Selective Requirement for SAGA in Hog1-Mediated Gene Expression Depending on the Severity of the External Osmostress Conditions[▽]

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Regulation of gene expression by the Hog1 stress-activated protein kinase is essential for proper cell adaptation to osmstress. Hog1 coordinates an extensive transcriptional program through the modulation of transcription. To identify systematically novel components of the transcriptional machinery required for osmstress-mediated gene expression, we performed an exhaustive genome-wide genetic screening, searching for mutations that render cells osmosensitive at high osmolarity and that are associated with reduced expression of osmoresponsive genes. The SAGA and Mediator complexes were identified as putative novel regulators of osmstress-mediated transcription. Interestingly, whereas Mediator is essential for osmstress gene expression, the requirement for SAGA is different depending on the strength of the extracellular osmotic conditions. At mild osmolarity, SAGA mutants show only very slight defects on RNA polymerase II (Pol II) recruitment and gene expression, whereas at severe osmotic conditions, SAGA mutants show completely impaired RNA Pol II recruitment and transcription of osmoresponsive genes. Thus, our results define an essential role for Mediator in osmstress gene expression and a selective role for SAGA under severe osmstress. Our results indicate that the requirement for a transcriptional complex to regulate a promoter might be determined by the strength of the stimuli perceived by the cell through the regulation of interactions between transcriptional complexes.

In eukaryotic cells, stress-activated protein kinases (SAPKs) play an essential role for proper cell adaptation to extracellular stimuli (16). Exposure of cells to high osmolarity results in rapid activation of a conserved family of SAPKs, which includes mammalian p38 and yeast Hog1 (5, 26). In *Saccharomyces cerevisiae*, the SAPK Hog1 elicits the program for cell adaptation required for cell survival in response to osmstress, which includes a global change in gene expression (5, 11, 30).

It has been reported that SAPKs can modify gene regulation by direct phosphorylation of transcription factors, both activators and repressors, and can modulate factors involved in chromatin remodeling and structure (5, 16). In yeast, Hog1 participates initially to make cells competent for gene expression (23), and in addition, it is intimately recruited to chromatin in response to osmstress (2). Once bound to chromatin, apart from its role in elongation (20), Hog1 is able to regulate transcription initiation by several mechanisms: direct phosphorylation of several transcription factors (i.e., Sko1 and Smp1) (6, 21, 21), recruitment of the Rpd3 histone deacetylase complex, which promotes the modification of the chromatin at the promoters (7), and stimulation of the recruitment of RNA polymerase II (Pol II) (1). Thus, the specific chromatin association of Hog1 with stress-responsive promoters has shown an important function for this kinase in the regulation of transcription initiation.

In eukaryotic cells, transcription initiation by RNA polymerase II is a highly regulated process that requires the coordinated activities of a large number of factors. An important and conserved class of factors consists of coactivators, multiprotein complexes that are recruited to cognate promoters, such as SAGA (Spt-Ada-Gcn5), TFIID, and Mediator. These coactivators lead to Pol II recruitment and subsequent preinitiation complex (PIC) formation to facilitate transcription initiation (14). SAGA is a multisubunit cofactor for Pol II transcription that regulates chromatin, and it is required to deliver TATA-binding protein to promoters (27). The molecular architecture of SAGA has depicted different subcomplexes with distinct regulatory functions (31). It has been reported that SAGA and TFIID make overlapping contributions to the expression of Pol II-transcribed genes (17). However, TFIID function seems to predominate in the transcription of ~90% of the yeast genome, whereas SAGA might have an important role in the transcription of ~10% of the genes, largely stress-induced and highly regulated genes (13). Strikingly, genes that are commonly up-regulated during general environmental stress are strongly biased toward being SAGA dominated. This suggests that SAGA might be particularly important for genes that respond to stress. There are several aspects of SAGA that are still uncertain, such as what determines SAGA specificity for certain genes and its relationship in time and space with other components of the PIC (e.g., SWI/SNF, Mediator, TFIID, etc.). For instance, in the *GAL1* promoter, SAGA recruitment by Gal4 precedes that of Mediator (4), whereas at the *HO* gene, there is simultaneous recruitment of SWI/SNF and Mediator which precedes SAGA recruitment (9). Actually, recent results suggest that Mediator is not a stoichiometric component of the basic Pol II machinery but rather a complex selec-

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tively required by specific activators. Furthermore, it is also found in some inactive promoters prior to transcription to mark regulatory regions ahead of input stimulatory signals (3, 8). Thus, it seems that the ordered assembly of the PIC varies depending on specific promoters and activators.

In response to osmotic stress, the ATF/CREB-related transcription factor Sko1 regulates several genes under the control of the Hog1 mitogen-activated protein kinase (MAPK) (19, 29, 21). Interestingly, Hog1 phosphorylation switches Sko1 activity from a repressing to an activating state, which involves the recruitment of the SWI/SNF and SAGA complexes (22). However, the relevance of SAGA in osmotic stress transcription and how it is targeted to the osmotic stress promoters remain unclear.

In this work, by an exhaustive genetic approach, we have defined the roles of the SAGA and Mediator complexes in osmoadaptation. SAGA and Mediator are targeted by Hog1 at the osmosensitive genes, where they play a critical role in osmotic stress gene expression. Interestingly, whereas Mediator is essential for proper gene induction under any osmotic stress condition, the role of SAGA at the promoters seems to be stress dependent, which results in a differential promoter regulation in response to the strength of the stimuli perceived by the cell.

