Sko1 to the Cyc8-Tup1 corepressor.

As Cyc8-Tup1 represses genes in many diverse pathways, it has been presumed that pathway-specific regulation upon environmental stress occurs by inactivation of the relevant DNA binding repressor and hence no recruitment of Cyc8-Tup1 (Smith and Johnson, 2000). Here, we demonstrate that the role of Sko1 goes beyond its repressor function. Under conditions of osmotic stress, Sko1 is bound to target promoters, it is required to recruit SAGA and SWI/SNF, and it co-occupies promoters together with both coactivator complexes. Most convincingly, a Gal4-Sko1 fusion protein that confers Cyc8-Tup1-dependent repression in normal conditions (Proft and Serrano, 1999) activates transcription from the *GAL1* promoter in a stress-, Hog1-, and Sko1-phosphorylation-dependent fashion. The Gal4-Sko1 fusion protein permits a specific assay of Sko1 function without the complications of other activators that bind ATF/CREB sites and affect Sko1-dependent promoters, such as Gcn4 (Pascual-Ahuir et al., 2001b), Aca1, Aca2 (Garcia-Gimeno and Struhl, 2000; Rep et al., 2001), Yap1 (Rep et al., 2001), and an unidentified CRE binding activator (Garcia-Gimeno and Struhl, 2000). Although Cyc8-Tup1 modestly affects activation by Sko1 under inducing conditions (about 2-fold), loss of the corepressor is not sufficient to turn Sko1 into an activator. Instead, Sko1-mediated activation absolutely requires Hog1 function and the presence of three Hog1-phosphorylation sites in Sko1. We conclude that Hog1 triggers a transcriptional switch by phosphorylating Sko1 and converting it from a repressor to an activator.

We further demonstrate that Sko1 together with Tup1 is promoter bound even when they are fully activated. Therefore, release from Sko1 mediated repression is not achieved by disrupting Sko1 binding or Cyc8-Tup1 recruitment. We note, however, that Sko1 binding is reduced upon stress at transcriptionally active promoters at least in the case of *GRE2* and *HAL1*, but interestingly this diminished Sko1 occupancy is sufficient to recruit the Tup1 corepressor to the same level as observed for the repressed promoters. It is likely that the additional targeting of coactivator complexes is the critical event in this stress-induced transcriptional switch.

A Single Transcription Factor, Sko1, Mediates Recruitment of Cyc8-Tup1, Hog1, SAGA, and SWI/SNF

Sko1 is required for the physical associations of Cyc8-Tup1 corepressor, Hog1 kinase, SAGA histone acetylase

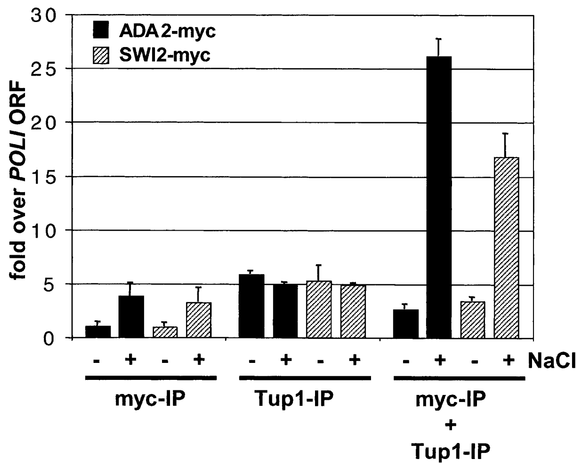

Figure 5. Tup1 Co-Occupies the Activated *GRE2* Promoter together with SAGA or SWI/SNF In Vivo

Sequential ChIP analysis using 18Myc-Ada2 (K8135; black bars) or 18Myc-Swi2 (K8126) containing cells that were untreated or treated with 0.4 M NaCl for 5 min as indicated. Simple immunoprecipitations (myc-IP, Tup1-IP) were performed to follow SAGA, SWI/SNF, and Tup1 occupancy at the *GRE2* promoter. Sequential ChIP was performed by a first precipitation of myc-tagged Ada2 or Swi2, followed by a second precipitation of natural Tup1. Immunoprecipitation efficiencies are presented as the fold over the *POL1* coding sequence control.

complex, and SWI/SNF nucleosome-remodeling complex to target promoters in vivo. However, Sko1 differentially regulates the recruitment of these factors. Cyc8-Tup1 recruitment by Sko1 is not regulated by stress, and it presumably involves a physical association (direct or indirect) between Sko1 and Tup1 (Pascual-Ahuir et al., 2001a). The N-terminal 315 residues of Sko1 are particularly important for the Sko1-Tup1 interaction, although other regions of Sko1 also contribute to this interaction (Pascual-Ahuir et al., 2001a).

