Published in final edited form as:
Mol Microbiol. 2011 April ; 80(2): 407–422. doi:10.1111/j.1365-2958.2011.07583.x.

Activator-independent transcription of Snf1-dependent genes in mutants lacking histone tails

Juan J. Infante[1], **G. Lynn Law**[1], **I-Ting Wang**[1], **Hsin-Wen Ella Chang**[1], and **Elton T. Young**[*]
Department of Biochemistry, University of Washington, Seattle, WA 98195, USA

Summary

Transcriptional regulation of Snf1-dependent genes occurs in part by histone-acetylation-dependent binding of the transcription factor Adr1. Analysis of previously published microarray data indicated unscheduled transcription of a large number of Snf1- and Adr1-dependent genes when either the histone H3 or H4 tail was deleted. Quantitative real time PCR confirmed that the tails were important to preserve stringent transcriptional repression of Snf1-dependent genes when glucose was present. The absence of the tails allowed Adr1 and RNA Polymerase II to bind promoters in normally inhibitory conditions. The promoters escaped glucose repression to a limited extent and the weak constitutive *ADH2* transcription induced by deletion of the histone tails was transcription factor- and Snf1-independent. These effects were apparently due to a permissive chromatin structure that allowed transcription in the absence of repression mediated by the histone tails. Deleting *REG1*, and thus activating Snf1 in the H3 tail mutant enhanced transcription in repressing conditions, indicating that Snf1 and the H3 tail influence transcription independently. Deleting *REG1* in the histone H4 tail mutant appeared to be lethal, even in the absence of Snf1, suggesting that Reg1 and the H4 tail have redundant functions that are important for cell viability.

Introduction

Chromatin structure poses a barrier to transcription by RNA Polymerase II (RNA Pol II). Eukaryotic organisms have evolved mechanisms that use this barrier to transcription to regulate gene expression (Wu & Grunstein, 2000, Jenuwein & Allis, 2001). The structure of chromatin is modulated by post-translational modifications of histones, the highly conserved proteins that constitute the central structure of the nucleosome core particle. Many proteins interact with histones and induce these modifications that in turn affect the dynamic properties of nucleosome core particles (Wu & Grunstein, 2000, Saha *et al.*, 2006). These proteins include histone acetyl transferases (HATs), histone deacetylases (HDACs), and ATP-dependent chromatin remodeling complexes (CRCs) and target primarily the unstructured N-terminal tails of the histones (Millar & Grunstein, 2006). HATs and HDACs regulate the charge of the tails to create a dynamic equilibrium between the presence and absence of acetyl groups on lysine residues. The modulation of charge influences the interactions between histones and DNA that in turn can change the location of the nucleosomes on the DNA (Zheng & Hayes, 2003). The tails interact *in vitro* with the DNA to enhance the stability of the nucleosome at a particular translational position (Yang *et al.*,

[*]Address correspondence to: Elton T. Young, Department of Biochemistry, Box 357350, University of Washington, Seattle, WA 98195-7350. Phone: (206) 543-6517. Fax: (206) 685-1792; ety@u.washington.edu..
[1]Present addresses: Juan J. Infante: Bioorganic Research and Services-Bionaturis, Doctor Duarte de Acosta, 5d, 11500 El Puerto de Santa María, Cádiz, Spain.; G. Lynn Law: Department of Microbiology, University of Washington, Seattle, WA 98195, USA; I-Ting Wang: Neuroscience Graduate Group, Department of Genetics, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; Hsin-Wen Ella Chang: Department of Molecular Biology and GEnetics Cornell University, Ithaca, NJ, USA.

2007). This translational movement can affect the accessibility of specific binding sites to transcription factors (Brower-Toland *et al.*, 2005). For example, specific patterns of acetylation in the histone tails are recognized by CRCs that alter the translational position of the nucleosome core particle (Saha et al., 2006), making a previously occluded binding site accessible to a DNA-binding protein or *vice versa*. Both DNA sequence and chromatin binding proteins can influence the position of nucleosomes on the DNA (discussed in (Caserta *et al.*, 2009, Travers *et al.*, 2009)).

We analyzed the consequences of deleting or mutating the histone tails for gene expression and promoter architecture of glucose-repressed genes in *Saccharomyces cerevisiae* (*S. cerevisiae*). The genes analyzed were those whose derepression in the absence of glucose is mediated by the transcription factors Adr1 and Cat8, which act downstream of the Snf1 kinase, the yeast homolog of the mammalian AMP-activated protein kinase (AMPK). Adr1 activates more than 100 genes whose encoded enzymes are required for non-fermentative metabolic pathways (Young *et al.*, 2003). In the presence of glucose the activity of Adr1 is inhibited by phosphorylation of the DNA binding and regulatory domains (Kacherovsky *et al.*, 2008; Ratnakumar *et al.*, 2009; Ratnakumar & Young, 2010). In the absence of glucose, Snf1 promotes the dephosphorylation and activation of Adr1. The yeast 14-3-3 or Bmh proteins bind to the regulatory domain when S230 is phosphorylated and this inhibits a nearby activation domain (Parua *et al.*, 2010). By promoting Ser230 dephosphorylation Snf1 counteracts the inhibitory role of Bmh. At the *ADH2* promoter (*ADH2prm*), Adr1 and Cat8 cooperate to bind DNA, recruit coactivators, remodel chromatin and activate transcription (Tachibana *et al.*, 2005, Biddick *et al.*, 2008). At the promoters of genes encoding glycolytic and gluconeogenic enzymes, such as the *FBP1* promoter (*FBP1prm*), only Cat8 is needed for close to maximal derepression. A HAT-dependent increase in histone H3- and H4-tail acetylation facilitates both chromatin remodeling and transcriptional activation shortly after glucose is exhausted in a set of co-regulated promoters including *ADH2prm* (Agricola *et al.*, 2006).

Surprisingly, Adr1 and Cat8 can bind in repressing conditions in a mutant lacking two HATs, Hda1 and Rpd3 (Δ*hdac*) (Tachibana *et al.*, 2007, Verdone *et al.*, 2002). Activator binding in the Δ*hdac* mutant in repressing conditions is associated with HAT-dependent hyperacetylation of promoter nucleosomes similar to that observed in derepressing conditions (Verdone et al., 2002), suggesting that histone hypoacetylation inhibits activator binding in repressing conditions. Although activator binding and promoter remodeling take place, and a stable pre-initiation complex (PIC) is recruited in the Δ*hdac* mutant in repressing conditions, there is very little transcription (Tachibana et al., 2007, Verdone et al., 2002). The inactive “poised” PIC contains SAGA (Spt-Ada-Gcn5 Acetyltransferase complex), Mediator, Swi/Snf (Swi/Snf chromatin remodeling complex), and RNA Pol II partially phosphorylated on Ser5 of the carboxy-terminal domain (Tachibana et al., 2007). The inactive “poised” complex can be activated in the Δ*hdac* mutant in repressing conditions by activating Snf1 and by activating Adr1 (Tachibana et al., 2007). Thus PIC recruitment is necessary but not sufficient for efficient transcription.

Although increased acetylation of the histone tails is associated with increased activator binding in the Δ*hdac* mutant, it is possible that enhanced acetylation of other proteins contributes to or is responsible for activator binding and PIC formation. To test this possibility we analyzed activator and RNA Pol II binding, gene expression and chromatin remodeling in mutants lacking the histone H3 and H4 N-terminal tails or containing Gln substitutions of the Lys residues in the tails. Our analysis shows that the absence of the H3- or H4-tails leads to specific up-regulation of Snf1-dependent genes. Adr1 binding sites became accessible at the *ADH2* promoter when the histone tails were altered and well-positioned nucleosomes were lost or became unstable, allowing the binding of Adr1.

However, the low level of constitutive (glucose-resistant) ADH2 expression induced by these alterations was independent of the presence of Adr1 and Cat8 and independent of Snf1, indicating that RNA Pol II is able to actively engage Adr1-dependent promoters in the absence of an activator if the chromatin structure is permissive.

Results

Deletion of either H3- or H4-tail causes constitutive expression of glucose-repressed genes

Since there was previous evidence for a role of the histone H3- and H4-tails in glucose regulation (Agricola et al., 2006, Tachibana et al., 2007, Verdone et al., 2002), we looked for the effect on genes whose expression is dependent on either the transcription factors Adr1 and Cat8 or the upstream kinase Snf1 in the dataset of the first transcriptome analysis published for mutants deleted for these histone tails (Sabet *et al.*, 2003). This genome-wide expression study showed up-regulation of a significant number of genes when the H3-tail was deleted, suggesting that the H3-tail has a global role in repression in glucose-rich media (Table 1). In contrast, deletion of the H4-tail either increased or decreased expression of a very similar proportion of genes.

We analyzed the data corresponding to the specific subset of genes whose derepression depends on Adr1, Cat8, or Snf1 (Young et al., 2003) and found a significant difference between the observed number of genes affected by either tail deletion and the expected distribution if the different subsets followed the same trend observed genome-wide (Table 1 and Table S2). Specifically, in each of these subsets there was a significantly higher proportion of genes whose expression was up-regulated by the deletion of either the H3- or H4-tail than expected. We also looked for the effect of the histone tail deletions in a subset of genes whose regulation is totally independent of the carbon source in order to estimate the specificity of the contribution of the tail deletions to the Snf1-dependent genes. The genes chosen for this subset are regulated by the unfolded protein response and are up-regulated when the endoplasmic reticulum is subjected to acute stress (Travers *et al.*, 2000). This subset was also up-regulated by both tail deletions in yeast growing in the absence of stress (Table 1). However, the proportion of genes in this subset that was affected was lower than the proportion of glucose-regulated genes. When we only considered those genes in which direct binding and regulation of transcription by Adr1, Cat8, or both factors have been confirmed (Tachibana et al., 2005), the array data of Sabet (Sabet et al., 2003) showed a very high proportion of genes up-regulated by deletion of the H3-tail. It also showed a significant but lower proportion of genes up-regulated by deletion of the H4-tail only when Adr1 participates in their regulation (Table 1).