MATERIALS AND METHODS

Yeast strains and plasmids. (i) **Yeast strains.** Wild-type strain BY4741 (*MATa his3-Δ1 leu2-Δ0 met15-Δ0 ura3-Δ0*) and derivatives containing chromosomally integrated *ADA2-TAP*, *ADA1-TAP*, *SPT20-TAP*, *SPT3-TAP*, *MED2-TAP* and *SPT8-TAP*, YEN233 (*MATa SPT20-MYC9-KAN*), YMZ45 (*MATa SRB4-HA6-HIS3*), YMZ53 (*MATa hog1::kanMX4 SRB4-HA6-HIS3*), YMZ33 (*MATa HOG1-HA6-HIS3*), YMZ34 (*MATa spt20::kanMX4 HOG1-HA6-HIS3*), YEN225 (*MATa pgd1::kanMX4 HOG1-HA6-HIS3*), YEN239 (*MATa SPT20-HA6-HIS3*), YEN240 (*MATa pgd1::kanMX4 SPT20-HA6-HIS3*), YMZ46 (*MATa spt20::kanMX4 SRB4-HA6-HIS3*), YMZ48 (*MATa HOG1-MYC18-KAN SRB4-HA6-HIS3*), YEN252 (*MATa spt20::kanMX4 SRB4-MYC13-TRP*), YMZ65 (*MATa sin4::kanMX4 SPT20-HA6-HIS*), YMZ66 (*MATa TBPI-MYC18-HIS*), YMZ68 (*MATa spt20::kanMX4 TBPI-MYC18-HIS*), YMZ69 (*MATa spt20::kanMX4 MED2-TAP*). The strain YEN234 (*MATa ura3 leu2 trp1 his3 hog1::TRP1 spt20-MYC9-KAN*) is derived from wild-type S288C. Strain SGY190 (*SRB4-MYC13-TRP*) and the *taf1^{ts}*, *taf2^{ts}*, and *srb4^{ts}* strains and their respective wild-type strains were kindly provided by M. R. Green (University of Massachusetts).

(ii) **Plasmids.** The pRS426TEG1, pRS426TEG1-Hog1, pRS426TEG-Srb4 plasmids, expressing glutathione *S*-transferase (GST), GST-Hog1, and GST-Srb4, were described previously (1).

High-throughput osmotic screen. An ordered array of 4,644 *MATa* viable haploid yeast gene deletion mutants (*Saccharomyces* Gene Deletion Project, obtained from EUROSCARF) in duplicate was replica pinned onto yeast extract-peptone-dextrose (YPD) and YPD plus 2.2 M sorbitol. The screen was performed by using an automated system. For automated arraying, yeast cells were transferred using the Biomek FX robot and a 384-floating-pin replicator (Biomek FX HDR 384-pin plate) as described previously (28). The screen was performed two times, and plates were incubated at 30°C for 3 days before scoring.

Chromatin immunoprecipitation. Chromatin immunoprecipitation was performed as described previously (2, 15). Yeast cultures were grown to early log phase (optical density at 600 nm [OD₆₀₀] = 0.6 to 1.0) before aliquots of the culture were exposed to osmotic-stress treatment (0.4 M or 1.2 M NaCl) for various lengths of time. For cross-linking, yeast cells were treated with 1% formaldehyde for 20 min at room temperature. Primer mixes were adjusted for balanced signals. We used oligonucleotides to amplify regions of *STL1* (−181/+117)/(−372/−112) and +1000/+1280) to analyze binding of the Pol II, Hog1, SAGA, and Mediator proteins to the promoter or coding region, respectively. As internal controls, *TEL1* (chromosome VI; coordinates 269606 to 269783) and *GAL1* (−273/+132) were used. Immunoprecipitation efficiencies were calculated in triplicate by dividing the amount of PCR product in the immunoprecipitated sample by the amount of control. Data are presented as *n*-fold immunoprecipitation relative to results for the *TEL1* or *GAL1* control.

GST pull-down experiments. To analyze the association of Hog1 with components of the SAGA complex, two milligrams of yeast extract, from cells expressing GST or GST-Hog1 and specific TAP-tagged proteins, in buffer A (50 mM Tris-HCl [pH 7.5], 15 mM EDTA, 15 mM EGTA, 0.1% Triton X-100, 150 mM NaCl, 2 mM dithiothreitol plus antiproteases and phosphatase inhibitors) was incubated with 50 μl of glutathione Sepharose 4B beads overnight at 4°C. To analyze the association of SAGA with Mediator, two milligrams of yeast extract from cells expressing GST or GST-Srb4 and Spt20-Myc was treated as above. The beads were washed extensively with buffer A, resuspended in loading buffer, and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The antibody used to detect the TAP-tagged proteins was the PAP antibody from Sigma. Similarly, coimmunoprecipitation of Hog1 with Mediator proteins was performed with two milligrams of yeast extract from cells expressing GST or GST-Hog1 and Srb4-Myc and treated as above. The presence of Mediator in the coimmunoprecipitates was detected with the monoclonal Myc antibody.

Gel filtration analysis. One liter of culture of logarithmically growing cells expressing tagged Srb4-Myc or Med2-TAP was subjected to osmotic stress (1.2 M NaCl, 100 min) and harvested by centrifugation, and cells were lysed in the presence of buffer A. Crude extracts were cleared, and the supernatant was passed through a 0.45-μm filter. Five milligrams of protein was loaded onto a Superose 6 HR 10/30 column (Amersham Pharmacia Biotech). The flow rate was adjusted to 0.3 ml/min, and 0.5-ml fractions were collected. Elution profiles of tagged proteins were analyzed by Western analysis and compared to the elution profile of known standards.

Northern blot analysis. Yeast strains were grown to mid-log phase in rich medium and then subjected to osmotic shock (0.4 M NaCl, 1.2 M NaCl, or 1.8 M sorbitol) for various lengths of time. Total RNA and expression of specific genes were analyzed using labeled PCR fragments containing the entire open reading frame (ORF) of *STL1* (1.7 kbp), *CTT1* (1.7 kbp), *GRE2* (1.0 kbp), *RPL28* (0.45 kbp), *HSP12* (0.3 kbp), or *TRX1* (0.3 kbp) or the noncoding exon of *RDN18-1* (1.8 kbp). Signals were quantified using a Fujifilm BAS-5000 phosphorimager.