Hog1 is the terminal MAP kinase of the HOG signaling pathway, which is rapidly and specifically activated upon hyperosmotic stress (Gustin et al., 1998). Upon activation, Hog1 is imported into the nucleus (Ferrigno et al., 1998; Reiser et al., 1999) where it associates with at least two transcription factors, Sko1 (Proft et al., 2001) and Hot1 (Rep et al., 1999; Alepuz et al., 2001). Our results indicate that Sko1 is required for Hog1 association with promoters in response to osmotic stress, and that the Hog1-interaction region of Sko1 is sufficient for transcriptional activation. Although phosphorylation of Sko1 is critical for activation, the Hog1 phosphorylation sites are not required for the Hog1-Sko1 association. Thus, Sko1 recruitment of Hog1 to promoters is regulated by osmotic stress but is unaffected by phosphorylation, whereas the transcriptional switch of Sko1 to an activator fully depends on phosphorylation. Hog1 can also be directed to certain promoters (e.g., *GPD1*) by the Hot1 activator (Alepuz et al., 2001). Hence, activated Hog1 can be recruited to distinct classes of promoters by different DNA binding proteins, whereupon it forms a structural part of upstream-bound transcriptional activating complexes. The functional role of Hog1 in these complexes, however, remained to be defined.

One possible role of Hog1 in transcriptional activation is the recruitment of chromatin-modifying complexes. Indeed, both Sko1 and Hog1 are required to target the SAGA and SWI/SNF coactivator complexes to target promoters in response to hyperosmotic shock. The association of SAGA and SWI/SNF to target promoters occurs with indistinguishable kinetics and correlates very well with increasing histone H3 acetylation and occupancy of TBP. In general, increased association of Hog1, SAGA, SWI/SNF, and TBP occurs within 2–3 min after stress exposure and is maximal at 5 min. These observations are consistent with parallel rather than ordered recruitment of these factors at the stress-regulated genes, although this remains to be proven. While Cyc8-Tup1 and Hog1 are very likely to be targeted to promoters by direct interaction with Sko1, it remains to be shown whether Sko1 directly interacts with SAGA and/or SWI/SNF. Other yeast activators can directly interact with these coactivator complexes in vitro and recruit them to promoters in vivo (Cosma et al., 1999; Natarajan et al., 1999; Neely et al., 1999; Yudkovsky et al., 1999; Kuo et al., 2000; Bhaumik and Green, 2001; Brown et al., 2001; Larschan and Winston, 2001). Thus,

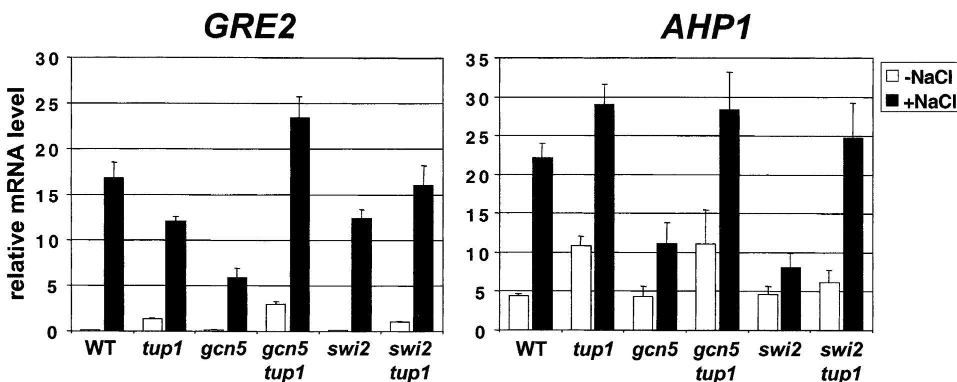

Figure 6. SAGA and SWI/SNF Coactivators Are Important for Osmotic Stress-Induced Transcription in the Presence of Cyc8-Tup1

Reverse transcriptase analysis of *GRE2* and *AHP1* mRNA levels under noninducing (−NaCl) and inducing (+NaCl; 0.4 M NaCl for 10 min) conditions in wild-type (FT5), *gcn5* (JDY191), *swi2* (JDY193), *tup1* (MAP69), *gcn5 tup1* (MAP70), and *swi2 tup1* (MAP71) strains. Levels of mRNA are given relative to the *TBP1* control.

