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Yeast Mpk1 Mitogen-Activated Protein Kinase Activates Transcription through Swi4/Swi6 by a Noncatalytic Mechanism That Requires Upstream Signal▽†

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The cell wall integrity mitogen-activated protein kinase (MAPK) cascade of *Saccharomyces cerevisiae* drives changes in gene expression in response to cell wall stress. We show that the MAPK of this pathway (Mpk1) and its pseudokinase paralog (Mlp1) use a noncatalytic mechanism to activate transcription of the *FKS2* gene. Transcriptional activation of *FKS2* was dependent on the Swi4/Swi6 (SBF) transcription factor and on an activating signal to Mpk1 but not on protein kinase activity. Activated (phosphorylated) Mpk1 and Mlp1 were detected in a complex with Swi4 and Swi6 at the *FKS2* promoter. Mpk1 association with Swi4 in vivo required phosphorylation of Mpk1. Promoter association of Mpk1 and the Swi4 DNA-binding subunit of SBF were codependent but did not require Swi6, indicating that the MAPK confers DNA-binding ability to Swi4. Based on these data, we propose a model in which phosphorylated Mpk1 or Mlp1 forms a dimeric complex with Swi4 that is competent to associate with the *FKS2* promoter. This complex then recruits Swi6 to activate transcription. Finally, we show that human ERK5, a functional ortholog of Mpk1, is similarly capable of driving *FKS2* expression in the absence of protein kinase activity, suggesting that this mammalian MAPK may also have a noncatalytic function in vivo.

The cell wall of the budding yeast *Saccharomyces cerevisiae* is required to maintain cell shape and integrity (13, 41). The cell must remodel this rigid structure during vegetative growth and during pheromone-induced morphogenesis. Wall remodeling is monitored and regulated by the cell wall integrity (CWI) signaling pathway controlled by the Rho1p GTPase (reviewed in reference 43). Two essential functions have been identified for Rho1p. First, it serves as an integral regulatory subunit of the 1,3-β-glucan synthase (GS) complex stimulating GS activity in a GTP-dependent manner. A second essential function of Rho1 is to bind and activate protein kinase C, which is encoded by *PKC1*. Loss of Pkc1 function or of any of the components of the mitogen-activated protein kinase (MAPK) cascade under its control results in a cell lysis defect that is attributable to a deficiency in cell wall construction. The MAPK cascade is a linear pathway comprised of a MEKK (Bck1), a pair of redundant MEKs (Mkk1/2), and a MAPK (Mpk1/Slt2). Mpk1 is a functional homolog of human ERK5 (65), a MAPK that is activated in response to growth factors as well as physical and chemical stresses (1, 68).

CWI signaling is induced in response to a variety of cell wall stressors. First, signaling is activated persistently in response to growth at elevated temperatures (e.g., 37 to 39°C) (37), consistent with the finding that null mutants in many of the pathway components display cell lysis defects only when cultivated at high temperatures. Second, hypo-osmotic shock induces a rapid but transient activation of signaling (16, 37). Third, treatment with mating pheromone stimulates signaling at a time that is coincident with the onset of morphogenesis (12). Finally, CWI signaling is also stimulated by agents that interfere with cell wall biogenesis, such as the chitin antagonist calcofluor white (39), Congo red, caffeine, or zymolyase (18, 47).

One consequence of CWI signaling is activation of the Rlm1 transcription factor (19, 67) through phosphorylation by Mpk1 (35). Rlm1 regulates the transcription of a wide array of cell wall metabolism genes (23, 36, 55). However, the *FKS2* gene, which encodes one of two alternative catalytic subunits of the GS complex, is an unusual transcriptional target of CWI signaling because it is induced independently of Rlm1 (36, 70).

A second transcription factor that plays a poorly defined role in CWI signaling is SBF (7, 44), a dimeric G_1 regulator comprised of Swi4 and Swi6 (reviewed in reference 11). Swi4 is the sequence-specific DNA binding subunit (64), but Swi6 is required for DNA binding (4, 6, 58). SBF is essential to normal regulation of G_1-specific transcription, but genetic and biochemical evidence suggests that SBF also participates in CWI signaling as a target of Mpk1. First, the cell lysis defect of an *mpk1*Δ mutant is suppressed by overexpression of Swi4 (44). Second, both *swi4*Δ and *swi6*Δ mutants are hypersensitive to calcofluor white, supporting a role for SBF in cell wall biogenesis (33). Third, Mpk1 associates with SBF in vivo, as judged by coprecipitation experiments (44), and with Swi4 (but not Swi6) in vitro (7). Fourth, Swi6 is phosphorylated in vivo and in vitro by Mpk1 in response to cell wall stress (44).

Here, we find that CWI signaling drives expression of the *FKS2* gene through SBF. Although Mpk1 must be in the active (phosphorylated) conformation to induce *FKS2* expression, its protein kinase activity is not required. Indeed, the pseudoki-

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TABLE 1. *S. cerevisiae* strains

Strain	Relevant genotype[a]	Reference or source
1788	*MAT***a**/*MAT*α EG123 *leu2-3,112 trp1-1 ura3-52 his4 can1*^{r}	I. Herskowitz
DL454	*MAT***a** EG123 *mpk1*Δ::*TRP1*	42
DL3145	*MAT***a**/*MAT*α EG123 *swi4*Δ::*TRP1*/*swi4*Δ::*TRP1*	This study
DL3148	*MAT***a**/*MAT*α EG123 *swi6*Δ::*LEU2*/*swi6*Δ::*LEU2*	This study
DL3166	*MAT***a**/*MAT*α EG123 *mlp1*Δ::*URA3*/*mlp1*Δ::*URA3 mpk1*Δ::*TRP1*/*mpk1*Δ::*TRP1*	This study
DL3183	*MAT***a** S288c *mpk1*Δ::*G418 mlp1*Δ::*G418*	This study; Research Genetics
DL3187	*MAT***a** S288c (BY4741) *his3*Δ *leu2*Δ *ura3*Δ *lys2*Δ	Research Genetics
DL3193	*MAT***a**/*MAT*α S288c (BY4743)	Research Genetics
DL3194	*MAT***a**/*MAT*α S288c *mlp1*Δ::*G418*/*mlp1*Δ::*G418*	This study; Research Genetics
DL3195	*MAT***a**/*MAT*α S288c *mpk1*Δ::*G418*/*mpk1*Δ::*G418*	This study; Research Genetics
DL3196	*MAT***a**/*MAT*α S288c *mpk1*Δ::*G418*/*mpk1*Δ::*G418 mlp1*Δ::*G418*/*mlp1*Δ::*G418*	This study; Research Genetics
DL3233	*MAT***a** S288c *swi6*Δ::*G418*	Research Genetics
DL3405	*MAT***a** S288c *swi4*Δ::*G418*	Research Genetics
DL3529	*MAT***a**/*MAT*α EG123 *bck1*Δ::*G418*/*bck1*Δ::*G418*	Research Genetics
DL3541	*MAT***a**/α S288c *mkk1*Δ::*G418*/*mkk1*Δ::*G418 mkk2*Δ::*G418*/*mkk2*Δ::*G418*	This study; Research Genetics
SFY526	*MAT***a** *gal4-542 gal80-538 ura3-52 ura3*::*GAL1-lacZ ade2-101 leu2-3,112 his3-200 trp1-905 lys2-801 can*^{R}	Clontech

[a] Strain background EG123 is described by Siliciano and Tatchell (61).

nase paralog of Mpk1, Mlp1 (for *M*pk-*l*ike *p*rotein), is also capable of inducing *FKS2* expression. This novel noncatalytic function involves stable association of Mpk1 or Mlp1 with SBF at the *FKS2* promoter. Transcriptional activity of *FKS2* has been conserved in mammalian ERK5, suggesting that this MAPK may also have noncatalytic functions in metazoan cells.

