Interplay between chromatin modulators and histone acetylation regulates the formation of accessible chromatin in the upstream regulatory region of fission yeast \textit{fbp1}

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Numerous noncoding RNA transcripts are detected in eukaryotic cells. Non-coding RNAs transcribed across gene promoters are involved in the regulation of mRNA transcription via chromatin modulation. This function of noncoding RNA transcription was first demonstrated for the fission yeast \textit{fbp1} gene, where a cascade of noncoding RNA transcription events induces chromatin remodeling to facilitate transcription factor binding. We recently demonstrated that the noncoding RNAs from the \textit{fbp1} upstream region facilitate binding of the transcription activator Atf1 and thereby promote histone acetylation. Histone acetylation by histone acetyl transferases (HATs) and ATP-dependent chromatin remodelers (ADCRs) are implicated in chromatin remodeling, but the interplay between HATs and ADCRs in this process has not been fully elucidated. Here, we examine the roles played by two distinct ADCRs, Snf22 and Hrp3, and by the HAT Gcn5 in the transcriptional activation of \textit{fbp1}. Snf22 and Hrp3 redundantly promote disassembly of chromatin in the \textit{fbp1} upstream region. Gcn5 critically contributes to nucleosome eviction in the absence of either Snf22 or Hrp3, presumably by recruiting Hrp3 in \textit{snf22}\textsuperscript{Δ} cells and Snf22 in \textit{hrp3}\textsuperscript{Δ} cells. Conversely, Gcn5-dependent histone H3 acetylation is impaired in \textit{snf22}\textsuperscript{Δ}/\textit{hrp3}\textsuperscript{Δ} cells, suggesting that both redundant ADCRs induce recruitment of Gcn5 to the chromatin array in the \textit{fbp1} upstream region. These results reveal a previously unappreciated interplay between ADCRs and histone acetylation in which histone acetylation facilitates recruitment of ADCRs, while ADCRs are required for histone acetylation.

\textbf{Key words:} ATP-dependent chromatin remodeler, histone acetyl transferase, Snf22, Hrp3, Gcn5

\textbf{INTRODUCTION}

Chromatin structure consisting of chromosomal DNA bound to histone-octamers plays important roles in the regulation of gene expression and genome stability. Generally, chromatin compaction inhibits many DNA-related reactions, such as transcription, by preventing the access of trans-acting DNA-binding factors to their binding sites in the DNA (Wolffe, 1997). Past studies have revealed two major classes of chromatin modifying machinery. The first class is histone modifications such as acetylation and methylation. Histone acetyltransferases (HATs) add acetyl groups that are usually associated with a derepressed chromatin state (Grant et al., 1998; Kuo and Allis, 1998). The second class of chromatin modulators is ATP-dependent chromatin remodelers (ADCRs). ADCRs alter chromatin structure by sliding or disassembling nucleosomes in an ATP-dependent manner (Travers, 1999). The yeast Swi2/Snf2 family ADCR contains a helicase/ATPase motif as a catalytic domain and a bromodomain, which recognize acetylated histone (Chandy et al., 2006; Hassan et al., 2006). Thus, pro-
teins carrying bromodomains, including Swi2/Snf2 family ADCRs, serve as a decoder for the histone acetylation. A further class of ADCRs implicated in chromatin remodeling is the CHD family ADCR that possesses two chromodomains (Travers, 1999), which recognize methylated histones (Flanagan et al., 2005). A recent study revealed the importance of this domain in enzymatic activity but not in chromatin localization of this enzyme (Morettini et al., 2011).

