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 tup1Δ tup12Δ 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 Snf6 protein (11,12). This Snf6–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 Snf6–Tup1 complex is supposed to regulate transcription by modulating the stability of the basal transcription machinery (11,16,17), and by organizing...
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 suggested elsewhere (24).

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 32P using a random priming kit (Amersham-Pharmacia, Piscataway, NJ). The nucleotide sequences of the primers used for fbp1+ and cam1+ are as described (38). The primer sequences for cta3+ were as follows: cta3-5’, CGAACATTGCCTTCCTCC; and cta3-3’, GGTGCCGAACAAATTC.

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 micrococal 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.

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 H2O2).

Northern blot analysis

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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. 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 Na3VO4, 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 (H2O2) 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 ± 0.3/104 cells, n = 4) or presence (2.3 ± 0.3/104 cells, n = 4) of sorbitol was very similar to that between ade6-M375 and ade6-M469 in the absence (2.3 ± 0.3/104 cells, n = 4) or presence (2.2 ± 0.6/104 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 H2O2) 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...
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), cgs1Δ (K142), wis1Δ (K123), spc1Δ (JK157), atf1Δ (K186) and pcr1Δ (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 was clearly observed in wild-type and cgs1Δ strains (Fig. 2A indicated by an arrowhead). This hypersensitive site is as best weakly detected in mutants for SAPK components (wis1Δ and spc1Δ), and is absent in strains defective in the Atf1·Pcr1 activator (atf1Δ and pcr1Δ), in
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 tupΔA 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 tupΔA cells, we analyzed ade6′ transcription in the wild-type and tupΔA strain by northern analysis. As shown in Figure 2B, ade6-M26 transcription was activated in the tupΔA 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′-TTAGGTAA-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 H2O2). However, loss of Tup11 and Tup12 (tupΔA 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 MNa-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 tupΔA cells in the absence or presence of various stresses, in agreement with the constitutive activation of cta3+ transcription in the tupΔA cells. We identified a putative stress response element (STRE) sequence in the cta3+ promoter region (Fig. 3B and C, indicated by arrowheads) only when the cells were treated with KCl, 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
stress response. As reported elsewhere, Tup11 and Tup12 repress chromatin remodeling in the \textit{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 \textit{fbp1} promoter in response to stresses other than glucose starvation. As previously reported (38), the chromatin structure in the \textit{fbp1} promoter is markedly altered in response to stresses other than 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 \textit{fbp1} promoter (Fig. 4A). Similarly, no significant transcripts could be detected in \textit{fbp1} (Fig. 4B). On the other hand, the treatment of the \textit{tup}\textit{DD} strain with sorbitol and nitrogen starvation resulted in marked changes in chromatin structure around the \textit{fbp1} promoter (Fig. 4A) and dramatic activation of \textit{fbp1} transcription (Fig. 4B), similar to that observed in wild-type cells cultured in derepressed (glucose starved) conditions (38). Unlike the constitutive effects of \textit{tup}\textit{DD} on the chromatin and transcription in the \textit{cta3} promoter, the \textit{fbp1} promoter region in \textit{tup}\textit{DD} cells still exhibits repressed chromatin and transcription status under conditions with glucose. Thus, the regulation of the \textit{fbp1} promoter might be more complex than that of the \textit{cta3} promoter. Presumably, Rst2 protein may be involved in such complex regulation of the \textit{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
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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