RESULTS

SAGA is required for cell survival at high osmolarity. The ability of cells to survive at high osmolarity depends on the HOG signaling pathway and the control of gene expression exerted by the Hog1 MAPK. The identification of specific transcription factors under the control of the MAPK has shed some light on how gene expression is controlled by this MAPK. However, novel mechanisms involving the direct effect of the MAPK at responsive promoters have been shown to play a critical role in order for cells to properly execute the gene expression program required for adaptation to stress. To identify systematically novel activities required for full expression of the gene program required for cell survival upon osmotic stress, we performed an exhaustive genome-wide genetic screening, searching for mutations that render cells osmosensitive at high osmolarity.

Thus, we performed a high-throughput screen by robotically pinning an ordered array of ≈4,700 haploid yeast deletion mutants onto YPD or YPD plus 2.2 M sorbitol. Osmosensitive mutants lead to the formation of smaller colonies when grown on sorbitol-containing medium. The screen was performed twice by using an automated system, and a total of 179 mutants were scored as osmosensitive in both cases. In addition, we also scored the mutant cells for defective expression of an osmotic stress gene reporter system (*STL1::LacZ*) to link specific mutations to regulation of gene expression (not shown). Some of the mutants identified were already known as important genes for the response to osmotic stress (e.g., *HOG1*, *PBS2*, and *GPD1* genes). Analysis of this large-scale data set was performed using the Internet-based tool “FunSpec” (<http://funspec.med.utoronto.ca>), which identified protein complexes enriched on the list of osmosensitive mutant strains (25). As

TABLE 1. Protein complexes of osmotic stress-sensitive deletion strains^a

Category	P value	No. of genes	
		Input	Total
Histone deacetylase complexes	1.24E-02	5	12
SAGA complex	3.00E-02	5	14
Transcription complexes/transcriptosome	1.13E-04	16	193
Translation complexes	1.30E-03	16	240
Gim complexes	3.15E-03	4	5
Mitochondrial processing complexes	8.98E-02	3	4
Mitochondrial ribosomes	2.52E-03	6	49
Class C Vps protein complex	4.69E-03	2	4
Tubulin-associated motor proteins	6.61E-03	3	14

^a MIPS protein complexes (315 categories). Mutants of the SAGA complex are overrepresented in a high-throughput osmotic sensitivity screen. *P* values represent the probability that the intersection of a given list with any given functional category occurs by chance. Input, input cluster in a given category; total, total in a given category.

shown in Table 1, several complexes without a previously defined role in the osmostress response were identified: protein complexes involved in different biological processes, such as cytoskeleton protein binding (Gim complexes), regulation of translation (mitochondrial processing complexes), and vacuole biogenesis (class C Vps protein complex). We also found different complexes related to regulation of gene expression that were required for cell viability upon high osmolarity: the previously characterized Rpd3 histone deacetylase complex (7) and two major transcriptional complexes, the SAGA and Mediator complexes.

To characterize in more detail the relevance of SAGA for survival at high osmolarity, we performed a comprehensive phenotypic analysis of a full set of SAGA mutants for growth at high osmolarity (1.2 M NaCl or 2.2 M sorbitol). As shown in Fig. 1, deletion of *ADA1*, *SPT7*, and *SPT20* strongly affected cell growth at high osmolarity and deletion of *ADA2*, *ADA3*, *SPT3*, and *SPT8* caused a somewhat reduced survival at high osmolarity, whereas deletion of other components of SAGA resulted in cells with minor defects in growth at high osmolarity. It is worth noting that mutations that affect SAGA structural integrity (31) (i.e., *Spt20*, *Spt7*, and *Ada1*) are essential for survival at high osmolarity.

Mutation of SAGA affects gene expression in response to high osmolarity. The requirement of SAGA for cell survival at high osmolarity prompted us to analyze the expression of several osmosensitive genes upon stress. In response to mild osmostress conditions (0.4 M NaCl), mutations in SAGA genes resulted in cells that displayed a slight delay in expression of several osmosensitive genes (i.e., *STL1* and *CTT1*) but without a general decrease on gene expression (Fig. 1B; also data not shown). Correspondingly, these mutant cells did not display clear growth defects at 0.4 M NaCl (data not shown). However, because SAGA-deficient strains were unable to grow under severe osmotic conditions (1.2 M NaCl), we tested whether expression of specific osmosensitive genes was affected with severe osmolarity. Indeed, expression of *STL1*, *CTT1*, and *GRE2* was strongly reduced by deletion of *SPT20*, to an extent similar to that of elimination of HOG signaling by deletion of the *PBS2* MAPK kinase (Fig. 1C). Similar results were observed when cells were subjected to 1.8 M sorbitol (Fig.

1D). Therefore, the SAGA complex is critical for osmostress gene expression under severe osmolarity but is dispensable for transcription under mild osmotic conditions.

