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Fission yeast global repressors regulate the specificity of chromatin alteration in response to distinct environmental stresses

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ABSTRACT

The specific induction of genes in response to distinct environmental stress is vital for all eukaryotes. To study the mechanisms that result in selective gene responses, we examined the role of the fission yeast Tup1 family repressors in chromatin regulation. We found that chromatin structure around a cAMP-responsive element (CRE)-like sequence in *ade6-M26* that is bound by Atf1·Pcr1 transcriptional activation was altered in response to osmotic stress but not to heat and oxidative stresses. Such chromatin structure alteration occurred later than the Atf1 phosphorylation but correlated well with stress-induced transcriptional activation at *ade6-M26*. This chromatin structure alteration required components for the stress-activated protein kinase (SAPK) cascade and both subunits of the *M26*-binding CREB/ATF-type protein Atf1·Pcr1. Cation stress and glucose starvation selectively caused chromatin structure alteration around CRE-like sequences in *cta3*$^+$ and *fbp1*$^+$ promoters, respectively, in correlation with transcriptional activation. However, the *tup11*Δ *tup12*Δ double deletion mutants lost the selectivity of stress responses of chromatin structure and transcriptional regulation of *cta3*$^+$ and *fbp1*$^+$. These data indicate that the Tup1-like repressors regulate the chromatin structure to ensure the specificity of gene activation in response to particular stresses. Such a role for these proteins may serve as a paradigm for the regulation of stress response in higher eukaryotes.

INTRODUCTION

Environmental stresses induce distinct cellular responses of particular stress-responsive genes, which play pivotal roles in the stress response of eukaryotic cells. Signals from stress sensors to the particular transcription factors are transduced by evolutionarily conserved stress-activated protein kinase (SAPK) cascades (1–3), which are also involved in induction of apoptosis and inflammation in mammalian cells. Counterparts of the mammalian SAPK family, such as p38 and SAPK/c-Jun N-terminal kinase (JNK), are present in both *Saccharomyces cerevisiae* (Hog1) and *Schizosaccharomyces pombe* (Spc1/Sty1). The mammalian p38 and *S.pombe* Spc1/Sty1 kinase cascades are activated by a range of environmental stresses (4–7), while the *S.cerevisiae* Hog1 cascade is dedicated to response to hyperosmolarity (8) and heat stress (9). Thus, in mammalian and *S.pombe* cells, the same SAPK modules transmit various stress response signals, which raise the question about the mechanism of selective activation of distinct sets of genes by various environmental stresses.

The mechanisms for such selective gene activation by SAPK cascades have been investigated with respect to both positive and negative controls of transcription activators. The former positive controls involve at least in part the formation of multienzyme complexes between SAPKs and scaffold proteins that insulate the bound SAPK against gene activation by irrelevant stress signals (10). The latter negative controls have been demonstrated by recent studies on the roles of *S.cerevisiae* and *S.pombe* Tup1 family global repressors in the stress response.

The *S.cerevisiae* Tup1 repressor forms a complex with the Ssn6 protein (11,12). This Ssn6–Tup1 complex regulates the expression of numerous genes by interacting with a variety of sequence-specific DNA-binding proteins (13), and it is involved in the repression of genes regulated by cell type, glucose, oxygen, DNA damage and other cellular stress signals (14,15). The Ssn6–Tup1 complex is supposed to regulate transcription by modulating the stability of the basal transcription machinery (11,16,17), and by organizing

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repressive chromatin structure (18–21). In some Hog1-dependent osmostress genes such as *ENA1* and *GRE2*, Sko1 recruits the Ssn6–Tup1 repressor that represses gene expression (22). Importantly, phosphorylation of Sko1 by Hog1 causes disruption of the interaction between Sko1 and Ssn6–Tup1, and then induces a subset of osmostress genes (23). Therefore, these osmostress genes are mainly upregulated by selective derepression, rather than by gene activation as suggested elsewhere (24).

The fission yeast *S.pombe* has two partially redundant counterparts (Tup11 and Tup12) of Tup1. They have been shown to be involved in transcription repression of the *fbp1*$^+$ gene encoding the fructose-1,6-bisphosphatase (25,26) and the *cta3*$^+$ gene encoding the cation-transporting P-type ATPase (24). These loci have cAMP-responsive element (CRE)-like sequences in the promoter regions, and are activated specifically in response to glucose starvation (*fbp1*$^+$) and elevated cation concentration (*cta3*$^+$). The CRE-like sites function as binding sites for the CREB/ATF transcription factor Atf1·Pcr1, which is activated by the *S.pombe* SAPK cascade in response to various environmental stresses (7,27–31). Thus, Tup11 and Tup12 antagonize the function of the CRE·Atf1·Pcr1 transcription activation complex on CRE-like sites in the *fbp1*$^+$ and *cta3*$^+$ promoters. Interestingly, double deletion mutants of *tup11*$^+$ and *tup12*$^+$ genes confer loss of selective gene activation to distinct environmental stresses. Consequently, *fbp1*$^+$ and *cta3*$^+$ genes are non-selectively activated under various stresses (24). Therefore, it is suggested that Tup1 family global repressors may function as 'guardians of specificity' to prevent induction of those genes in response to irrelevant stress signals.