MATERIALS AND METHODS

Strains, growth conditions, and transformations. The *S. cerevisiae* strains used in this study are listed in Table 1. Yeast cultures were grown in YEPD medium (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose) with or without 10% sorbitol, or in SD medium (0.67% yeast nitrogen base, 2% glucose) supplemented with the appropriate nutrients to select for plasmids and gene replacements. *Escherichia coli* DH5α was used to propagate all plasmids. *E. coli* cells were cultured in Luria broth medium (1% Bacto tryptone, 0.5% Bacto yeast extract, 1% NaCl) and transformed to carbenicillin resistance by standard methods. Two-hybrid tests were conducted using yeast strain SFY526 (Clontech Laboratories) in cultures grown to mid-log phase. β-Galactosidase assays for promoter-*lacZ* fusion expression experiments and for two-hybrid analyses were conducted as described previously (70).

Plasmids. Plasmids used in this study are listed in Table 2. A plasmid consisting of *FKS2* residues −540 to −375 [*FSK2*(−540 to −375)]-*CYC1-lacZ* (plasmid p2052) was constructed by replacing the 134-bp SmaI/XhoI fragment of the *CYC1* promoter of pLGΔ-312 (26) with a 165-bp PCR-amplified *FKS2* fragment, resulting in fusion of the *FKS2* promoter sequences to the *CYC1* minimal promoter. Three SBF-binding site mutant alleles of *FKS2* were constructed by PCR overlap mutagenesis (32) of p2052: an A to T at residue −386 (plasmid p2053), a G to C at residue −388 (p2054), and a C to G at residue −389 (p2055). A plasmid consisting of *FKS2*(−706 to −360)-*CYC1-lacZ* (plasmid p1070), which bears a *LEU2* marker, was created by replacement of the *URA3* marker in plasmid p916 (70) by the method of Cross (15). A plasmid consisting of *CLN2*(−600 to −400)-*CYC1-lacZ* (plasmid p2066) was constructed by replacing the 134-bp SmaI/XhoI fragment of the *CYC1* promoter of pLGΔ-312 (26) with a 200-bp PCR-amplified *CLN2* fragment, resulting in fusion of the *CLN2* promoter sequences containing multiple SBF-binding sites to the *CYC1* minimal promoter.

PCR overlap mutagenesis was used to construct point mutants in *MPK1-3xHA* (where HA is hemagglutinin) and *MLP1-3xHA*. These alleles (*mpk1-K54R-3xHA* and *mlp1-Y192F-3xHA*), their wild-type counterparts, and *mpk1-T190A Y192F-3xHA* (*mpk1-TA/YF-3xHA*) (37) were subcloned from a *URA3*-based multicopy plasmid (YEp352) (31) into *LEU2*-based multicopy (YEp351) (31) and centromeric (pRS315) (60) plasmids, using SalI/EcoRI for *MPK1* alleles and XbaI/SacI for *MLP1* alleles. Mutant alleles of *MPK1* (*mpk1-TA/YF* and *mpk1-K54R*) were tagged with the FLAG epitope by subcloning a SacI/SacII fragment bearing the mutant regions from the appropriate YEp351 clones into YEp351(*MPK1-FLAG*) (40).

SWI4-6HIS was created by PCR amplification of the *SWI4* coding region using primers that include a SpeI site in the upstream primer and an XhoI site in the downstream primer. The downstream primer also included a six copies of the HIS tag adjacent to the termination codon. This fragment was cloned into pUT36 (plasmid p2415) (49), creating pUT36(*SWI4-6HIS*) (p2418), which expresses Swi4-6HIS under the control of the *MET25* promoter and is selectable by the *URA3* marker.

ERK5-6HIS was subcloned from pUT36(ERK5-6HIS) (49) (gift of P. Piper) using BamHI and SalI into vector pUT34 (plasmid p2348), creating pUT34(ERK5-6HIS) (p2349), which expresses ERK5-6HIS under the control of the *MET25* promoter and is selectable by the *HIS3* marker. QuikChange site-directed mutagenesis (66) was used to create pUT34(ERK5-T219A Y221F-6HIS) (p2350) and pUT34(ERK5-K84R-6HIS) (p2351).

A Gal4 activation domain (AD) fusion to Swi4 for two-hybrid analysis was constructed in pGAD424 (Clontech Technologies) (plasmid p1173) by PCR amplification of the coding region using primers that include an SalI site in the upstream primer and a BglII site in the downstream primer. The Gal4 DNA-binding domain (DBD) fusion to the catalytic domain of Mpk1 residues 1 to 328 [pGBT9-Mpk1(1-328)]; plasmid (p2248) was the gift of M. Molina. All PCR-amplified sequences were confirmed by DNA sequence analysis across the entire amplified region.

Genomic deletions of *SWI4* and *SWI6*. To delete the genomic copy of *SWI4*, 595 bp of sequence 5′ to the *SWI4* start codon and 614 bp of sequence 3′ of the *SWI4* stop codon were amplified in separate PCRs from genomic DNA of yeast strain 1788. The 5′ fragment was amplified with primers that placed an NotI site at the end adjacent to the *SWI4* coding sequence and a PstI site at the opposite end. The 3′ fragment was amplified with primers that placed an SalI site adjacent to the *SWI4* coding sequence and a PstI site at the opposite end. These fragments were ligated in a three-molecule reaction to the NotI and SalI sites of the integrative plasmid pRS304 (60) to create a unique PstI site between the fragments. The resulting plasmid, pRS304(*swi4*Δ::*TRP1*) (p2344), was linearized with PstI and used to transform yeast strains to tryptophan prototrophy.

To delete the genomic copy of *SWI6*, 615 bp of sequence 5′ to the *SWI6* start codon and 611 bp of sequence 3′ of the *SWI6* stop codon were amplified in separate PCRs from genomic DNA of yeast strain 1788. The 5′ fragment was amplified with primers that placed an NotI site at the end adjacent to the *SWI6* coding sequence and a PstI site at the opposite end. The 3′ fragment was amplified with primers that placed an SalI site adjacent to the *SWI6* coding sequence and a PstI site at the opposite end. These fragments were ligated in a three-molecule reaction to the NotI and SalI sites of the integrative plasmid pRS305 (60) to create a unique PstI site between the fragments. The resulting plasmid, pRS305(*swi6*Δ::*LEU2*) (p2345), was linearized with PstI and used to transform yeast strains to leucine prototrophy.

ChIP. Chromatin immunoprecipitation (ChIP) assays were conducted as described by Hecht and Grunstein (30), with the following modifications. Yeast cells expressing HA-tagged proteins of interest and multicopy plasmids containing various alleles of *FKS2* were grown in YEPD medium at room temperature or at 39°C to an A_{600} of 1.5. DNA was cross-linked to proteins by the addition of

