Interplay between chromatin modulators and histone acetylation regulates the formation of accessible chromatin in the upstream regulatory region of fission yeast *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 *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 *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 *fbp1*. Snf22 and Hrp3 redundantly promote disassembly of chromatin in the *fbp1* upstream region. Gcn5 critically contributes to nucleosome eviction in the absence of either Snf22 or Hrp3, presumably by recruiting Hrp3 in *snf22Δ* cells and Snf22 in *hrp3Δ* cells. Conversely, Gcn5-dependent histone H3 acetylation is impaired in *snf22Δ/hrp3Δ* cells, suggesting that both redundant ADCRs induce recruitment of Gcn5 to the chromatin array in the *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.

Key words: ATP-dependent chromatin remodeler, histone acetyl transferase, Snf22, Hrp3, Gcn5

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-

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teins carrying bromodomains, including Swi2/Snf2 family ADCRs, serve as a decoder for the histone acetylation. A further class of ADCR 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 CCATT 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 Gcn5 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-

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

Strain	Genotype	Reference
SPH1	<i>h</i> – <i>leu1-32</i>	(Yamada et al., 2004)
SPH98	<i>h</i> + <i>ade6-M26 ura4-D18 his5-303 gcn5::ura4</i>	(Yamada et al., 2004)
SPH100	<i>h</i> + <i>ade6-M26 snf22::ura4 ura4-D18 his5-303</i>	(Yamada et al., 2004)
SPH101	<i>h</i> + <i>ade6-M26 snf22::ura4 gcn5::ura4 ura4-D18 his5-303</i>	(Yamada et al., 2004)
SPH102	<i>h</i> + <i>ade6-M26 hrp1::ura4 ura4-D18 his5-303</i>	(Hirota et al., 2008b)
SPH103	<i>h</i> + <i>ade6-M26 hrp3::ura4 ura4-D18 his5-303</i>	(Hirota et al., 2008b)
SPH105	<i>h</i> + <i>ade6-M26 hrp3::ura4 gcn5::ura4 his5-303 ura4-D18</i>	(Hirota et al., 2008b)
SPH111	<i>h</i> – <i>ade6-M26 leu1-32 ura4-D18 fbp1(EcoT22::UAS1-2)</i>	(Hirota et al., 2008a)
SPH112	<i>h</i> + <i>ade6-M26 hrp3::ura4 snf22::ura4 his5-303 ura4-D18</i>	(Hirota et al., 2008b)
SPH116	<i>h</i> – <i>ade6-M26 leu1-32 ura4-D18 fbp1(EcoT22::nmt-terminator)</i>	(Hirota et al., 2008a)
SPH243	<i>h</i> – <i>leu1-32 snf21-36</i>	(Yamada et al., 2008)
SPH331	<i>h</i> – <i>leu1-32 snf21-36 snf22::kanMX6</i>	This study
SPH332	<i>h</i> + <i>ura4-D18 leu1-32 swr1::kanMX6</i>	This study
SPH333	<i>h</i> + <i>ade6-M26 snf22::ura4 ura4-D18 his5-303 swr1::kanMX6</i>	This study
SPH334	<i>h</i> + <i>ura4-D18 leu1-32 arp8::kanMX6</i>	This study
SPH335	<i>h</i> + <i>ade6-M26 snf22::ura4 ura4-D18 his5-303 arp8::kanMX6</i>	This study

amplified DNA fragments, and the DNA fragments were further labeled with ^{32}P 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 (Yasuikikai, 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 *Sma*I and *Eco*RI. This fragment was inserted into *Sma*I and *Eco*RI 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 EDTA, 20 mM Tris-HCl [pH 8.0]) and incubated at 30 °C for 10 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 M 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, Darmstadt, 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 immunopre-

cipitation (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(GGGATGAAAACAATCAACCTC and GGAATGCAGCAACGAAAATC), UAS2(GGGTGGAAATG-AGTCCGC and GTTCCGCGAATCATAAGCC), TATA(CG-CGGAACATAACATAGCG and GCTAGAAACCGAGTG-GTG), and prp3(GCACAGTCGTTGTACAAATTCGTATTCCC, and ACGATTCTAAACGCCTCTTGTACGATCC).

