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Reciprocal Nuclear Shuttling of Two Antagonizing Zn Finger Proteins Modulates Tup Family Corepressor Function To Repress Chromatin Remodeling[▽]

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The *Schizosaccharomyces pombe* global corepressors Tup11 and Tup12, which are orthologs of *Saccharomyces cerevisiae* Tup1, are involved in glucose-dependent transcriptional repression and chromatin alteration of the *fbp1*$^+$ gene. The *fbp1*$^+$ promoter contains two regulatory elements, UAS1 and UAS2, one of which (UAS2) serves as a binding site for two antagonizing C_2H_2 Zn finger transcription factors, the Rst2 activator and the Scr1 repressor. In this study, we analyzed the role of Tup proteins and Scr1 in chromatin remodeling at *fbp1*$^+$ during glucose repression. We found that Scr1, cooperating with Tup11 and Tup12, functions to maintain the chromatin of the *fbp1*$^+$ promoter in a transcriptionally inactive state under glucose-rich conditions. Consistent with this notion, Scr1 is quickly exported from the nucleus to the cytoplasm at the initial stage of derepression, immediately after glucose starvation, at which time Rst2 is known to be imported into the nucleus. In addition, chromatin immunoprecipitation assays revealed a switching of Scr1 to Rst2 bound at UAS2 during glucose derepression. On the other hand, Tup11 and Tup12 persist in the nucleus and bind to the *fbp1*$^+$ promoter under both derepressed and repressed conditions. These observations suggest that Tup1-like proteins recruited to the *fbp1*$^+$ promoter are controlled by either of two antagonizing C_2H_2 Zn finger proteins. We propose that the actions of Tup11 and Tup12 are regulated by reciprocal nuclear shuttling of the two antagonizing Zn finger proteins in response to the extracellular glucose concentration. This notion provides new insights into the molecular mechanisms of the Tup family corepressors in gene regulation.

A proper response to extracellular stresses is vital for the homeostasis of biological systems. Therefore, transcriptional regulation in response to stress signaling must be rigorously controlled. Transcription preferentially occurs in accessible chromatin domains, where acetylation of histones and local chromatin remodeling are induced to facilitate the recruitment of transcriptional regulators and RNA polymerases onto DNA. Such local chromatin accessibility is under the regulation of transcription activators and repressors that can bind specifically to *cis*-acting regulatory elements and subsequently recruit coactivators and corepressors, respectively (26, 34, 41).

The *Saccharomyces cerevisiae* Tup1 protein is a global corepressor with WD40 repeats that can interact with Ssn6 (35, 47) and has been suggested to be a potential yeast ortholog of Groucho (reviewed in reference 4) family corepressors (7). The Ssn6-Tup1 complex regulates the expression of numerous genes controlled by a variety of DNA binding proteins involved in transcriptional repression under the control of cell type, glucose, DNA damage, and other cellular stress signals (36, 49). Tup1 binds to histones, histone deacetylases, transcription regulators, and RNA polymerase II (5, 12, 35, 51, 53). This suggests potential roles of the Ssn6-Tup1 complex in transcriptional regulation by altering chromatin or the stability of the transcription machinery. In fact, the Ssn6-Tup1 complex has been shown to establish repressive chromatin structures around promoters (3, 8, 9) and to inhibit the function of the basal transcription machinery (24, 35, 56). Tup1 is recruited to the promoters of target genes via interactions with various sequence-specific DNA binding repressors. For example, Tup1 is recruited by the Mig1 repressor to glucose-repressed genes (46).

The *Schizosaccharomyces pombe* (fission yeast) Tup11 and Tup12 proteins are redundant counterparts of Tup1 which are involved in transcriptional glucose repression of the *fbp1*$^+$ gene, encoding fructose-1,6-bisphosphatase (20, 30). Furthermore, Tup11 and Tup12 are required for the proper induction of chromatin alteration and later activation of transcription for specific environmental stresses at the *fbp1*$^+$ and *cta3*$^+$ promoters (15). The closest *S. pombe* homolog of Mig1 is Scr1, a C_2H_2 Zn finger protein that represses the transcription of *inv1*$^+$ (44) and *fbp1*$^+$ (31). Note that Mig1 and Scr1 are highly conserved around their C_2H_2 Zn finger domains (Fig. 1A). In addition, an *scr1*$^+$ deletion displays genetic interactions with deletion of either *tup11*$^+$ or *tup12*$^+$ (20).

Transcription of the *fbp1*$^+$ gene is regulated in response to environmental glucose (17–19, 48). Exposure of fission yeast cells to a high concentration of extracellular glucose results in an intracellular cyclic AMP (cAMP) signal (2, 25, 29) to activate the cAMP-dependent kinase protein kinase A (PKA) by dissociation of the regulatory subunit Cgs1 from the catalytic subunit Pka1 (reviewed in reference 55). The activated PKA signal then represses the transcription of genes, including *fbp1*$^+$ (2, 17, 21), by inhibiting the C_2H_2 Zn finger transcrip-

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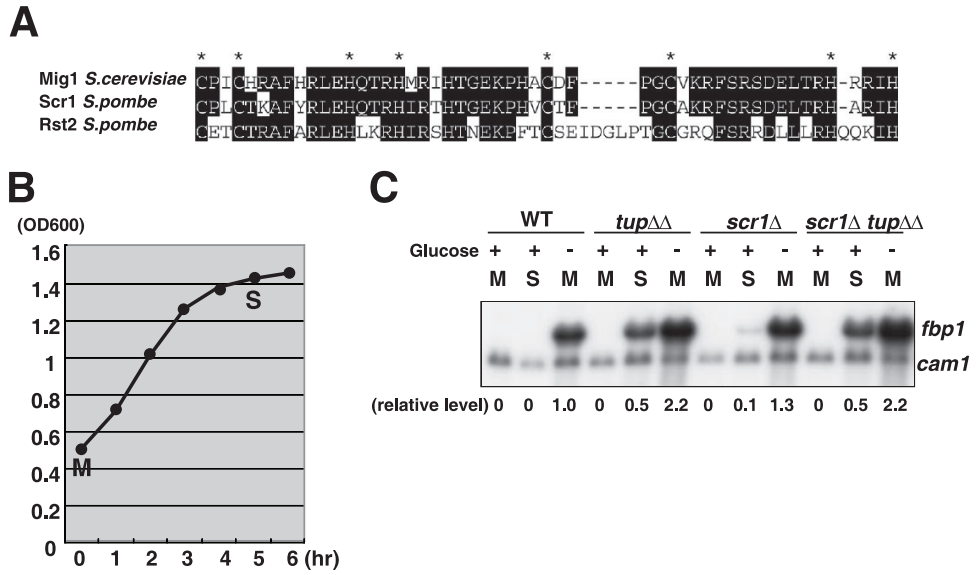