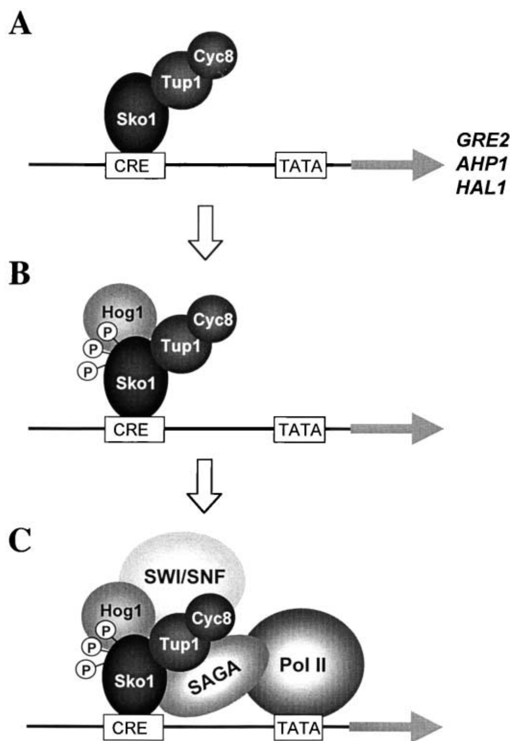

Figure 7. Model of the Repression/Activation Switch of Sko1-Cyc8-Tup1 Triggered by the Stress-Activated Hog1 MAP Kinase

(A) Active repression of Sko1-regulated promoters under normal (no stress) growth conditions. Sko1 is bound to upstream CRE sequences of osmotically inducible genes and recruits the Cyc8-Tup1 corepressor, which inhibits preinitiation complex formation and actively prevents transcription.

(B) Upon hyperosmotic stress, Hog1 kinase translocates to the nucleus, whereupon it is recruited by Sko1 to target promoters. Hog1 interacts with and multiply phosphorylates the Sko1 N-terminal (1–315) domain. This phosphorylation event is a prerequisite for the further recruitment of chromatin modifying complexes.

(C) Complete promoter activation is reached a few minutes after challenge by osmotic stress. The complex of phosphorylated Sko1-Cyc8-Tup1-Hog1 recruits SWI/SNF and SAGA chromatin-modifying complexes which promote RNA polymerase II binding and transcriptional activation. Recruitment requires Sko1, Tup1, and Hog1.

our results strongly imply that the upstream-bound activation complex, which includes phosphorylated Sko1, Hog1, and Cyc8-Tup1, interacts with SWI/SNF and SAGA (Figure 7).

Cyc8-Tup1 Is Associated with Transcriptionally Active Promoters and Is Important for SAGA and SWI/SNF Recruitment

Repressor-mediated and artificial recruitment of Cyc8-Tup1 represses transcription of target promoters (Keleher et al., 1992; Tzamarias and Struhl, 1994), leading to the view that derepression of Cyc8-Tup1-regulated genes upon environmental stress is due to removal of the corepressor from the promoter (Smith and Johnson, 2000). In striking contrast to this expectation, Cyc8-Tup1 remains bound to all three Sko1-bound promoters investigated here that are transcriptionally activated in response to osmotic stress. As we presume that near-complete Cyc8-Tup1 occupancy of the promoter is required for efficient repression, this suggests that Cyc8-Tup1 is present at near stoichometric levels at a transcriptionally active promoter. Cyc8-Tup1 also remains associated with other stress-regulated genes that utilize distinct DNA binding repressors (Mig1 and Rox1) to recruit the corepressor (Papamichos-Chronakis et al., 2002), indicating that transcriptional derepression of Cyc8-Tup1-regulated genes is not generally due to removal of the corepressor. The persistence of Cyc8-Tup1 corepressor during transcriptional inducing conditions is distinct from the situation with nuclear hormone receptors, in which the activating ligand results in dissociation of corepressor complexes and association of distinct coactivator complexes.

Cyc8-Tup1 not only remains bound to Sko1-regulated promoters in response to conditions of hyperosmolarity, but it is important for stress-induced recruitment of SAGA and SWI/SNF. In the absence of Cyc8-Tup1, Sko1 binds normally to promoters (data not shown), and we assume that Sko1 also normally recruits Hog1 kinase, because Hog1-dependent induction of *GRE2* is observed in *tup1* or *cyc8* mutant strains (Rep et al., 2001; Figures 4 and 6). The fact that stress-induced SAGA and SWI/SNF recruitment is significantly reduced in the absence of Cyc8-Tup1, even though Sko1 and Hog1 are fully bound to promoters, strongly argues that Cyc8-Tup1 directly participates in targeting both SAGA and SWI/SNF. This is furthermore supported by our finding that Tup1 physically co-occupies a stress-activated promoter together with both coactivator complexes. Other studies have independently reached this conclusion and also identified Cti6 as a protein that links Cyc8-Tup1 to SAGA (Papamichos-Chronakis et al., 2002). As SWI/SNF and SAGA recruitment occurs only upon osmotic stress, our results suggest that the Sko1-Cyc8-Tup1 repressor complex becomes competent to recruit SAGA and SWI/SNF in a switch that is triggered by Hog1 phosphorylation of Sko1 (Figure 7). Our results do not address the mechanism by which the activated Sko1-Cyc8-Tup1 complex recruits SAGA and SWI/SNF, and we disfavor a model in which Tup1 directly interacts with SAGA and/or SWI/SNF.