Antibodies The following antibodies were used in this study. Anti-total histone H3 (catalog no. ab1791, Abcam, UK), Anti-H3ac (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-Kan^R* and *arp8-Kan^R* were generated from genomic PCR products combined with *KanMX* selection marker gene. Genomic DNA sequences were amplified using the following primers for *swr1*: 5'-GCGGC-CGCTCTAGAAGCCTCGCAACTATACTTGTCC-3' and 5'-AGCCCGGGGGATCCACCCACTGTGAGTAACCAACAG-3' plus 5'-TCGATACCGTCGACCGGGGCACATTGATGAGTACATG-3' and 5'-GTACCGGGCCCCCCCCACCAATACAGAGACCAGCTTC-3' for the left arm and right arm, respectively. Similarly, *arp8* sequences were amplified using the following primers: 5'-GCGGCCGCTCTAGAACGCAATTCCCTAAAGCCGAG-3' and 5'-AGCCCGGGGGATCCAGCGGAGTCTCGCCATATATAC-3' plus 5'-TCGATACCGTCGACCCCTGGAAGAGCGTATCCATTCC-3' and 5'-GTACCGGGCCCCCCCCGAAGGCTCC-TTGCTTCGG-3' for the left arm and right arm, respectively. Left arm and right arm was inserted into *Spe*I and *Xho*I site of pBS-KanMX to create *swr1-Kan^R* or *arp8-Kan^R* using GENEART Seamless Cloning (Invitrogen). 5 × 10⁷ fission yeast cells were transformed by 5 µg of *swr1-Kan^R* or *arp8-Kan^R* 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

mIcnRNA transcription-mediated histone acetylation converts chromatin into an accessible state in the *fbp1* upstream region To gain insights into the link between mIcnRNA 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. Micrococcal 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 transcrip-

tional 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

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 (2×10^7 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).

al., 2008a). 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-c 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.

Fig. 3. Chromatin structure around *fbp1* upstream region in chromatin modulators deletion cells. (A) Indicated cells were cultured as described in Fig. 1. Chromatin analysis was performed as in Fig. 1. The white and gray arrowheads indicate the region of MNase sensitive site around UAS1 appeared in wild-type cells at 10 min after glucose starvation. The white arrowhead shows appearance of MNase sensitive site in wild-type cells, while the gray arrowhead shows impaired chromatin remodeling around UAS1 in *snf22Δ* cells. The black and white dashed lines indicate MNase sensitive sites around TATA box appeared at 60–180 min after glucose starvation. (B) The intensities of the band corresponding to an MNase hypersensitive site at TATA box (enclosed by white box in A) were quantified and the ratios of band intensity at the TATA box to that of the total lane were calculated. 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, *gcn5Δ* cells exhibited only slightly reduced and delayed chromatin structure alterations (Fig. 3A, black dashed line in *gcn5Δ* lanes). Intensity of the MNase sensitive band 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 *snf22Δ/gcn5Δ* 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 *snf21-36^{ts}* mutant (Yamada et al., 2008). The *snf21-36^{ts}* mutant cells exhibited slightly reduced *fbp1* mRNA transcription at the restrictive temperature (37 °C). Interestingly, *snf22Δ* and *snf22Δ / snf21-36^{ts}* 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 (2×10^7 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 mlonRNAs. 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 mlonRNAs 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 mlonRNAs. Under glucose starvation conditions (-Glucose), RNA polymerase II initiates mlonRNA transcription. This RNA polymerase II interacts with HAT and induces histone acetylation around the initiation site. (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 H2A.Z plays a role in *fbp1* transcriptional activation. To this end, we disrupted *swr1*, which encodes a histone-variant exchanger that efficiently replaces canonical histone H2A with histone H2A.Z in nucleosome arrays (Mochizuki and Yamamoto, 1992; Luk et al., 2010). The *swr1Δ* cells exhibited no defect in *fbp1* induction upon glucose starvation (Supplementary Fig. S1). Moreover, *snf22Δ/swr1Δ* and *snf22Δ* cells exhibited very similar defects in *fbp1* induction (Supplementary Fig. S1). We next examined the H2A.Z 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 H2A.Z 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/gcn5Δ* 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 Tup11/12-dependent and independent mechanisms (Takemata et al., 2016). In the Tup11/12 dependent mechanism, mlonRNAs interact with Tup11/12 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 Tup11/12-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.,

2010; Weiner et al., 2010). 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. Gcn5 might facilitate both activities, because Gcn5 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 recognizes and binds to acetylated histone (Grant et al., 1998; Chandy et al., 2006; Hassan et al., 2006). The next question is how Gcn5 is involved in the regulation of Hrp3. It is possible that some proteins carrying bromodomains recruit Hrp3. Another possibility is that Gcn5 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 Gcn5 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 Gcn5 has a limited impact on histone H4 acetylation and concurrent loss of Gcn5 and Mst2 results in synthetic defects in the meiotic recombination at the CRE-related hotspot *ade6-M26* (Hirota et al., 2008b). 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.

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