The *Schizosaccharomyces pombe fbp1* gene has served as an excellent model system to study transcriptional regulatory mechanisms. Transcription of the *fbp1* gene is regulated in response to environmental glucose (Hoffman and Winston, 1989, 1990, 1991; Vassarotti and Friesen, 1985). Under glucose starvation conditions, two critical transcription factors, Atf1 and Rst2, bind to upstream activating sequences (UAS1 and UAS2) to activate *fbp1* expression (Neely and Hoffman, 2000; Janoo et al., 2001; Hirota et al., 2006). The UAS1 and UAS2 consist of a cAMP response element (CRE) and a stress response element (STRE) respectively. Moreover, the CCAT box binding factor (CBF), which includes Php5 that possesses a histone-fold domain, is required for *fbp1* activation (Janoo et al., 2001). CBF binds to the UAS1-UAS2 region with a peak at UAS2 in glucose-starved cells (Asada et al., 2015). The Tup11/12 global co-repressors also bind to the *fbp1* upstream region and regulate transcription to ensure a stress-specific response through chromatin regulation (Hirota et al., 2004, 2006). We also identified a cascade of long non-coding RNA (lncRNA) transcription across the *fbp1* upstream region. The lncRNAs have been referred to as ‘metabolic stress-induced long non-coding RNA (mlonRNA)’ (Galipon et al., 2013; Miki et al., 2016), and this mlonRNA transcription is involved in the chromatin opening and the binding of transcription activators at the *fbp1* promoter (Hirota et al., 2008a; Takemata et al., 2016). Our recent study demonstrated that the mlonRNAs interact with Tup11/12 corepressors to antagonize the repressive function of Tup11/12 and thereby enhance binding of Atf1, which in turn induces histone acetylation through the recruitment of Gen5 HAT (Takemata et al., 2016). Having established the functional link between mlonRNAs, transcription activators and histone acetylation, the roles played by ADCRs in these processes remain to be elucidated. Here, we reveal a cooperative and interdependent relationship between ADCRs and histone acetylation in the chromatin remodeling for *fbp1* regulation.

**MATERIALS AND METHODS**

**Strains and media** *S. pombe* strains used in this study are listed in Table 1. Standard rich yeast extract medium YEL (2% glucose) was used for culturing cells (Gutz et al., 1974). YER medium (yeast extract with 6% glucose) and YED medium (yeast extract with 0.1% glucose plus 3% glycerol) was used for glucose repression and derepression, respectively (Hirota et al., 2003). All culture media used in this study contain 100 mg/L of adenine. Transformation was performed by the lithium acetate method as previously described (Hirota et al., 2001).

**Northern blot analysis** The probes to detect transcripts of *fbp1*+ and *cam1*+ were prepared from PCR-

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<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
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<tr>
<td>SPH1</td>
<td>h− leu1-32</td>
<td>(Yamada et al., 2004)</td>
</tr>
<tr>
<td>SPH98</td>
<td>h+ ade6-M26 ura4-D18 his5-303 gen5::ura4</td>
<td>(Yamada et al., 2004)</td>
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<td>SPH100</td>
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<td>SPH102</td>
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<td>(Hirota et al., 2008b)</td>
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<td>SPH103</td>
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<td>SPH243</td>
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<td>This study</td>
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<td>This study</td>
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<td>This study</td>
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amplified DNA fragments, and the DNA fragments were further labeled with 32P using a random-priming kit (GE healthcare, NJ). The nucleotide sequences of the primers used for fbp1' and cam1' probes were as described (Hirota et al., 2003). Total RNA was prepared from S. pombe cells by homogenizing cells in 0.3 ml of extraction buffer (0.5 M NaCl, 1% SDS, 10 mM EDTA, 0.2 M Tris-HCl [pH 8.0]), 0.3 ml of phenol-chloroform and 0.5 g of glass beads using multi beads shocker (Yasukikai, JP). 

For the Northern blot analysis, 10 μg of total RNA was denatured with formamide, electrophoresed in 1.5% agarose gels containing formaldehyde and blotted to a charged Nylon membrane (Biodyne B membrane, PALL, NY).

**Plasmid construction** A 1.5 kb fragment of KanMX selection marker gene (Bähler et al., 1998) was digested from pFA6a-KanMX by SmaI and EcoRI. This fragment was inserted into SmaI and EcoRI site of pBluescript KS+ to create pBS-KanMX.