It is known that, for instance, expression of *STL1* and *CTT1* depends on different transcription factors (i.e., Hot1 and Msn2/Msn4, respectively). To confirm that under severe stress conditions (1.2 M NaCl), expression of these genes is mediated by the known transcription factors, we tested *STL1* and *CTT1* gene expression in deficient *hot1Δ* and *msn2Δ msn4Δ* strains. Indeed, expression of *STL1* and *CTT1* also depends on Hot1 and Msn2/Msn4, respectively, in response to severe stress

FIG. 1. SAGA complex is essential for osmostress gene expression at high osmolarity. (A) Mutations on specific SAGA components render cells osmosensitive. Wild type (wt) and indicated mutant strains were spotted on YPD plates without (Control) or with 1.2 M NaCl (NaCl) or 2.2 M sorbitol (Sorbitol). Growth was scored after 3 days. (B) Deletion of genes encoding components of SAGA complex causes delay in transcription under mild osmostress conditions. The wild-type strain and indicated mutant strains were grown to mid-log phase in rich medium and then subjected to osmotic shock (0.4 M NaCl) for indicated times. Total RNA was assayed by Northern blotting for *STL1*, *CTT1*, and *RPL28* (as a loading control). (C and D) Deletion of genes encoding components of SAGA complex abolishes transcription under severe osmostress conditions. Wild-type and mutant strains were grown to mid-log phase in rich medium and then subjected to osmotic shock (1.2 M NaCl or 1.8 M sorbitol) for indicated times. Total RNA was assayed as for panel B.

FIG. 2. SAGA is required under severe stress conditions irrespective of the activator responsible for osmotic stress gene induction. (A) Wild-type (wt) and *hot1Δ* strains were grown to mid-log phase in rich medium and then subjected to osmotic shock (1.2 M NaCl) for indicated times. Total RNA was assayed by Northern blotting for *STL1* and *RDN18-1* (as a loading control). (B) Wild-type and *msn2/4Δ* strains were grown as for panel A. Total RNA was assayed by Northern blotting for *CTT1* and *RDN18-1* (as a loading control).

(Fig. 2). Therefore, SAGA is required under severe stress irrespective of the activator responsible for gene induction.

It was proposed that stress-responsive genes were controlled by SAGA rather than TFIID (13). Correspondingly, mutation of specific components of the TFIID complex, such as *TAF1* and *TAF2*, did not alter osmotic stress gene expression at high osmolarity (Fig. 3). Under the same conditions, expression of the TFIID-dependent gene (*TRX1*) was abolished in the *taf1ts* mutant (Fig. 3, lower panel). These results are in agreement with a previous report that showed that in response to osmotic stress, *CTT1* expression was not affected by inactivation of *TAF1* or *TAF5* (18).

FIG. 3. Osmotic stress gene expression is not affected by mutations of TFIID complex. Wild-type (wt), *taf1ts*, or *taf2ts* strain was grown at 25°C until an OD₆₆₀ of 0.5 was reached, shifted to 37°C for 90 min, and then subjected to osmotic stress (1.2 M NaCl) for indicated times. Total RNA was assayed by Northern blotting for *STL1*, *CTT1*, and *RDN18-1* (as a loading control). Expression of TFIID-dependent gene *TRX1* was monitored by Northern blotting with the wild-type or *taf1ts* strain without NaCl treatment (lower panel).

FIG. 4. Mediator is critical for gene expression in response to osmotic stress. (A) Deletion of genes encoding for components of Mediator complex render cells osmosensitive. The wild-type (wt) and indicated mutant strain were spotted on YPD plates with or without 1.2 M NaCl (NaCl) or 2.2 M sorbitol (Sorbitol). Growth was scored after 3 days. (B) Deletion of genes encoding Mediator subunits causes impaired transcription already under mild osmotic stress conditions. Wild-type and indicated mutant strains were grown to mid-log phase in rich medium and then subjected to osmotic shock (0.4 M NaCl) for indicated times. Total RNA was assayed by Northern blotting for *STL1*, *CTT1*, *HSP12*, and *RPL28* (as a loading control). (C and D) Inactivation of *Srb4* Mediator subunit causes impaired transcription under mild and severe osmotic stress conditions. Wild-type and *srb4ts* strains were grown at 25°C until an OD₆₆₀ of 0.5 was reached and shifted to 37°C for 90 min. Cultures were then subjected to osmotic stress (0.4 or 1.2 M NaCl) for indicated times. Total RNA was assayed as for panel B.

The Mediator complex is essential for Hog1-dependent gene expression. Mediator is a highly conserved complex that is a component of the transcriptional machinery (14). Because the initial screen yielded as osmosensitive mutants basic subunits of the Mediator complex, we tested the relevance of Mediator for osmotic stress-mediated gene expression. Initially, we tested cell survival upon high osmolarity and osmotic stress gene expression in several mediator mutants (i.e., *pgd1Δ*, *med2Δ*, *gal11Δ*, and *srb4-1* mutants). As shown in Fig. 4A and B, mutant cells

FIG. 5. Hog1 is required for recruitment of SAGA, Mediator, and RNA Pol II to osmoresponsive promoters under mild and severe osmotic conditions. (A) Recruitment of SAGA, Mediator, and RNA Pol II depends on Hog1 MAPK under mild osmotic conditions. Wild-type (wt) or *hog1Δ* mutant strain expressing Spt20-Myc (left) or Srb4-HA (center) or non-epitope tagged (right) was grown to mid-log phase and treated with 0.4 M NaCl for 5 min. Proteins were immunoprecipitated with anti-Myc, HA, or RNA Pol II antibody. Binding to *STL1* promoter was determined by ChIP. *n*-fold induction of treated (open bars) or untreated (filled bars) cultures normalized to a telomere- or *GAL1*-specific band (internal controls) is shown. (B) Recruitment of SAGA, Mediator, and RNA Pol II depends on Hog1 MAPK under severe osmotic conditions. Strains as in panel A were grown and treated with 1.2 M NaCl for 75 min. Samples for ChIP analyses were processed as for panel A.

were sensitive to osmotic stress and already displayed a dramatic impairment of gene induction under mild osmotic conditions (0.4 M NaCl). Similar results were obtained with the *SRB4-1* mutant (*srb4⁴⁵*), in which gene expression is dramatically affected at both 0.4 and 1.2 M NaCl (Fig. 4C and D). Thus, in contrast to the case with SAGA, the Mediator complex is fully required for Hog1-induced gene expression under any osmotic stress condition.