The data reported above suggest that both tails are specifically involved in the glucose-repression pathway controlled by the Snf1 kinase and the downstream transcriptional activators Adr1 and Cat8. We quantified the mRNA levels of several Snf1-dependent genes in mutants lacking either histone H3- or H4-tail (Δ*h3t* and Δ*h4t*, respectively) by quantitative real-time reverse transcriptase PCR (qPCR). We confirmed that both tail deletions cause the low-level, constitutive activation of *ADH2*, *ACS1*, *ADY2*, *FBP1*, *MLS1*, and perhaps *MDH2*, which are representatives of Snf1-dependent genes. Fig. 1A shows their expression in repressing growth conditions relative to a strain containing wildtype (wt) histone genes. The increase in *ADH2* mRNA levels in the tail mutants is about 6-fold. Derepression results in an ~400-fold increase in *ADH2* mRNA levels in a strain with wt histone tails (Fig. 1B). Thus, the *ADH2* mRNA levels in the Δ*h3t* mutant represent only about 1.5% of the derepressed level. The extent of relief from repression of the other genes is also minor. *MDH2* repression was not affected by the tail deletions in the array data and was the least affected as determined by qPCR. Derepressed expression of these genes was

reduced in the tail mutants. Fig. 1B shows their derepressed expression relative to wt in repressing conditions. *ADH2* was the least affected (two-fold reduced) and the strongly Cat8-dependent genes *FBP1* and *MLS1* were more strongly affected (about four-fold reduced). In summary deletion of the H3 and H4 tails alters regulation of Snf1-dependent genes in two ways. The genes are released to a limited extent from the stringent glucose repression that normally prevails and their maximal derepression is reduced.

Deletion of the histone H3 and H4 tails allows Adr1, but not Cat8, to bind in repressing conditions

A repressive chromatin structure due to hypoacetylated histones appears to prevent Adr1 from binding in the presence of glucose (Verdone et al., 2002, Tachibana et al., 2007). When the HDACs Rpd3 and Hda1 are absent, Adr1 and Cat8 can bind in the presence of glucose but transcription is still strongly glucose-repressed, suggesting that hyperacetylation of the histone tails allows Adr1 and Cat8 binding but post-binding step(s) in glucose repression are still operative. Hypoacetylated histone tails interact with neighboring DNA and increase the stability of a specific nucleosome position (Yang et al., 2007). If hyperacetylation of the histone tails, and not acetylation of some other substrate allows Adr1 to bind in repressing conditions, deletion of the tails might also create a permissive chromatin structure.

Adr1 binding in the tail mutants was studied by chromatin immunoprecipitation (ChIP) after epitope tagging Adr1 at its C-terminus. As expected, Adr1 binding was not detectable in the wildtype (wt) parent strain grown in repressing conditions and could be readily detected after derepression (Fig. 2A). However, in a Δ*h3t* mutant Adr1 was bound to several promoters (*ADH2, ADY2, FBP1*; Fig. 2A) at similar levels in repressing and derepressing conditions, a dramatic difference compared to its binding in cells with wt histones where binding is restricted to derepressing conditions. Moreover, Adr1 binding in the Δ*h3t* mutant in repressing conditions was at a level similar to its binding in cells with wt histones in derepressing conditions. A similar result was observed with Δ*h4t* (data not shown). These results indicate that the restriction on Adr1 binding in repressing conditions is absent when the histone H3 or H4 tails are missing, as was observed in a mutant in which the Rpd3 and Hda1 deaectylases are deleted (Verdone et al., 2002,Tachibana et al., 2007),

These results suggest that the H3- and H4-tails restrict the accessibility of the Adr1-binding site during glucose repression. Since Adr1 binding also occurs in repressive growth conditions in the Δ*hdac* mutant (Verdone et al., 2002, Tachibana et al., 2007), hypoacetylation of the H3- and H4-tails may contribute to glucose repression. In agreement with this interpretation, substitution of all lysines by glutamines in either tail, which mimics the acetylated state of lysine, also led to weak constitutive activation of *ADH2* at a level similar to that observed in the tail-less mutants (Fig. 1C). Expression of *ADY2, ACS1, FBP1*, and *MLS1* was less affected by the K-to-Q substitutions than by deletion of the tails. This apparent promoter specificity may indicate that transcription factor binding to the *ADH2* promoter is more sensitive to histone acetylation than the other promoters tested.

Several genes dependent on Cat8 for their expression were weakly activated in repressing conditions in the tail mutants (Table 1, Table S2**, and** Fig. 1A). Therefore, we asked whether deletion of the H3-tail also allowed Cat8 binding in repressing conditions. Cat8 was epitope-tagged at its C-terminus and binding was measured by ChIP. In derepressing conditions Cat8 was detected at the promoters of *ADH2* and *FBP1* as expected (Tachibana *et al.*, 2005). However, in repressing conditions we found that, unlike for Adr1, the Δ*H3t* deletion did not allow Cat8 binding to any of the promoters tested at a level above background (Fig. 2B).

Low-level, constitutive ADH2 activation caused by the tail deletion is independent of the transcription factors Adr1 and Cat8 and the Snf1 kinase

The high level of Adr1 binding in repressing conditions in Δ*h3t* was surprising in light of the low level of gene expression. A similar phenomenon, a high level of promoter occupancy but very weak transcription, was observed in a Δ*hdac* mutant: (Verdone *et al.*, 2002, Tachibana *et al.*, 2007). An important difference between the two situations is the high level of Cat8 binding in the Δ*hdac* mutant (Tachibana *et al.*, 2007) but lack of detectable Cat8 binding in the histone tail mutants (Fig. 2B). Since *ADH2* expression is dependent on both Adr1 and Cat8, the lack of Cat8 binding to the *ADH2prm* in the tail mutants in repressing conditions might explain the relatively weak transcription. To determine whether transcription in the tail mutants in repressing conditions is transcription factor-dependent, we deleted both Adr1 and Cat8 in a strain lacking the H3-tail. Surprisingly, *ADH2* transcription in the H3-tail mutant in repressing conditions was unaffected by the deletions and *FBP1* transcription was only reduced about 30% (Fig. 2C).

Binding and activity of both Adr1 and Cat8 in derepressing conditions are dependent on Snf1 (Schuller, 2003, Young *et al.*, 2003, Young *et al.*, 2002) and their binding is Snf1-dependent in repressing conditions in the Δ*hdac* mutant (Tachibana *et al.*, 2007). Although Snf1 is primarily inactive in the presence of glucose (Hardie *et al.*, 1998, Hong & Carlson, 2007, Rubenstein *et al.*, 2008, Orlova *et al.*, 2008), the Snf1-dependent binding of Adr1 and Cat8 in the Δ*hdac* mutant suggests that loss of HDAC activity may constitute a stress that activates Snf1 (Hong & Carlson, 2007). Surprisingly, expression of *ADH2* in the H3-and H4-tail mutants in repressing conditions was independent of Snf1 (Fig. 2C). In contrast *FBP1* expression was reduced about two-fold in the tail mutants in the absence of Snf1 but it was still significantly higher than in a strain with wt histones. These results are consistent with those showing that *ADH2* expression is independent of the transcription factors Adr1 and Cat8 (Fig. 2C), both of which are Snf1-dependent for activity. When the tail mutants were derepressed by depleting glucose from the media, the increase in *ADH2* activity was Snf1-dependent (**data not shown**), indicating that the major pathway of derepression, which is Snf1-dependent, is still functional in the absence of the H3 tail.

In the Δ*hdac* mutant Adr1 binding is accompanied by recruitment of an inactive PIC containing RNA Pol II (Tachibana et al., 2007). To determine if similar recruitment of RNA Pol II occurs in the Δ*h3t* mutant we did ChIP for the largest subunit of RNA Pol II, Rpb1, to measure promoter occupancy at *ADH2, ADY2*, and *FBP1* (Fig. 2D). The level of RNA Pol II in the H3-tail mutant in repressing conditions was significantly higher than the background binding in the wt strain in repressing conditions at the promoters of *ADH2* and *ADY2*. Upon derepression RNA Pol II levels increased in the tail mutants and reached a level similar to that in the wt parent at the *ADH2prm* but not at the promoter of *ADY2*, in agreement with the lower level of derepression of *ADY2* (Fig. 1B). The occupancy by RNA Pol II at the *ADH2prm* in repressing conditions was higher than would be predicted based on the low level of *ADH2* mRNA (Fig. 2A). This observation suggests that RNA Pol II is bound but that some step in transcription is still repressed, as it is in the Δ*hdac* mutant (Tachibana et al., 2007). In contrast, the tail deletion did not lead to a detectable increase in RNA Pol II binding at the *FBP1prm* in repressing conditions despite the observation of significant transcription. The reason for this difference might be partial stabilization of RNA Pol II binding or PIC formation because of the presence of Adr1 in the promoters of *ADH2* and *ADY2* but the absence of the major activator of *FBP1*, Cat8, at the *FBP1prm* in the tail mutant (Fig. 2B).