**Chromatin analysis and Chromatin Immunoprecipitation (ChIP)** Isolation of crude chromatin from cells and digestion with MNase were performed according to the method as described previously (Hirota et al., 2007) with slight modifications as described below. Fixed amount of cells (0.1 gram wet weight) were suspended in preincubation buffer (0.7 M β-mercaptoethanol, 3 mM MgCl₂, 0.25 mM EGTA, 0.25 mM EDTA, 1 mM Pefabloc SK) for 5 min (for snf22Δ strain 15 min) and washed once with 1 ml of ice-cold wash buffer (1.0 M sorbitol, 10 mM EDTA). Cells were centrifuged and resuspended in 0.5 ml of incubation buffer (0.75 mM sorbitol, 37.5 mM Tris-HCl [pH 7.5], 1.25% Glucose, 6.25 mM EDTA). 0.5 ml of freshly prepared incubation buffer containing 2.5 mg/ml (for snf22Δ strain 5 mg/ml) of zymolyase 100T (seikagaku cooperation, JP) was added and mixed well. The cells were incubated at 30 °C for 5 min (for snf22Δ strain 15 min) and washed once with 1 ml of ice-cold wash buffer. Resultant spheroplasts were suspended well by pipetting in 1.4 ml of lysis buffer (18% Ficoll 400, 10 mM KH₂PO₄, 10 mM K₂HPO₄, 1 mM MgCl₂, 0.25 mM EGTA, 0.25 mM EDTA, 1 mM Pefabloc SC [Roche, Mannheim, Germany]). After centrifugation at 14000 rpm for 30 min at 4 °C, the crude nuclear pellet was resuspended in 1.5 ml of buffer A (10 mM Tris-HCl [pH 8.0], 150 mM NaCl, 5 mM KCl, 1 mM EDTA, 1 mM Pefabloc SC [Roche, Mannheim, Germany]). After addition of CaCl₂ (5 mM final concentration), 0.5 ml aliquots of crude nuclear suspension were digested with different amounts of MNase (0, 20, and 50 U/ml) at 37 °C for 5 min. The reaction was terminated by adding 25 mM EDTA, and DNA was purified by the incubation with 1% SDS and 20 μg of proteinase K (Merck, Damstadt, Germany) at 55 °C followed by phenol-chloroform extraction. The DNA samples were analyzed by Southern blot as described previously (Hirota et al., 2003). Chromatin immunoprecipitation (ChIP) and quantification of ChIP DNA sample was performed as described previously (Asada et al., 2015). Following Primer sets were used in the quantitative PCR. UAS1(GGGATGAAAAAATCACAACCTC and GGAATGGCAAGCAAGAAATC), UAS2(GGTTGGAGGGGGGATCGCCGC and GTTCCGGGAATCATAAGG), TATAI(CGC-GGGAAATACATACGGC and GCTTAGAAACCCGAGTTG), and Fpb3(GACAGTCTGTGTACAAATTCTGTATT CCC, and ACGATCCTAAACCGCTTTGTTAGTC).

**Antibodies** The following antibodies were used in this study. Anti-total histone H3 (catalog no. ab1791, Abcam, UK), Anti-H3a (catalog no. #06-599, Millipore) and Anti-H4ac (catalog no. #06-866, Millipore).

**Disruption of the fission yeast swr1 and arp8 genes** The disruption constructs for swr1 and arp8 genes, swr1-KanR and arp8-KanR were generated from genomic PCR products combined with KanMX selection marker gene. Genomic DNA sequences were amplified using the following primers for swr1: 5'-GGGCGG-CGCTCTAGAAGCCTCGCAACTATAC-3' and 5'-AGCCGGGGGATCCACCCGACATT-GATGAGTACATG-3' and 5'-GGTACCAGGGCCCGCCCGGACATT-CAATACAGAGACCAGCTTC-3' for the left arm and right arm, respectively. Similarly, arp8 sequences were amplified using the following primers: 5'-GGGGGATCCACCCGACATT-GATGAGTACATG-3' and 5'-GGTACCAGGGCCCGCCCGGACATT-CAATACAGAGACCAGCTTC-3' for the left arm and right arm, respectively. Left arm and right arm was inserted into SpeI and XhoI site of pBS-KanMX to create swr1-KanR or arp8-KanR using GENEART Seamless Cloning (Invitrogen). 5 × 10⁷ fission yeast cells were transformed by 5 μg of swr1-KanR or arp8-KanR and spread on YE agar plate. After 16 hours, transformed cells were replica-plated on YE containing G418 (100 μg/ml) agar plate. Successful gene disruptions were confirmed by PCR using primers flanking the swr1 or arp8 genes.