In *S.pombe* meiosis, the CRE·Atf1·Pcr1 complex activates homologous recombination at the *ade6-M26* (*M26*) recombination hotspot (32), which accompanies a CRE-like sequence created by a single G to T transversion in the *ade6* coding region (33,34). Components of the *S.pombe* SAPK pathway, such as the SAPK kinase Wis1 and the SAPK Spc1/Sty1, are required for activation of the *M26* recombination hotspot (27). We previously demonstrated that the CRE·Atf1·Pcr1 complex induces local alteration of chromatin structure at the *M26* recombination hotspot during meiosis [(35) and our unpublished observation]. In addition, components of the SAPK cascade are also needed for chromatin changes at *M26* (36). Possibly, the SAPK-activated CRE·Atf1·Pcr1 complex helps to create an open chromatin configuration around *M26* as a prerequisite to access of meiotic recombination initiating proteins, such as Rec12 that catalyzes meiosis-specific recombination-initiating DNA double-strand breaks (37). Importantly, Tup11 and Tup12 are required for such chromatin changes in the *M26* recombination hotspot as well as chromatin alteration in the *fbp1*$^+$ promoter under derepressed conditions (38). Thus, the *M26* site, like the *fbp1*$^+$ and *cta3*$^+$ promoters, provides a good system to analyze the functions of Tup1 family repressors in chromatin regulation.

Here we report that particular environmental stresses cause alteration of chromatin structure around CRE-like sequences in the *M26* site, and *fbp1*$^+$ and *cta3*$^+$ promoters, correlating well with distinct transcription activation of these genes. In addition, we found that Tup11 and Tup12 function as specificity factors to ensure proper gene responses to particular stress signals by selectively allowing chromatin opening in response to distinct environment stresses.

MATERIALS AND METHODS

Fission yeast strains, genetic methods and media

The *S.pombe* strains used in this study are listed in Table 1. General genetic procedures of *S.pombe* were carried out as described (39). Minimal medium (SD) (40) was used for the culture of *S.pombe* unless otherwise stated. Construction of the strains was carried out by mating haploids on sporulation medium (SPA) (39) followed by tetrad dissections. Standard rich yeast extract medium (YEL) (39) was used for culturing cells with various stress-inducing agents (1.2 M sorbitol, 0.9 M KCl, 42°C or H_2O_2).

Northern blot analysis

The probes to detect transcripts of *cta3*$^+$, *fbp1*$^+$ and *cam1*$^+$ were prepared from PCR-amplified DNA fragments, and the DNA fragments were further labeled with ^{32}P using a random priming kit (Amersham-Pharmacia, Piscataway, NJ). The nucleotide sequences of the primers used for *fbp1*$^+$ and *cam1*$^+$ were as described (38). The primer sequences for *cta3*$^+$ were as follows: cta3-5′, CGAACATTGGCTTCTCC; and cta3-3′, GGTTGCGTAACAAATTCC.

The probe to detect the *ade6*$^+$ transcript was prepared from a DNA fragment as described (41). Total RNA was prepared from *S.pombe* cells according to the method described elsewhere (42). For the northern blot analysis, 10 μg of total RNA was denatured with formamide, separated in 1.5% agarose gels containing formaldehyde (43), and blotted to a charged Nylon membrane (Biodyne B membrane, PALL, EA).

Chromatin analysis

Analysis of chromatin structure by indirect end labeling was done according to the method of Mizuno *et al*. (35). The DNA samples were analyzed by Southern analysis as described below. To analyze chromatin around the *cta3*$^+$ promoter, the micrococcal nuclease (MNase)-treated DNA was digested with HpaII and separated by electrophoresis in a 1.5% agarose gel (40 cm long) containing TAE buffer. The separated DNA fragments were alkali-transferred to charged Nylon membranes (Biodyne B membrane, PALL, EA). The probe used for the indirect end labeling of the *cta3*$^+$ region was the same probe used for the northern analysis as described above. For the *ade6*$^+$ or *fbp1*$^+$ locus, the MNase-treated DNA was digested with XhoI or ClaI, respectively, followed by Southern analysis using the probe as described (35,38).

Determination of recombination rate

Each of the diploid strains D25 (M26/M469) and D26 (M375/M469) were cultured in YEL for 20 h from a single colony. Cells were then transferred to YEL containing 1.2 M sorbitol, cultured for 24 h and spread on an SD ± adenine plate. Recombination rates (R) around *M26* are calculated as follows: R = colony number on plates lacking adenine/colony number on plates containing adenine.