In summary, our results suggest that although Adr1 is bound to several promoters in repressing conditions in the tail mutants, it is not necessary to recruit RNA Pol II. Rather, transcription occurred independently of Adr1, Cat8, and Snf1. However, RNA Pol II at the

ADH2prm in repressing conditions appeared to be mostly inactive as indicated by the low level of transcription (Fig. 1A) compared to RNA Pol II occupancy in the H3-tail mutant (Fig. 2D), suggesting that some coactivator is absent or modification of the complex cannot occur in the presence of glucose, similar to the situation in an Δ*hdac* mutant (Tachibana et al., 2007). However, the Snf1-independent Adr1 binding in the tail mutants is in contrast with the situation in the Δ*hdac* mutant in which Adr1 binding is Snf1-dependent (Tachibana et al., 2007). This difference suggests that Snf1 has a role in Adr1 binding that is not essential when the tails are deleted. Another important difference is the extent of derepresion in the two situations. Derepression occurs normally in the Δ*hdac* strain but is severely restricted in the histone tail mutants. Thus, the tails have an important positive as well as negative role in Adr1-dependent gene regulation.

Snf1 and the histone tails affect different aspects of ADH2 repression

The low level of *ADH2* transcription in the histone tail mutants despite the presence of Adr1 and RNA pol II at the promoter suggests that a major step of glucose repression is still functional when either histone tail is missing. A major pathway of repression of Snf1-dependent genes is mediated by the essential protein phosphatase Glc7. The Glc7 phosphatase activity relevant to glucose repression can be eliminated by deleting Reg1, a non-essential targeting subunit. In the absence of Glc7 phosphatase activity, Snf1 is constitutively phosphorylated and active in the presence of glucose (Sanz *et al.*, 2000) although it may not be fully activated (Orlova et al., 2008). We deleted *REG1* in strains lacking the H3- or H4-tail to assess genetically the interaction of the tail mutants with Snf1. As expected (Dombek *et al.*, 1993), deleting *REG1* in a strain with intact H3 resulted in significant ADH II activity in repressing conditions as detected by *in situ* staining for ADH activity after electrophoresis of cell extracts (Fig. 3). The constitutive ADH II activity increased dramatically when the H3-tail was deleted in the Δ*reg1*mutant (Fig. 3), suggesting that the H3-tail has a role in repressing transcription that cannot be overcome by activation of Snf1. Synergistic relief from glucose repression when Snf1 was activated by deleting *REG1* in the absence of the H3 tail was confirmed by β-galactosidase assays using a *lacZ* reporter gene under the control of the *ADH2prm* (Fig. 3**, bottom**). Similar results were obtained when *ADH2* expression was measured by qPCR in strains with a *REG1* deletion in the presence and absence of the H3-tail (Table 2). The enhanced *ADH2* expression caused by deleting *REG1* in the Δ*h3t* mutant required Snf1 because the expression was reduced to that of the Δ*h3t* single mutant when *SNF1* was deleted (Table 2). These results are similar to those in the Δ*hdac* mutant where the Δ*reg1*Δ*hdac* combination synergistically activated *ADH2* transcription (Tachibana et al., 2007).

We also observed that *FBP1* expression was enhanced in repressing conditions when *REG1* was deleted (Table 2), suggesting that activation of Snf1 activates Cat8 which in turn activates *FBP1*. However, combining activation of Snf1 (by deleting *REG1*) with deletion of the H3-tail did not increase expression of *FBP1* suggesting that activation of Snf1 can fully overcome the restriction to transcriptional activation imposed by the presence of the H3-tail. The contrast between the effect of deleting *REG1* in the presence and absence of the H3 tail at these two promoters suggests that the histone tails may act differently at different promoters.

Phosphorylation of H3-Ser10 by Snf1 and subsequent K14 acetylation by Gcn5 has been proposed to relieve repression of *INO1* expression (Lo *et al.*, 2001). To test whether this mechanism could be involved in the activation of *ADH2* expression we measured ADHII activity and the activity of an *ADH2*/lacZ reporter gene in a strain in which the H3-S10A allele was the only copy of histone H3. The activity of *ADH2* was unaffected as determined by both assays (**data not shown**). We also combined a deletion of *REG1* with a histone H3-S10A allele to test the possibility that the S10A allele in the presence of active Snf1 might

allow Adr1 binding and transcriptional activation as in the Δ*hdac* mutant (Tachibana *et al.*, 2007). However, *ADH2* was expressed to the same extent in the Δ*reg1* mutants carrying either a wt-H3 or the H3-S10A mutant histone (Table 2) suggesting that Snf1 promotes transcriptional activation of *ADH2* independently of H3-Ser10 phosphorylation.

To test the possibility that the histone H4 tail contains a target of Snf1 we attempted to delete *REG1* in a strain with a Δ*h4t* allele. However, we were unable to delete *REG1* in this strain unless it also carried a plasmid with an intact H4 (*HHF1*) gene (Fig. 4 and **data not shown**). This result suggests that activating Snf1 in a strain lacking the H4-tail causes a severe growth defect. In support of this conclusion, we observed that the H4-tail mutant was defective for growth on glycerol, a carbon source that activates Snf1. The H3-tail mutant was not glycerol-negative (**data not shown**) and the slow-growth phenotype of a Δ*reg1*Δ*h3t* double mutant was cured by deleting Snf1 (Fig. 4). In contrast, the apparent growth defect of a Δ*reg1*Δ*h4t* double mutant was not cured by deleting *SNF1* (Fig. 4). These results indicate that the growth defect caused by deleting *REG1* in the H3-tail mutant acts through Snf1, but in the case of the H4-tail mutant Reg1 may have a Snf1-independent function.

Activator-independent remodeling of ADH2prm

A significant perturbation of the nucleosome architecture of the *ADH2prm* in the Δ*h3t* mutant might explain binding of Adr1 and RNA Pol II in repressing conditions, as well as the weak constitutive activation of transcription. The *ADH2prm* has three well-positioned nucleosomes as indicated by Micrococcal nuclease (MNase) mapping (Di Mauro *et al.*, 2002, Verdone *et al.*, 1996, Tachibana et al., 2007) (see Fig. 6A and Fig. S1 for a depiction of the *ADH2* promoter nucleosomes). The promoter nucleosomes are remodeled in an Adr1- and Cat8-dependent, but transcription-independent manner upon derepression (Verdone *et al.*, 1997, Biddick *et al.*, 2008b) including a small 3′-translation of the N(−1) TATA-box containing nucleosome (Di Mauro *et al.*, 2002, Verdone *et al.*, 1996, Tachibana *et al.*, 2007). A supercoiling assay (Simpson *et al.*, 1985) using the *ADH2prm* on a small episomal plasmid indicated that on average about one nucleosome is lost upon derepression in a wt strain and a similar loss occurred in a Δ*hdac* mutant in repressing conditions (Tachibana et al., 2007).

To address the influence of histone tails on the nucleosome density at the *ADH2prm* and to compare this situation with the Δ*hdac* mutation, we performed a supercoiling assay using DNA prepared from the Δ*h3t* mutant and its parent strain, each carrying an *ADH2prm*-containing minichromosome (Fig. 5A and B). There was a decrease in nucleosome density of the *ADH2prm*-containing minicircle upon derepression of the parent strain, albeit a smaller change than was seen in the strain studied previously (Fig. 5B **inset**). Reminiscent of the previous results with the Δ*hdac* mutant, the supercoiled plasmid isolated from the Δ*h3t* strain grown in repressing conditions had about the same nucleosome density as the plasmid from the wt strain after derepression, and even lower nucleosome density in derepressed conditions. Since Adr1 can bind in both the Δ*h3t* (Fig. 2) and Δ*hdac* mutants in repressing conditions (Verdone et al., 2002), we asked whether the change in nucleosome density in the Δ*hdac* strain was Adr1-dependent. In the strain with wt histones, the change in nucleosome density that accompanied derepression was Adr1-dependent (Fig. 5C and D). In contrast, the repressed Δ*adr1*Δ*hdac* strain had a lower nucleosome density in repressing conditions and further loss occurred upon derepression (Fig. 5E and F), suggesting that if the histone tails are hyperacetylated, Adr1 is not necessary to recruit activities that remodel the nucleosomes at the promoter. Thus, in both the Δ*h3t* and Δ*hdac* mutant nucleosome eviction or alteration apparently occurs without requiring an activator.

We also analyzed the *ADH2prm* using a nucleosome-scanning assay (NuSA) because both position and relative occupancy can be determined by this technique (Sekinger *et al.*, 2005).

We first used NuSA combined with ChIP (ChIP-NuSA) to analyze the histone composition of individual promoter nucleosomes (Sekinger et al., 2005). ChIP-NuSA for histones H3 (Fig. S2), H4, H2B, and the variant histone Htz1 confirmed that the N(−1), N(−2), and N(+1) nucleosomes contain these histones as expected (**data not shown**). When the *ADH2prm* in the Δ*h3t* and Δ*h4t* strains on glucose was analyzed, the results indicated that it was as remodeled in the tail mutants as in the derepressed parent strain (Fig. 6B) as judged by the decrease in relative nucleosome occupancy (decrease in resistance to MNase digestion) and altered positions of the N(−1) and N(−2) nucleosomes. As a control we performed a NuSA using a derepressed culture of a strain lacking both *ADR1* and *CAT8*. This NuSA pattern matched that of the wt strain grown in repressing conditions, confirming that the remodeling is transcription-factor-dependent when the histone tails are present. These results confirm those obtained with the supercoiling assay (Fig. 5) showing that when the H3 or H4 tail was deleted, complete remodeling was observed on glucose. In a Δ*reg1* strain the loss of protection of DNA bound by the nucleosomes in repressing conditions was intermediate between that observed in repressing and derepressing conditions in a wt strain (Fig. 6C). In particular the TATA-containing nucleosome N(−1) did not experience the same increase in accessibility in repressing conditions as was observed when the cells were derepressed. This might explain the inability of a *REG1* deletion to induce full derepression of *ADH2* on glucose (Table 2). These results suggest that additional derepression-dependent remodeling may be needed for full activation of the *ADH2prm*.