Although Cyc8-Tup1 is important for recruitment of SAGA and SWI/SNF to Sko1-regulated promoters, it is not essential for transcriptional activation of these genes (Proft and Serrano, 1999; Pascual-Ahuir et al., 2001b; Rep et al., 2001; this study). In the absence of Cyc8-Tup1, Hog1 phosphorylation converts Sko1 repressor into an activator that can stimulate transcription without recruiting the coactivator complexes. In wild-type cells, however, Cyc8-Tup1 augments the Sko1 repressor-activator switch by recruiting additional coactivator complexes. These additional coactivator complexes might be important to counteract the inherently repressive function of Cyc8-Tup1. In agreement with this, we show that both SAGA and SWI/SNF play positive roles in osmotic stress-induced transcription in the presence of Cyc8-Tup1, but not in its absence. In this regard, two-hybrid experiments indicate that both Cyc8- and Tup1-VP16 activation domain fusions can stimulate transcription when targeted to a promoter (Tzamarias and Struhl, 1994, 1995).

What is the biological purpose of having Cyc8-Tup1 involved in recruiting SWI/SNF and SAGA to promoters

in response to stress? We speculate that this feature provides a general mechanism for yeast cells to achieve a very rapid on/off switch of transcriptional activity in response to a wide variety of changing environmental conditions. In particular, Sko1-Cyc8-Tup1 can switch between a transcriptional stimulator and an active repressor without ever leaving the promoter. Such a mechanism is especially relevant when cells have adapted to or are no longer subject to the stress condition, in which case it is desirable to immediately repress transcription. In such a situation, it would likely take more time to remove SWI/SNF and SAGA and re-recruit Cyc8-Tup1 than to simply remove the coactivator complexes.

For osmotic-inducible genes, the on/off transcriptional switch is mediated by a single DNA binding factor (Sko1) whose activity is governed by a single effector (Hog1 kinase) that lies at the end of a signal transduction cascade. Hog1 plays multiple roles in the process. Hog1 phosphorylation is required to convert Sko1 into an activator, and this conversion is accompanied by the association of Hog1 with target promoters. In addition, Hog1 phosphorylation appears to counteract Cyc8-Tup1 repressor function, because Sko1 phosphorylation sites are less important for activation in the absence of Cyc8-Tup1. Lastly, Hog1 alters the Sko1-Cyc8-Tup1 complex such that SAGA and SWI/SNF are recruited to target promoters in a manner dependent on Cyc8-Tup1. Taken together, our results indicate unexpected complexity of a transcriptional switch regulated by a MAP kinase in response to environmental stress.

Experimental Procedures

Yeast Strains

The following yeast strains derived from W303-1A (*MATa ura3 leu2 trp1 his3 ade2*) were used for chromatin immunoprecipitation. MAP37 (*3HA-SKO1*) (Proft et al., 2001), MAP36 (*hog1::KAN MX*, *3HA-SKO1*) (Proft et al., 2001), MAP59 (*tup1::KAN MX*, *3HA-SKO1*), MAP19 (*sko1::KAN MX*) (Proft and Serrano, 1999), MAP51 (*3HA-HOG1*), MAP54 (*sko1::KAN MX*, *3HA-HOG1*), K8135 (*ADA2-18Myc*) (Cosma et al., 1999), MAP62 (*hog1::KAN MX*, *ADA2-18Myc*), MAP63 (*sko1::KAN MX*, *ADA2-18Myc*), MAP66 (*tup1::KAN MX*, *ADA2-18Myc*), K8126 (*SWI2-18Myc*) (Cosma et al., 1999), MAP64 (*hog1::KAN MX*, *SWI2-18Myc*), MAP65 (*sko1::KAN MX*, *SWI2-18Myc*), MAP67 (*tup1::KAN MX*, *SWI2-18Myc*). *SPT20*-tagged strain YDH254 (*MAT*α *ura3-*Δ*0 leu2-*Δ*0 his3-*Δ*1 lys2-*Δ*0* Δ*gal4::KAN SPT20-3Myc*) was a kind gift of Dan Hall. Gene disruptions using the *loxP-KAN MX-loxP* cassette (Guldener et al., 1996) were confirmed by genomic PCR. *SKO1* and *HOG1* genes were NH_2-terminally tagged at their chromosomal loci with 3HA epitopes (Schneider et al., 1995). HA-Sko1 is fully functional (Proft et al., 2001), and fusion protein levels are not affected by *hog1* or *tup1* deletions as confirmed by Western blotting using anti HA-antibody. HA-Hog1 confers wild-type growth rate under high osmolarity conditions and therefore is functional. HA-Hog1, Ada2-Myc, and Swi2-Myc protein levels were identical in wild-type and all the deletion strains used in this work as determined by Western blotting.