Recruitment of SAGA, Mediator, and Pol II to osmotic stress promoters upon stress is mediated by Hog1. The relevance of SAGA and Mediator in osmotic stress gene expression prompted us to analyze the presence of these complexes at osmoresponsive promoters. To study the binding of SAGA and Mediator to osmotic stress promoters and the relevance of Hog1 in their recruitment, we utilized chromatin immunoprecipitation (ChIP) and followed the binding of the transcription machinery at the osmoresponsive *STL1*, *GRE2*, or *CTT1* promoter before or after osmotic stress. Chromatin from wild-type and *hog1Δ* yeast strains expressing functional epitope-tagged Spt20 or Srb4 components of the SAGA and Mediator complexes from their natural locus were immunoprecipitated and analyzed by PCR. As shown in Fig. 5, Spt20, Srb4, and Pol II (Rpb1) were present at osmoresponsive genes only in response to osmotic stress, and their recruitment to osmotic stress promoters was completely dependent on Hog1. It is worth noting that binding of

FIG. 6. Recruitment of SAGA, Mediator, and RNA Pol II at osmoresponsive promoters occurs after Hog1 binding to *STL1* promoter. Wild-type strains carrying Hog1-Myc, Spt20-Myc, or Srb4-HA were grown to mid-log phase and treated with 1.2 M NaCl for indicated times. Proteins were immunoprecipitated with anti-Myc, HA, or RNA Pol II antibody (W8G16). Binding to *STL1* promoter was determined by ChIP. Relative intensities of PCR fragments normalized to the telomere-specific band (internal control) are shown. Data represent means and standard deviations for three independent experiments.

SAGA, Mediator, and Pol II occurred under mild and severe osmotic stress conditions (Fig. 5A and B). Thus, Hog1 is essential for the recruitment of SAGA, Mediator, and Pol II upon osmotic stress.

To analyze the kinetics of the recruitment of the transcription components (Hog1, SAGA, Mediator, and Pol II) at the osmoresponsive promoters, we followed their binding to the *STL1* promoter in response to high osmolarity. Although binding of Hog1 to *STL1* seemed to precede binding of the other components, subsequent binding of SAGA and Mediator was virtually identical to Pol II entry at both 0.4 M NaCl (not shown) and 1.2 M NaCl (Fig. 6). Taken together, our data support the idea that PIC assembly at osmoresponsive promoters depends on the presence of the MAPK and that once it is recruited to the stress promoters, binding of the transcription complexes is rapidly stimulated to induce gene expression.

Recruitment of Mediator depends on Hog1 and SAGA under severe osmotic stress conditions. To establish the order of recruitment of SAGA and Mediator with respect to Hog1 at osmotic stress promoters, we performed ChIP analysis using several mutant strains. Initially, we tested whether binding of Hog1 to promoters was dependent on SAGA or Mediator in addition to the Hot1 transcription factors. Chromatin from wild-type, *hot1Δ*, *spt20Δ*, and *pgd1Δ* yeast strains expressing functional epitope-tagged Hog1 from the natural locus was immunoprecipitated with antibodies against the hemagglutinin (HA) epitope and analyzed by PCR. As shown in Fig. 7A, binding of Hog1 to the *STL1* promoter in response to mild osmotic stress conditions was abolished by deletion of *HOT1*, which encodes the transcription factor that mediates *STL1* expression upon stress (2), whereas it was not affected by deletion of *SPT20* or *PGD1* (left panel). Similar results were obtained when binding of Hog1 was analyzed under severe osmotic stress conditions (right panel). This indicates that Hog1 recruitment to osmotic stress promoters is independent of SAGA

FIG. 7. Interaction of Hog1 with *STL1* promoter initiates sequential recruitment of proteins at osmostress promoters. (A) Recruitment of Hog1 to osmostress promoters is independent of SAGA and Mediator complexes. Wild-type, *hot1Δ*, *spt20Δ*, or *pgd1Δ* strain expressing Hog1-HA was grown and subjected to 0.4 M NaCl for 5 min (left panel) or 1.2 M NaCl for 75 min (right panel). Binding to *STL1* promoter was determined by ChIP. *n*-fold induction of treated (open bars) or untreated (filled bars) cultures normalized to a telomere-specific band (internal control) is shown. (B) SAGA and Mediator complexes are recruited independently to osmostress promoters at mild osmostress conditions. Wild-type, *pgd1Δ*, or *sin4Δ* strain expressing Spt20-HA (left) or wild-type or *spt20Δ* strain expressing Srb4-HA (right) was grown and subjected to osmostress (0.4 M NaCl for 5 min). Binding to *STL1* promoter was determined as for panel A. (C) Recruitment of Mediator to osmostress promoters is reduced in SAGA mutants under severe osmostress conditions. Strains as in panel B were grown and treated with 1.2 M NaCl for 75 min. Binding to *STL1* promoter was determined as for panel A.

and Mediator, and it is consistent with the previous observation that binding of Hog1 precedes binding of the two complexes at the promoters and the fact that Hog1 is essential for their recruitment (Fig. 5 and 6).