A small peak of MNase-resistant DNA is usually observed in the nucleosome-free (nfr) region between the N(−1) and N(−2) nucleosomes (Fig. 6B, and (Tachibana et al., 2007,Biddick et al., 2008)). The ChIP-NuSA results (Fig. S2 and **data not shown**) indicate that this DNA is protected by histones. Its MNase-resistance is intermediate between that of a nucleosome and free DNA and it appears to contain a shorter stretch of DNA than is contained within a nucleosome (**data not shown**). It could represent an altered nucleosome either alone or complexed with another protein as was recently reported for a nucleosome-RSC complex in the *GAL1,10* promoter (Floer *et al.*, 2010). Its presence is unaffected by deletion of the H3 and H4 tails.

Discussion

These results demonstrate the importance of the histone tails in maintaining a repressive state of chromatin at Adr1-dependent promoters. The similar phenotypes of the histone tail mutants and the Δ*hdac* mutant with regard to Adr1 binding and recruitment of a mostly inactive RNA pol II suggest that hyperacetylation of the histone tails and not increased acetylation of another protein most likely explains activator binding and PIC recruitment in the Δ*hdac* mutant.

Although the transcriptional activator Adr1 and RNA Pol II were able to gain access to glucose-repressed promoters when the histone tails were altered (Fig. 2), they were incapable of inducing the high level of transcription that is observed when glucose is absent from the media, showing that the mechanism of glucose repression operates at several levels including a step after RNA Pol II has been recruited (Tachibana *et al.*, 2007). When the H3 or H4 tail was deleted, the binding site occluded by the presence of nucleosome N(−1) apparently became accessible because Adr1 occupied the *ADH2prm* in the presence of glucose to the same extent that it binds in a derepressed wt strain (Fig. 2A and **data not shown**). However, although the chromatin state of the *ADH2prm* in the repressed Δ*h3t* and Δ*h4t* strains resembled that of a derepressed wt strain (Fig. 6B) and the Adr1 binding site appeared to be fully occupied (Fig. 2A), *ADH2* transcript levels reached only 1.5% of the level attained upon derepression (Fig. 1). Surprisingly, we found that this weak transcriptional activity was independent of Adr1, Cat8, and Snf1 (Fig. 2C). Dispensability of

transcriptional activators, and Adr1 in particular, has been observed when histone reassembly is altered by mutation of Spt6, a histone H3-H4 chaperone (Adkins & Tyler, 2006). Both defective reassembly, and loss of the H3 and H4 tails appears to lead to an altered chromatin state that allows activator-independent RNA Pol II binding and transcription. In the former case, a high level of transcription may be maintained after inactivating Spt6 because Adr1 was previously activated by derepression.

The permissive chromatin structure at the *ADH2prm* created by deletion of the H3 tail led to a transcriptional state that resembles the state of genes that are occupied by RNA Pol II but remain inactive (Zeitlinger *et al.*, 2007, Ivaldi *et al.*, 2007, Tachibana *et al.*, 2007). In these complexes RNA Pol II appears to be paused downstream of the start site. In the tail deletion mutants grown in repressing conditions, we observed Adr1-independent transcription at a lower level than would be predicted by the level of RNA Pol II bound to the promoter (Fig. 2D). The inability of Adr1 to more effectively activate transcription was not due solely to the absence of the histone tail because a high level of *ADH2* mRNA was observed in the Δ*h3t* mutant when glucose was removed from the media (Fig. 1B) and an *ADH2*/lacZ reporter showed a high level of Snf1-dependent derepression in the Δ*h3t* mutant strain (**data not shown**). In a Δ*hdac* mutant, both Adr1 and Cat8 are bound to the *ADH2prm*, have recruited a PIC consisting of coactivators and RNA Pol II, but are unable to activate transcription (Tachibana *et al.*, 2007). This poised and inactive PIC can be activated by activating Snf1, or by activating Adr1. Indeed, we found similarly that activation of Snf1 by deleting *REG1* could increase the weak constitutive expression of *ADH2* in the tail mutants (Table 2 and **data not shown**).

Glucose-resistant *ADH2* transcription is Snf1-independent in the histone H3 and H4 tail mutants (Fig. 2C), but Snf1-dependent in the Δ*hdac* mutant (Tachibana *et al.*, 2007), suggesting that there may be an important Snf1 target within the tails. Ser10 of Histone H3 seemed a likely possibility because Snf1 appears to act by phosphorylating H3-Ser10 to induce *INO1* expression (Lo *et al.*, 2001). However, the S10A mutation had no effect on *ADH2* regulation, apparently eliminating its phosphorylation by Snf1 as a prerequisite of Adr1 binding and transcription activation. *HIS3* expression is also dependent on Snf1 but, analogous to *ADH2* expression, the dependence on Snf1 does not involve phosphorylation of Ser10 on histone H3 (Liu *et al.*, 2005). Recent studies have not reproduced the dependence of *INO1* induction on Ser10 phosphorylation (Shirra *et al.*, 2005) so its importance, if any, is uncertain. An alternative site of Snf1 action in the histone tails is suggested by research demonstrating that Ser36 in the tail of mammalian H2B is phosphorylated by AMPK in response to stress and that this modification is related to gene activation (Bungard *et al.*, 2010). In yeast H2B Threonine is present at an analogous position but its phosphorylation status has not been reported and the importance of the H2B tail for expression of Snf1-dependent genes is unknown.

Some aspect of the histone H4 tail might play a role in growth on a non-fermentable carbon source because its deletion, but not deletion of the H3 tail, prevents growth on glycerol (**data not shown**). This role may involve the Glc7 phosphatase because deleting *REG1*, which activates Glc7, was lethal in the absence of the H4 tail, but not in the absence of the H3 tail. Surprisingly, deleting Snf1 did not rescue the inviability of the Δ*reg1*Δ*h4t* double mutant. Thus, Reg1 appears to have a role independent of Snf1 that might be related to chromatin. This role may involve histone gene expression because a histone gene transcription factor, Spt10, is synthetically lethal in the absence of Reg1 (Eriksson *et al.*, 2005, Hess & Winston, 2005).

The chromatin architecture of the *ADH2prm* is significantly altered in the histone tail deletion mutants and this may account for the binding of Adr1 and RNA Pol II. Similar

changes in chromatin architecture occur in the Δ*hdac* mutant and these changes were independent of Adr1. In both cases promoter nucleosomes have apparently been evicted. Since recruitment of the CRCs SAGA and Swi/Snf to the *ADH2prm* is normally dependent on Adr1 (Biddick et al., 2008b), these results suggest that significant perturbations in promoter architecture can occur without these chromatin modifying activities. Alternatively, in the absence of the histone tails the CRCs may be able to bind and alter the chromatin without activator-dependent recruitment. The increase in accessibility of DNA occluded by nucleosomes induced by the histone tail deletion may accomplish the role of the missing step in chromatin remodeling that normally occurs downstream of Snf1 activation in derepressing conditions. This would explain the synergistic effect on *ADH2* expression described in Table 2 and Fig. 3.

In summary we have shown that deletion of the H3 or H4 tail allows Adr1 and RNA Pol II to bind the promoters in repressing conditions when chromatin structure normally precludes such binding. Activator- and Snf1-independent transcription occurred at a low level in the absence of the H3 and H4 tails. However, the RNA Pol II bound at the *ADH2prm* appeared to be mostly inactive, suggesting that, as observed in a Δ*hdac* mutant, there is something missing from the PIC that is necessary for efficient transcription. Alternatively or in addition, incomplete chromatin remodeling might prevent complete activation. We conclude that when glucose is present, hypoacetylated histone H3 and H4 tails are essential to avoid Snf1-independent transcriptional noise and the unscheduled binding of activators. However, the inactive state of the Snf1 kinase prevents the formation of highly active transcriptional complexes on glucose even when chromatin is not a barrier for transcription. At the same time, Snf1-independent factors, or Snf1 activated to a higher level in the absence of glucose may be required to complete chromatin remodeling of the *ADH2prm*.

Experimental Procedures

Yeast strains, plasmids, and growth conditions

The *S. cerevisiae* strains used in all experiments with histone mutants were derived from MX1-4C, in which both chromosomal copies of the histone H3 and H4 genes have been deleted (Morgan *et al.*, 1991) and the only source of histones is a centromeric plasmid containing one copy of the histone H3 and H4 genes encoding either wt or mutant versions of the histones (Table 3). Other strains are derived from W303. The plasmids used in this study are listed in Table 3. Deletion and tagging of chromosomal genes used published methods and plasmids (Knop *et al.*, 1999, Guldener *et al.*, 1996). For glucose-repression, yeast strains were grown in YP media (1% Yeast extract, 2% Peptone) containing 5% glucose and kept under OD_{600}=1.0. Glucose-repression was maintained in synthetic media containing 5% glucose to maintain selection for plasmids (Sherman, 1991). Derepression medium was YP containing 0.05% glucose. Growth conditions on plates for plasmid selection or with 5-fluoro-orotic acid (FOA) were described elsewhere (Young et al., 2003, Voronkova *et al.*, 2006). All cultures were grown at 30 °C.