For analyzing *LacZ* expression (Figure 3), SFY526 (*MATa ura3 his3 ade2 lys2 trp1 leu2 gal4 gal80 URA3::GAL1-lacZ*) (Bartel et al., 1993) and its derivatives MAP34 (*cyc8::KAN MX*) (Proft and Serrano, 1999) and MAP57 (*hog1::KAN MX*) were used. Yeast strains containing appropriate plasmids were grown to saturation in synthetic complete medium without tryptophan and diluted into YPD medium, and exponentially growing cells were then subjected or not to salt shock (0.4 M NaCl for 45 min). β-galactosidase assays were performed as described previously (Gaxiola et al., 1992), and results are presented as mean values obtained from three independent transformants measured in duplicate.

For expression analyses (Figure 6), wild-type strain FT5 (*MAT*α *ura3-52 leu2::PET56 trp1-*Δ*63 his3-*Δ*200*) and strains JDY191 (FT5 with *gcn5::LEU2*; Deckert and Struhl, 2002), JDY193 (FT5 with *swi2::LEU2*; Deckert and Struhl, 2002), MAP69 (FT5 with *tup1::KAN MX*), MAP70 (JDY191 with *tup1::KAN MX*), and MAP71 (JDY193 with *tup1::KAN MX*) were used.

DNA Molecules

Yeast expression plasmids harboring *GST-SKO1* (full length), *GST-SKO1* (315–647), *GST-SKO1* (486–647), and *GST-SKO1* (1–315) under control of the *CUP1* promoter have been described previously (Pascual-Ahuir et al., 2001a), as has the *GAL4-SKO1* expression plasmid pMP235 (Proft and Serrano, 1999). The *SKO1(E)* NH_2-terminal domain (1–315) including S108, T113, S126 to alanine mutations (Proft et al., 2001) was inserted into pYEX-4T. The *GAL4-SKO1(E)* fusion was constructed by inserting the *SKO1* ORF (SmaI/SalI) harboring S108, T113, S126 to alanine mutations into the two-hybrid vector pGBT9 (Clontech). Accordingly, *SKO1*(1–315) and *SKO1E*(1–315) were inserted into pGBT9.

Chromatin Immunoprecipitation

Cells were grown in YPD medium and NaCl was added from a 5 M stock solution to a final concentration of 0.4 M. Chromatin preparation and immunoprecipitation were performed as described previously (Kuras and Struhl, 1999), except that insoluble total chromatin was separated from the soluble fraction by spinning for 2 min in a microcentrifuge. After sonication, soluble chromatin fragments were obtained by spinning for 1 hr in a microcentrifuge. The following antibodies were used for immunoprecipitation: α-HA (12CA5 ascites), polyclonal α-TBP (Kuras and Struhl, 1999), polyclonal α-Myc (06340, Upstate Biotechnology), polyclonal α-Tup1 (gift from S.Y. Roth and D. Edmondson), and α-acetylated H3 (against acetylated histone H3 at K9 and 14, Upstate Biotechnology). PCR primers were designed to amplify the following promoters or ORF regions: *GRE2* ($-310/-145$, spanning both CRE elements); *GRE2* ($-168/+3$, spanning the TATA box); *AHP1* (–474/–328, spanning the CRE element); *AHP1* (–213/+52, spanning the TATA box); *HAL1* (–230/–146, spanning the CRE element); *HAL1* (–170/+52, spanning the TATA box); and Pol1 ORF (+2499/+2717). Quantitative PCR analyses were performed in real time using an Applied Biosystems 7700 sequence detector, using the *POL1* coding sequence as a negative control in all experiments. Each immunoprecipitation was performed twice from different chromatin samples, and the immunoprecipitation efficiency was calculated in triplicate by dividing the amount of PCR product from the immunoprecipitated sample by the amount of PCR product in the input sample prior to immunoprecipitation. All data are presented as fold immunoprecipitation efficiency over the *POL1* coding sequence control. In the case of acetylated H3, relative histone acetylation was calculated by correcting each immunoprecipitated sample for the total DNA input.

For sequential chromatin immunoprecipitations analysis, immunoprecipitated material using α-myc antibody was eluted in FA-lysis buffer (Kuras and Struhl, 1999) containing 1 mg/ml myc peptide (Roche). 90% of the recovered chromatin was subjected to a second immunoprecipitation using α-Tup1 antibody in FA-lysis buffer containing 25 μg/ml Lambda DNA, 50 μg/ml tRNA (*E. coli*), and 5 mg/ml BSA.