FIG. 1. Tup11-Tup12 and Scr1 act in the same pathway to regulate *fbp1*$^+$ expression. (A) Alignment of C_2H_2 Zn finger domains in *S. pombe* Scr1 and Rst2 and *S. cerevisiae* Mig1 by ClustalW (45; http://www.ebi.ac.uk/clustalw/). Identical amino acid residues are shaded. (B) Cells of the wild-type strain (K131) were cultured in YER (containing 6% glucose), and the density of the culture was monitored by the optical density at 600 nm (OD_{600}). Cells were harvested at the times indicated by "M" and "S." (C) Results of Northern analysis. Cells of the wild-type strain (WT; K131) and the *tup11*Δ *tup12*Δ (*tup*ΔΔ; PKH40), *scr1*Δ (PKH164), and *scr1*Δ *tup11*Δ *tup12*Δ (PKH186) mutant strains were cultured in YER (M, glucose +) and collected at the densities indicated in panel B. Glucose starvation was carried out by transferring a portion of the mid-log-phase cells to YED (containing 0.1% glucose and 3% glycerol) and culturing them for 3 h (M, glucose −). Expression of *fbp1*$^+$ was analyzed by Northern blotting. Expression of *cam1*$^+$ (43) was also analyzed and used as an internal control to normalize the expression levels of *fbp1*$^+$.

tion activator Rst2 (13, 23). Thus, the Rst2 C_2H_2 Zn finger protein is assumed to have an antagonizing role to that of the C_2H_2 Zn finger repressor protein Scr1. Interestingly, Rst2 has significant homology to Mig1 and Scr1 at the C_2H_2 Zn finger domain (Fig. 1A).

PKA activation is also antagonistic to a pathway of stress-activated protein kinases (SAPKs), i.e., Spc1/Sty1. Glucose starvation stimulates the SAPK pathway, leading to the derepression of *fbp1*$^+$ transcription (22, 40, 42). The SAPK signal is mediated by the CREB/ATF-type transcription factor Atf1 (22, 39, 42, 52), a basic leucine zipper (bZIP) protein forming a heterodimer with another bZIP protein, Pcr1 (50).

Gene regulation of *fbp1*$^+$ requires two upstream *cis*-acting elements, called UAS1 and UAS2, which include cAMP response element-like and stress response element (STRE)-like DNA sequences (31). Aft1-Pcr1 and Rst2 can bind to the UAS1 and UAS2 sequences, respectively (13, 31). A protein complex is formed on UAS2 in an Scr1-dependent manner, suggesting a direct or indirect interaction of the Scr1 repressor with UAS2 (31). Thus, UAS2 appears to serve as a common binding site for the antagonizing C_2H_2 Zn finger Rst2 activator and Scr1 repressor.

We previously reported that Tup11 and Tup12, together with Rst2, are involved in the chromatin opening at *fbp1*$^+$ during glucose derepression (15). It has been reported that *scr1*$^+$ displays a genetic interaction with *tup11*$^+$ and *tup12*$^+$ in glucose repression at *fbp1*$^+$. Thus, the Tup11-Tup12 corepressors may repress chromatin alteration at *fbp1*$^+$ with Scr1. However, cooperative mechanisms of Scr1 and Tup11-Tup12 in the chromatin regulation at *fbp1*$^+$ remain to be elucidated.

In this study, we present evidence for reciprocal nuclear translocation of the two counteracting Zn finger proteins Scr1 and Rst2, with which the Tup11-Tup12 proteins function to induce proper chromatin responses of the *fbp1*$^+$ promoter during glucose repression and derepression. We discuss a model for the regulation of transcriptional repression by Tup family corepressors and C_2H_2 Zn finger proteins.

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 were carried out as described previously (11). Minimal medium (SD) (38) was used for the culture of *S. pombe* unless stated otherwise. Strain construction was carried out by mating haploids on sporulation medium (SPA) (11), followed by tetrad dissection. The standard rich yeast extract medium YEL (with 2% glucose) (11) was used for culturing cells. YER medium (yeast extract with 6% glucose) and YED medium (yeast extract with 0.1% glucose plus 3% glycerol) were used for glucose repression and derepression, respectively. Transformation was performed by the lithium acetate method as previously described (16). All strains were grown in 200 ml of YEL in 2-liter flasks at 30°C. To select kanamycin-resistant (Kan^r) colonies, culture suspensions were inoculated onto YE plates, incubated for 16 h, and then replica plated onto YE plates containing 100 μg/ml of Geneticin (Sigma). For the construction of strains expressing proteins with epitope tags, we employed a PCR-based integration method (1). These strains expressing fusion proteins (Scr1-FLAG, Scr1-green fluorescent protein [Scr1-GFP], Tup11-FLAG, etc.) can properly repress and induce *fbp1*$^+$ transcription, indicating that the fusion proteins are functional.

Northern blot analysis. The probes to detect transcripts of *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*$^+$ probes were as described previously (15). Total RNA was prepared from *S. pombe* cells according to a method described elsewhere (6). For Northern blot analysis, 10 μg of total RNA was denatured with formamide, electrophoresed in 1.5% agarose gels containing formaldehyde (37), and blotted onto a charged nylon membrane (Biodyne B membrane; Pall).

TABLE 1. *S. pombe* strains used in this study

Strain	Genotype[a]
K131	*h⁻ ade6-M26 leu1-32*
PKH40	*h⁻ ade6-M26 tup11::ura4 tup12::ura4 leu1-32 ura4-D18*
PKH164	*h⁻ ade6-M26 scr1::ura4 ura4-D18*
PKH166	*h⁻ ade6-M26 tup11-3flag≪kanMX6 leu1-32*
PKH167	*h⁻ ade6-M26 tup12-3flag≪kanMX6 leu1-32*
PKH168	*h⁻ ade6-M26 tup11-GFP≪kanMX6 leu1-32*
PKH169	*h⁻ ade6-M26 scr1::ura4 tup11-3flag≪kanMX6 ura4-D18*
PKH170	*h⁻ ade6-M26 scr1::ura4 tup12-3flag≪kanMX6 ura4-D18*
PKH171	*h⁻ ade6-M26 scr1-3flag≪kanMX6 ura4-D18*
PKH186	*h⁻ ade6-M26 tup11::ura4 tup12::ura4 scr1::ura4 leu1-32 ura4-D18*
PKH187	*h⁻ ade6-M26 scr1-GFP≪kanMX6 leu1-32*
PKH188	*h⁻ ade6-M26 tup12-GFP≪kanMX6 leu1-32*
PKH241	*h⁻ ade6-M26 scr1-GFP≪kanMX6 spc1::ura4 ura4-D18*
PKH243	*h⁻ ade6-M26 scr1-GFP≪kanMX6 pka1::ura4 ura4-D18 leu1-32 his7-366*
PKH246	*h⁻ ade6-M26 scr1-GFP≪kanMX6 cgs1::ura4 ura4-D18 leu1-32*
PKH251	*h⁺ ade6-M26 scr1-GFP≪kanMX6 atf1::ura4 ura4-D18 leu1-32 his3-D1*
PKH418	*h⁺ ade6-M26 scr1-GFP≪kanMX6 tor1::ura4 ura4-D18*
PKH453	*h⁻ ade6-M26 rst2-3flag≪kanMX6 leu1-32*

[a] ≪ indicates linking of the marker gene to the inserted gene.