Table 1. *Schizosaccharomyces pombe* strains used in this study

Strain		Genotype
K123	h^-	*ade6-M26 wis1::ura4$^+$ ura4-D18 leu1-32*
K127	h^-	*ade6-M375 leu1-32*
K131	h^-	*ade6-M26 leu1-32*
K142	h^-	*ade6-M26 cgs1::ura4$^+$ ura4-D18 leu1-32*
K186	h^-	*ade6-M26 atf1::ura4$^+$ ura4-D18 his3-D1 leu1-32*
K188	h^-	*ade6-M26 pcr1::his3$^+$ ura4-D18 his3-D1 leu1-32*
JK40	h^-	*ade6-M26 leu1-32 ura4-D18 tup11::ura4$^+$ tup12::ura4$^+$*
JK90	h^-	*ade6-M375 leu1-32 ura4-D18 tup11::ura4$^+$ tup12::ura4$^+$*
JK157	h^-	*ade6-M26 spc1::ura4$^+$ ura4-D18*
D20	h^+/h^-	*ade6-M26/ade6-M26 his5-303/his5$^+$ leu1-32/leu1$^+$*
D25	h^+/h^-	*ade6-M26/ade6-469 his5-303/his5$^+$ leu1-32/leu1$^+$ ura4-D18/ura4-D18*
D26	h^+/h^-	*ade6-M375/ade6-469 his5-303/his5+ leu1-32/leu1+ ura4-D18/ura4D18*

Detection of Atf1 by western blotting

We essentially used a previously described protocol (44), except for a few modifications. Briefly, cells were harvested using a nitrocellulose filter (Millipore, USA), suspended in 50 µl of STOP buffer and boiled for 3 min, and then disrupted with glass beads in 200 µl of HB buffer. Each suspension was separated by SDS–PAGE. Polyclonal anti-Atf1 antibody (this study) was raised in rabbits with recombinant His-tagged Atf1 protein, which had been expressed in *Escherichia coli* BL-21 strain carrying pET15b/Atf1 and purified by a TALON metal affinity column (Clonetech) according to the manufacturer's procedure. The serum was affinity purified using the purified recombinant His-tagged Atf1 protein. The anti-Atf1 antibody was used as the primary antibody (1:1000), and goat anti-rabbit immunoglobulin G (IgG) Fc fragment conjugated with horseradish peroxidase (Amersham-Pharmacia, Piscataway, NJ) was used for detection on the membrane.

Phosphatase treatment

Atf1 was enriched in the precipitate after a centrifugation of the cell suspension described above. The precipitated sample was washed five times with the alkaline phosphate buffer indicated by the manufacturer (Takara, Kyoto Japan) and suspended in the same buffer. The suspension was incubated with 22 U of calf intestinal alkaline phosphate (Takara, Kyoto, Japan) at 37°C for 60 min, either with or without inhibitor mixture (10 mM EGTA, 0.1 M Na_3VO_4, 50 mM β-glycerophosphate, 15 mM *p*-nitrophenyl phosphate). The suspension was then precipitated by centrifugation and precipitates were boiled with SDS–PAGE sample buffer. Western analysis was done as described above.

RESULTS AND DISCUSSION

Osmotic stress induces chromatin alteration and transcription, but not recombination, at CRE-like sites in *ade6-M26*

To examine the relationship between chromatin structure around CRE-like sequences and cellular stress response, we first examined the chromatin structure of *ade6-M26* in cells exposed to various extracellular stresses. Indirect end labeling analysis with MNase-treated chromatin from haploid cells revealed the positions of individual nucleosomes and nuclease-hypersensitive sites in the *ade6-M26* region. Haploid K131 (h^- *ade6-M26 leu1-32*) cells were cultured in a rich medium (YEL) to mid-log phase, and then treated with various cellular stresses such as osmotic (sorbitol), cation (KCl), heat (42°C) and oxidative (H_2O_2) stresses.

The chromatin around the *M26* site is protected from MNase digestion in untreated cells, although a hypersensitive site appears at the *M26* site and positioning of the nearby nucleosomes become perturbed when cells are treated with 1.2 M sorbitol for 90 min (Fig. 1A, arrowheads). The changes in chromatin configuration became remarkable 60–90 min after the medium shift. The kinetics of these changes in chromatin occur at a relatively slower rate than that of Spc1-dependent phosphorylation of Atf1 in response to the sorbitol treatment (Fig. 1B), which normally occurs within 5 min after receiving the sorbitol osmotic stress, but similar to the kinetics of transcriptional activation at *ade6-M26* (Fig. 1C). The *ade6-M26* transcripts became smaller and more abundant in response to sorbitol treatment, suggesting the usage of a distinct transcription start site in cells adapting to osmotic stress.

Unlike the transcriptional activation, the sorbitol treatment did not influence interallelic recombination (gene conversion) frequency between *ade6-M26* and *ade6-M469* recombination markers. The recombination frequency between *ade6-M26* and *ade6-M469* in the absence ($2.3 \pm 0.3/10^4$ cells, $n = 4$) or presence ($2.3 \pm 0.3/10^4$ cells, $n = 4$) of sorbitol was very similar to that between *ade6-M375* and *ade6-M469* in the absence ($2.3 \pm 0.3/10^4$ cells, $n = 4$) or presence ($2.2 \pm 0.6/10^4$ cells, $n = 4$) of sorbitol. These data are not surprising, as proteins required for meiotic recombination initiation such as Rec12 are not expressed during the *S.pombe* mitotic cell cycle (45). These results also indicate that changes in chromatin structure itself cannot activate the *M26* recombination hotspot. Presumably, chromatin remodeling at *M26* is required, but not sufficient for the activation of the *M26* recombination hotspot, which ensures access of the meiotic recombination machinery to the chromosomal DNA.