Coprecipitation Assays

Full-length and truncated GST-Sko1 fusion proteins were expressed in yeast strain MAP51 (W303-1A with *3HA-HOG1*). Fusion gene expression was induced by addition of $CuSO_4$ to a final concentration of 0.5 mM for 1 hr, and GST pull-downs from cell-free extracts were performed as described previously (Pascual-Ahuir et al., 2001a). GST and GST-Sko1 fusion proteins were detected by Western blot using α-GST polyclonal antibody (Z-5, Santa Cruz Biotechnology, 1:5000), and HA-Hog1 was detected using α-HA monoclonal (12CA5) antibody (1:10,000).

Analysis of mRNA Levels

Yeast strains were grown in YPD to $OD_{600} = 0.8$ and were treated or not with 0.4 M NaCl for 10 min. Total RNA was extracted from 50 ml culture by acid phenol treatment (Iyer and Struhl, 1996) and

DNase digested (RQ1 DNase, Promega). Total RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen) following the manufacturer's instructions and RNase (Roche) treated. As a control, each sample was additionally mock treated (without reverse transcriptase). Appropriately diluted samples were analyzed by quantitative PCR in real time using primers amplifying *GRE2* (nucleotides 1816–2098), *AHP1* (nucleotides 1138–1308), and *TBP1* (nucleotides 521–677).

Acknowledgments

We thank Ramon Serrano for support during the initial phase of this work, Pia Cosma and Kim Nasmyth for myc-tagged Ada2 and Swi2 strains, Jutta Deckert for *gcn5* and *swi2* mutant strains, Daniel Hall for myc-tagged Spt20 strain, Sharon Roth and Diane Edmondson for Tup1 antisera, Gauri Dhavan, Paul Mason, and Zarmik Moqtaderi for help with sequential chromatin immunoprecipitation and reverse transcriptase procedures, and Amparo Pascual-Ahuir for stimulating discussion of the results. This work was supported by an EMBO Long Term Fellowship to M.P. and research grants to K.S. from the National Institutes of Health (GM30186 and GM53720).

Received: February 5, 2002
Revised: May 30, 2002

References

Alepuz, M.P., Jovanovic, A., Reiser, V., and Ammerer, G. (2001). Stress-induced MAP kinase Hog1 is part of transcription activation complexes. Mol. Cell *7*, 767–777.

Balciunas, D., and Ronne, H. (1995). Three subunits of the RNA polymerase II mediator complex are involved in glucose repression. Nucleic Acids Res. *23*, 4421–4425.

Bartel, P.L., Chien, C.-T., Sternglanz, R., and Fields, S. (1993). Elimination of false positives that arise in using the two-hybrid system. Biotechniques *14*, 920–924.

Bhaumik, S.R., and Green, M.R. (2001). SAGA is an essential *in vivo* target of the yeast acidic activator Gal4p. Genes Dev. *15*, 1935–1945.

Bone, J.R., and Roth, S.Y. (2001). Recruitment of the yeast Tup1p-Ssn6p repressor is associated with localized decreases in histone acetylation. J. Biol. Chem. *276*, 1808–1813.

Brown, C.E., Howe, L., Sousa, K., Alley, S.C., Carrozza, M.J., Tan, S., and Workman, J.L. (2001). Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. Science *292*, 2333–2337.

Conlan, R.S., Gounalaki, N., Hatzis, P., and Tzamarias, D. (1999). The Tup1-Cyc8 protein complex can shift from a transcriptional co-repressor to a transcriptional co-activator. J. Biol. Chem. *274*, 205–210.

Cosma, M.P., Tanaka, T., and Nasmyth, K. (1999). Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. Cell *97*, 299–311.

De Vit, M.J., Waddle, J.A., and Johnston, M. (1997). Regulated nuclear translocation of the Mig1 glucose repressor. Mol. Biol. Cell *8*, 1603–1618.

Deckert, J., and Struhl, K. (2001). Histone acetylation at promoters is differentially affected by activators and repressors. Mol. Cell. Biol. *21*, 2726–2735.

Deckert, J., and Struhl, K. (2002). Targeted recruitment of Rpd3 histone deacetylase represses transcription by inhibiting recruitment of Swi/Snf, SAGA, and TBP. Mol. Cell. Biol. *22*, in press.

DeRisi, J.L., Iyer, V.R., and Brown, P.O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. Science *278*, 680–686.

Edmondson, D.G., Smith, M.M., and Roth, S.Y. (1996). Repression domain of the yeast global repressor TUP1 interacts directly with histones H3 and H4. Genes Dev. *10*, 1247–1259.