Chromatin analysis. Analysis of chromatin structure by indirect end labeling was done according to the method of Mizuno et al. (28). The DNA samples were analyzed by Southern analysis as described below. To analyze chromatin around the *fbp1*$^+$ promoter, the micrococcal nuclease (MNase)-treated DNA was digested with ClaI and separated by electrophoresis in a 1.5% agarose gel (40-cm long) containing Tris-acetate-EDTA buffer. The separated DNA fragments were alkali transferred to charged nylon membranes (Biodyne B membrane; Pall). The probe used for indirect end labeling of the *fbp1*$^+$ region was the same probe used for Northern analysis, as described above.

Chromatin immunoprecipitation. Chromatin immunoprecipitation (ChIP) was performed as described previously (54), with slight modifications, as briefly described below. Fifty milliliters of culture was incubated with 1.4 ml of a 37% formaldehyde solution for 15 min at room temperature, and 2.5 ml of 2.5 M glycine was added and incubated for 5 min. After centrifugation, collected cells were washed twice with cold Tris-buffered saline. The cells were mixed with 400 μl of lysis 140 buffer (0.1% sodium deoxycholate, 1 mM EDTA, 50 mM HEPES-KOH [pH 7.5], 140 mM NaCl, 1% Triton X-100), and 0.6 ml of zirconia beads was added. After disruption of the cells using a multibead shocker (Yasuikikai, Japan), the suspension was sonicated five times for 30 s each and centrifuged at 4°C for 10 min, and the supernatant was collected as a whole-cell extract. Three hundred microliters of whole-cell extract was mixed with 4 μl of anti-FLAG antibody M2 (Sigma) and 40 μl of DYNA protein A beads (DYNAL) and allowed to immunoprecipitate at 4°C overnight. The precipitates were washed with lysis 140 buffer twice and with lysis 500 buffer (0.1% sodium deoxycholate,1 mM EDTA, 50 mM HEPES-KOH [pH 7.5], 500 mM NaCl, 1% Triton X-100) once and then further washed with wash buffer (0.5% sodium deoxycholate, 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, 10 mM Tris-HCl [pH 8.0]) twice and with TE (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) once. The well-washed precipitates were mixed with 100 μl of elution buffer (10 mM EDTA, 1% sodium dodecyl sulfate, 50 mM Tris-HCl [pH 8.0]), and the immunoprecipitated protein-DNA complexes were eluted at 65°C for 15 min (IP sample). The IP sample or 3 μl of whole-cell extract was mixed with 150 μl or 250 μl of 1% sodium dodecyl sulfate-containing TE buffer and incubated at 37°C for 8 h. After incubation, the temperature was shifted to 65°C, and the sample was further incubated overnight. After incubation, DNA was phenol-chloroform extracted from each of the samples and slot blotted onto a charged nylon membrane (Biodyne B membrane; Pall), followed by Southern blot analysis to quantify the DNA content. Quantification was conducted using a Fuji BAS2000 image analyzer by obtaining a calibration curve with various concentrations of input genome sample. The probes were amplified from *S. pombe* genomic DNA by PCR, using primer sets fbp1-1 (ACGATCTAACGAAACAGGAA and CCCTTTGTGGACATTTAG AC), fbp1-2 (GAAAATTCCACGGGACATTAG and CCCTTCCTATTAGCA ATAAGG), fbp1-3 (GGGATGAAAACAATCAACCTC and GGAATGCAGC AACGAAAATC), fbp1-4 (GATTTTCGTTGCTGCATTCC and CCTATGAT TTGATGTCTAGC), fbp1-5 (GCTAGACATGAAATGATACC and CATTCC ACCCTATTCATC), fbp1-6 (GGGTGGAATGAGTCCGC and GTTCCGCG AATCATAAGCC), fbp1-7 (CGCGGAACTAAACATAGCG and GCTAGAA ACCGAGTGGTG), fbp1-8 (GCCCAACTTAACTCAGCTC and GCTTCTGA TTGTATCGGCG), and fbp1-9 (CGCCGATACAATCAGAAGC and CGATG AGTTTGCAGCATCC).

To investigate whether Scr1-3Flag and Rst2-3Flag bind to UAS2 in the *fbp1*$^+$ promoter, the primer set fbp1-6 was used together with the control primer set ade10 (ACGTAGCAAACAAAGCAG and CTAATTCCTACAGAACTG).

Fluorescence microscopy. The cells were collected by filtration and suspended with 5 μl of culture on a slide glass. Fluorescence images of living cells were taken with a cooled charge-coupled device camera and stored digitally using MetaMorph software (Universal Imaging, Downingtown, PA). For fixed samples, the culture was centrifuged for 5 s, and the pelleted cells were suspended in 70% ethanol, washed with phosphate-buffered saline, and suspended in 5 μl of phosphate-buffered saline containing Hoechst 33342 dye (0.1 mg/ml).

RESULTS

Tup11-Tup12 corepressors and the Scr1 repressor function in the same pathway to repress chromatin remodeling around the *fbp1*$^+$ promoter. The fission yeast Tup11-Tup12 corepressors are required for glucose repression of *fbp1*$^+$ transcription, possibly collaborating with the C_2H_2 Zn finger Scr1 repressor (20, 30, 31). To investigate their cooperative roles in chromatin regulation, we first investigated the genetic relationship between the *tup11*Δ *tup12*Δ double deletion and *scr1*Δ deletion strains. Both the *tup11*Δ *tup12*Δ and *scr1*Δ strains were cultured to mid-log phase (Fig. 1C, lanes M) or early stationary phase (lanes S) in YER medium containing 6% glucose (for glucose-rich conditions). The cells in mid-log phase were cultured further for 3 h in YED medium containing 0.1% glucose (for glucose-starved conditions). As previously reported (15, 20), a robust transcriptional activation of *fbp1*$^+$ was detected by Northern analysis after glucose starvation of wild-type cells. The *tup11*Δ *tup12*Δ strain exhibited a slight derepression of *fbp1*$^+$ transcription in glucose-fed cells at early stationary phase.

The *scr1*Δ mutation also conferred a slight induction of *fbp1*$^+$ transcription, even at early stationary phase (relative ratios compared to the derepressed level in the wild type, 0.5 and 0.1 for the *tup11*Δ *tup12*Δ and *scr1*Δ strains, respectively), whereas no induction was observed in wild-type cells under the same conditions (Fig. 1C, lanes S and +). The expression levels of *fbp1*$^+$ in the *tup11*Δ *tup12*Δ and *scr1*Δ strains were 2.2 and 1.4 times higher, respectively, than that in the wild type at mid-log phase under glucose-starved conditions (Fig. 1C, lanes M and −). Thus, the effects of the *tup11*Δ *tup12*Δ double deletion on *fbp1*$^+$ glucose repression are much more severe than those of the *scr1*Δ single deletion. More importantly, the phenotype of a triple deletion mutant deleted for *tup11*$^+$, *tup12*$^+$, and *scr1*$^+$ was similar to the case for the *tup11*Δ *tup12*Δ mutant at the mid-log and early stationary phases. Thus, Scr1 appears to act in the same pathway as Tup11 and Tup12.