**RESULTS** mlonRNA transcription-mediated histone acetylation converts chromatin into an accessible state in the fbp1 upstream region To gain insights into the link between mlonRNA transcription across the fbp1 upstream region and chromatin modulation mechanisms, we first examined the changes in the chromatin state upstream of fbp1 during glucose starvation. Micrococal nuclease (MNase) sensitive sites reflecting an open chromatin state first appeared near UAS1 (a black arrow head in Fig. 1A), and then the chromatin around the
TATA box progressively converted into an open state (a long dashed line in Fig. 1A), when massive induction of \( fbp1 \) was observed (Fig. 1A, 1B). Prior to full transcriptional activation of \( fbp1 \), long noncoding RNAs initiated from \( fbp1 \) upstream sites (referred to as mlonRNA-a, b and c) were detected as previously reported (Hirota et al., 2003).

Fig. 1. mlonRNA RNAs transcription preceding to \( fbp1 \) mRNA transcription and stepwise and progressive chromatin remodeling at \( fbp1 \) upstream. (A) The chromatin structure altered from 5' to 3' region of \( fbp1^+ \) promoter. The wild-type cells were grown to mid log phase (2 x 10^7 cells/ml) in YER medium containing 6% glucose and transferred to YED containing 0.1% glucose and 3% glycerol. The cells were harvested at time points indicated in the figure. Chromatin was analyzed as described previously (Hirota et al., 2003). The open arrow indicates the \( fbp1^+ \) coding regions. The black arrow head indicates an MNase sensitive site around UAS1 appeared at 10 min after glucose starvation. The long dashed line indicates MNase sensitive sites around TATA box appeared at 60–180 min after glucose starvation. (B) \( fbp1 \) transcripts were analyzed by northern analysis. Expression of \( cam1^+ \) was used as an internal control (Takeda and Yamamoto, 1987). (C) The binding of histone H3 to the \( fbp1 \) locus was examined by ChIP analysis. The wild-type cells were cultured as described in A. DNA of the input samples and ChIP samples were quantified as previously described (Hirota et al., 2006). (D) Acetylation of histone H3 and H4 was examined by ChIP using antibody for acetylated histone H3 and H4 respectively. (Upper panels) Vertical and horizontal axes represent ChIP efficiency (%) and time after glucose starvation, respectively. (Lower panels) ChIP efficiency of acetylated histone was divided by that of H3 and indicated in the Vertical axis. (E) Schematic representation of control strain and terminator inserted strain. A sequence lacking terminator activity (sequence between UAS1 and UAS2) or containing the \( nmt \)-terminator was inserted at \( EcoT22I \) site, located between UAS2 and the TATA box. RNA Polymerase II transcription is arrested between UAS2 and the TATA box as previously reported (Hirota et al., 2008a). Total histone H3 and histone H3 acetylation levels at UAS1 and the TATA box in the control- or terminator-inserted cells were examined as in C and D. ChIP efficiency for \( prp3 \) gene was used for normalization as previously described (Takemata et al., 2016). ChIP efficiency of acetylated histone H3 was divided by that of total H3 (Ac-histones/total). Fold enrichment after glucose starvation was calculated. The error bars show the standard deviations. \( p \)-value was calculated by student's t-test. * \( p < 0.05 \), ** \( p < 0.01 \) and ns (not significant).
Consistent with this observation, as well as our recent report (Takemata et al., 2016), ChIP analysis showed that histone H3 binding at UAS1 and UAS2 was decreased after glucose starvation (Fig. 1C), and acetylation levels of histone H3 and H4 peaked at 30 min after glucose starvation at UAS1 and UAS2 (Fig. 1D). Since our recent study revealed that mlonRNAs transcribed at fbp1 upstream facilitates binding of transcription factor Atf1, which is implicated in histone acetylation (Takemata et al., 2016), we assumed that the transient elevation of histone acetylation might be caused by mlonRNA transcription. To test this assumption, we inserted a transcriptional terminator sequence between UAS2 and the TATA box to arrest mlonRNAs transcription downstream of UAS2 (Fig. 1E), and measured the histone H3 acetylation at TATA box after glucose starvation. In the control cells that contain an insert of a similar size, but lacking a terminator sequence, histone acetylation was significantly increased and histone binding was decreased at 30 min after glucose starvation at both UAS1 and TATA box. On the other hand, in the terminator-inserted strain, histone acetylation and histone eviction was dramatically impaired at the TATA box, but not at UAS1 (Fig. 1E). These data indicate that mlonRNA transcription is required for histone acetylation and following disassembly of nucleosomes in this region.