Ferrigno, P., Posas, F., Koepp, D., Saito, H., and Silver, P. (1998). Regulated nucleo-cytoplasmic exchange of HOG1 MAPK requires the importin β homologs NMD5 and XPO1. EMBO J. *17*, 5606–5617.

Garcia-Gimeno, M.A., and Struhl, K. (2000). Aca1 and Aca2, ATF/CREB activators in *Saccharomyces cerevisiae*, are important for carbon-source utilization but not the response to stress. Mol. Cell. Biol. *20*, 4340–4349.

Gaxiola, R., de Larrinoa, I.F., Villalba, J.M., and Serrano, R. (1992). A novel and conserved salt-induced protein is an important determinant of salt tolerance in yeast. EMBO J. *11*, 3157–3164.

Gromoller, A., and Lehming, N. (2000). Srb7p is a physical and physiological target of Tup1p. EMBO J. *19*, 6845–6852.

Guldener, U., Heck, S., Fielder, T., Beinhauer, J., and Hegemann, J.H. (1996). A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res. *24*, 2519–2524.

Gustin, M.C., Albertyn, J., Alexander, M., and Davenport, K. (1998). MAP kinase pathways in the yeast *Saccharomyces cerevisiae*. Mol. Biol. Rev. *62*, 1264–1300.

Han, S.J., Lee, J.S., Kang, J.S., and Kim, Y.J. (2001). Med9/Cse2 and Gal11 modules are required for transcriptional repression of distinct group of genes. J. Biol. Chem. *276*, 37020–37026.

Huang, M., Zhou, Z., and Elledge, S.J. (1998). The DNA replication and damage checkpoint pathways induce transcription by inhibition of the Crt1 repressor. Cell *94*, 595–605.

Iyer, V., and Struhl, K. (1996). Absolute mRNA levels and transcriptional initiation rates in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *93*, 5208–5212.

Johnson, P.R., Swanson, R., Rakhilina, L., and Hochstrasser, M. (1998). Degradation signal masking by heterodimerization of MATa2 and MATa1 blocks their mutual destruction by the ubiquitin-proteasome pathway. Cell *94*, 217–227.

Keleher, C.A., Redd, M.J., Schultz, J., Carlson, M., and Johnson, A.D. (1992). Ssn6-Tup1 is a general repressor of transcription in yeast. Cell *68*, 709–719.

Komachi, K., Redd, M.J., and Johnson, A.D. (1994). The WD repeats of Tup1 interact with the homeo domain protein a2. Genes Dev. *8*, 2857–2867.

Kuchin, S., and Carlson, M. (1998). Functional relationships of Srb10-Srb11 kinase, carboxy-terminal domain kinase CTDK-1, and transcriptional corepressor Ssn6-Tup1. Mol. Cell. Biol. *18*, 1163–1171.

Kuchin, S., Yeghiayan, P., and Carlson, M. (1995). Cyclin-dependent protein kinase and cyclin homologs SSN3 and SSN8 contribute to transcriptional control in yeast. Proc. Natl. Acad. Sci. USA *92*, 4006–4010.

Kuo, M.-H., vom Baur, E., Struhl, K., and Allis, C.D. (2000). Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. Mol. Cell *6*, 1309–1320.

Kuras, L., and Struhl, K. (1999). Binding of TBP to promoters *in vivo* is stimulated by activators and requires Pol II holoenzyme. Nature *399*, 609–612.

Larschan, E., and Winston, F. (2001). The *S. cerevisiae* SAGA complex functions *in vivo* as a coactivator for transcriptional activation by Gal4. Genes Dev. *15*, 1946–1956.

Natarajan, K., Jackson, B.M., Zhou, H., Winston, F., and Hinnebusch, A.G. (1999). Transcriptional activation by Gcn4 involves independent interactions with the Swi/Snf complex and the Srb/mediator. Mol. Cell *4*, 657–664.

Neely, K.E., Hassan, A.H., Wallberg, A.E., Steger, D.J., Cairns, B.R., Wright, A.P., and Workman, J.L. (1999). Activation domain-mediated targeting of the SWI/SNF complex to promoters stimulates transcription from nucleosome arrays. Mol. Cell *4*, 649–655.

Nehlin, J.O., Carlberg, M., and Ronne, H. (1992). Yeast *SKO1* gene encodes a bZIP protein that binds to the CRE motif and acts as a repressor of transcription. Nucleic Acids Res. *20*, 5271–5278.

Papamichos-Chronakis, M., Conlan, R.S., Gounalaki, N., Copf, T., and Tzamarias, D. (2000). Hrs1/Med3: a Cyc8-Tup1 corepressor target in the RNA polymerase II holoenzyme. J. Biol. Chem. *275*, 8397–8403.