Chromatin Immunoprecipitation (ChIP) and mRNA quantification

ChIP was performed as described (Tachibana *et al.*, 2005). One mg of whole-cell extract was immunoprecipitated with 2 μg of F7-antihemagglutinin monoclonal antibody (Santa Cruz Biotechnology). Quantification of specific sequences in both input and immunoprecipitated (IP) DNA preparations was done by qPCR using Power SYBR Green master mix (Applied Biosystems) in a PTC-200 Thermocycler coupled to a Chromo 4 continuous fluorescence detector (MJ-Research). Opticon 3 software (MJ-Research) was used for the data analysis. Occupancy of a protein at the promoter is expressed as fold-increase of the IP/input ratio of the amount of the specific amplicon for the promoter

sequence over the IP/input ratio corresponding to the amplicon for the telomeric sequence TEL-VI-R. For the analysis of gene expression, total RNA samples were purified as described (Collart & Oliveiro, 1993) and DNAse I-treated with the Ambion DNAse free Kit. We used SSII-reverse transcriptase (Invitrogen) for the reverse-transcription of 1μg of RNA as described (Collart & Oliveiro, 1993). Quantification of mRNA levels was done by qPCR as described above and in (Tachibana *et al.*, 2007). The primers used for both ChIP and expression analyses are in Table S1.

In-gel ADH assays and β-galactosidase assays

In-gel ADH assays were performed by *in situ* staining after electrophoresis of whole cell extracts on non-denaturing polyacrylamide gels (Dombek et al., 1993). β-galactosidase assays were performed on permeabilized cells (Guarente, 1983). The *ADH2*/lacZ reporter plasmid was pBGM18 (Table 3; (Young *et al.*, 2000)). Activities are expressed in Miller units.

Nucleosome Scanning Assays (NuSA)

NuSA was performed as described in Tachibana (Tachibana *et al.*, 2007) using 200 ml cultures of cells grown in either repressing or derepressing medium for 4 hr or 6 hr for W303 cells or cells derived from MX1-4C, respectively. Fold-enrichment values were normalized to either total DNA, or amplicons from *CEN3* or *ACT1* as described in Biddick (Biddick *et al.*, 2008). The amplicon used for *ACT1* normalization was demonstrated to be within a region in the *ACT1* gene that was protected by nucleosomes during the MNase digestion (**data not shown**). No detectable difference was noted using the different procedures for normalization. The total DNA concentration was determined using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). When doing NuSA-ChIP assays, the MNase digestions were stopped by shifting the reactions to 4 °C and adding 18 μl of 0.5M EDTA and 7 μl of 200 mM EGTA. The samples were frozen at −70 °C until the next step. SDS was not added at this point since it would interfere with the antibody binding in subsequent steps. One 600 μl-aliquot from the MNase digestion step was used for each NuSA- ChIP. As outlined in the ChIP procedure referenced above protease inhibitors and PMSF were added to each 600μl sample. Appropriate amounts of stock solutions were added to make the final volume of each aliquot 800 μl in 1X of Buffer L (ChIP Buffer; 50mM Hepes, pH 7.5, 140mM NaCl, 1% triton X-100, 0.1M sodium deoxycholate and a mixture of protease inhibitors). The samples were sonicated (4X 20 sec pulses with low, constant power using a microprobe), centrifuged for 10min at 10000 xg, 4 °C in a table-top microfuge, and the supernatant was transferred to a new tube with PMSF. The antibody was added and incubated overnight at 4 °C with rocking. For histone immunoprecipitation, 5 μg of antibody per sample were used (H3: Upstate mAb 05-499; H2B: Upstate mAb 07-371; H4: Abcam ab 7311-200). For HA–immunoprecipitation, 10μl of HA antibody (#SC7392, Santa Cruz Biotechnology) were used. From this step on the ChIP procedure referenced above was used. The final ChIP samples were diluted approximately 20-fold in 1mM Tris pH 8.0. These diluted NuSA-ChIP samples were analyzed using the same method described for the NuSA samples.

Supercoiling assays

Supercoiling assays were performed as described in Tachibana (Tachibana *et al.*, 2007) using the pLLTY1 plasmid, carrying the −640 to +135 region of the *ADH2* gene. Repressing and derepressing media were the same as described above. Derepression was carried out for 4 hr (W303-derived strains) or for 6hr for cells derived from the MX1-4C strain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The research was supported by a grant from the General Medical Sciences Institute of the National Institutes of Health, GM26079 to ETY. J.J. Infante was the recipient of a postdoctoral fellowship from the Ministry of Science and Innovation of Spain. We thank R. Morse, S. Berger, and M. Grunstein for the generous provision of strains and plasmids.

Bibiography

Adkins MW, Tyler JK. Transcriptional activators are dispensable for transcription in the absence of Spt6-mediated chromatin reassembly of promoter regions. Mol Cell. 2006; 21:405–416. [PubMed: 16455495]

Agricola E, Verdone L, Di Mauro E, Caserta M. H4 acetylation does not replace H3 acetylation in chromatin remodelling and transcription activation of Adr1-dependent genes. Mol Microbiol. 2006; 62:1433–1446. [PubMed: 17121596]

Biddick R, Law G, Chin K, Young E. The transcriptional coactivators SAGA, SWI/SNF and mediator make distinct contributions to activation of glucose-repressed genes. J. Biol. Chem. 2008b

Biddick RK, Law GL, Young ET. Adr1 and Cat8 mediate coactivator recruitment and chromatin remodeling at glucose-regulated genes. Plos One. 2008; 3:e1436. [PubMed: 18197247]

Brower-Toland B, Wacker DA, Fulbright RM, Lis JT, Kraus WL, Wang MD. Specific contributions of histone tails and their acetylation to the mechanical stability of nucleosomes. J Mol Biol. 2005; 346:135–146. [PubMed: 15663933]

Bungard D, Fuerth BJ, Zeng PY, Faubert B, Maas NL, Viollet B, Carling D, Thompson CB, Jones RG, Berger SL. Signaling kinase AMPK activates stress-promoted transcription via histone H2B phosphorylation. Science. 2010; 329:1201–1205. [PubMed: 20647423]

Caserta M, Agricola E, Churcher M, Hiriart E, Verdone L, Di Mauro E, Travers A. A translational signature for nucleosome positioning in vivo. Nucleic Acids Res. 2009; 37:5309–5321. [PubMed: 19596807]

Collart, M.; Oliveiro, S. Current Protocols in Molecular Biology. In: Ausubel, F.; Brent, R.; Kingston, RT.; Moode, DD.; Seidman, JG.; Smith, JA.; Struhl, K., editors. Current Protocols in Molecular Biology. Greene/Wiley Interscience; New York: 1993.

Di Mauro E, Verdone L, Chiappini B, Caserta M. In vivo changes of nucleosome positioning in the pretranscription state. J Biol Chem. 2002; 277:7002–7009. [PubMed: 11748218]

Dombek KM, Camier S, Young ET. ADH2 expression is repressed by REG1 independently of mutations that alter the phosphorylation of the yeast transcription factor ADR1. Molecular And Cellular Biology. 1993; 13:4391–4399. [PubMed: 8321238]

Eriksson PR, Mendiratta G, Mclaughlin NB, Wolfsberg TG, Marino-Ramirez L, Pompa TA, Jainerin M, Landsman D, Shen CH, Clark DJ. Global regulation by the yeast Spt10 protein is mediated through chromatin structure and the histone upstream activating sequence elements. Molecular And Cellular Biology. 2005; 25:9127–9137. [PubMed: 16199888]

Floer M, Wang X, Prabhu V, Berrozpe G, Narayan S, Spagna D, Alvarez D, Kendall J, Krasnitz A, Stepansky A, Hicks J, Bryant GO, Ptashne M. A RSC/nucleosome complex determines chromatin architecture and facilitates activator binding. Cell. 2010; 141:407–418. [PubMed: 20434983]

Guarente L. Yeast promoters and lacZ fusions designed to study expression of cloned genes in yeast. 1983:181–191.

Guldener U, Heck S, Fielder T, Beinhauer J, Hegemann JH. A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res. 1996; 24:2519–2524. [PubMed: 8692690]

Hardie DG, Carling D, Carlson M. The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Annu Rev Biochem. 1998; 67:821–855. [PubMed: 9759505]

Hess D, Winston F. Evidence that Spt10 and Spt21 of Saccharomyces cerevisiae play distinct roles in vivo and functionally interact with MCB-binding factor, SCB-binding factor and Snf1. Genetics. 2005; 170:87–94. [PubMed: 15744051]

Hong SP, Carlson M. Regulation of snf1 protein kinase in response to environmental stress. J Biol Chem. 2007; 282:16838–16845. [PubMed: 17438333]

Ivaldi MS, Karam CS, Corces VG. Phosphorylation of histone H3 at Ser10 facilitates RNA polymerase II release from promoter-proximal pausing in Drosophila. Genes Dev. 2007; 21:2818–2831. [PubMed: 17942706]

Jenuwein T, Allis CD. Translating the histone code. Science. 2001; 293:1074–1080. [PubMed: 11498575]

Kacherovsky N, Tachibana C, Amos E, Fox D 3rd, Young ET. Promoter binding by the Adr1 transcriptional activator may be regulated by phosphorylation in the DNA-binding region. Plos One. 2008; 3:e3213. [PubMed: 18791642]

Knop M, Siegers K, Pereira G, Zachariae W, Winsor B, Nasmyth K, Schiebel E. Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. Yeast. 1999; 15:963–972. [PubMed: 10407276]

Liu Y, Xu X, Singh-Rodriguez S, Zhao Y, Kuo MH. Histone H3 Ser10 phosphorylation-independent function of Snf1 and Reg1 proteins rescues a gcn5-Mutant in HIS3 expression. Molecular And Cellular Biology. 2005; 25:10566–10579. [PubMed: 16287868]

Lo WS, Duggan L, Emre NC, Belotserkovskya R, Lane WS, Shiekhattar R, Berger SL. Snf1--A histone kinase that works in concert with the histone acetyltransferase Gcn5 to regulate transcription. Science. 2001; 293:1142–1146. [PubMed: 11498592]

Millar CB, Grunstein M. Genome-wide patterns of histone modifications in yeast. Nat Rev Mol Cell Biol. 2006; 7:657–666. [PubMed: 16912715]