To establish the relationship between SAGA and Mediator complexes, we performed ChIP analysis of the SAGA compo-

FIG. 8. Recruitment of SAGA and Mediator complexes also occurs at coding regions of stress-responsive gene upon induction. (A) SAGA complex is recruited to the coding region of *STL1* upon osmotic stress. Wild-type strains expressing Ada2-Myc or Spt20-Myc were grown and subjected to mild (0.4 M NaCl for 5 min, left) or severe (1.2 M NaCl for 75 min, right) osmotic stress. Binding to the *STL1* ORF was determined by ChIP. *n*-fold induction of treated (open bars) or untreated (filled bars) cultures normalized to a telomere- or *GAL1*-specific band (internal controls) is shown. (B) Mediator complex is recruited at the coding region of osmostress genes. Wild-type and *spt20Δ* strains expressing Srb4-HA were grown and subjected to mild (0.4 M NaCl for 5 min, left) or severe (1.2 M NaCl for 75 min, right) osmotic stress. Binding to the *STL1* ORF was determined as for panel A. (C) Binding of Tbp1 is restricted to promoter regions of osmostress genes. The wild-type strain expressing Tbp1-Myc was grown and subjected to mild (0.4 M NaCl for 5 min, left) or severe (1.2 M NaCl for 75 min, right) osmotic stress. Binding to the *STL1* promoter and ORF was determined as for panel A.

nent Spt20 on wild-type, *pgd1Δ*, and *sin4Δ* strains and analyzed binding of Srb4 to wild-type and *spt20Δ* strains. ChIP analyses showed that binding of the SAGA subunit Spt20 to *STL1* upon osmostress was independent of the presence of Mediator under either mild or severe stress conditions (Fig. 7B and C). Similar results were observed for Ada2 (data not shown). Interestingly, although binding of the Srb4 Mediator subunit to *STL1* was almost identical for wild-type and *spt20Δ* strains under mild osmostress (0.4 M NaCl), binding of Srb4 was significantly reduced in the *spt20Δ* cells where they were ex-

FIG. 9. Hog1 coprecipitates with SAGA and Mediator complexes. (A) TAP-tagged Ada2, Ada1, Spt20, Spt3 or Spt8 strain expressing GST or GST-Hog1 was subjected to brief osmotic shock (10 min, 0.4 M NaCl). GST proteins were pulled down by glutathione-Sepharose 4B, and the presence of TAP proteins was probed by immunoblotting using anti-TAP (PAP; Sigma) (upper panel). Prec., precipitated proteins. Total extract represents <20% of total input protein (middle panel). Amounts of precipitated GST proteins were detected using anti-GST (lower panel). (B) Interaction of SAGA with Hog1 is conserved in Mediator-deficient strain. Wild-type (wt) or *pgd1Δ* strain bearing Spt20-HA was transformed with plasmid expressing GST or GST-Hog1 and grown in selective medium. Cultures were either untreated or treated with 0.4 M NaCl for 10 min. GST proteins were pulled down as in panel A. (C) Interaction of Mediator with Hog1 is impaired in SAGA-deficient strain under severe osmotic stress conditions. Wild-type or *spt20Δ* strain bearing Srb4-Myc was transformed with plasmid expressing GST or GST-Hog1 and grown in selective medium. Cultures were either untreated or treated with 0.4 M NaCl for 10 min or 1.2 M NaCl for 100 min. GST proteins were pulled down as in panel A.

posed to severe osmotic stress (1.2 M NaCl) (Fig. 7B and C). Therefore, in addition to the requirement of the Hog1 MAPK for binding, SAGA is required for efficient recruitment of Mediator under severe stress conditions.

Recently it was reported that Hog1 is recruited to coding regions of stress-responsive genes upon activation (20). In addition, recent reports have shown that Mediator is present on many coding regions of actively transcribed genes (3, 8). Global ChIP studies have shown that the SAGA subunit Gcn5

seems to be enriched on the ORFs of transcribing genes, although to a lesser extent than in promoters (24). Interestingly, a recent report has shown that several SAGA subunits are present on the *GAL1* ORF upon galactose induction and on the *ARG1* and *ARG4* ORFs in response to amino acid deprivation (10). We have followed binding of Mediator and SAGA by ChIP analyses and found that under mild and severe osmotic stress conditions, both complexes are recruited to the coding region of *STL1* (Fig. 8A and B). Under the same conditions,

binding of Tbp1 is restricted to promoters (Fig. 8C). It is worth noting that binding of Mediator to the *STL1* ORF is reduced in an *spt20Δ* strain under severe osmostress conditions, which is consistent with the diminished recruitment observed at the promoter (Fig. 7C and 8B).

Therefore, in addition to the requirement of the Hog1 MAPK to promote binding of Mediator to stress-responsive genes, SAGA is required for efficient recruitment of Mediator under severe stress conditions.

Binding of Hog1 to Mediator is reduced in SAGA-deficient cells. SAGA is recruited to different promoters through association to specific activators (27). We next tested whether Hog1 was able to interact with the SAGA complex by performing GST pull-down experiments with extracts from osmotically stressed cells expressing GST-Hog1 and chromosomally TAP-tagged versions of Ada2, Ada1, Spt7, Spt20, Spt3, and Spt8. In all cases, GST-Hog1 but not the GST control coprecipitated the TAP-tagged SAGA component (Fig. 9A). To analyze whether interaction of Hog1 with SAGA was mediated by the Mediator, we performed GST pull-down experiments with extracts from wild-type and *pgd1Δ* strains expressing GST-Hog1 and chromosomally HA-tagged Spt20. Hog1 coprecipitated Spt20 similarly with the two strains (Fig. 9B). Therefore, these results indicate that Hog1 associates with the SAGA complex and this association is independent of the presence of Mediator, which is in agreement with the ChIP data.