We further analyzed the chromatin structure around the *fbp1*$^+$ promoter under the conditions described above. In the wild-type strain grown under glucose-rich conditions as previously reported (15), chromatin around the UAS1 and UAS2-TATA sites was relatively resistant to MNase digestion, pre-

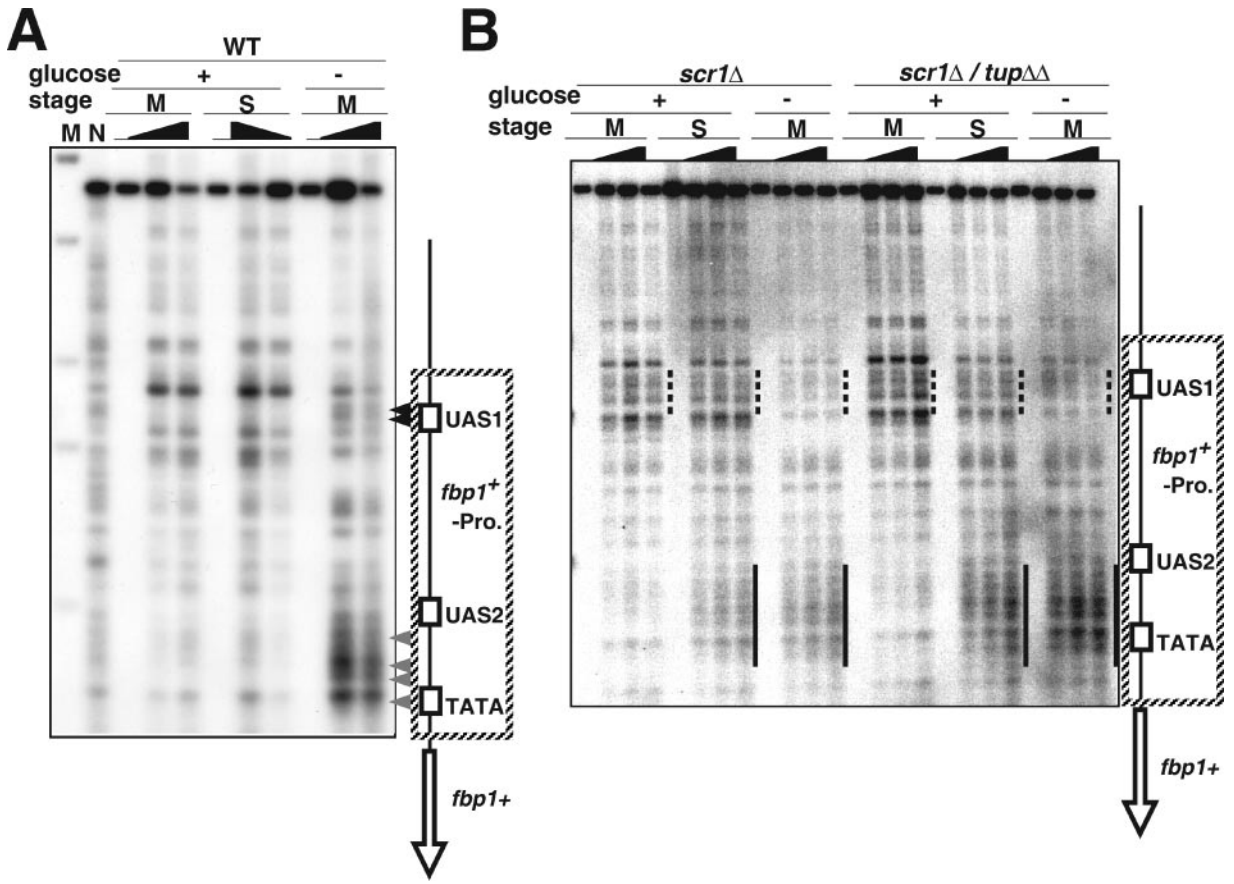


FIG. 2. Tup11-Tup12 and Scr1 act together to regulate the chromatin structure in the *fbp1*$^+$ promoter. (A and B) Chromatin structures around the *fbp1*$^+$ promoter in wild-type (WT), *scr1*Δ, and *scr1*Δ *tup11*Δ *tup12*Δ (*scr1*Δ *tup*ΔΔ) strains, using the same strains as those described in the legend to Fig. 1C. Lanes contain chromatin from mid-log-phase (M) and early-stationary-phase (S) cells. The isolated chromatin was digested with 0, 20, 30, or 50 units/ml of MNase at 37°C for 5 min. Purified DNA was digested with ClaI and analyzed by Southern blot analysis as described in Materials and Methods. Black and gray arrowheads indicate regions with MNase-sensitive sites within UAS1 (the open square labeled UAS1, positions −1566 to −1573 from the first A of the *fbp1*$^+$ open reading frame) and around UAS2 (the open square labeled UAS2, positions −926 to −938), respectively.

senting only a couple of bands around the UAS1 site (Fig. 2A). Under glucose-starved conditions at mid-log phase, some faint bands appeared in the UAS1 region (Fig. 2A, filled arrowheads), and several hypersensitive sites were generated around the UAS2-TATA region (Fig. 2A, gray arrowheads).

On the other hand, in the *scr1*Δ and *scr1*Δ *tup11*Δ *tup12*Δ strains, some sensitive sites appeared constitutively in the UAS1 region from the mid-log to early stationary stages, even when the cells were cultured in a glucose-rich medium (Fig. 2B, dashed lines). For the chromatin from *scr1*Δ *tup11*Δ