Interestingly, relatively few changes in chromatin structure could be detected at *ade6-M26* in cells subjected to cation (0.9 M KCl), heat shock (42°C) or oxidative (6 mM H_2O_2) stresses even 90 min after the treatments (Fig. 1D and E), and transcriptional activation of *ade6-M26* was not observed by these stresses (Fig. 1F). Thus, the chromatin around *M26* is

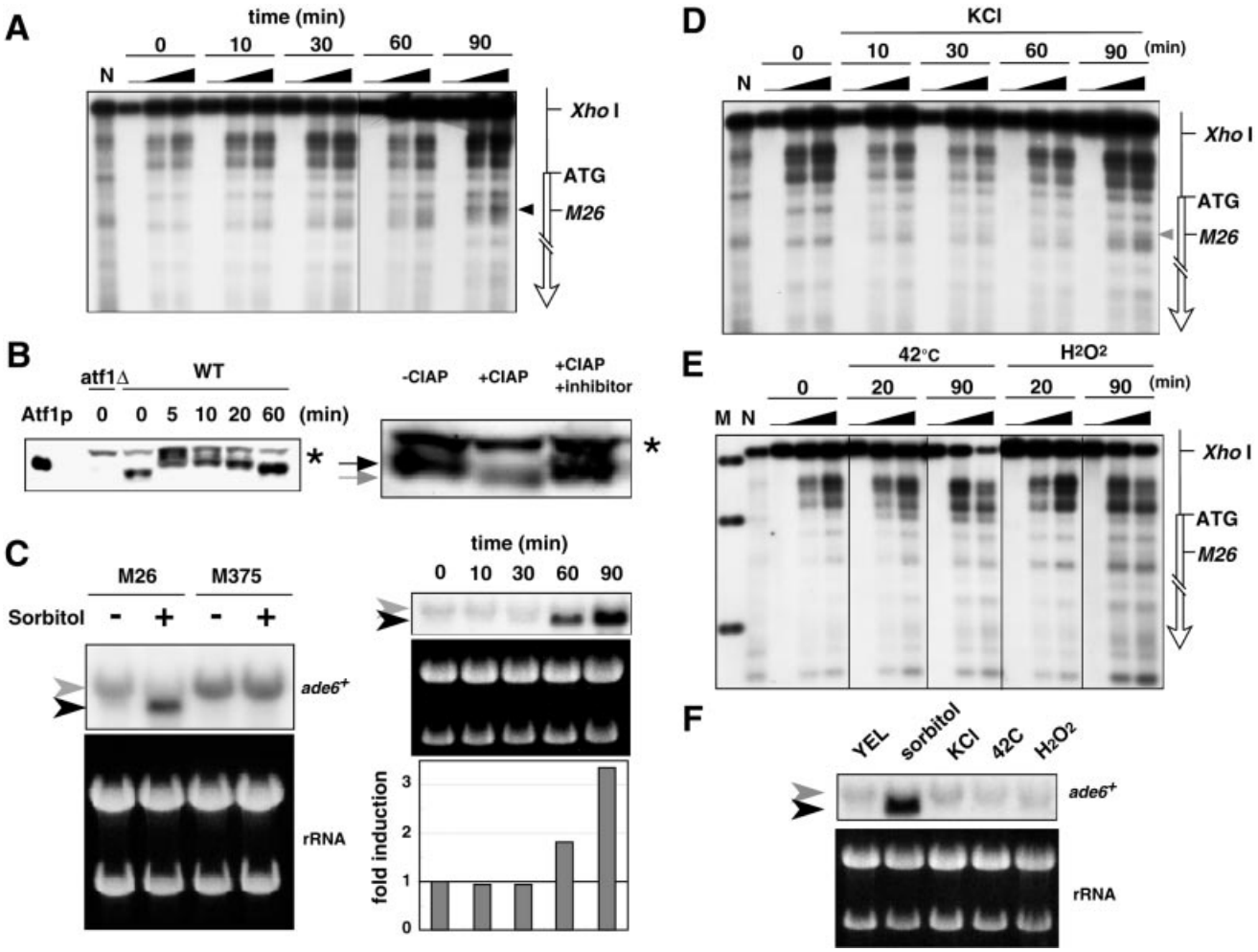

Figure 1. Chromatin structure at *M26* is altered in response to osmotic stress. (**A**, **D** and **E**) *M26* strain K131 was cultured in YEL to mid-log phase and transferred to YEL containing 1.2 M sorbitol (A), 0.9 M KCl (D), 6 mM H_2O_2 (E) or YEL pre-warmed to 42°C (E). Cells were harvested at the indicated time after the culture transfer. The arrowheads show MNase-sensitive sites surrounding the *M26* mutation site. (**B**) K131 (wild-type) or K186 (*atf1*Δ) cells were cultured in YEL and shifted to YEL containing 1.2 M sorbitol. The cells were harvested at the indicated time after the osmotic shock. The protein samples were subjected to SDS–PAGE and western blot to detect the phosphorylation state of Atf1. The His_6 tag of *E.coli*-expressed Atf1 protein (a positive loading control), which includes some spacer peptide sequences, reduces the mobility slightly. The extract from the *atf1*Δ strain shows a non-specific band (indicated by asterisks). The 10 min time point sample was treated by calf intestine alkaline phosphatase (CIAP) in the presence or absence of phosphatase inhibitors. The black and gray arrows indicate the bands of Atf1 in the phosphorylated or unphosphorylated state. (**C**) The *M26* and *M375* strains (K131 and K127, respectively) were cultured in YEL to mid-log phase and transferred to YEL containing 1.2 M sorbitol. Cells were harvested 90 min after osmotic shock. Left panel: the transcriptional shift of *ade6-M26* through time 0–90 min after osmotic shock. Right panel: transcription levels were quantified using a Fuji BAS2000 scanner. (**F**) The *M26* transcriptional shift was examined subsequent to the indicated stresses in K131 cells. Cells were harvested 90 min after each stress. rRNA was detected by ethidium bromide staining as a loading control. Arrows in (C) and (F) indicate two distinct transcripts from the *ade6* locus, the smaller of which is induced by osmotic stress.