We then tested the binding of Hog1 to Mediator. We performed GST pull-down experiments using extracts from cells subjected to osmostress expressing GST-Hog1 and chromosomally Myc-tagged *Srb4*. As shown in Fig. 9C, Hog1 precipitates with *Srb4* and the interaction is more efficient when cells are subjected to osmostress. Similar results were obtained when yeast extracts were treated with DNase, suggesting that binding occurs in non-chromatin-associated complexes (Fig. 10B). Based on the ChIP data, we predicted that binding of Mediator to Hog1 should be similar in a wild-type strain and a SAGA-deficient strain under mild osmostress conditions, but it could be reduced under severe osmostress conditions. To test this prediction, we analyzed interaction of Hog1 with *Srb4* in wild-type and *spt20Δ* strains at 0.4 M and 1.2 M NaCl. Indeed, binding of Mediator to Hog1 is not affected by deletion of *spt20* under mild osmostress conditions (0.4 M NaCl), but it is dramatically reduced under severe osmotic conditions (1.2 M NaCl) (Fig. 9C). Gel filtration analyses showed that under severe stress conditions, the integrity of the Mediator complex in a SAGA mutant is maintained (Fig. 10A). It is worth noting that SAGA and Mediator still interact under severe stress conditions, and this interaction does not seem to be affected by deletion of *HOG1* (Fig. 10C). Thus, taken together, we propose that SAGA is critical in promoting the interaction of Mediator with Hog1 under severe osmostress conditions.

Recruitment of RNA Pol II to osmostress promoters depends on SAGA under severe osmostress conditions. The fact that gene expression and cell survival are severely affected by inactivation of Mediator, whereas SAGA mutants are affected only under severe osmostress conditions, suggests that the recruitment of RNA Pol II could be impaired either by the lack of Mediator or due to severe osmostress in SAGA-deficient cells.

Thus, we initially tested binding of Pol II (Rpb1) to osmo-

FIG. 10. (A) Integrity of Mediator complex is maintained in *spt20Δ* mutant strain under severe stress conditions. Extracts from *SrB4-Myc*- and *Med2-TAP*-tagged strains were subjected to gel filtration through Superose 6 HR 10/30 column. Fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and proteins detected with antibodies against Myc and TAP epitopes. wt, wild type. (B) Coimmunoprecipitation of Mediator with Hog1 occurs in non-chromatin-associated complexes. The wild-type strain bearing *SrB4-Myc* was transformed with plasmid expressing GST or GST-Hog1 and grown in selective medium. Cultures were treated with either 0.4 M NaCl for 10 min or 1.2 M NaCl for 100 min. Protein extracts were untreated (–) or treated (+) with DNase for 30 min at 37°C before GST proteins were pulled down by glutathione-Sepharose beads. *SrB4-Myc*-containing (top panel) or GST-containing (bottom panel) proteins were detected by Western blotting using antibodies against Myc and GST. Prec., precipitated proteins. Total extract represents <20% of total input protein (middle panel). (C) SAGA and Mediator complexes coprecipitate in Hog1-independent manner. Wild-type or *hog1Δ* strain bearing *Spt20-Myc* was transformed with plasmid expressing GST or GST-*SrB4* and grown in selective medium. Cultures were treated with 1.2 M NaCl for 100 min. GST proteins were pulled down by glutathione-Sepharose beads. *Spt20-Myc*-containing (top panel) or GST-containing (bottom panel) proteins were detected by Western blotting using antibodies against Myc and GST. Total extract represents <20% of total input protein (middle panel).

FIG. 11. Whereas Mediator is essential to recruit Pol II at the *STL1* promoter upon stress, SAGA is required selectively under severe osmotic stress conditions. (A) Recruitment of Pol II is impaired by deletion of Mediator *PGD1* gene even under mild osmotic stress conditions. Wild-type or *pgd1Δ* strain was grown to mid-log phase and subjected to mild osmotic stress treatment (0.4 M NaCl for 5 min). Pol II was immunoprecipitated with W8G16 antibody. Binding to *STL1* promoter was determined by ChIP. *n*-fold induction of treated (open bars) or untreated (filled bars) cultures normalized to a telomere-specific band (internal control) is shown. (B) Recruitment of RNA Pol II to promoters is slightly delayed in a SAGA mutant under mild osmotic stress conditions. Wild-type (filled circles) or *spt20Δ* (open circles) strain was treated with 0.4 M NaCl for indicated times. ChIP analyses were performed as for panel A. Data from one representative experiment out of three are shown. (C) Recruitment of RNA Pol II to promoters is dramatically impaired in a SAGA mutant under severe osmotic stress conditions. Strains as in panel B were treated with 1.2 M NaCl for indicated times. ChIP analyses were performed as for panel A. Data from one representative experiment out of three are shown. (D) Recruitment of Tbp1 at the *STL1* promoter is abolished under severe osmotic stress in SAGA-deficient cells. Wild-type or *spt20Δ* strain expressing Tbp1-Myc was grown and subjected to severe osmotic stress (1.2 M NaCl) for indicated times. Binding to *STL1* promoter was determined by ChIP. *n*-fold-induction of treated or untreated cultures normalized to a *GAL1*-specific band (internal control) is shown.

responsive promoters by ChIP in *gal11Δ*, *pgd1Δ*, and *srb4-1* strains. As expected, binding of Pol II to *STL1* and *GRE2* was severely impaired even under mild osmotic stress conditions in Mediator mutant cells (Fig. 11A; also data not shown). We then performed a time course analysis of the recruitment of Pol II to osmotic stress promoters with a wild-type strain and a SAGA-deficient (*spt20Δ*) strain under mild and severe osmotic stress conditions. In contrast to the requirement for Mediator, recruitment of Pol II was strongly dependent on the strength of the osmotic conditions. In a SAGA mutant strain, binding of polymerase to *STL1* was only delayed under mild stress (0.4 M NaCl), whereas it was dramatically impaired when cells were subjected to 1.2 M NaCl (Fig. 11B and C). Correspondingly, binding of Tbp1 was also impaired under severe osmotic stress conditions in a SAGA-deficient mutant (Fig. 11D). Thus, whereas Mediator is essential to making possible Pol II recruitment upon stress, SAGA is required for Pol II recruitment and gene expression selectively under severe osmotic stress conditions.