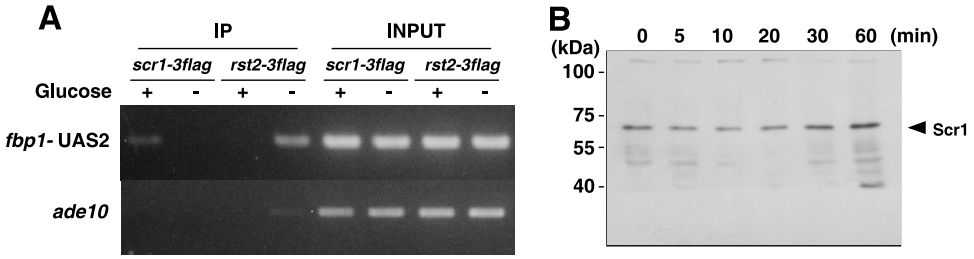
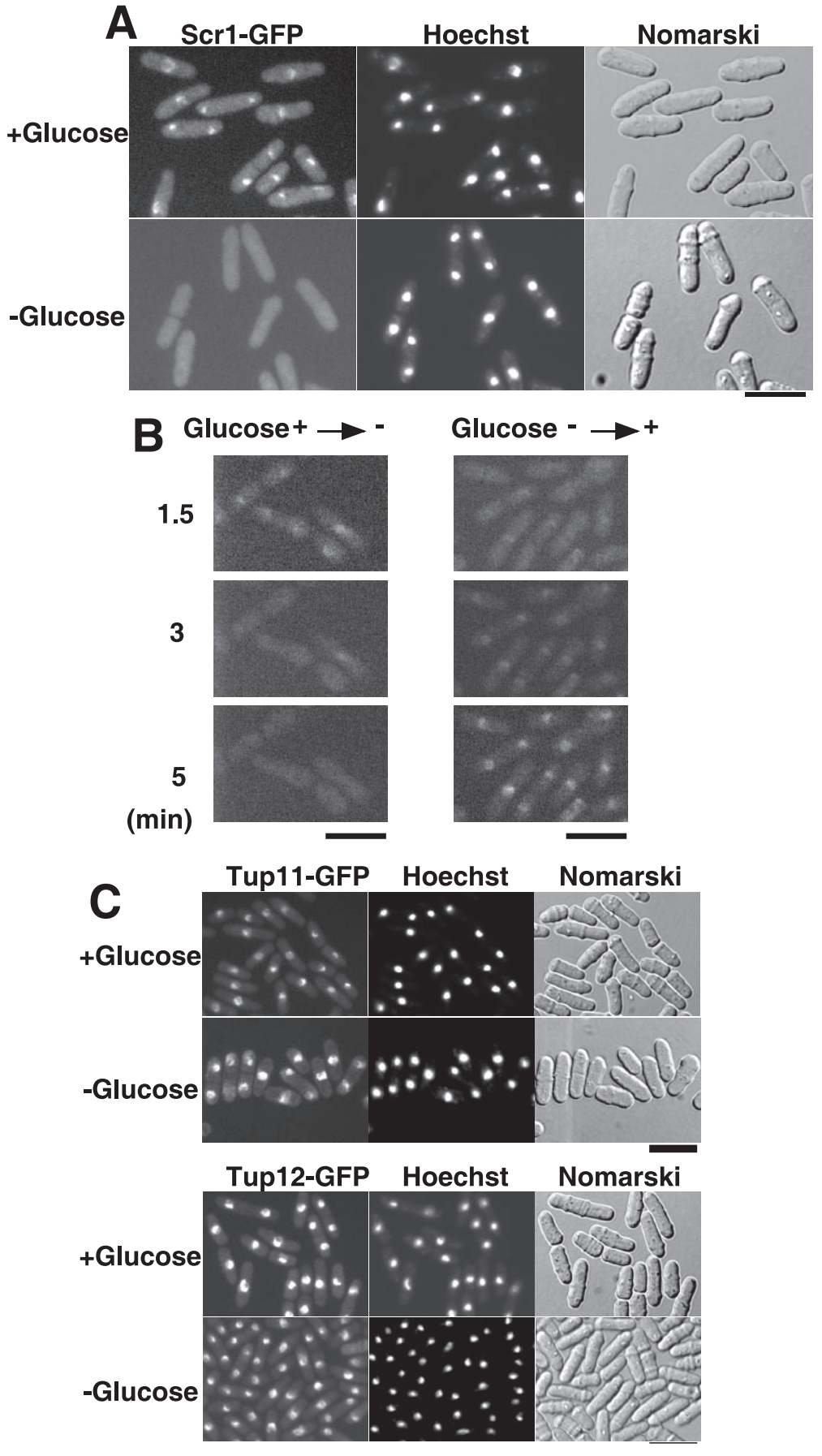


FIG. 3. Scr1 and Rst2 bind to UAS2 in the *fbp1*$^+$ promoter under repressing and derepressing conditions, respectively. (A) Cells of *scr1-3flag* (PKH171) and *rst2-3flag* (PKH453) strains were cultured in YER to mid-log phase, and portions were shifted to YED medium and cultured for 60 min. ChIP experiments were conducted as described in Materials and Methods. The binding of Scr1-3FLAG and Rst2-3FLAG to UAS2 in the *fbp1*$^+$ promoter was detected by PCR. Whole genomic DNA (1% input) was amplified at the same time. The primer set amplifying the *ade10* locus was used as a control. (B) Cells of the *scr1-3flag* (PKH171) strain were cultured in YER to mid-log phase, and portions were shifted to YED medium and harvested at the indicated times after the medium shift. Protein samples were prepared from the same amount of cells (5×10^7 cells). The arrowhead indicates the band corresponding to Scr1-3Flag.


A
Scr1-GFP
Hoechst
Nomarski
+Glucose
-Glucose
B
Glucose+ → -
Glucose - → +
1.5
3
5
(min)
C
Tup11-GFP
Hoechst
Nomarski
+Glucose
-Glucose
Tup12-GFP
Hoechst
Nomarski
+Glucose
-Glucose

*tup12*Δ cells grown to early stationary phase under glucose-rich conditions, several hypersensitive sites appeared around the UAS2-TATA region (Fig. 2B, thick lines), similar to those for the glucose-starved wild-type cells. Chromatin from the *scr1*Δ mutant cells exhibited a similar but weaker pattern of hypersensitive sites around the UAS2-TATA region, especially under glucose-rich conditions at early stationary phase (Fig. 2B, thick lines). Under the same conditions, the *scr1*Δ mutant and *scr1*Δ *tup11*Δ *tup12*Δ triple mutant exhibited a chromatin configuration very similar to that of glucose-starved wild-type cells. These results indicate that the Scr1 repressor and Tup11-Tup12 corepressors function in the same pathway to establish or maintain transcriptionally repressed chromatin in the *fbp1*$^+$ region.

Exchange of Scr1/Rst2 on UAS2 in response to glucose starvation. Both the Scr1 repressor and the Rst2 activator are C_2H_2 Zn finger proteins that appear to bind UAS2 in the *fbp1*$^+$ promoter (13, 31). However, Scr1 and Rst2 have opposite roles in regulating *fbp1*$^+$ transcription, and the Rst2 activator is thought to act to create a more open chromatin structure in the *fbp1*$^+$ promoter region, in opposition to the action of Tup11-Tup12 (15). To address how these related proteins function antagonistically while sharing the same binding site, we first conducted ChIP analysis and examined the binding of the Scr1 repressor and the Rst2 activator to UAS2 in the *fbp1*$^+$ promoter. We found that Rst2 binds to UAS2 under glucose-starved conditions, while Scr1 binds to UAS2 under glucose-rich conditions (Fig. 3A). This result indicates that Rst2 replaces Scr1 at UAS2 in response to glucose starvation.