altered selectively in response to osmotic stress. In DNA containing a control allele *ade6-M375* (*M375*) having no CRE-like sequence, but with the identical termination codon adjacent to the position of the one created by the *M26* mutation (33,46), no such chromatin alteration was observed in response to osmotic stress (Fig. 2A). This suggests that the CRE-like sequence is required for the chromatin alteration induced by osmotic stress.

Chromatin alteration at *M26* induced by osmotic stress requires components of the Wis1–Spc1 SAPK cascade

We previously reported that chromatin alteration at the *M26* site during meiosis is regulated antagonistically by the SAPK cascade and cAMP-dependent kinase (PKA) pathway (36). We next examined if the SAPK cascade and the PKA pathway regulate chromatin alteration at the *M26* site induced by osmotic stress. Wild-type (K131), *cgs1*Δ (K142), *wis1*Δ (K123), *spc1*Δ (JK157), *atf1*Δ (K186) and *pcr1*Δ (K188) cells were cultured in YE to mid-log phase, and further cultured in YE containing 1.2 M sorbitol for 90 min to induce chromatin alteration. The appearance of the MNase-sensitive site around *M26* site is clearly observed in wild-type and *cgs1*Δ strains (Fig. 2A indicated by an arrowhead). This hypersensitive site is as best weakly detected in mutants for SAPK components (*wis1*Δ and *spc1*Δ), and is absent in strains defective in the Atf1·Pcr1 activator (*atf1*Δ and *pcr1*Δ), in

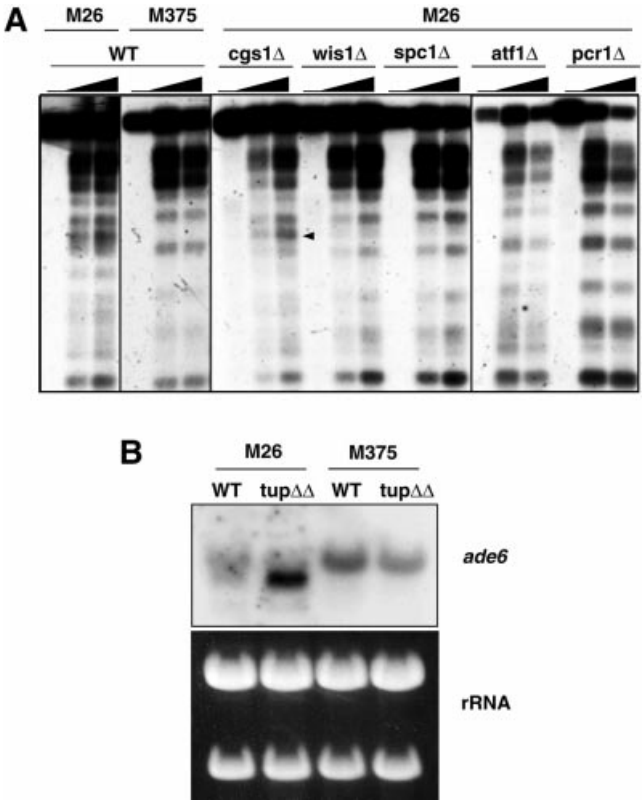

Figure 2. Chromatin structure alteration at *M26* induced by osmotic stress requires the Wis1–Spc1 SAPK cascade. (**A**) The cells of strains K131 (*ade6-M26*), K127 (*ade6-M375*), K142 (*cgs1*Δ *ade6-M26*), K123 (*wis1*Δ *ade6-M26*), JK157 (*spc1*Δ *ade6-M26*), K186 (*atf1*Δ *ade6-M26*) and K188 (*pcr1*Δ *ade6-M26*) were cultured in YEL to mid-log phase and transferred to YEL containing 1.2 M sorbitol. The cells were harvested 90 min after osmotic shock and chromatin structure was examined as described in Materials and Methods. The arrowhead indicates the position of the sensitive site around the *M26* site. (**B**) Constitutive transcriptional activation and alteration of the transcriptional start site of *ade6-M26* in a *tup*ΔΔ strain. The cells of strains K131 (*ade6-M26*), JK40 (*ade6-M26 tup*ΔΔ), K127 (*ade6-M375*) and JK90 (*ade6-M375 tup*ΔΔ) were cultured in YEL. The RNA from each of the strains was analyzed by northern blotting.