TABLE 2. Plasmids

Plasmid	Description	Reference or source
pRS315	*LEU2*-based centromeric plasmid	60
YEp351	*LEU2*-based 2μm plasmid	31
YEp352	*URA3*-based 2μm plasmid	31
p740	YEp351[*MPK1*]	37
p777	YEp351[*MPK1-3xHA*]	37
p778	YEp351[*mpk1*(*T190 Y192F*)*-3xHA*]	37
p903	pLGΔ-312; *CYC1-lacZ* reporter vector	26
p904	pLGΔ-178; *CYC1-lacZ* reporter vector	26
p916	*FKS2*(−706 to −360)*-CYC1-lacZ* in p903	70
p950	YEp352[*FKS2*]	70
p1070	*FKS2*(−706 to −360)*-CYC1-lacZ* with *LEU2* marker; derived from p916	This study
p1150	YEp24(*SWI4*)	Joseph Gray
p1172	pGBT9; 2-hybrid vector with Gal4 DBD	Clontech
p1173	pGAD424; 2-hybrid vector with Gal4 AD	Clontech
p1366	*PRM5*(*YIL117c*)*-lacZ*	35
p2022	YEp351[*MLP1-3xHA*]	This study
p2024	YEp351[*mlp1*(*Y192F*)*-3xHA*]	This study
p2052	*FKS2*(−540 to −375)*-CYC1-lacZ* with *URA3* marker	This study
p2053	*FKS2*(−540 to −375)*-CYC1-lacZ* with SBF-binding site mutation at −386T	This study
p2054	*FKS2*(−540 to −375)*-CYC1-lacZ* with SBF-binding site mutation at −388C	This study
p2055	*FKS2*(−540 to −375)*-CYC1-lacZ* with SBF-binding site mutation at −389G	This study
p2066	*CLN2*(−600 to −400)*-CYC1-lacZ*	This study
p2119	YEp351[*mpk1*(*K54R*)*-3xHA*]	This study
p2188	pRS315[*MPK1-3xHA*]	This study
p2190	pRS315[*mpk1*(*T190A Y192F*)*-3xHA*]	This study
p2193	pRS315[*mpk1*(*K54R*)*-3xHA*]	This study
p2248	pGBT9 *MPK1*(*1-328*)	62
p2313	YEp351[*MPK1-FLAG*]	40
p2316	YEp351[*mpk1*(*T190A Y192F*)*-FLAG*]	This study
p2317	YEp351[*mpk1*(*K54R*)*-FLAG*]	This study
p2339	BG1805-*GAL1-SWI4-ZZ-HA-6HIS*	24
p2341	BG1805-*GAL1-SWI6-ZZ-HA-6HIS*	24
p2344	pRS304[*swi4*Δ::*TRP1*]	This study
p2345	pRS305[*swi6*Δ::*LEU2*]	This study
p2346	pRS315[*MLP1-3xHA*]	This study
p2347	pRS315[*mlp1*(*Y192F*)*-3xHA*]	This study
p2348	pUT34; *HIS3*-based *MET25* promoter	49
p2349	pUT34[ERK5-*6HIS*]	This study
p2350	pUT34[ERK5(T219A Y221F)-*6HIS*]	This study
p2351	pUT34[ERK5(K84R)-*6HIS*]	This study
p2352	pGAD424 *SWI4*(*1-1093*)	This study
p2415	pUT36; *URA3*-based *MET25* promoter	49
p2418	pUT36 (*SWI4-6HIS*)	This study

formaldehyde to 1%, followed by incubation at 30°C for 1 h. Chromatin was sheared using a Branson Sonifier 450 (output setting 5) in 10-s bursts for a total of 90 s so as to generate fragments of an approximately mean length of 500 bp. Mouse monoclonal antibody 12CA5 (BabCo) was used for immunoprecipitation of 500 μg of protein. PCR (35 cycles) was used for detection of coprecipitated DNA sequences. For *FKS2-CYC1-lacZ* promoter association, one primer of the pair was homologous to *CYC1* sequence to avoid interference from the endogenous *FKS2* promoter. This was critical for experiments testing Mpk1-HA and Mlp1-HA association to the SBF-site mutant allele of the *FKS2* promoter. A primer pair for amplification of part of the *DYN1* gene was used as a negative control for genomic ChIP experiments. For primer sequences, see Table S1 in the supplemental material.

Coimmunoprecipitation. Protein extractions and coimmunoprecipitation of yeast strain DL454 (*mpk1*Δ) expressing Swi4-His_6 with Mpk1-FLAG (from 300 μg of protein) were conducted as described previously (37) using mouse monoclonal anti-FLAG (M2) affinity beads (Sigma) or protein A affinity beads (no antibody controls; Sigma). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (on 7.5% gels) of samples representing 100 μg of initial protein was followed by immunoblotting to detect either Mpk1-FLAG (M2 anti-FLAG antibody; Sigma) or Swi4-His_6 (mouse monoclonal tetra-His antibody; Qiagen). Input controls represented 50 μg of initial protein.

RESULTS

Cell wall stress induction of *FKS2* requires SBF. Expression of the *FKS2* gene is induced in response to a variety of cell wall stressing agents including elevated growth temperature, Congo red, and calcofluor white (70; also K.-Y. Kim, unpublished data). A *lacZ* transcriptional reporter bearing the *FKS2* promoter region between residues −706 and −360 fused to the basal *CYC1* promoter (*FKS2-lacZ*) is responsive to thermal stress (70) and to expression of a constitutive form of the CWI MEK, Mkk1, but its induction is independent of the Rlm1 transcription factor (36). A consensus SBF-binding site (SCB; CACGAAA) resides within the responsive region of the *FKS2* promoter (−385 to −391). To assess the contribution of this site to cell wall stress-induced activation of the *FKS2* promoter, we mutated three residues predicted to abolish SBF binding (5). Mutant promoters were tested in the context of a shorter *FKS2-lacZ* reporter plasmid (with residues −540 to −375). All three mutations abolished induction of this reporter at increased temperatures or following Congo red treatment (Fig. 1A), indicating that the SCB is necessary for the response. Because elevated growth temperature was the most effective of several cell wall stressors for induction of *FKS2* expression, subsequent experiments were conducted using that stress. Thermal induction of *FKS2-lacZ* was similarly abolished in *swi4*Δ and *swi6*Δ mutants (Fig. 1B), indicating the requirement for both subunits of SBF in this response. Because the *swi4*Δ mutant has an osmotically remedial growth defect at elevated temperatures (44, 50), it was necessary to conduct this experiment in the presence of sorbitol for osmotic support, which diminished somewhat the stress response in the wild-type strain. In contrast to these results, a reporter that is activated by cell wall stress through the Rlm1 transcription factor (*PRM5-lacZ*) was induced normally at an elevated temperature in *swi4*Δ and *swi6*Δ mutants (Fig. 1C), indicating that Rlm1-mediated transcription is not dependent on these factors and that the mutant strains are not generally impaired for transcriptional induction at high temperature.

It has been demonstrated previously that a *pkc1* mutation does not affect expression of an SBF-driven cell cycle-regulated promoter (33). Consistent with this, we found that an SBF-driven reporter that is regulated periodically through the cell cycle (*CLN2-lacZ*) showed no activation in response to cell wall stress induced either by high temperatures or by Congo red (Fig. 1D), revealing that the SCB in *FKS2* functions differently than the SCBs in cell cycle-regulated genes.

Thermal induction of *FKS2* requires either the Mpk1 MAPK or its pseudokinase paralog, Mlp1. To determine which elements of the CWI signaling pathway are required for thermal induction of *FKS2* through SBF, we examined mutants in the