To examine whether the Scr1-Rst2 switch in response to glucose starvation is regulated by posttranslational modifications (e.g., phosphorylation) or degradation of the Scr1 protein, we examined the level of Scr1 protein in the course of glucose starvation by Western blotting. As shown in Fig. 3B, no visible changes in protein level or mobility, which would be suggestive of a posttranslational modification, were detected. However, we cannot exclude the possibility of posttranslational modifications without mobility shifts, and hence it still remains unsolved whether Scr1-Rst2 switching is regulated via posttranslational protein modification.

Nuclear export of the Scr1 repressor. Higuchi et al. reported that the function of Rst2 is regulated by its nuclear localization (13). Rst2 is exported from the nucleus in response to glucose/cAMP signaling in a PKA pathway-dependent manner. These observations led us to speculate that switching between Scr1 and Rst2 on UAS2 may be controlled by nuclear import and export.

To test this possibility, we constructed a strain expressing Scr1-GFP and analyzed the protein's subcellular localization. We found that Scr1-GFP localizes to the nucleus under glucose-rich conditions and to the cytoplasm under glucose-starved conditions (Fig. 4A). A time-lapse examination of Scr1-GFP revealed that Scr1-GFP in the nucleus under glucose-rich conditions is exported quickly to the cytoplasm within 5 min of exposure to glucose-starved conditions (Fig. 4B). We further confirmed that nuclear import of Scr1-GFP occurs immediately after the reexposure of cells to glucose (Fig. 4B). Moreover, we examined the behavior of Tup11-GFP and Tup12-GFP. As shown in Fig. 4C, Tup11-GFP and Tup12-GFP were found in the nucleus under either glucose-rich or glucose starvation conditions, indicating that Tup11 and Tup12 are not regulated by changes in localization (Fig. 4C).

Since the nuclear localization of Rst2 is regulated by PKA phosphorylation (13), we further tested the localization of Scr1-GFP in strains lacking a functioning mitogen-activated protein (MAP) kinase pathway (*spc1*Δ and *atf1*Δ strains), PKA pathway (*cgs1*Δ and *pka1*Δ strains) (reviewed in reference 55), or TOR pathway (*tor1*Δ strain) (27). We detected proper localization patterns of Scr1-GFP in all mutant strains (Fig. 5), indicating that these intracellular signaling pathways do not regulate the nuclear localization of Scr1.

Tup11 and Tup12 occupy UAS2 in the *fbp1*$^+$ promoter even under derepressed conditions. The observations described above led us to speculate that under glucose-repressing conditions, Tup11 and Tup12 are recruited to UAS2 by Scr1, which is preloaded on UAS2 in place of Rst2. To test this notion, we analyzed the binding of Tup11 and Tup12 to the *fbp1*$^+$ promoter by ChIP analysis. For quantitative analysis, the 5′ promoter region of *fbp1*$^+$ was divided into segments of ~250 bp (Fig. 6A), and the probe for each region was used in slot blot analysis to measure the ChIP efficiency at that segment.

We found that the FLAG-tagged versions of Tup11 and Tup12 were enriched in the UAS2 region. Their occupancy on DNA was significantly higher under derepressed conditions (Fig. 6B and C). Considering the amount of whole genomic DNA in the input control, the IP efficiencies of Tup11 and Tup12 around UAS2 were estimated to be ~1.0% and ~0.2% under derepressed and repressed conditions, respectively. We further examined the requirement of Scr1 in the binding of Tup11 and Tup12 to UAS2 (Fig. 6C) and found that the Scr1 repressor was dispensable for their binding. Therefore, Scr1 is not required for the recruitment of Tup11 and Tup12 to the *fbp1*$^+$ promoter. Tup1 and Groucho cannot bind to DNA directly (reviewed in reference 4), so other DNA binding proteins may be required for the recruitment of Tup11and Tup12 to DNA. Several proteins with zinc fingers related to those of Rst2 and Scr1 are encoded by the *S. pombe* genome and may contribute to the multiple band shifts observed with the UAS2 probe (31).

FIG. 4. Scr1 translocates from the nucleus to the cytoplasm upon glucose starvation. (A) Cells of *scr1-GFP* strain PKH187 were cultured in YER to mid-log phase, and a portion was transferred to YED. The cells were fixed and observed as described in Materials and Methods. Bar, 10 μm. (B) Live observation of Scr1-GFP. Cells of the *scr1-GFP* strain (PKH 187) were transferred from YER to YED (glucose positive to glucose negative) or from YED to YER (glucose negative to glucose positive). The amount of time after the medium change is indicated. Bars, 10 μm. (C) Tup11 and Tup12 persist in the nucleus under repressing and derepressing conditions. Cells of the *tup11-GFP* (PKH168) and *tup12-GFP* (PKH188) strains were cultured, fixed, and observed as described for panel A. Bars, 10 μm.

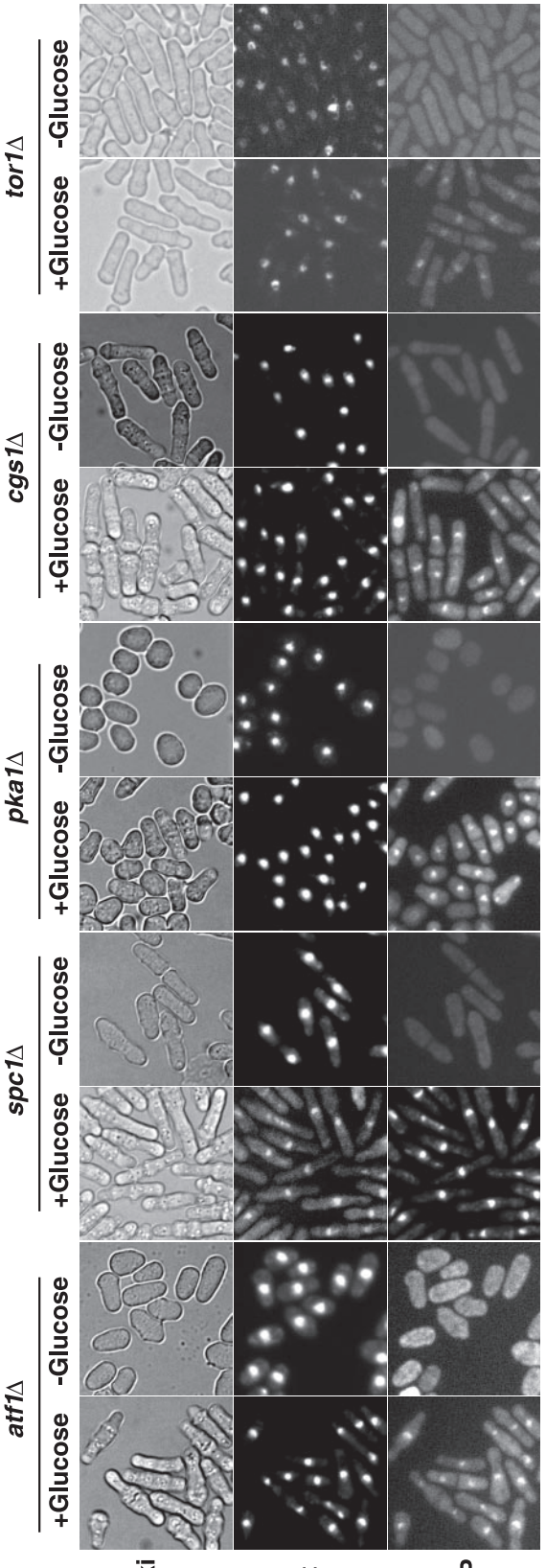


FIG. 5. *scr11-GFP* strains lacking *pka1* (PKH243), *cgs1* (PKH246), *atf1* (PKH251), *spc1* (PKH241), or *tor1* (PKH418) were cultured, fixed, and observed as described in the legend to Fig. 4A. Bar, 10 μm.