Snf22 and Gcn5 are required for chromatin remodeling at fbp1 Since our recent study revealed that histone H3 acetylation at fbp1 is mediated by the Gcn5 HAT (Takemata et al., 2016), we examined the role of Gcn5 in chromatin remodeling at fbp1. Additionally, we also examined the role of the Snf22 ADCR. After glucose starvation, gcn5Δ cells exhibited reduced induction of fbp1 mRNA, while snf22Δ exhibited impaired mlonRNA transcription and delayed expression of fbp1 mRNA, suggesting pivotal roles of Snf22 and Gcn5 in the regulation of fbp1 transcription (Fig. 2). Consistently, snf22Δ cells exhibited little chromatin alterations around UAS1 (Fig. 3A, white and gray arrows in wild-type and snf22Δ lanes, respectively) and partial chromatin opening at the fbp1 TATA box (Fig. 3A, white dashed line in snf22Δ lanes).

Fig. 2. Measurement of fbp1 transcription by northern blot analysis in chromatin modulators deletion cells. (A) Indicated cells were cultured as described in Fig. 1. The transcripts of fbp1 were analyzed by northern analysis. (B) The band intensity of fbp1 mRNA was quantified and normalized to that of cam1 internal control. The fbp1-mRNA/cam1 ratio was normalized to the 120 min wild-type value to define the relative mRNA level (Y-axis). The error bars show the standard deviation from three independent experiments.
indicating that Snf22 is involved in the chromatin remodeling at *fbp1* upstream regulatory region and promoter TATA region. Interestingly, *gcn5*Δ cells exhibited only slightly reduced and delayed chromatin structure alterations (Fig. 3A, black dashed line in *gcn5*Δ lanes). Intensity of the MNase sensitive site at TATA (surrounded by white box) was quantified (Fig. 3B). These results indicate that Snf22 plays a pivotal role, while Gcn5 has a minor role, in the regulation of chromatin at *fbp1*. We next examined the genetic relationship between *snf22* and *gcn5*. The *snf22Δgcn5Δ* cells exhibited critically reduced *fbp1* mRNA transcription and completely impaired chromatin structure alterations (Fig. 2, 3). These results indicate that Gcn5 plays a critical role in the absence of Snf22 with regard to promoter nucleosome eviction during *fbp1* transcriptional activation.
CHD1 family ADCR Hrp3 serves as a backup for Snf22 in the regulation of chromatin state at *fbp1*. We next explored the mechanisms that induce chromatin remodeling in *snf22Δ* cells. First, we tested the role of another class of Snf2-related ADCR, Snf21. Since Snf21 is essential for cell viability, we used a *snf21-36ts* mutant (Yamada et al., 2008). The *snf21-36ts* mutant cells exhibited slightly reduced *fbp1* mRNA transcription at the restrictive temperature (37 °C). Interestingly, *snf22Δ* and *snf22Δ/ snf21-36ts* cells exhibited a