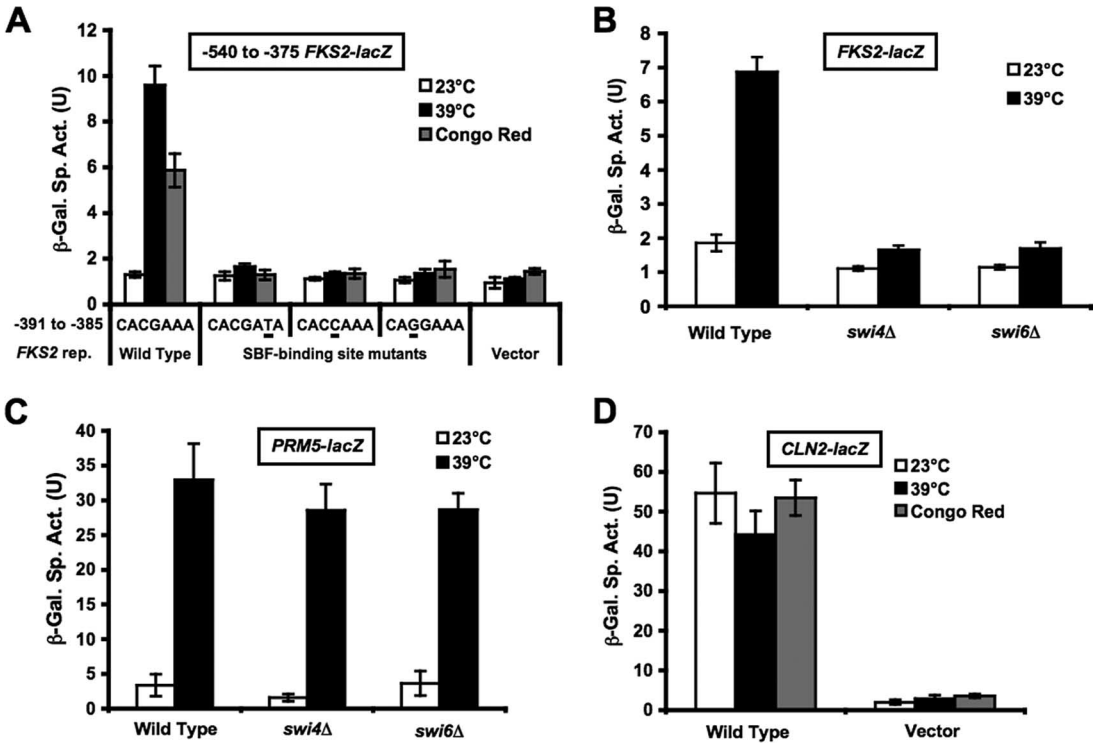

FIG. 1. Cell wall stress induction of *FKS2* expression is dependent on an SBF-binding site and SBF. (A) The SBF-binding site in the *FKS2* promoter is required for cell wall stress-induced activation. *FKS2-lacZ* reporter plasmids bearing point mutations in a predicted SBF-binding site (wild-type, p2052; −386T, p2053; −388C, p2054; and −389G, p2055) were transformed into wild-type yeast strain 1788. Transformants were grown to saturation at 23°C in SD-uracil medium. Cultures were diluted into 3 ml of YEPD medium so that subsequent incubation at 23°C, 39°C, or 23°C with 50 μg/ml Congo red for 15 h resulted in mid-log-phase cultures (A_{600} of 1.0 to 1.5). β-Galactosidase (β-Gal) activity was measured in crude extracts. (B) Mutants in *SWI4* and *SWI6* are defective for thermal activation of *FKS2* expression. An *FKS2-lacZ* reporter plasmid (p2052) was transformed into wild-type (1788), *swi4*Δ (DL3145), or *swi6*Δ (DL3148) strains. Transformants were treated as described in panel A, except that cultures were diluted into YEPD medium containing 10% sorbitol for osmotic support. (C) Mutants in *SWI4* and *SWI6* are competent for thermal activation of *PRM5* expression. A *PRM5-lacZ* reporter plasmid (p1366) was transformed into wild-type (1788), *swi4*Δ (DL3145), or *swi6*Δ (DL3148) strains. Transformants were treated as described in panel A, except that cultures were diluted into YEPD medium containing 10% sorbitol for osmotic support. (D) A cell cycle-regulated SCB reporter is not induced in response to cell wall stress. A *CLN2-lacZ* reporter plasmid (p2066) and its parent vector (p904) were transformed into the wild-type strain (1788). Transformants were treated as described in panel A. Each value represents the mean and standard deviation from three independent transformants. Sp. Act., specific activity; U, unit.

MAPK (*mpk1*Δ), the MEK (*mkk1*Δ/*mkk2*Δ), and the MEKK (*bck1*Δ) of this pathway. As above, these mutants were exposed to thermal stress in the presence of sorbitol for osmotic support. Although the *bck1*Δ and *mkk1*Δ/*mkk2*Δ mutants were defective for *FKS2* induction, we were surprised to find that the *mpk1*Δ mutant was only modestly impaired (Fig. 2A). This was unexpected because the MAPK branch of the CWI signaling pathway has been thought to be linear, with Mpk1 being the sole MAPK activated through this pathway (43).

A paralog of Mpk1, encoded by the *MLP1* gene (67), exists within the yeast genome as a consequence of an ancestral whole-genome duplication (38). Mlp1 shares 53% amino acid sequence identity with Mpk1. However, Mlp1 lacks two catalytic domain residues recognized to be critical for protein kinase activity. First, a universally conserved Lys residue within subdomain II of all protein kinases, which is important for ATP positioning (28), is mutated to an Arg residue in Mlp1 (residue 54). This mutation is often engineered to create catalytically inactive forms of protein kinases. Second, a universally conserved Asp residue within the triplet DFG in subdomain VII is mutated to an Asn residue in Mlp1 (residue 171). This residue normally coordinates a magnesium ion that is also critical for ATP positioning (28), further suggesting that Mlp1 does not have the capability to bind ATP in a catalytically productive manner. Additionally, we have been unable to detect protein kinase activity associated with immunoprecipitated Mlp1 using in vitro substrates of Mpk1 (unpublished results), supporting the conclusion that Mlp1 is a pseudokinase. In addition to the differences in the ATP-binding site, a conserved Thr residue within the dual phosphorylation site of MAPK activation loops (TXY) is mutated to a Lys residue in Mlp1 (residue 190), further indicating that it is not a true MAPK. However, because phosphorylation of the activation loop Tyr residue by MEKs precedes that of the Thr residue (21, 29), it is possible that Mlp1 is phosphorylated on Tyr192 by Mkk1/2.

We tested an *mlp1*Δ mutation individually and in combination with *mpk1*Δ for thermal induction of *FKS2* expression (Fig. 2A). Although the *mlp1*Δ mutant was not compromised, the double mutant *mlp1*Δ *mpk1*Δ was incapable of inducing *FKS2* expression, indicating that these proteins serve an overlapping function for activation of transcription through SBF.