Morgan BA, Mittman BA, Smith MM. The highly conserved N-terminal domains of histones H3 and H4 are required for normal cell cycle progression. Molecular And Cellular Biology. 1991; 11:4111–4120. [PubMed: 2072911]

Orlova M, Barrett L, Kuchin S. Detection of endogenous Snf1 and its activation state: application to Saccharomyces and Candida species. Yeast. 2008; 25:745–754. [PubMed: 18949820]

Parua PK, Ratnakumar S, Braun KA, Dombek KM, Arms E, Ryan PM, Young ET. 14-3-3 (Bmh) proteins inhibit transcription activation by Adr1 through direct binding to its regulatory domain. Molecular And Cellular Biology. 2010; 30:5273–5283. [PubMed: 20855531]

Ratnakumar S, Kacherovsky N, Arms E, Young ET. Snf1 controls the activity of adr1 through dephosphorylation of Ser230. Genetics. 2009; 182:735–745. [PubMed: 19398770]

Ratnakumar S, Young ET. Snf1 dependence of peroxisomal gene expression is mediated by Adr1. J Biol Chem. 2010; 285:10703–10714. [PubMed: 20139423]

Rubenstein EM, Mccartney RR, Zhang C, Shokat KM, Shirra MK, Arndt KM, Schmidt MC. Access denied: Snf1 activation loop phosphorylation is controlled by availability of the phosphorylated threonine 210 to the PP1 phosphatase. J Biol Chem. 2008; 283:222–230. [PubMed: 17991748]

Sabet N, Tong F, Madigan JP, Volo S, Smith MM, Morse RH. Global and specific transcriptional repression by the histone H3 amino terminus in yeast. Proc Natl Acad Sci U S A. 2003; 100:4084–4089. [PubMed: 12649325]

Saha A, Wittmeyer J, Cairns BR. Chromatin remodelling: the industrial revolution of DNA around histones. Nat Rev Mol Cell Biol. 2006; 7:437–447. [PubMed: 16723979]

Sanz P, Alms GR, Haystead TA, Carlson M. Regulatory interactions between the Reg1-Glc7 protein phosphatase and the Snf1 protein kinase. Molecular And Cellular Biology. 2000; 20:1321–1328. [PubMed: 10648618]

Schuller HJ. Transcriptional control of nonfermentative metabolism in the yeast Saccharomyces cerevisiae. Curr Genet. 2003; 43:139–160. [PubMed: 12715202]

Segal E, Fondufe-Mittendorf Y, Chen L, Thastrom A, Field Y, Moore IK, Wang JP, Widom J. A genomic code for nucleosome positioning. Nature. 2006; 442:772–778. [PubMed: 16862119]

Sekinger EA, Moqtaderi Z, Struhl K. Intrinsic histone-DNA interactions and low nucleosome density are important for preferential accessibility of promoter regions in yeast. Mol Cell. 2005; 18:735–748. [PubMed: 15949447]

Sherman F. Getting started with yeast. Methods Enzymol. 1991; 194:3–21. [PubMed: 2005794]

Shirra MK, Rogers SE, Alexander DE, Arndt KM. The Snf1 protein kinase and Sit4 protein phosphatase have opposing functions in regulating TATA-binding protein association with the Saccharomyces cerevisiae INO1 promoter. Genetics. 2005; 169:1957–1972. [PubMed: 15716495]

Simpson RT, Thoma F, Brubaker JM. Chromatin reconstituted from tandemly repeated cloned DNA fragments and core histones: a model system for study of higher order structure. Cell. 1985; 42:799–808. [PubMed: 2996776]

Tachibana C, Biddick R, Law GL, Young ET. A poised initiation complex is activated by SNF1. J Biol Chem. 2007; 282:37308–37315. [PubMed: 17974563]

Tachibana C, Yoo JY, Tagne JB, Kacherovsky N, Lee TI, Young ET. Combined global localization analysis and transcriptome data identify genes that are directly coregulated by Adr1 and Cat8. Molecular And Cellular Biology. 2005; 25:2138–2146. [PubMed: 15743812]

Travers A, Caserta M, Churcher M, Hiriart E, Di Mauro E. Nucleosome positioning--what do we really know? Mol Biosyst. 2009; 5:1582–1592. [PubMed: 19795076]

Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell. 2000; 101:249–258. [PubMed: 10847680]

Verdone L, Camilloni G, Di Mauro E, Caserta M. Chromatin remodeling during saccharomyces cerevisiae ADH2 gene activation. Molecular And Cellular Biology. 1996; 16:1978–1988. [PubMed: 8628264]

Verdone L, Cesari F, Denis CL, Di Mauro E, Caserta M. Factors affecting Saccharomyces cerevisiae ADH2 chromatin remodeling and transcription. J Biol Chem. 1997; 272:30828–30834. [PubMed: 9388226]

Verdone L, Wu J, Van Riper K, Kacherovsky N, Vogelauer M, Young ET, Grunstein M, Di Mauro E, Caserta M. Hyperacetylation of chromatin at the ADH2 promoter allows Adr1 to bind in repressed conditions. Embo J. 2002; 21:1101–1111. [PubMed: 11867538]

Voronkova V, Kacherovsky N, Tachibana C, Yu D, Young ET. Snf1-dependent and Snf1-independent pathways of constitutive ADH2 expression in Saccharomyces cerevisiae. Genetics. 2006; 172:2123–2138. [PubMed: 16415371]

Wu J, Grunstein M. 25 years after the nucleosome model: chromatin modifications. Trends Biochem Sci. 2000; 25:619–623. [PubMed: 11116189]

Yang Z, Zheng C, Hayes JJ. The core histone tail domains contribute to sequence-dependent nucleosome positioning. J Biol Chem. 2007; 282:7930–7938. [PubMed: 17234628]

Young ET, Dombek KM, Tachibana C, Ideker T. Multiple pathways are co-regulated by the protein kinase Snf1 and the transcription factors Adr1 and Cat8. J Biol Chem. 2003; 278:26146–26158. [PubMed: 12676948]

Young ET, Kacherovsky N, Van Riper K. Snf1 protein kinase regulates Adr1 binding to chromatin but not transcription activation. J Biol Chem. 2002; 277:38095–38103. [PubMed: 12167649]

Young ET, Sloan J, Miller B, Li N, Van Riper K, Dombek KM. Evolution of a glucose-regulated ADH gene in the genus Saccharomyces. Gene. 2000; 245:299–309. [PubMed: 10717481]

Zeitlinger J, Stark A, Kellis M, Hong JW, Nechaev S, Adelman K, Levine M, Young RA. RNA polymerase stalling at developmental control genes in the Drosophila melanogaster embryo. Nat Genet. 2007; 39:1512–1516. [PubMed: 17994019]

Zheng C, Hayes JJ. Structures and interactions of the core histone tail domains. Biopolymers. 2003; 68:539–546. [PubMed: 12666178]

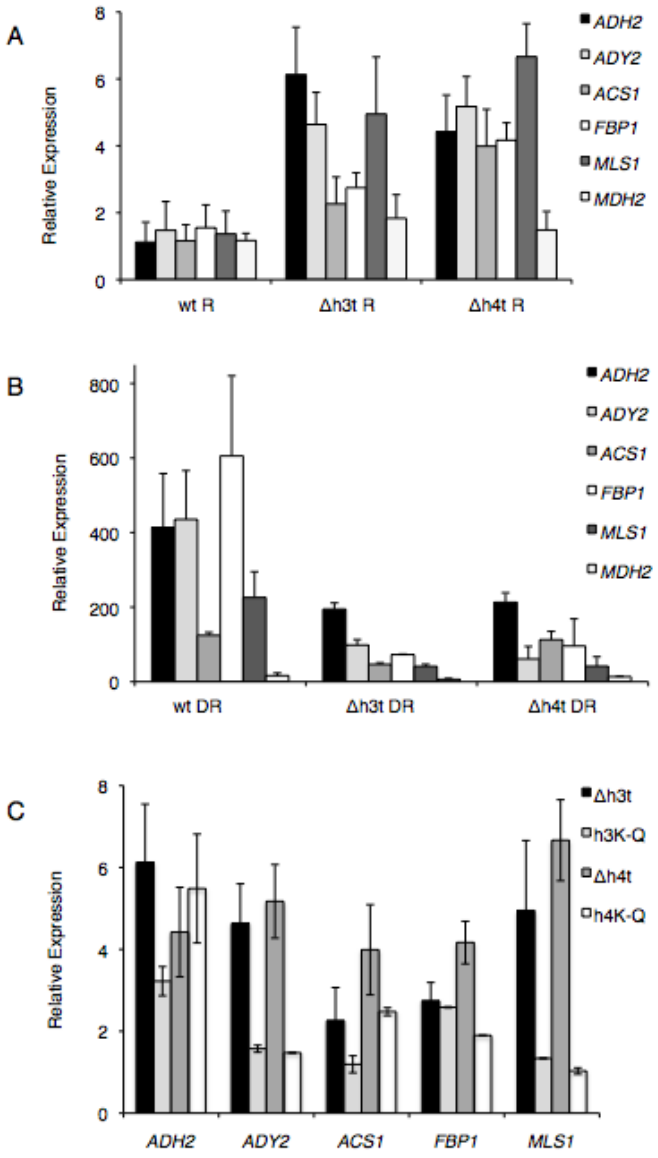

Figure 1. Histone tail deletions alter transcriptional regulation of Adr1- and Cat8 -dependent genes

mRNA levels were determined by qPCR, normalized to *ACT1* mRNA levels and expressed as fold-increase over wt, repressed. A. Repressed growth conditions. The dotted line represents the level of wt expression (1.0). B. Derepressed growth conditions. Error bars represent sd of three biological replicates. Wt, Δ*h3t* and Δ*h4t* indicate wt H3-H4 and deletions of amino acids 4-30 in H3 and amino acids 4-28 in H4, carried on plasmids in strains JJY200, JJY430, and JJY428, respectively. **C**. Repressed mRNA levels measured in strains deleted for the H3-H4 genes and carrying plasmids for expression of histone tails in which all of the lysines have been replaced by glutamines in H3 and H4 (plasmids pRM200 HHT2KtoQ-HHF2 and pRM200 HHT2-HHF2KtoQ, respectively). The dotted line represents the level of wt expression (1.0).