Papamichos-Chronakis, M., Petrakis, T., Ktistaki, E., and Tzamarias, D. (2002). Permanently bound corepressor complex facilitates SAGA-mediated transcriptional activation. Mol. Cell, in press.

Pascual-Ahuir, A., Posas, F., Serrano, R., and Proft, M. (2001a).

Multiple levels of control regulate the yeast cAMP-response-element-binding protein repressor Sko1p in response to stress. J. Biol. Chem. *276*, 37373–37378.

Pascual-Ahuir, A., Serrano, R., and Proft, M. (2001b). Sko1p-repressor and Gcn4p-activator antagonistically modulate stress-regulated transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *21*, 16–25.

Proft, M., and Serrano, R. (1999). Repressors and upstream repressing sequences of the stress-regulation *ENA1* gene in *Saccharomyces cerevisiae*: bZIP protein Sko1p confers HOG-dependent osmotic regulation. Mol. Cell. Biol. *19*, 537–546.

Proft, M., Pascual-Ahuir, A., de Nadal, E., Arino, J., Serrano, R., and Posas, F. (2001). Regulation of the Sko1 transcriptional repressor by the Hog MAP kinase in response to osmotic stress. EMBO J. *20*, 1123–1133.

Reiser, V., Ruis, H., and Ammerer, G. (1999). Kinase activity-dependent nuclear export opposes stress-induced nuclear accumulation and retention of Hog1 mitogen-activated protein kinase in the budding yeast *Saccharomyces cerevisiae*. Mol. Biol. Cell *10*, 1147–1161.

Rep, M., Reiser, V., Gartner, U., Thevelein, J.M., Hohmann, S., Ammerer, G., and Ruis, H. (1999). Osmotic stress-induced gene expression in *Saccharomyces cerevisiae* requires Msn1p and the novel nuclear factor Hot1p. Mol. Cell. Biol. *19*, 5474–5485.

Rep, M., Proft, M., Remize, F., Tamas, M., Serrano, R., Thevelein, J.M., and Hohmann, S. (2001). The *Saccharomyces cerevisiae* Sko1p transcription factor mediates HOG pathway-dependent osmotic regulation of a set of genes encoding enzymes implicated in protection from oxidative damage. Mol. Microbiol. *40*, 1067–1083.

Schneider, B.L., Seufert, W., Steiner, B., Yang, Q.H., and Futcher, A.B. (1995). Use of polymerase chain reaction epitope tagging for protein tagging in *Saccharomyces cerevisiae*. Yeast *11*, 1265–1274.

Smith, R.L., and Johnson, A.D. (2000). Turning genes off by Ssn6-Tup1: a conserved system of transcriptional repression in eukaryotes. Trends Biochem. Sci. *25*, 325–330.

Song, W., Treich, I., Qian, N., Kuchin, S., and Carlson, M. (1996). SSN genes that affect transcriptional repression in *Saccharomyces cerevisiae* encode SIN4, ROX3 and SRB proteins associated with RNA polymerase II. Mol. Cell. Biol. *16*, 115–120.

Tzamarias, D., and Struhl, K. (1994). Functional dissection of the yeast Cyc8-Tup1 transcriptional corepressor complex. Nature *369*, 758–761.

Tzamarias, D., and Struhl, K. (1995). Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 co-repressor complex to differentially regulated promoters. Genes Dev. *9*, 821–831.

Vincent, A.C., and Struhl, K. (1992). ACR1, a yeast ATF/CREB repressor. Mol. Cell. Biol. *12*, 5394–5405.

Wahi, M., and Johnson, A.D. (1995). Identification of genes required for a2 repression in *Saccharomyces cerevisiae*. Genetics *140*, 79–90.

Watson, A.D., Edmondson, D.G., Bone, J.R., Mukai, Y., Yu, Y., Du, W., Stillman, D.J., and Roth, S.Y. (2000). Ssn6-Tup1 interacts with class I histone deacetylases required for repression. Genes Dev. *14*, 2737–2744.

Wu, J., Suka, N., Carlson, M., and Grunstein, M. (2001). TUP1 utilizes histone H3/H2B-specific HDA1 deacetylase to repress gene activity in yeast. Mol. Cell *7*, 117–126.

Yudkovsky, N., Logie, C., Hahn, S., and Peterson, C.L. (1999). Recruitment of the SWI/SNF chromatin remodeling complex by transcriptional activators. Genes Dev. *13*, 2369–2374.

Zaman, Z., Ansari, A.Z., Koh, S.S., Young, R.A., and Ptashne, M. (2001). Interaction of a transcriptional repressor with the RNA polymerase II holoenzyme plays a crucial role in repression. Proc. Natl. Acad. Sci. USA *98*, 2550–2554.