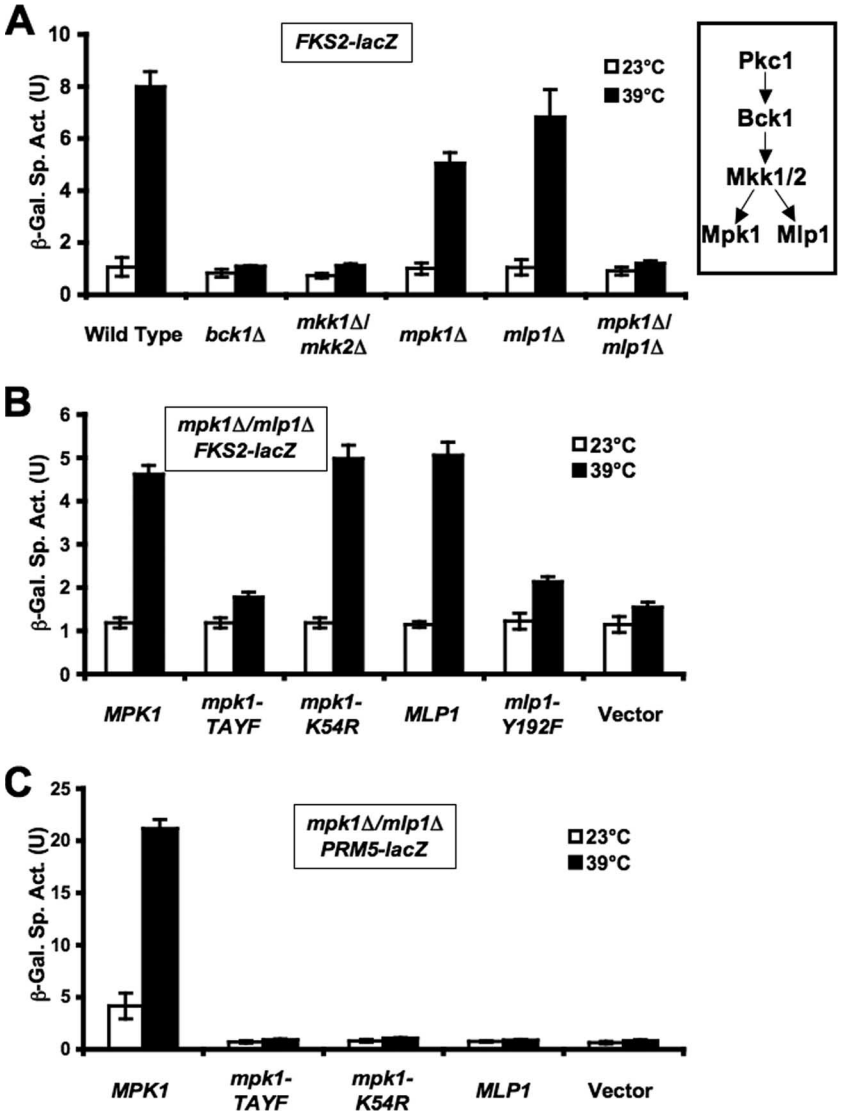

FIG. 2. CWI requirements for thermal induction of *FKS2* and *PRM5*. (A) Induction of *FKS2* expression is dependent on either Mpk1 or its pseudokinase paralog, Mlp1. An *FKS2-lacZ* reporter plasmid (p2052) was transformed into the wild-type (DL3193), *bck1*Δ (DL3529), *mkk1*Δ *mkk2*Δ (DL3541), *mpk1*Δ (DL3195), *mlp1*Δ (DL3194), or *mpk1*Δ *mlp1*Δ (DL3196) strain. Transformants were cultured as described in the legend of Fig. 1 in the presence of YEPD medium containing 10% sorbitol for osmotic support. β-Galactosidase (β-Gal) activity was measured in crude extracts. (B) Mpk1 does not require catalytic activity for thermal induction of *FKS2*, but Mpk1 and Mlp1 must be phosphorylated. An *FKS2-lacZ* reporter plasmid (p2052) was cotransformed with centromeric plasmids bearing *MPK1* (p2188), *mpk1-TA/YF* (p2190), *mpk1-K54R* (p2193), *MLP1* (p2346), *mlp1-Y192F* (p2347), or vector (pRS315) into an *mpk1*Δ *mlp1*Δ strain (DL3196). Transformants were treated as above. (C) Mpk1 catalytic activity is required for thermal induction of *PRM5*. A *PRM5-lacZ* reporter plasmid (p1366) was cotransformed with the indicated plasmids from panel B into an *mpk1*Δ *mlp1*Δ strain (DL3196). Transformants were treated as above, except that culture time was 5 h rather than 15 h. Each value represents the mean and standard deviation from three independent transformants. Sp. Act., specific activity; U, unit.

This surprising result also indicated that Mlp1 drives *FKS2* expression through a noncatalytic mechanism.

Because protein kinase activity is not required for Mlp1 to induce *FKS2* expression, we asked if Mpk1 requires its protein kinase activity for this function. To address this, two mutant forms of Mpk1 were constructed. The first was *mpk1-K54R*, a mutation within the ATP-binding site, which blocks catalytic activity by interfering with ATP positioning and is analogous to Arg54 found naturally in *MLP1*. This allele fails to complement the cell lysis defect of an *mpk1*Δ mutant (46) and is devoid of detectable protein kinase activity (44, 69). The second mutant form, *mpk1-TA/YF*, eliminates the two phosphorylation sites within the activation loop of Mpk1 that are normally phosphorylated by Mkk1/2. This allele similarly fails to complement the cell lysis defect of an *mpk1*Δ mutant (42) and is devoid of detectable protein kinase activity (37). These forms were introduced into an *mpk1*Δ *mlp1*Δ double mutant on centromeric plasmids. Figure 2B shows that although the *mpk1-TA/YF* mutant was blocked for thermal induction of *FKS2*, the *mpk1-K54R* mutant was not, indicating that Mpk1 must be in the active (phosphorylated) conformation to drive transcription through SBF but need not be catalytically active.

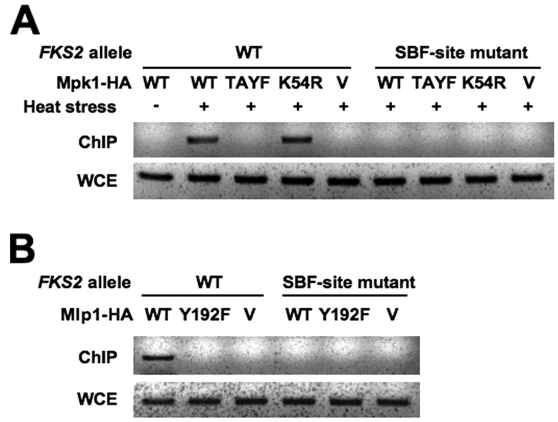

FIG. 3. Phosphorylated Mpk1 and Mlp1 bind to the *FKS2* promoter. (A) ChIP analysis of the *FKS2* promoter with Mpk1-HA. A wild-type yeast strain (1788) was cotransformed with *FKS2-lacZ* reporter plasmids (p2052, wild-type; or p2053, SBF-site mutant −386T) and multicopy plasmids expressing the indicated HA-tagged *MPK1* allele (wild-type, p777; *mpk1-TA/YF*, p778; *mpk1-K54R*, p2119; or vector, YEp351). Transformants were cultivated in YEPD medium at 23°C or subjected to thermal stress at 39°C for 15 h prior to ChIP analysis using primers designed to detect only the plasmid-borne *FKS2* promoter. PCRs from whole-cell extracts (WCE) are also shown. (B) ChIP analysis of the *FKS2* promoter with Mlp1-HA. Wild-type yeast strain 1788 was cotransformed with *FKS2-lacZ* reporter plasmids from panel A and multicopy plasmids expressing the indicated HA-tagged *MLP1* allele (wild-type, p2022; *mlp1-Y192F*, p2024; or vector, YEp351). Transformants were cultivated and subjected to thermal stress prior to ChIP analysis, as above. WT, wild type; TAYF, T190A Y192F.

A mutant form of Mlp1 that eliminates its lone potential phosphorylation site (Y192F) was similarly blocked for *FKS2* induction (Fig. 2B), suggesting that Mlp1 must also be in the active conformation to drive transcription. These results are in contrast to cell wall stress induction of *PRM5* transcription, which requires phosphorylation of the Rlm1 transcription factor by Mpk1 (35). Mutations in Mpk1 that either block its phosphorylation by Mkk1/2 or interfere with its catalytic activity block thermal induction of *PRM5* (Fig. 2C). Additionally, although Mlp1 can associate with Rlm1 (67), it cannot substitute for Mpk1 in the activation of this transcription factor (Fig. 2C).

Mpk1 and Mlp1 associate with the *FKS2* promoter through Swi4. Because Mpk1 and Mlp1 must be in the active conformation but do not require catalytic activity to drive *FKS2* expression through SBF, we considered a model in which these proteins act by stable association with SBF on the *FKS2* promoter. We used ChIP analysis to test this model. Cells expressing epitope-tagged forms of Mpk1 were first exposed to mild heat stress to activate CWI signaling (37). Proteins were then cross-linked to DNA with formaldehyde, followed by cell lysis and DNA fragmentation. Mpk1-HA was immunoprecipitated from extracts, and coprecipitating *FKS2* promoter sequence was detected by PCR. Wild-type Mpk1-HA was detected in a complex with the *FKS2* promoter only after its activation by heat stress (Fig. 3A). The ATP-binding site mutant form of Mpk1 (Mpk1-K54R-HA) was also detected in a promoter complex. However, we failed to detect the phosphorylation site mutant form of Mpk1 (Mpk1-TA/YF-HA) bound to the *FKS2* promoter even though this mutant protein is maintained at normal levels (37). To determine if this association was dependent on the SBF-binding site, the plasmid bearing the wild-type *FKS2* promoter was replaced with one bearing the mutant SBF-binding site form described above. Mpk1-HA was not detected in association with this mutant promoter. Similarly to Mpk1-HA, wild-type Mlp1-HA was detected in a complex with the *FKS2* promoter, but the phosphorylation site mutant of this protein (Mlp1-Y192F-HA) failed to bind the promoter (Fig. 3B). These results are consistent with the transcriptional data presented above and indicate that phosphorylated Mpk1 and Mlp1 drive transcription of *FKS2* through association with the SCB at the promoter.