Fig. 4. Snf22 and Hrp3 are required for histone H3 acetylation in the *fbp1* upstream region. (A) Acetylation of histone H3 was examined by ChIP using antibody for acetylated histone H3. Indicated cells were cultured to mid log phase (2 × 10⁷ cells/ml) in YER medium containing 6% glucose (Glucose+) and transferred to YED containing 0.1% glucose and 3% glycerol and further cultured for 30 min (Glucose-). ChIP efficiency for *prp3* gene was used for normalization as previously (Takemata et al., 2016). Fold enrichment after glucose starvation was calculated. The error bars show the standard deviation from three independent experiments. (B–D) Schematic representations of the models from this study. (B) A model of histone acetylation by *mlon*RNAs. In glucose rich conditions (+Glucose), Atf1 binding to UAS1 is suppressed by the repressive function of Tup-proteins. In glucose starvation conditions (-Glucose), transcription of *mlon*-RNAs is activated and they interact and antagonize Tup-proteins. Then, Atf1 is allowed to bind to UAS1 and induces histone acetylation and subsequent chromatin remodeling. (C) A model of histone acetylation by RNA Polymerase II (Pol II) transcribing *mlon*RNAs. Under glucose starvation conditions (-Glucose), transcription of *mlon*RNAs is activated and they interact and antagonize Tup-proteins. Then, Atf1 is allowed to bind to UAS1 and induces histone acetylation and subsequent chromatin remodeling. (D) Interplay between ADCRs and histone acetylation. ADCRs, Snf22 and Hrp3 are critical activities for *fbp1* chromatin remodeling. The histone acetylation by HAT, including Gcn5, is dependent on Snf22 and Hrp3 ADCR, while this acetylation or HAT enzyme itself recruits ADCRs.
similar fbp1 transcriptional defect, indicating that Snf21 and Snf22 play a role in the same pathway (Supplementary Fig. S1). Secondly, we tested the possibility that a histone variant H2AZ plays a role in fbp1 transcriptional activation. To this end, we disrupted sur1, which encodes a histone-variant exchanger that efficiently replaces canonical histone H2A with histone H2AZ in nucleosome arrays (Mochizuki and Yamamoto, 1992; Luk et al., 2010). The sur1Δ cells exhibited no defect in fbp1 induction upon glucose starvation (Supplementary Fig. S1). Moreover, snf22Δ/sur1Δ and snf22Δ cells exhibited very similar defects in fbp1 induction (Supplementary Fig. S1). We next examined the H2AZ removal process by Ino80 (Morrison and Shen, 2009). To this end, we disrupted arp8, which encodes a subunit of the Ino80 complex and plays critical role for this complex. The snf22Δ/arp8Δ cells and snf22Δ exhibited very similar defects in fbp1 induction (Supplementary Fig. S1). These data suggest that incorporation and removal of histone variant H2AZ are not involved in fbp1 transcriptional control. We next analyzed the role of the Hrp3 CHD1 family ADCR, which is involved in the regulation of nucleosome positioning in fission yeast (Hennig et al., 2012; Pointner et al., 2012; Shim et al., 2012; Touat-Todeschini et al., 2012). After glucose starvation, hrp3Δ cells exhibited reduced induction of fbp1 mRNA (Fig. 2A). Moreover, the snf22Δ/hrp3Δ cells exhibited critically reduced fbp1 mRNA transcription, suggesting that function of Hrp3 in fbp1 chromatin regulation is redundant with that of Snf22 (Fig. 2). Indeed, chromatin alterations at the fbp1 upstream and TATA box regions were critically impaired in the snf22Δ/hrp3Δ cells (Fig. 3).

Gcn5 plays pivotal roles to induce fbp1 chromatin remodeling in hrp3Δ cells Because both Gcn5 and Hrp3 have critical functions for chromatin remodeling in the absence of Snf22, we next investigated the genetic relationship between Hrp3 and Gcn5. Both single mutant cells exhibited mild defects in fbp1 mRNA induction upon glucose starvation (Fig. 2). Moreover, these exhibited limited defects in chromatin remodeling in the fbp1 upstream region (Fig. 3). Strikingly, hrp3Δ gcen5Δ cells exhibited little fbp1 mRNA induction and were completely impaired for chromatin remodeling (Fig. 2, 3). These data suggest that Gcn5-mediated histone acetylation might play pivotal roles in the absence of Hrp3. Taken together, concurrent loss of any two of the Snf22, Hrp3 and Gcn5 proteins results in loss of chromatin remodeling at the fbp1 upstream region and TATA box during glucose starvation (Fig. 3), suggesting a complex interplay among these factors.

Snf22 and Hrp3 are required for Gcn5-mediated histone H3 acetylation Having established the critical role of Gcn5 in the regulation of chromatin at fbp1 in the absence of either Snf22 or Hrp3, we next asked if Gcn5-mediated acetylation is affected by Snf22 and Hrp3. To address this issue, we investigated histone acetylation at UAS1, UAS2 and the TATA box upon glucose starvation. Interestingly, in snf22Δ cells, histone H3 acetylation was partially reduced at UAS1 and critically reduced to basal levels at UAS2 and the TATA box, indicating a pivotal role of Snf22 in histone H3 acetylation (Fig. 4A). The residual histone H3 acetylation at UAS1 in snf22Δ cells was completely reduced to basal level by depleting Hrp3, suggesting that Snf22 plays a major role for histone acetylation and Hrp3 serves as a backup for Snf22.