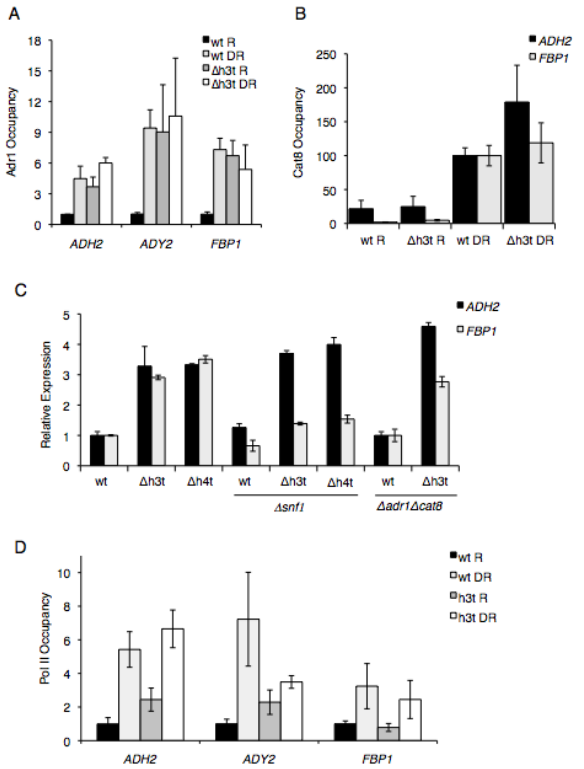

Figure 2. Binding and activity of transcription components in histone tail mutants

A. ChIP for Adr1-HA at the *ADH2*, *ADY2* and *FBP1* promoters in wt or Δ*h3t* strains (JJY200A-HA and JJY430A-HA, respectively; Table 3) grown in repressing (R) or derepressing conditions for 6h (DR). **B.** ChIP for Cat8-TAP-HA at *ADH2prm* and *FBP1prm* in wt or Δ*h3t* strains (LLTY79 and LLTY80, respectively; Table 3) grown in repressed or derepressed conditions for 6h. **C**. *ADH2* and *FBP1* mRNA levels in wt and tail mutants (Δ*h3t* and Δ*h4t*) in combination with either Δ*snf1* or Δ*adr1*Δ*cat8* (see Table 3 for strains). Cells were grown in repressing conditions and mRNA levels were determined as in Fig. 1. **D**. ChIP for Rpb1 at *ADH2*, *ADY2* and *FBP1* promoters. Cells were grown in repressing conditions. Error bars represent the sd of three biological replicates in each panel.

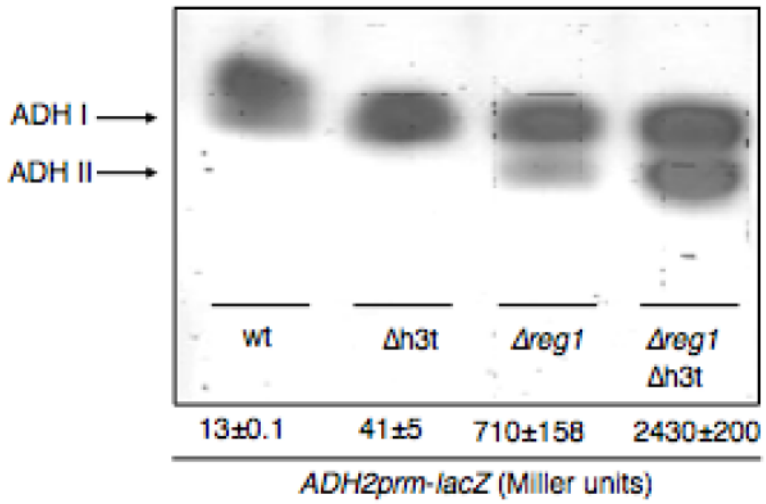

Figure 3. Synergistic effect of Δ*h3t* and Δ*reg1* deletions on the derepression of *ADH2*

In-gel ADH assay of extracts from yeast cells growing in repressing synthetic medium lacking uracil. ADHI, glucose-induced ADH isozyme derived from *ADH1*; ADHII, glucose-repressed ADH isozyme derived from *ADH2*. Indicated at the bottom of each lane □□□□□□β-galactosidase activities expressed in Miller units (average and sd of three biological replicates) of a *lacZ* reporter gene driven by the *ADH2* promoter on plasmid pBGM18 carried in each strain. Strains and plasmids are listed in Table 3**.**

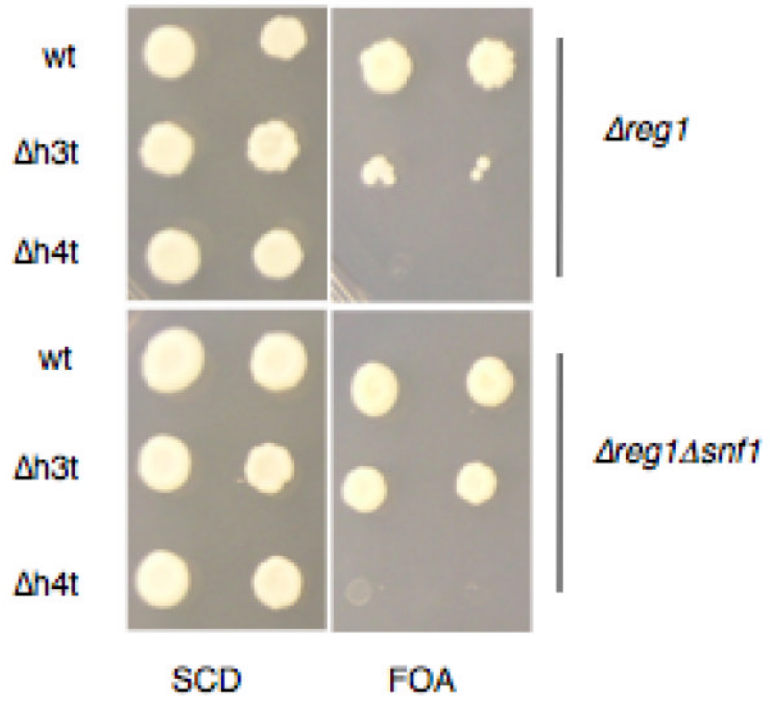

Figure 4. Interaction between activation of Snf1 on glucose and deletion of the H3- and H4-N terminal tails

Yeast cells with the indicated genotypes (see Table 3 for strains) were grown for ten generations in complete repressing medium containing uracil and then plated at various dilutions on either synthetic medium with glucose (SCD) or the same medium with FOA in order to test their ability to lose the *URA3*-plasmid carrying wt-histones (plasmid pMS329). The 10X or 1X dilutions are shown. The higher dilutions showed that approximately the same number of cells were plated from each strain.

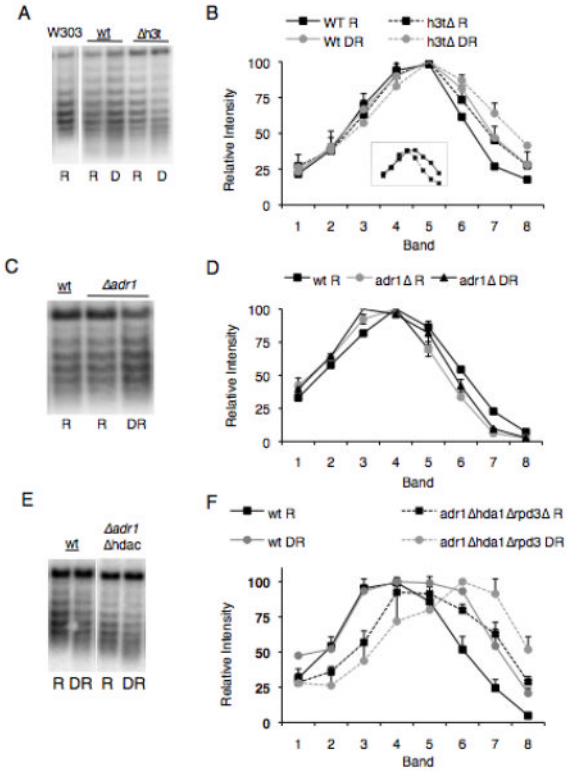

Figure 5. Loss of the histone H3 tail is sufficient for chromatin remodeling on glucose at the *ADH2prm*

Supercoiling analysis of a plasmid with the *ADH2* promoter (pLLTY1) was performed as described in Experimental Procedures. **A.** Samples from W303-CH1a (LLYT4), MX1-4C (wt; JJY200) strain or Δ*h3t* (JJY430) strain were repressed (R) or derepressed for 6h (DR). **B.** Quantitation of supercoiling assays in a gel similar to that shown in A. The topoisomer distributions were quantified using phosphorimaging. The data are presented as the relative intensity for each of eight bands. Error bars represent the s.d. of two independent experiments in this panel and in panels D and F. A shift to the right represents a decrease in overall nucleosome density. The inset shows the results from the W303 strain, repressed (R, dashed line) and derepressed for 4h (DR, solid line). **C, D.** Samples from wt (LLTY4) or Δ*adr1 (*LLTY6*)* grown in either repressing (R) or depressing conditions for 4h (DR). **E, F.** Samples from wt (W303; LLTY4) or Δ*adr1*Δ*hdac (*LLTY111*)* grown in either repressing (R) or depressing conditions for 6h (DR).