To determine the specific requirements for complex formation on the native *FKS2* promoter, we conducted genomic ChIP analysis in *swi4*Δ, *swi6*Δ, and *mpk1*Δ/*mlp1*Δ mutants. As was observed using the plasmid-borne *FKS2* promoter, wild-type Mpk1-HA and its ATP-binding site mutant form bound to the genomic *FKS2* promoter in response to activation by heat stress (Fig. 4A). By contrast, the phosphorylation site mutant form of Mpk1-HA failed to bind *FKS2*. Mpk1-HA did not associate with the *FKS2* promoter in an *swi4*Δ mutant but was still capable of association in the *swi6*Δ mutant (Fig. 4A). The latter result was somewhat surprising because Swi4 requires Swi6 to bind DNA at cell cycle-regulated promoters (4, 6, 58), and this suggests that Mpk1 can replace that function of Swi6 in this context. Swi4-HA associated with the *FKS2* promoter in an *swi6*Δ mutant but not in an *mpk1*Δ *mlp1*Δ mutant (Fig. 4B), supporting the conclusion that Mpk1 (or Mlp1), but not Swi6, confers DNA-binding ability to Swi4 in this promoter context. Thus, Mpk1/Mlp1 and Swi4 promoter binding is codependent. Finally, because Swi6 is required for thermal induction of *FKS2* expression, we examined the requirements for Swi6-HA promoter binding. We found that Swi6 association with the *FKS2* promoter requires both Mpk1/Mlp1 and Swi4 (Fig. 4C). Taken together, these results support a model in which phosphorylated Mpk1 (or Mlp1) in stable complex with Swi4 is competent to bind the *FKS2* promoter at the SBF binding site. However, transcriptional activation of *FKS2* requires the further recruitment of Swi6 to the complex.

Association of Mpk1 with Swi4 is regulated by the phosphorylation state of Mpk1. Phosphorylation of Mpk1 might regulate its association with Swi4, indicating that formation of the heterodimeric complex is the key step controlling DNA binding. Alternatively, Mpk1 might reside constitutively in complex with Swi4 and induce a conformational shift in response to activation that allows DNA binding of the complex. Therefore, we examined the regulation of the interaction of these proteins by two-hybrid analysis and by coimmunoprecipitation. Because Mpk1 possesses a transcriptional activation region within its C-terminal domain (62) that would interfere with two-hybrid analysis, we used a truncated form [Gal4DBD-Mpk1(1-328)] that possesses only the Mpk1 catalytic domain. A full-length Swi4 fusion (Gal4AD-Swi4) displayed interaction with Mpk1 but only in response to thermal stress (Fig. 5A), revealing that the interaction is regulated by the activation state of Mpk1.

We next tested for coimmunoprecipitation of Mpk1 and Swi4. Epitope-tagged forms of Mpk1 were immunoprecipitated from extracts of cells that had been either exposed to thermal stress or grown under nonstress conditions. Epitope-

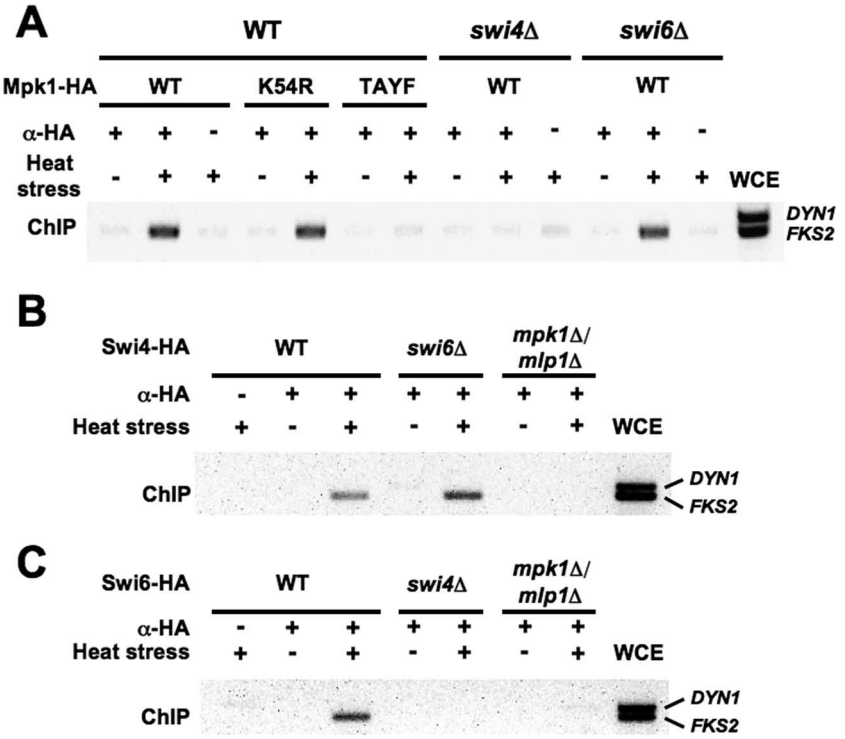

FIG. 4. Interdependent recruitment of Mpk1 and Swi4 to the *FKS2* promoter. (A) Genomic ChIP demonstrates that association of Mpk1-HA with the *FKS2* promoter is dependent on Swi4 but not Swi6. Yeast strains (wild-type, DL3187; *swi4*Δ, DL3405; or *swi6*Δ, DL3233) were transformed with a multicopy plasmid bearing the indicated *MPK1-HA* allele and subjected to thermal stress prior to ChIP analysis as described in the legend of Fig. 3, except that strains were cultivated in the presence of 10% sorbitol. An additional primer pair for PCR amplification *DYN1* sequence was included as a negative control. (B) Association of Swi4-HA with the *FKS2* promoter is dependent on Mpk1/Mlp1 but not Swi6. Yeast strains (wild-type, DL3187; *swi6*Δ, DL3233; or *mpk1*Δ *mlp1*Δ, DL3183) were transformed with a multicopy plasmid bearing *SWI4-HA* (p2339) and treated as above. (C) Association of Swi6 with the *FKS2* promoter is dependent on both Swi4 and Mpk1/Mlp1. Yeast strains (wild-type, DL3187; *swi4*Δ, DL3405; or *mpk1*Δ *mlp1*Δ, DL3183) were transformed with a multicopy plasmid bearing *SWI6-HA* (p2341) and treated as above. WCE, whole-cell extract; WT, wild type; α, anti; TAYF, *mpk1-T190A Y192F*.

tagged Swi4 (Swi4-6His) coprecipitated with either wild-type Mpk1 (Mpk1-FLAG) or the ATP-binding site mutant form of Mpk1 (Mpk1-K54R-FLAG) with an increased signal under thermal stress conditions (Fig. 5B). However, Swi4 failed to associate with the phosphorylation site mutant form of Mpk1 (Mpk1-TA/YF-FLAG) under either condition, confirming that Mpk1 must be in the active (phosphorylated) state but does not require protein kinase activity to bind Swi4. Thus, we propose that Mpk1 (or Mlp1), activated by cell wall stress, associates with Swi4. This dimeric complex is competent to bind the SCB of the *FKS2* promoter. Finally, Swi6 associates with this ternary complex to drive transcription initiation (Fig. 6).

Human ERK5 drives *FKS2* expression through a noncatalytic mechanism. When expressed in yeast, the human ERK5 MAPK is activated in response to cell wall stress and suppresses the phenotypic defects of an *mpk1*Δ mutant (65). Additionally, ERK5 is capable of activating the Rlm1 transcription factor. We therefore asked if the noncatalytic transcriptional activation of SBF by Mpk1 and Mlp1 is also conserved in ERK5. Human ERK5 variants were expressed in an *mpk1*Δ *mlp1*Δ mutant and tested for their ability to activate *FKS2* transcription in response to thermal stress. Both the wild-type and ATP-binding site mutant (K84R) alleles of ERK5 were capable of activating *FKS2* reporter expression (Fig. 7). By contrast, a nonphosphorylatable form of ERK5 (T219A Y221F) failed to show an increase over the vector control. As was seen for Mpk1 and Mlp1, induction of *FKS2* by ERK5 was dependent on the SCB. We conclude that the noncatalytic transcriptional function of Mpk1 and Mlp1 is conserved in ERK5.