DISCUSSION

Modulation of gene expression is essential to respond to environmental changes. Control of gene expression by stress-

activated protein kinases is critical for adaptation and cell survival in response to environmental stresses. Here we have performed a systematic genetic analysis to identify novel components involved in gene expression regulation that are important for cell survival in response to osmotic stress. We have found three major transcriptional complexes required for cell viability under high osmolarity that are important for proper gene expression upon stress. These complexes correspond to the SAGA and Mediator complexes, in addition to the previously described Rpd3 complex, involved in Hog1-mediated transcription initiation (7).

SAGA is a multisubunit protein complex involved in several aspects of transcription, for instance, TATA-binding protein delivery. SAGA and TFIID both act as important transcriptional coactivators that share a common set of subunits, although they seem to regulate different classes of genes. Actually, in a genome-wide analysis, it was shown that whereas TFIID function was important for transcription of about 90% of all yeast genes in the whole genome, only 10% seemed to be dependent on SAGA. Interestingly, SAGA-regulated genes were involved in a number of stress responses, and it was suggested that SAGA might be particularly geared for tuning on genes that respond to stress

(13). Correspondingly, we found that whereas SAGA was important for proper osmostress responses, elimination of *TAF1* or *TAF2* (components of TFIID) did not affect stress genes, supporting the hypothesis of SAGA playing a specialized role in stress responses.

SAGA contains a number of components with specific catalytic activities; for instance, Gcn5 has histone acetylase activity, and Ubp8 plays a critical role in deubiquitination of H2B. Nevertheless, deletion of genes encoding these activities of SAGA has a more limited impact on global gene expression than deletion of central structural components of SAGA, such as *SPT3* (13). Systematic analysis of the osmostress responses in the mutants whose mutations correspond to the components of SAGA has shown that the structural entity of SAGA, rather than specific catalytic activities, is important for gene expression and survival upon stress.

It is worth noting that deletion of *SPT8* results in a phenotype similar to that with deletion of other SAGA subunits, which suggests that it is SAGA and not SALSA which is important for gene expression upon stress (data not shown).

Full Hog1-mediated transcriptional responses to osmostress require expression of genes under control of several transcription factors. Previous results showed that at least in Sko1-dependent genes, recruitment of SAGA was observed upon gene activation (22). However, the relevance of SAGA in osmostress transcription and how it was targeted to the osmostress promoters remained unclear. We have shown that SAGA is recruited to genes controlled by Sko1 but also by other transcription factors, such as Hot1 or Msn2 and Msn4. Thus, SAGA is important for expression of osmostress genes independently of the activator present on the promoter. It is worth noting that Hog1 is recruited to stress promoters upon stress (2), and once bound to osmoreponsive promoters, Hog1 acts as a transcriptional activator (1). ChIP analyses have shown that recruitment of Hog1 precedes and is independent of SAGA and Mediator, whereas recruitment of both complexes depends on Hog1. Correspondingly, Hog1 is able to coprecipitate with both SAGA and Mediator.

Recently it was shown that in addition to binding to osmostress promoters, Hog1 is also present on the coding regions of stress genes (20). The fact that Hog1 coprecipitates with and mediates recruitment of SAGA and Mediator suggested that binding of these complexes might not be limited to promoters. ChIP analyses have shown that indeed binding of SAGA and Mediator also occurs at the coding regions of osmostress genes. Along these lines, recent reports have shown that Mediator and SAGA are present on many coding regions of actively transcribed genes (8, 10, 3).

The Mediator complex plays an important role as a transcriptional coactivator (14). Actually, inactivation of some of the subunits of Mediator, such as *Srb4* (Med17), affects cell survival as a result of a global decrease in gene expression (12). Inactivation of other subunits has a much less dramatic effect. Here we show that deletion of nonessential components of Mediator results in cells with compromised survival upon osmostress and with a dramatic decrease of expression of osmostress genes. Furthermore, Mediator is recruited to osmostress promoters upon induction (as reported previously [8]), and its recruitment is dependent on Hog1. The crucial relevance of Mediator in osmostress is illustrated by the fact that

cells become osmosensitive even under mild stress conditions (data not shown).

The Mediator complex seems to be essential under both mild and severe stress conditions, whereas SAGA seems to be essential only under severe osmostress conditions. It is worth noting that SAGA is recruited under both mild and severe osmostress, and although it is essential only for cell survival under severe stress, the lack of SAGA already alters the normal pattern of gene expression at mild osmolarity. Thus, both SAGA and Mediator are recruited similarly to stress genes, and their recruitment depends on Hog1. Interestingly, although binding of SAGA is always observed upon osmostress independently of Mediator, binding of Mediator is reduced in a SAGA mutant strain under severe osmostress conditions. It is worth noting that SAGA and Mediator are able to interact under severe osmostress conditions, and this binding seem to be independent of Hog1. Taken together, we propose the following mechanism for the role of SAGA and Mediator in osmostress gene expression. Recruitment of Hog1 to promoters stimulates binding of SAGA and Mediator to promoters. Under mild stress conditions, there should be a redundant mechanism that leads to the recruitment of Mediator, both dependent on and independent of SAGA (possibly by direct binding with Pol II). Under severe stress conditions, SAGA provides the unique route that makes possible the recruitment of Mediator, which suggests that binding of Hog1 to Mediator is indirect and is mediated by SAGA, at least under severe osmostress. Under these conditions, the lack of SAGA reduces the amount of Mediator recruited to the promoters, Pol II recruitment is affected, and thus, transcription is strongly impaired. Consistent with this model, binding of Mediator to Hog1 is strongly reduced in cells deficient in SAGA only under severe stress conditions.

Thus, our results define an essential role for Mediator in osmostress gene expression and a selective role for SAGA under severe osmolarity conditions. In addition, our results indicate that the requirement for a transcriptional complex for the expression of a given promoter might be fine-tuned according to the strength of the stimuli perceived by the cell through the regulation of the interactions between transcriptional complexes.

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