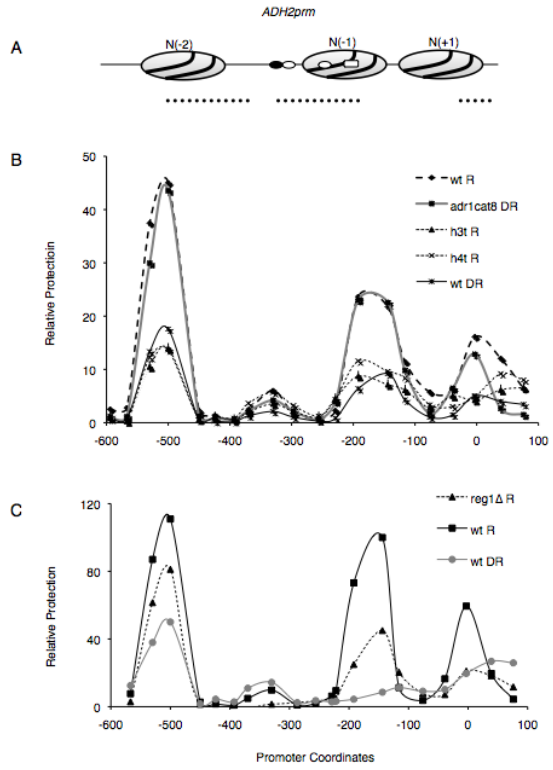

Figure 6. Chromatin architecture of the *ADH2prm* and the effect of histone-tail deletions and activation of Snf1 in repressing conditions

A. The location of *ADH2prm* nucleosomes in repressed cells. The white box indicates the location of the TATA box; white circles, Adr1 binding sites; black dot, Cat8 binding site. The dashes show the predicted positions of nucleosomes based exclusively on DNA sequence (Segal *et al.*, 2006). **B**. Nucleosome scanning assays of MX1-4C (wt histones) in repressing and derepressing conditions compared to the Δ*h3t* and Δ*h4t* strains in repressing conditions. A NuSA of a strain containing deletions of *ADR1* and *CAT8* is shown for comparison. **C.** Nucleosome scanning assays of Δ*reg1* in repressing conditions compared to its wt parent W303 in repressing and derepressing conditions. Relative protection was calculated by normalization to a *CEN3* amplicon and then to the wt repressed control sample (see Experimental Procedures for details). Two-to-five biological replicates were performed for each strain. The average standard deviation was less than 25%. See Fig. S1 for a detailed description of the sequence of the promoter and the nucleosome locations.

Table 1

Genes whose expression was affected more than two-fold by deletion of the histone tails in microarray analysis

Subset (number of genes)	up-regulated genes		down-regulated genes	
	Δh3t	*Δh4t*	*Δh3t*	*Δh4t*
Whole genome	590 (10%)	273 (5%)	47 (0.8%)	387 (6%)
Adr1-dependent (105)[a]	50 (48%)	26 (25%)	1 (1%)	6 (6%)
Cat8-dependent (247)[a]	61 (25%)	38 (15%)	10 (4%)	15 (6%)
Snf1-dependent (430)[a]	153 (36%)	111 (26%)	16 (4%)	22 (5%)
Adr1 direct targets (28)[b]	18 (64%)	5 (18%)	0	0
Cat8 direct targets (23)[b]	14 (61%)	1 (4%)	2 (9%)	2 (9%)
Adr1 & Cat8 direct targets (14)[b]	9 (64%)	2 (14%)	0	1 (7%)
Unfolded protein response (169)[c]	28 (17%)	29 (17%)	5 (3%)	3 (2%)

[a] The list of genes for the different subsets was obtained from (Young et al., 2003).

[b] The list of genes for the different subsets was obtained from (Tachibana et al., 2005).

[c] The list of genes for the different subsets was obtained from (Travers et al., 2000).

Table 2

Effect of combining REG1 and histone tail deletions on expression of Snf1-dependent genes

Relevant genotype[1]			Expression[2]	
REG1	***SNF1***	**Histones**	***ADH2***	***FBP1***
+	+	wt	1.0	1.0
−	+	wt	15	37
−	+	Δ *h3t*	23	29
−	+	*H3-S10A*	16	24
−	−	wt	1.3	1.1
−	−	Δ *h3t*	3.7	2.4

[1] Strains are listed in Table 3. Wt is JJY200; Δ*reg1* is JJY200r ; Δ*reg1* Δ*h3t* is JJY430r; Δ*reg1 H3-S10A* is JJYS10Ar; Δ*reg1* Δ*snf1* is JJY200rs; Δ*reg1* Δ*snf1* Δ*h3t* is JJY430rs.

[2] Yeast cultures were grown in repressing-rich medium (YPD). *ADH2* and *FBP1* mRNA levels were determined by qPCR, normalized to *ACT1*, and are expressed relative to expression of the same gene in wt cells grown in YPD. Values are the average of three biological replicates and the standard deviation was under 25% of the average.

Table 3

Strains and plasmids

Strain	Description
W303-CH1a	*MAT**a** ade2-1 can1-100 ura3-1 leu2-3,112 trp1-1 his3-11,115*
MX1-4C	*MAT**a** ura3-52 leu2-3,112 trp1-289 his31* Δ*(hhf1-hht1)*Δ*(hhf2-hht2)* pMS329 (Sabet et al., 2003)
CKY11-2 (Δ*reg1*)	W303-CH1a Δ*reg1::NAT1*
JJY200 (wt)	*MAT**a** ura3-52 leu2-3,112 trp1-289 his31* Δ*(hhf1-hht1)*Δ*(hhf2-hht2*pRM200) (Sabet et al., 2003)
JJY430 (Δ*h3t*)	*MAT**a** ura3-52 leu2-3,112 trp1-289 his31* Δ*(hhf1-hht1)*Δ*(hhf2-hht2*pRM430 (Sabet et al., 2003)
JJY428 (Δ*h4t*)	*MAT**a** ura3-52 leu2-3,112 trp1-289 his31* Δ*(hhf1-hht1)*Δ*(hhf2-hht2)* pGF29 (*hhf2*Δ*4-28)* (Sabet et al., 2003)
JJY200Hs	JJY200 Δ*snf1::ura3::HIS3*
JJY430Hs (Δ*h3t*)	JJY430 Δ*snf1::ura3::HIS3*
JJY428Hs (Δ*h4t*)	JJY428 Δ*snf1::ura3::HIS3*
JJY200ac	JJY200 Δ*adr1::NAT1,* Δ*cat8::KanMX*
JJY430ac	JJY430 Δ*adr1::NAT1,* Δ*cat8::KanMX*
JJY200r	JJY200 Δ*reg1::KanMX*
JJY430r	JJY430 Δ*reg1::KanMX*
JJYS10Ar	MX1-4C Δ*reg1::KanMX* pS10A
JJY200rs	JJY200 Δ*reg1::kanMx*Δ*snf1::NatMX*
JJY430rs	JJY430 Δ*reg1::kanMx,* Δ*snf1:: NatMX*
JJY428rs	MX1-4C Δ*reg1::kanMx,* Δ*snf1:: NatMX* pGF29 (*hhf2*Δ*4-28)*
JJY200A-HA	JJY200 *ADR1-3HA-KanMX*
JJY430A-HA	JJY430 *ADR1-3HA-KanMX*
JJY428A-HA	JJY428 *ADR1-3HA-KanMX*
JJY200 pMS329	MX1-4C pRM200
JJY430 pMS329	MX1-4C pRM430
JJY428 pMS329	MX1-4C pGF29 (*hhf2*Δ*4-28)*
LLTY4	W303-CH1a (pLLTY1,*TRP1*)
LLTY6	W303-CH1a *adr1::LEU2,*(pLLTY1, *TRP1*)
LLTY79	JJY200 *CAT8-3HA::KanMX*
LLTY80	JJY430 *CAT8-3HA::KanMX*
LLTY111	W303-CH1a Δ*rpd3::LEU2* Δ*hda::NatMX adr1::KanMX* (pLLTY1, *TRP1*) (aka Δ*hdac*) (Tachibana et al., 2007)
LLTY122	MX1-4C (pLLTY1,TRP)
LLTY124	*MAT**a*** ura3-52 *leu2-3,112 trp1-289 his3-11,115*Δ(hhf1-hht1)Δ(hhf2-hht2)(pLLTY21-*HIS3*) (pLLTY1,*TRP1*)

Plasmid	Description
pMS329	*CEN4, ARS1, URA3, HHT1-HHF1* (Sabet et al., 2003)
pRM200	*CEN4, ARS1, TRP1, HHT2-HHF2* (Sabet et al., 2003)
pRM430	*CEN4, ARS1, TRP1, hht2*Δ*4-30-HHF2* (Sabet et al., 2003)
pGF29	*CEN4, ARS1, TRP1, (HHT2-hhf2* Δ *4-28)*
pS10A	*CEN4, ARS1, TRP1, hht2 (H3 S10A)-HHF2* (Lo et al., 2001)
pBGM18	pRS316-based, *URA3 CEN4 ADH2* (from codon 109)-*lacZ* (Young et al., 2000)

Plasmid	Description
pLLTY21	*CEN4, ARS1, URA3, hht2* Δ *4-30-HHF2*
pNS460	CEN4, *ARS1, TRP1,* hht1K4,9,14,18,23,27Q-*HHF1* (Sabet et al., 2003)
pRM491	CEN4, *ARS1, TRP1*-HHT1-hhf1K5,8,12,16Q (Sabet et al., 2003)