DISCUSSION

A common mechanism by which MAPKs regulate gene expression in response to environmental stresses is through phosphorylation of transcription factors. However, protein kinases can also regulate transcription through the formation of stable interactions on the DNA. For example, the yeast Kss1 and Fus3 MAPKs function as repressors of transcription in the unphosphorylated state through association with the Ste12/Tec1 transcription factor complex (8, 14, 45). Inactive Kss1 binds directly to Ste12, recruiting the Dig1 and Dig2 transcriptional repressors to the DNA. Phosphorylation of Kss1 destabilizes this complex, causing derepression through a mechanism that does not require Kss1 protein kinase activity. Additionally, recent reports have revealed that some MAPKs can drive gene expression as integral components of transcription complexes (2, 3, 17, 51, 52, 53). This has been studied most thoroughly in the yeast Hog1 stress-activated protein kinase,

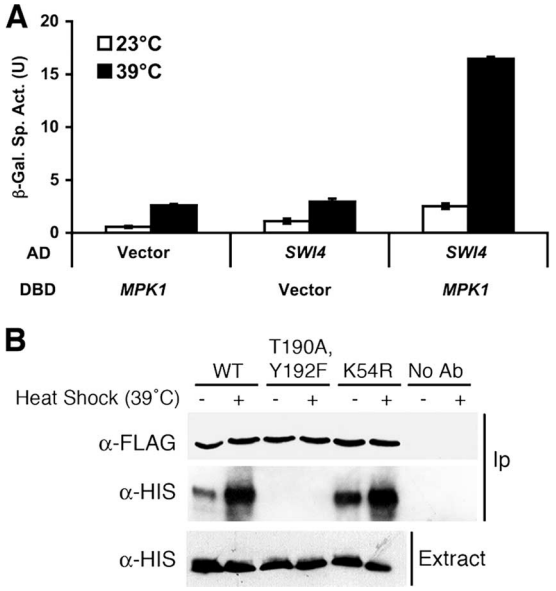

FIG. 5. Physical association of activated Mpk1 with Swi4. (A) Two-hybrid association of Mpk1 with Swi4 is dependent on activation of Mpk1. A Gal4DBD fusion to the catalytic domain of Mpk1, Mpk1(1-328) (plasmid p2248), was tested for interaction with a Gal4AD fusion to Swi4 (p2352) in yeast two-hybrid strain SFY526. Transformants were cultivated in selective medium for 15 h either at 23°C or 39°C. Vector controls (Gal4DBD vector, p1172; Gal4AD vector, p1173) are included for each fusion. Each value represents the mean and standard deviation from three independent transformants. (B) Coimmunoprecipitation (IP) of Swi4 with Mpk1 requires phosphorylation of Mpk1 but not its protein kinase activity. Yeast strain DL454 (*mpk1*Δ) was cotransformed with multicopy plasmids bearing the indicated FLAG-tagged *MPK1* allele (wild-type, p2313; *mpk1-TA/YF*, p2316; *mpk1-K54R*, p2317) and His-tagged *SWI4* allele (p2418). Transformants were cultivated to mid-log phase in selective medium lacking methionine (to induce expression of Swi4-His_6) and either subjected to heat stress for 1 h at 39°C or maintained at 25°C. Cell extracts (Input) and immunoprecipitates with anti-FLAG M2 affinity gel (Sigma) or protein A affinity gel (Sigma) or no-antibody controls were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and analyzed by immunoblotting with anti-FLAG or anti-His (Qiagen) antibodies. α, anti; WT, wild type; β-Gal, β-galactosidase; Sp. Act., specific activity; U, unit; Ab, antibody.

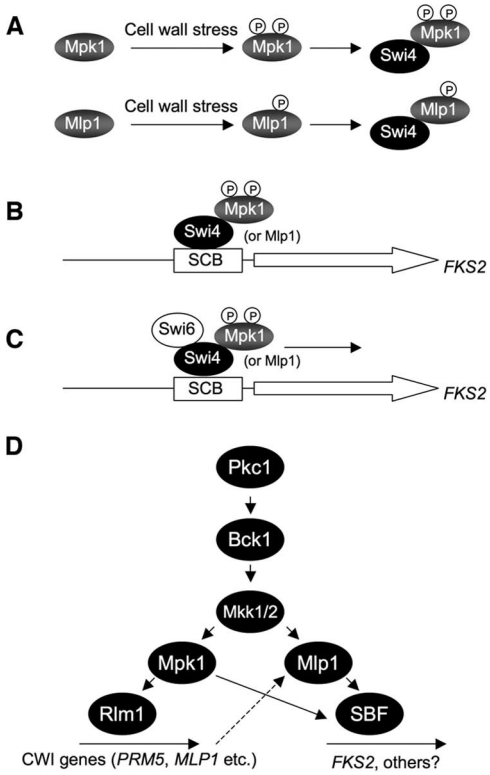

FIG. 6. Model for CWI-regulated induction of *FKS2* expression. (A) Cell wall stress results in phosphorylation and activation of the Mpk1 MAPK and the Mlp1 pseudokinase. Activated Mpk1 or Mlp1 binds Swi4. (B) The dimeric complex binds to the SBF-binding site within the *FKS2* promoter. (C) Swi6 engages the ternary complex to initiate transcription. (D) Model for activation of transcription by CWI signaling. Cell wall stress activates the Mpk1 MAPK and the Rlm1 transcription factor. *MLP1* expression is under the transcriptional control of Rlm1. Stress-induced Mlp1 is phosphorylated by MEKs (Mkk1/2) and stimulates *FKS2* transcription redundantly with Mpk1 by noncatalytic activation of SBF. P, phosphate.

which is activated by osmotic stress and uses several distinct mechanisms for inducing gene expression including recruitment of transcription factors, chromatin remodelers, and RNA polymerase to responsive promoters (reviewed in reference 20). All of these mechanisms require Hog1 protein kinase activity. In this study, we investigated the mechanism by which the yeast Mpk1 MAPK and its pseudokinase paralog, Mlp1, regulate gene expression.

Mpk1 and its pseudokinase paralog, Mlp1, function as transcriptional coactivators through a noncatalytic mechanism. The majority of transcriptional regulation through the CWI signaling pathway results from phosphorylation and activation of the Rlm1 transcription factor by Mpk1 (23, 35, 36, 55). We demonstrated that Mpk1 additionally uses a novel mechanism that does not require its protein kinase activity to drive expression of the *FKS2* gene in response to cell wall stress. This mechanism, which involves stable association with the *FKS2* promoter, was revealed through the discovery that Mpk1 is redundant with its catalytically inactive paralog Mlp1 for this specific function. Mpk1 and Mlp1 must nevertheless be activated through phosphorylation by their cognate MEKs (Mkk1/2).