DISCUSSION

In the present study, we characterized the roles of the Scr1 repressor and the Tup1-like corepressors Tup11 and Tup12 during glucose repression of *fbp1*$^+$. We found that Tup proteins function in concert with the Zn finger Scr1 repressor and Rst2 activator proteins, which undergo reciprocal nuclear shuttling and the resultant exchange of their occupancies at UAS2. Importantly, it turned out that Tup proteins persist at UAS2 under both repressed and derepressed conditions.

Switching of Zn finger proteins Scr1 and Rst2 to regulate the function of Tup11-Tup12 complexes. We showed that Scr1, a Zn finger repressor, and the Tup11-Tup12 corepressors function in concert to repress chromatin remodeling in the *fbp1*$^+$ promoter and the transcription of *fbp1*$^+$ (Fig. 1 and 2). Since we previously reported that Rst2, a Zn finger activator, acts to antagonize the function of Tup11-Tup12 in chromatin repression, it is supposed that Scr1 and Rst2 act toward Tup proteins in a mutually antagonizing manner (15). Interestingly, the Zn finger domains of *S. pombe* Rst2 and Scr1 and *S. cerevisiae* Mig1 are highly conserved (Fig. 1A), and they can bind to a common consensus sequence called STRE. Rst2 and Scr1 are supposed to share the binding site on the STRE-containing UAS2 regulatory element in the *fbp1*$^+$ promoter (13, 31). Thus, it is supposed that these Zn finger proteins compete with each other for the common binding site. In fact, our ChIP data demonstrate that the Scr1 repressor and Rst2 activator bind to UAS2 under repressing and derepressing conditions, respectively (Fig. 3A).

Interestingly, Scr1 rapidly translocates from the nucleus to the cytoplasm in response to glucose starvation (Fig. 4). This localization pattern is reciprocal to that of Rst2 (13). Therefore, such reciprocal nuclear shuttling can avoid conflicts between the Rst2 activator and the Scr1 repressor at an STRE in the *fbp1*$^+$ promoter. These results suggest that Tup proteins are regulated to be repressive or nonrepressive by exchanging the Zn finger proteins Scr1 and Rst2 (Fig. 7). This explains why the Rst2 activator is dispensable for *fbp1*$^+$ transcription per se in *tup11*Δ *tup12*Δ mutants (15). Presumably, Scr1 facilitates the ability of Tup proteins to repress *fbp1*$^+$ transcription under glucose-rich conditions, and once cells encounter glucose starvation conditions, Scr1 is replaced by Rst2 on UAS2. This may be the first step toward the activation of transcription, which might involve the subsequent action of the Aft1-Pcr1 activator at UAS1. It will be intriguing to learn whether or not similar reciprocal shuttling of antagonizing Zn finger proteins occurs to control transcriptional regulation in higher eukaryotes.

Tup11 and Tup12 persist at the *fbp1*$^+$ promoter under derepressing conditions. We have shown here that Tup11 and Tup12 bind persistently to UAS2 (Fig. 6). These results suggest that the major function of Tup proteins is to regulate chromatin configurations in repressive or nonrepressive states by replacing the Zn finger proteins Scr1 and Rst2 (Fig. 7) rather than by simply inducing transcriptional repression. This idea seems reasonable, since *S. cerevisiae* Tup1 also resides at promoters to activate chromatin alteration by recruiting a histone acetyltransferase complex (32), and more importantly, Tup1 occupancy at the *SUC2* promoter also increases under derepressing conditions (32).