which the chromatin structure resembles that of the *ade6-M375* negative control strain. These results indicate that the SAPK cascade and the Atf1·Pcr1 heterodimeric CREB/ATF-type protein regulate chromatin remodeling at *M26* in response to osmotic stress. Interestingly, unlike the case of meiotic chromatin alteration at *M26*, osmotic stress-induced chromatin alteration at *M26* does not require the Cgs1 function, which is involved in the activation of meiotic chromatin changes at *M26*. In addition, we observed wild-type levels of osmotic stress-induced chromatin alteration at *M26* in deletion mutants for *mei2*$^+$ and *mei3*$^+$ genes (data not shown), which are essential for the meiotic chromatin changes at *M26*. Such discrepancies in genetic requirements may suggest distinct regulatory pathways for chromatin alteration in meiotic and mitotic stress response.

We previously reported that *tup11*$^+$ and *tup12*$^+$ are involved in repression of chromatin remodeling at the *ade6-M26* locus (38). In the *tup*ΔΔ strain, the chromatin structure around *M26* was altered even in growing cells (38). To study the physiological significance of the *M26* chromatin alteration detected in *tup*ΔΔ cells, we analyzed *ade6*$^+$ transcription in the wild-type and *tup*ΔΔ strain by northern analysis. As shown in Figure 2B, *ade6-M26* transcription was activated in the *tup*ΔΔ strain and the size of the transcript became smaller as observed in *M26* (*tup11*$^+$*tup12*$^+$) cells subjected to osmotic stress (Fig. 1C), while transcription of the *ade6-M375* allele was not influenced by the loss of Tup11 and Tup12. Thus, the loss of the Tup proteins alters both the chromatin structure and transcription at this locus in an *M26*-dependent manner. These observations led us to speculate that Tup11 and Tup12 proteins may ensure the specificity of the transcriptional activation in response to stresses by regulating the local chromatin configuration.

Tup11 and Tup12 ensure selective response of chromatin configuration in the *cta3*$^+$ promoter to cation stress

As reported elsewhere (36), chromatin remodeling can occur meiotically at natural CRE-like sequences. Thus, we next examined the chromatin structure at natural CRE-like sequences in response to environmental stress. Greenall *et al*. (24) reported that the *cta3*$^+$ promoter harbors a CRE-like sequence 5′-TTACGTAA-3′, which can be a binding site for Atf1·Pcr1 heterodimer. The *cta3*$^+$ gene is markedly induced selectively by cation stress (24). This selectivity of the *cta3*$^+$ transcription activation by cation stress is abolished by the double deletion of *tup11*$^+$ and *tup12*$^+$ (24), which are also involved in repression of chromatin remodeling at the *ade6-M26* and *fbp1*$^+$ locus during mitosis and glucose repression, respectively (38). Thus, we next analyzed the chromatin structure around the *cta3*$^+$ promoter in response to various cellular stresses.

Transcription at *cta3*$^+$ is normally activated in response to cation stress (the KCl lane in Fig. 3A), but not by other stresses (sorbitol, heat shock and H_2O_2). However, loss of Tup11 and Tup12 (*tup*ΔΔ strain) leads to elevated *cta3*$^+$ transcription in either the absence or presence of those stresses. After the stress treatment of the wild-type cells for 15 min, we found that an MNase-sensitive site appeared around CRE-like site in the *cta3*$^+$ promoter region (Fig. 3B and C, indicated by arrowheads) only when the cells were treated with KCl, which is consistent with the activation of *cta3*$^+$ transcription in response to the KCl treatment. Importantly, the chromatin structure was constitutively remodeled in the *cta3*$^+$ promoter in the *tup*ΔΔ cells in the absence or presence of various stresses, in agreement with the constitutive activation of *cta3*$^+$ transcription in the *tup*ΔΔ cells. We identified a putative stress response element (STRE) sequence in the *cta3*$^+$ promoter region (Fig. 3B, indicated by an open square), which may be a target sequence for the binding of Scr1, an *S.pombe* counterpart of the *S.cerevisiae* Mig1 repressor that recruits the Tup1–Ssn6 complex to STRE sites. Therefore, it is possible that Scr1, or other related zinc finger proteins, recruits Tup11 and Tup12 to the *cta3*$^+$ promoter region, although this remains to be tested.