In addition to phosphorylated Mpk1 or Mlp1, we found that *FKS2* transcription in response to wall stress requires the Swi4/Swi6 (SBF) transcription factor. SBF regulates G_1 cyclin transcription in a cell cycle-periodic manner (11) but has been suggested to serve another function in the response to cell wall stress (7, 44). We found that association of phosphorylated Mpk1 and Mlp1 with the *FKS2* promoter is dependent on an SBF-binding site within the promoter. Moreover, promoter association of Mpk1 and Swi4 was codependent but independent of Swi6. This is in contrast to the promoters of SBF-regulated cell cycle genes, in which association of Swi4 with Swi6 by their C termini renders Swi4 competent to engage SBF-binding sites (4, 6, 58). Intramolecular association of the Swi4 C terminus with its DBD interferes with its ability to bind DNA, which is relieved by Swi6 association. Our results indicate that in the context of the *FKS2* promoter, Mpk1 (or Mlp1) replaces the function of Swi6 in allowing Swi4 to bind DNA. However, it is likely that Swi4 possesses separate binding sites

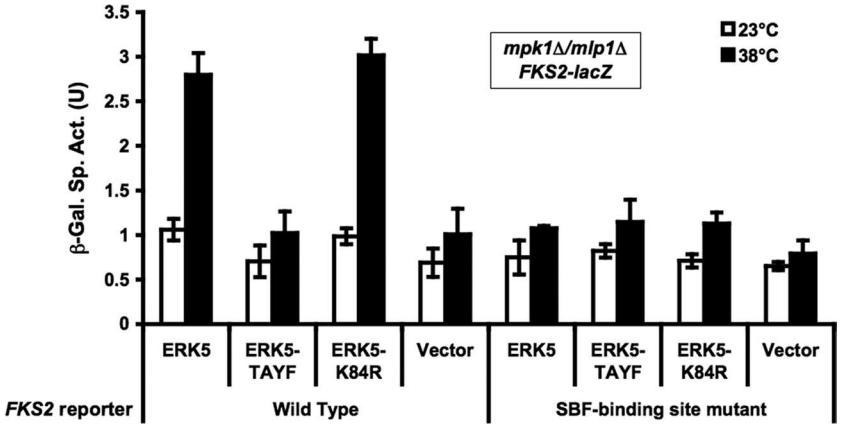

FIG. 7. The noncatalytic transcriptional function of Mpk1 and Mlp1 is conserved in human ERK5. *FKS2-lacZ* reporter plasmids (wild-type, p2052; SBF-binding site mutant, p2053) were cotransformed with ERK5 expression plasmids into an *mpk1*Δ *mlp1*Δ strain (DL3196). ERK5 plasmids were wild-type (ERK5; p2349), ERK5-T219A Y221F (TAYF; p2350), ERK5-K84R (p2351), or vector (pUT34; p2348). Transformants were cultured as described in the legend of Fig. 1 in the presence of YEPD medium containing 10% sorbitol for osmotic support. β-Galactosidase (β-Gal) activity was measured in crude extracts. Each value represents the mean and standard deviation from three independent transformants. Sp. Act., specific activity; U, unit.

for Mpk1 and Swi6 because Mpk1 forms a ternary complex with SBF in vivo and in vitro (7, 44). The difference between the SBF-binding site in the *FKS2* promoter, which is not regulated periodically through the cell cycle (48, 63), and the sites found in promoters of genes subject to G_1-specific transcriptional regulation must be defined outside of the core SBF-binding site, which is identical in *FKS2* and cell cycle-regulated genes. Perhaps the presence of a single SBF-binding site in *FKS2*, rather than the 2 to 10 sites found in cell cycle-regulated genes (5), contributes to this differential regulation.

Mpk1 has been shown previously to associate directly with Swi4 in vitro but not with Swi6 (7). We found that Mpk1 must be in the active conformation to associate with Swi4. MAPKs are activated by dual phosphorylation of neighboring Thr and Tyr residues in a TXY motif within their so-called activation loops, which is thought to relieve steric inhibition of protein substrate binding (28). The requirement for Mpk1 phosphorylation to bind Swi4 therefore suggests that the kinase engages Swi4 as though it is a substrate. Nevertheless, catalytic activity is not required for either DNA binding of the resulting complex or for transcriptional activation.

Although Swi6 is not required for Mpk1 or Swi4 to bind the *FKS2* promoter, we found that it is required for transcriptional activation and that Swi6 associates with the *FKS2* promoter in an Mpk1- and Swi4-dependent manner. Mpk1 is known to phosphorylate Swi6 in response to activation by cell wall stress (44). However, the consequence of phosphorylation has not been investigated. Our results demonstrate that this modification is not required for the involvement of Swi6 in *FKS2* induction. Indeed, loss of Mpk1 catalytic activity results in a small, but reproducible enhancement in transcriptional activation of *FKS2* (Fig. 2B). Mpk1 phosphorylation of Swi6 may be a negative regulatory modification, similar to phosphorylation of Swi6 by Cdc28, which results in its nuclear egress (25, 59). To our knowledge, this noncatalytic mode of transcriptional activation by a protein kinase (or a pseudokinase) is unprecedented.

The Mlp1 pseudokinase. Mlp1 is a paralog of Mpk1 that arose as a consequence of an ancestral whole-genome duplication (38). It was identified initially as a protein that interacts with the Rlm1 transcription factor (67). However, we demonstrated that Mlp1 neither contributes to Rlm1-driven transcription (Fig. 2C) nor interferes appreciably (K.-Y. Kim, unpublished). Although Mlp1 is not a catalytically active protein kinase, it possesses a Tyr residue within its activation loop that, as in MAPKs, is predicted to be phosphorylated in response to an activating signal. Mutation of this residue to Phe blocked the ability of Mlp1 to drive *FKS2* transcription and to associate with the *FKS2* promoter in response to cell wall stress. This result strongly suggests that tyrosine phosphorylation induces a conformational change in Mlp1 that allows it to bind Swi4 in a manner similar to activated Mpk1. As noted above, MAPKs are dually phosphorylated on neighboring Thr and Tyr residues. However, the activation loop Thr is replaced by a Lys residue in Mlp1. The significance of this change is not clear, but it evidently allows Mlp1 to be activated through a single phosphorylation event. This may alter its kinetics of activation by Mkk1/2 (34) or inactivation by the Msg5 and Sdp1 dual-specificity protein phosphatases (22, 27).

It is interesting that the *MLP1* gene is transcriptionally induced in response to cell wall stress (35, 36). Its induction results from phosphorylation and activation of the Rlm1 transcription factor by Mpk1. Thus, in the absence of Mpk1 or cell wall stress, very little Mlp1 is expressed (unpublished results). Nevertheless, it is evident that sufficient Mlp1 exists in the absence of Mpk1 to activate *FKS2* transcription to nearly normal levels (Fig. 2A). Figure 6C depicts a revised model of the CWI MAPK cascade based on these results.

Other characterized pseudokinases fall into two categories. Some, including mammalian STRAD (10), the ErbB3 epidermal growth factor receptor (9), and kinase suppressor of Ras (56), form complexes with active protein kinases (LKB1, ErbB, and Raf, respectively) and are critical to the regulation of their associated kinases. Others exist as pseudokinase domains

within active protein kinases, which also serve to regulate kinase activity. This group includes the yeast Gcn2 (54) and mammalian JAK kinases (57). Although we have not tested for a physical interaction between Mpk1 and Mlp1, our results clearly demonstrate that their functions are not interdependent.

What is the consequence of the absence of protein kinase activity in Mlp1? This is not yet clear, but it appears that mutational loss of catalytic activity in Mpk1 paralogs has evolved independently at least twice. *Saccharomyces castellii* is a yeast species which, like *S. cerevisiae*, arose after an ancestral whole-genome duplication event that was followed by deletion of most of the paralogous genes (38). Also like *S. cerevisiae*, *S. castellii* retained both copies of the *MPK1* gene and appears to have allowed one protein to evolve to a catalytically inactive state (Scas_678.13 [wolfe.gen.tcd.ie/browser]) but through a different set of mutations than those that inactivated *S. cerevisiae* Mlp1. This convergent evolution suggests that loss of catalytic activity in Mpk1 paralogs was the product of selection.

The noncatalytic mechanism for activation of *FKS2* expression is conserved in human ERK5. The human ERK5 MAPK complements loss of Mpk1 with respect to its cell lysis defect and transcriptional activation through Rlm1 (65). We found that this complementation extends to the noncatalytic transcriptional activation of *FKS2* through SBF. Although no Swi4 or Swi6 homologs are evident in metazoan genomes, our results raise the possibility that ERK5, or perhaps other MAPKs, possess previously unappreciated noncatalytic functions that require a signal from upstream kinases.

ACKNOWLEDGMENTS

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