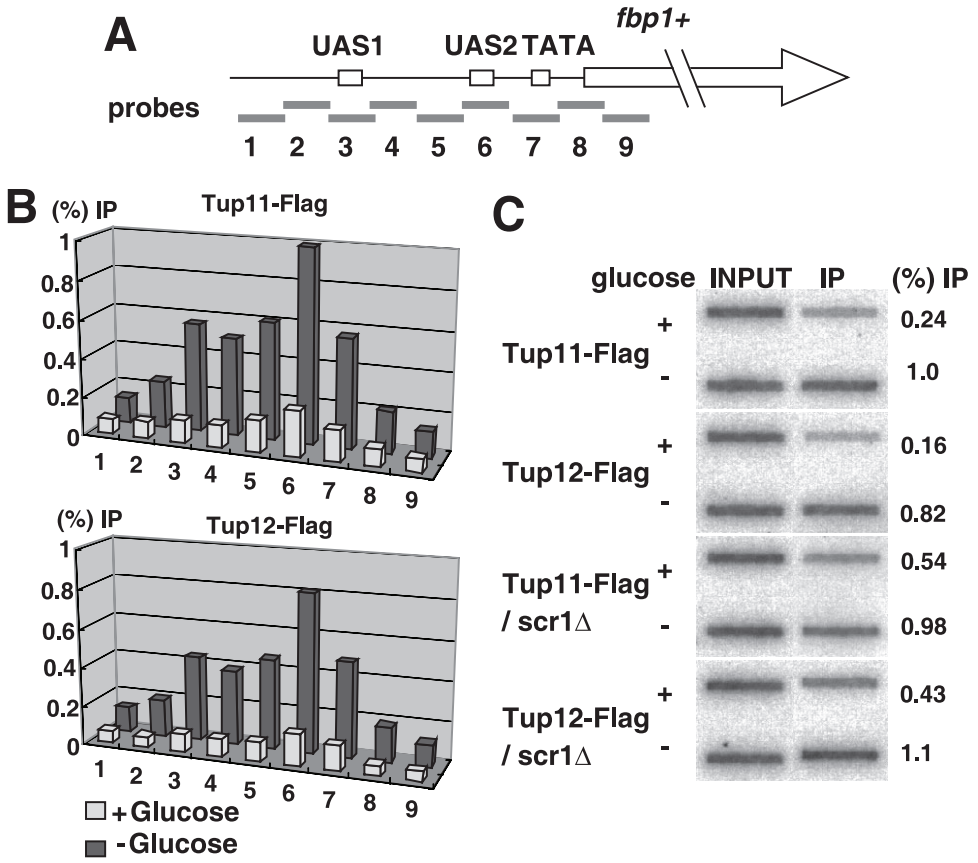


FIG. 6. Tup11-Tup12 binding to the UAS2 region of the *fbp1*$^+$ promoter. (A) Schematic drawing of the probes used to quantify DNA precipitated with Tup11 or Tup12. Probes 3, 6, and 7 contain UAS1, UAS2, and the TATA box, respectively. (B) Chromatin immunoprecipitation of Tup11 and Tup12. Cells of the *tup11-3flag* (PKH166) and *tup12-3flag* (PKH167) strains were cultured in YER to mid-log phase for the glucose-positive samples. One-half of each culture was transferred to YED and grown for 3 h for the glucose-negative samples. DNAs from 1% input and IP samples were quantified by slot blotting followed by hybridization with the above probes. IP efficiencies are presented (% IP). (C) Scr1 is dispensable for loading of Tup11-Tup12 onto the *fbp1*$^+$ promoter UAS2. The *tup11-3flag* strain lacking *scr1* (PKH169) and the *tup12-3flag* strain lacking *scr1* (PKH 170) were cultured as described for panel B. To quantify the binding of Tup11 and Tup12 to UAS2, probe 6 was used for hybridization.

The persistent binding of Tup proteins at the *fbp1*$^+$ promoter during derepression suggests that the binding of Tup proteins to a promoter does not necessarily result in transcriptional repression. Tup proteins may function as transcriptional activators and stay at the promoter to promote chromatin remodeling by recruiting SAGA and SWI/SNF remodeling complexes under derepressing conditions, as proposed in the case of *S. cerevisiae* (32, 33). This seems unlikely, though, since a robust transcriptional activation of *fbp1*$^+$ was observed in the *tup11*Δ *tup12*Δ strain. Alternatively, Tup proteins may function as transcriptional regulators that allow specific chromatin responses to distinct extracellular environments, as suggested previously (10). In fact, we previously demonstrated that the *tup11*Δ *tup12*Δ mutations conferred chromatin remodeling and unusual transcription activation at *fbp1*$^+$ in response to stresses that do not normally induce *fbp1*$^+$ transcription (14). We speculate that Tup proteins bound to the *fbp1*$^+$ UAS2 may prevent nonspecific chromatin remodeling that is not coupled to specific changes in extracellular circumstances. The exchange of the Zn finger proteins Scr1 and Rst2 may regulate the activity of Tup proteins to establish appropriate and specific transcriptional activation in the *fbp1*$^+$ promoter. As such, transcriptional activity does not simply correlate with the amount of Tup proteins bound to individual promoters. However, more studies will be needed to understand how the Tup proteins act to repress transcription under glucose-rich conditions yet allow high-level transcription under derepressing conditions, at which time they display increased occupancy at the *fbp1*$^+$ promoter.

Proper responses to extracellular stresses are vital for the survival of all eukaryotes. For the appropriate responses, biological systems have developed many signaling pathways to activate specific genes depending on the distinct environmental stresses. As discussed above, Tup proteins may act as a regulation center, interacting with both positive and negative regulators under distinct conditions to ensure the specificity of transcriptional activation in response to particular stresses. Such sophisticated gene regulation by Tup proteins, rather than their simple action as repressors, could also be important in higher eukaryotes, because the system consisting of MAP kinase pathways, PKA pathways, Tup proteins, and Zn finger

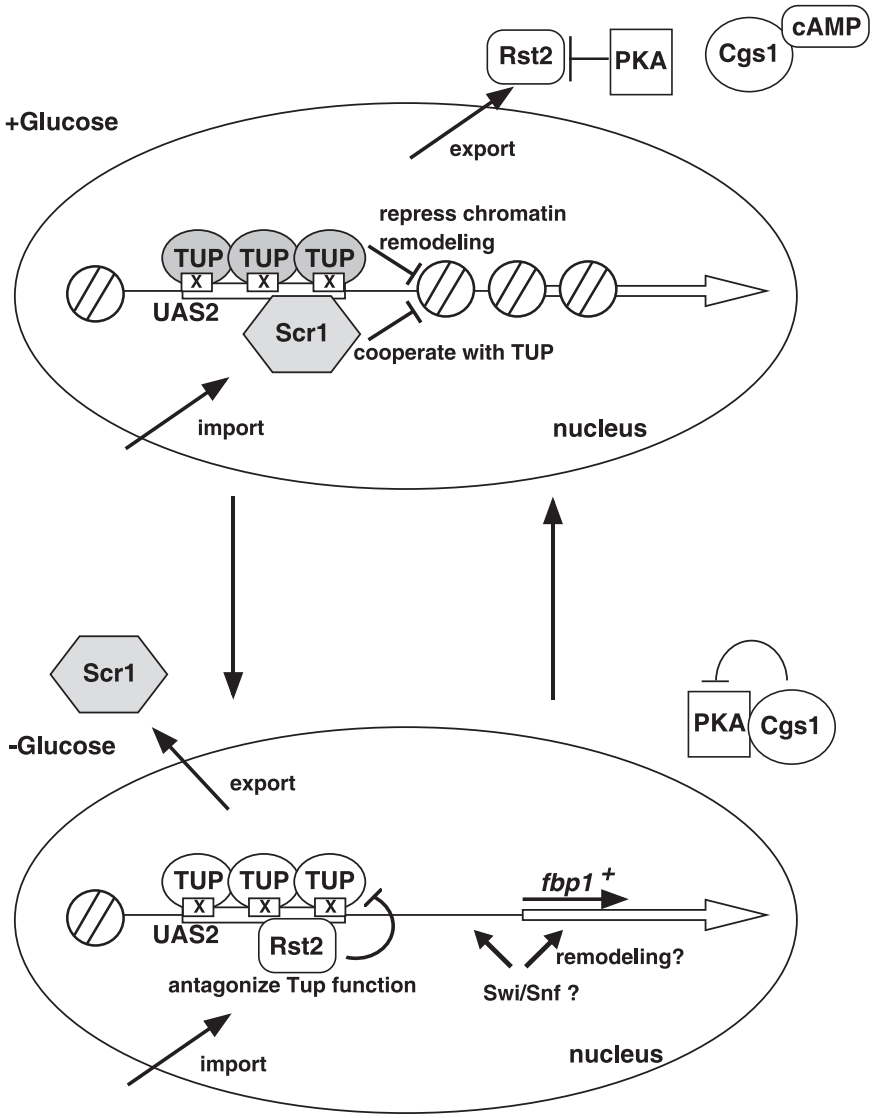


FIG. 7. Model of chromatin remodeling regulation around the *fbp1*$^+$ promoter region by switching between two antagonizing Zn finger proteins. See Discussion for details.

proteins is highly conserved. More precise molecular investigations of Tup proteins and their Zn finger partners will provide further understanding of genetic responses to environmental stresses.

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