Loss of Tup11 and Tup12 results in aberrant induction of *fbp1*$^+$ transcription in response to osmotic stress and nitrogen starvation

Finally, we examined the relationship between transcriptional activity and chromatin structure of the *fbp1*$^+$ promoter during

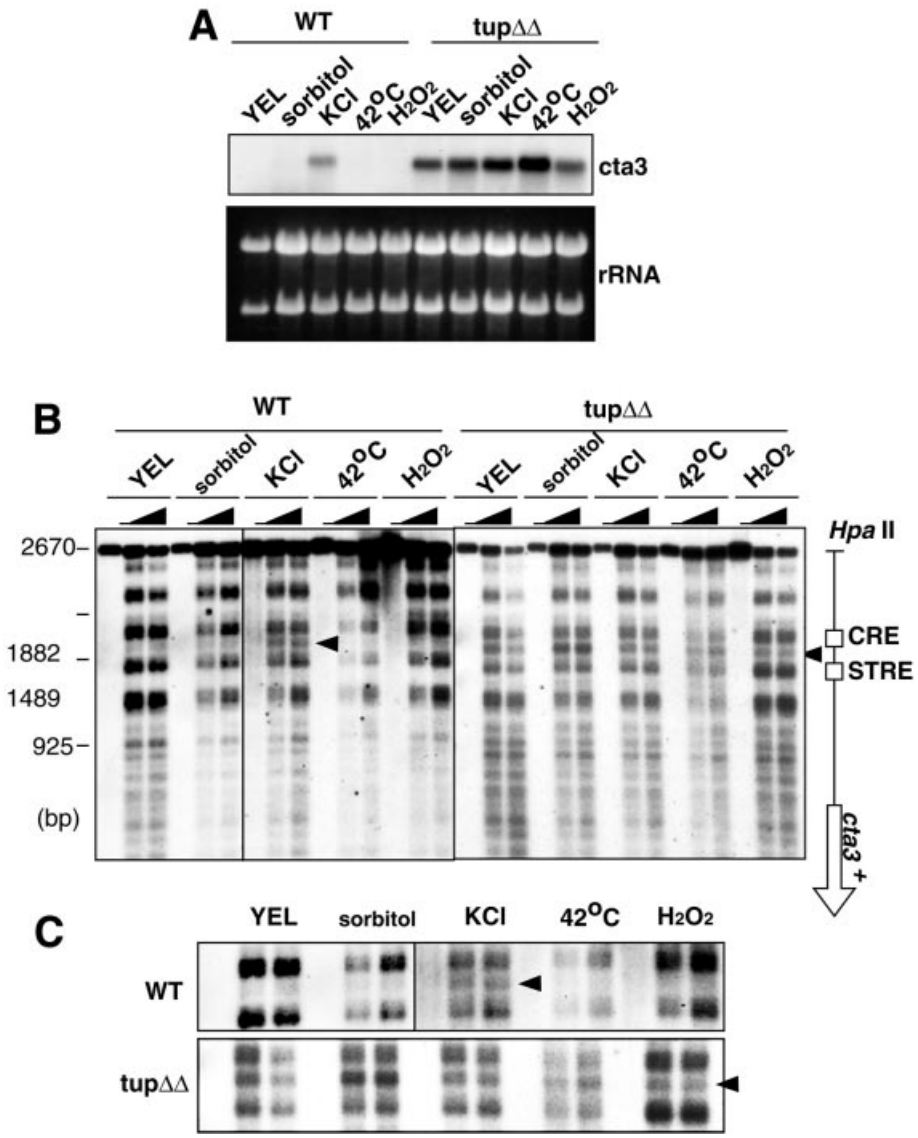

Figure 3. Selective response of chromatin structure around the *cta3*$^+$ promoter to cation stress and requirement of *tup11*$^+$ and *tup12*$^+$ for the chromatin structure regulation. Wild-type (K131) and *tup*ΔΔ (JK40) cells were cultured in YEL and a portion of each culture was harvested prior to stress treatment (YEL). The remainder was shifted as described in Figure 1. The cells were harvested 15 min after medium change. The cells were subjected to northern blot analysis to detect *cta3*$^+$ mRNA (**A**), or to chromatin analysis to examine chromatin structure (**B**). The open arrow indicates the *cta3*$^+$ coding regions. The arrowhead identifies a prominent MNase-sensitive site between a CRE (–1105 to –1098 bp relative to the translational start point) and an STRE (–741 to –736 bp) sequence, respectively. (**C**) Magnification of the MNase-sensitive pattern in the region of the CRE and STRE sequences.