DISCUSSION

Our recent study demonstrated the enhancer function of noncoding RNAs, which enhances transcription factor binding and thereby further induces histone acetylation as well as chromatin remodeling (Takemata et al., 2016). This current study addresses the role ADCRs to these processes. Here, we demonstrate the presence of a cooperative and interdependent relationship between two layers of chromatin modulators, ADCRs (Snf22 and Hrp3) and histone acetyltransferases.

Noncoding RNA transcription across the fbp1 upstream region induces histone acetylation at transcribed tracts We demonstrated that histone acetylation and chromatin remodeling precede the later disassembly of the TATA box nucleosome (Fig. 1). Concurrent with the transient elevation of histone acetylation, we detected the stepwise transcription of mlonRNAs a, b and c, initiated from sites far upstream of fbp1 promoter. These observations led us to test the hypothesis that mlonRNA transcription is involved in histone acetylation. Indeed, inhibition of the mlonRNAs transcription by inserting a transcription termination sequence significantly compromised hyperacetylation of histones downstream of the termination site, but not at the upstream of this site. These data support the above hypothesis and lead us to propose a possible model for the roles of mlonRNAs to establish an open chromatin state at fbp1 (Fig. 4B, 4C). Our recent study revealed that mlonRNAs facilitate binding of Atf1 by Tupil12-dependent and independent mechanisms (Takemata et al., 2016). In the Tupil12 dependent mechanism, mlonRNAs interact with Tupil12 to antagonize their repressive function and facilitate Atf1 binding, as demonstrated previously (Takemata et al., 2016). The enhanced binding of Atf1 may induce HAT recruitment to fbp1 upstream region (Fig. 4B). In the Tupil12-independent pathway, it is possible that RNA polymerase II complexes that are transcribing mlonRNAs may play roles in enhancing DNA accessibility, as reported previously (Gilchrist et al., 2016).
These RNA polymerase II complexes may interact with HATs, as is the case for the interaction between PCAF HAT and RNA polymerase II (Cho et al., 1998), to induce histone acetylation over the region covered by the mlonRNA transcripts (Fig. 4C). It is possible that enhancement of Atf1 binding may be further promoted by histone acetylation following chromatin remodeling.

The cooperative and interdependent relationship between ADCRs and histone acetylation

We demonstrated that two ADCRs Snf22 and Hrp3 play a critical role for the chromatin remodeling at fbp1 and the disassembly of promoter nucleosomes. Gen5 might facilitate both activities, because Gen5 was critical for residual Hrp3 or Snf22 activity in snf22Δ or hrp3Δ cells, respectively. This idea is consistent with the previous findings in budding yeast that the bromodomain of Snf2 re- cognizes and binds to acetylated histone (Grant et al., 1998; Chandy et al., 2006; Hassan et al., 2006). The next question is how Gen5 is involved in the regulation of Hrp3. It is possible that some proteins carrying bromodomains recruit Hrp3. Another possibility is that Gen5 recruits and directly regulates Hrp3 through a physical interaction. This idea is supported by the finding that the budding yeast Hrp3 homolog Chd1 is part of the SAGA complex (Pray-Grant et al., 2005). The histone acetylation might be induced redundantly by Gen5 and other HATs, since gcn5Δ cells exhibit mild defects in fbp1 mRNA induction. The NuA4 (nucleosomal acetyltransferase of histone H4) HAT (Mst1 and Mst2 in fission yeast) might be a plausible candidate, since loss of Gen5 has a limited impact on histone H4 acetylation and concurrent loss of Gen5 and Mst2 results in synthetic defects in fbp1 mRNA expression. Conversely, we observed that Snf22 and Hrp3 are involved in the histone acetylation at fbp1 upon glucose starvation. Thus, two classes of chromatin modulators, ADCRs (Snf22 and Hrp3) and histone acetyltransferases might have a cooperative and interdependent relationship (Fig. 4D), as suggested by this study. Further understanding of the mechanism of the noncoding RNA transcription as well as the interplay between chromatin modulators will provide important clues to understand the molecular basis of chromatin regulation by noncoding RNA transcription.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

REFERENCES