stress response. As reported elsewhere, Tup11 and Tup12 repress chromatin remodeling in the *fbp1*$^+$ promoter in glucose repression (38). This and the above data led us to speculate that the absence of Tup function might allow non-specific derepression of transcription and chromatin structure in the *fbp1*$^+$ promoter in response to stresses other than glucose starvation. As previously reported (38), the chromatin structure in the *fbp1*$^+$ promoter is markedly altered in response to glucose starvation. We observe that treatment of wild-type cells with 1.2 M sorbitol or with nitrogen starvation caused no significant changes in chromatin structure around the *fbp1*$^+$ promoter (Fig. 4A). Similarly, no significant transcripts could be detected in *fbp1*$^+$ (Fig. 4B). On the other hand, the treatment of the *tup*ΔΔ strain with sorbitol and nitrogen starvation resulted in marked changes in chromatin structure around the *fbp1*$^+$ promoter (Fig. 4A) and dramatic activation of *fbp1*$^+$ transcription (Fig. 4B), similar to that observed in wild-type cells cultured in derepressed (glucose starved) conditions (38). Unlike the constitutive effects of *tup*ΔΔ on the chromatin and transcription in the *cta3*$^+$ promoter, the *fbp1*$^+$ promoter region in *tup*ΔΔ cells still exhibits repressed chromatin and transcription status under conditions with glucose. Thus, the regulation of the *fbp1*$^+$ promoter might be more complex than that of the *cta3*$^+$ promoter. Presumably, Rst2 protein may be involved in such complex regulation of the *fbp1*$^+$ promoter in the presence of glucose.

The present data demonstrate that the selective response of transcription to various extracellular stresses in fission yeast is correlated with chromatin alterations of promoter regions affected by these stresses. In addition, it is suggested that

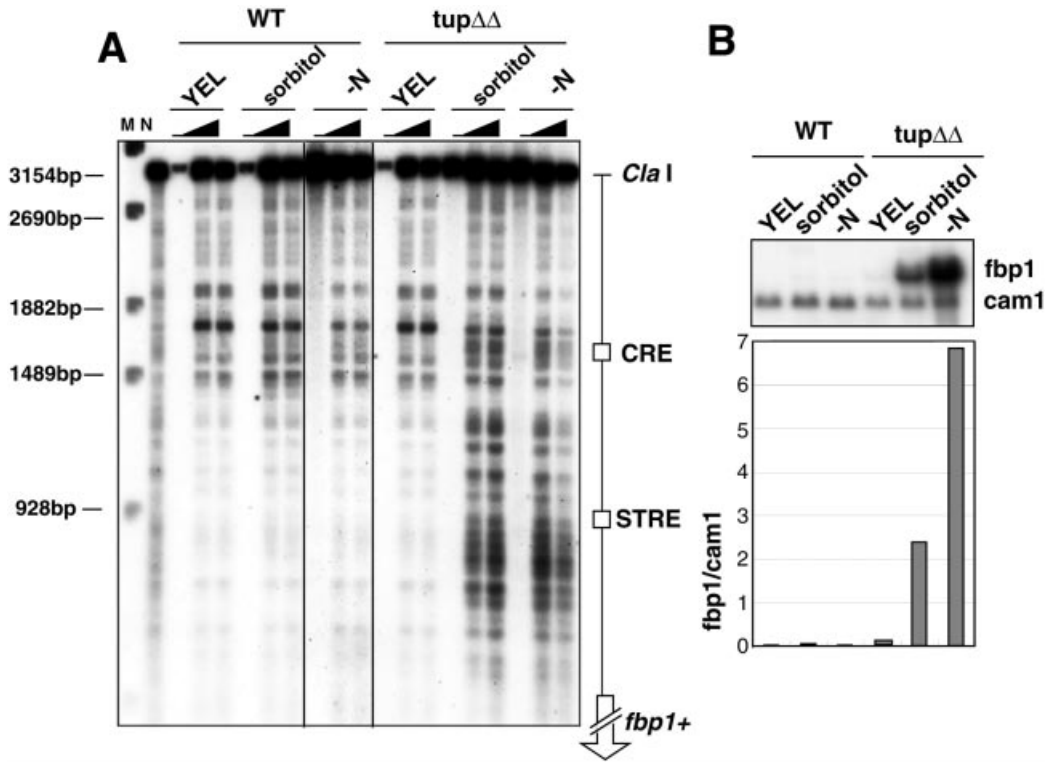

Figure 4. Requirement of Tup11 and Tup12 for the inhibition of chromatin structure alteration at the *fbp1*$^{+}$ promoter during stress response. (**A** and **B**) Wild-type (K131) and *tup*ΔΔ (JK40) cells were cultured in YEL and a portion was harvested (YEL). The remainder was shifted to YEL containing 1.2 M sorbitol or MM-N defined medium lacking nitrogen (–N). The cells shifted to YEL containing sorbitol were harvested 90 min after medium change, and the cells shifted to MM-N were harvested 3 h after medium change. Each sample was subjected to the chromatin analysis to see the chromatin structure (A), or northern blot analysis to detect *fbp1*$^{+}$ mRNA (B). The open arrow indicates the *fbp1*$^{+}$ coding regions. The open squares indicate the CRE (–1573 to –1566 bp relative to the translational start point) and STRE (–938 to –926 bp) sequences, respectively.

fission yeast Tup1-like repressors repress such chromatin remodeling. It is likely that the Tup1-like repressors, together with CREB/ATF-type proteins, the SAPK cascade and some other accessory factors (e.g. Scr1 and Rst2), govern selectivity of gene response to various stresses at the chromatin level. As components for these machineries are conserved in eukaryotes, this proposed function for Tup1 family repressors could also be true in higher eukaryotes.

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