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Global Role for Chromatin Remodeling Enzymes in Mitotic Gene Expression

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Summary

Regulation of eukaryotic gene expression requires ATP-dependent chromatin remodeling enzymes, such as SWI/SNF, and histone acetyltransferases, such as Gcn5p. Here we show that SWI/SNF remodeling controls recruitment of Gcn5p HAT activity to many genes in late mitosis and that these chromatin remodeling enzymes play a role in regulating mitotic exit. In contrast, interphase expression of *GAL1*, *HIS3*, *PHO5*, and *PHO8* is accompanied by SWI/SNF-independent recruitment of Gcn5p HAT activity. Surprisingly, prearresting cells in late mitosis imposes a requirement for SWI/SNF in recruiting Gcn5p HAT activity to the *GAL1* promoter, and *GAL1* expression also becomes dependent on both chromatin remodeling enzymes. We propose that SWI/SNF and Gcn5p are globally required for mitotic gene expression due to the condensed state of mitotic chromatin.

Introduction

The transcriptional machinery in eukaryotes faces a number of challenges in gaining access to target promoters. Not only must it contend with the 30–100 nm chromatin fibers prevalent in the interphase nucleus, but it must also be able to access a subset of genes that must be transcribed in the context of more highly condensed mitotic chromatin. In the budding yeast *Saccharomyces cerevisiae*, nearly 300 genes have been identified whose expression peaks during mitosis (Spellman et al., 1998). Furthermore, regulation of the mitosis to G1 cell cycle transition requires expression of a group of gene products in early telophase. This set of mitotically expressed genes includes *SIC1*, which encodes a CDK inhibitor that contributes to the deactivation of mitotic CDK/B cyclin kinase activity (Knapp et al., 1996). Mitotic expression of other gene products, such as Egt2p (Kovacech et al., 1996), promotes cytokinesis, and telophase expression of Cdc6p controls the assembly of prereplication complexes for the subsequent S phase (Piatti et al., 1995).

A key regulator of telophase gene expression and the efficiency of mitotic exit is the zinc finger protein, Swi5p (Toyn et al., 1997). Swi5p is expressed in the G2 phase of the yeast cell cycle (Nasmyth et al., 1990), where it is retained in the cytoplasm as a result of phosphorylation of its nuclear localization signal (NLS) (Moll et al., 1991). After dephosphorylation in late anaphase, Swi5p enters the nucleus and activates the expression of a number of genes in early telophase, including *SIC1* (Knapp et al., 1996), *CDC6* (Piatti et al., 1995), *EGT2* (Kovacech et al., 1996), *ASH1* (Bobola et al., 1996), *RME1* (Toone et al., 1995), *PCL2*, and *PCL9* (Nasmyth et al., 1990; Aerne et al., 1998). Swi5p also binds to the *HO* promoter during late mitosis, although *HO* is not expressed until late G1 (Cosma et al., 1999).

Ace2p is a zinc finger protein that is highly homologous to Swi5p and that regulates transcription of many of the same genes. These two proteins share 83% identity between their zinc finger domains, and they recognize similar binding sites in vitro (Dohrmann et al., 1992). Ace2p is also subject to similar cell cycle regulation as Swi5p, both in terms of expression during G2 and regulated nuclear localization during late anaphase (Dohrmann et al., 1992; O'Conallain et al., 1999). Despite these similarities, Swi5p and Ace2p play distinct roles in mitotic gene expression. Whereas Swi5p activates target genes such as *SIC1* and *PCL9* immediately after entering the nucleus in late anaphase, Ace2p is inactive during mitosis and can activate expression of these same genes only during early G1 (Aerne et al., 1998; McBride et al., 1999).

Two classes of highly conserved chromatin remodeling enzymes have been identified that play a major role in the regulation of transcription in eukaryotes. The first class includes enzymes that covalently modify the nucleosomal histones (acetylation, phosphorylation, methylation, and ADP-ribosylation; reviewed by Strahl and Allis, 2000), and the second class is composed of multisubunit complexes that use the energy of ATP hydrolysis to disrupt histone–DNA interactions (reviewed in Kingston and Narlikar, 1999; Vignali et al., 2000). The founding members of each class—SWI/SNF for the ATP-dependent remodeling enzymes and Gcn5p-containing complexes for the HATs—were originally characterized in yeast. Subsequently, multiple members of both classes have been identified and studied in *Drosophila*, mammals, and recently in *C. elegans*. In mammalian cells, hSWI/SNF is required for the functioning of heat shock factor (de La Serna et al., 2000), steroid receptors (Fryer and Archer, 1998), and for proper cell cycle control (Zhang et al., 2000). In yeast, SWI/SNF and Gcn5p control expression of a subset of highly inducible genes, and recent DNA microarray studies of asynchronous *swi/snf* or *gcn5* cells have indicated that expression of ~3% of yeast genes appear to depend on one or the other of these activities (Holstege et al., 1998; Sudarsanam et al., 2000).

Recently, two studies have shown that Swi5p potentiates transcription of the *HO* gene by sequentially recruiting chromatin remodeling enzymes during late mitosis (Cosma et al., 1999; Krebs et al., 1999). When Swi5p binds in late anaphase to two sites within the far upstream regulatory region of the *HO* gene, it rapidly triggers the recruitment of the SWI/SNF chromatin remodeling complex. The prior recruitment of SWI/SNF is required for subsequent telophase recruitment of a GCN5/ADA2 histone acetyltransferase complex that acetylates nucleosomes within a ~1 kb domain of the *HO* upstream region. This sequence of events is required

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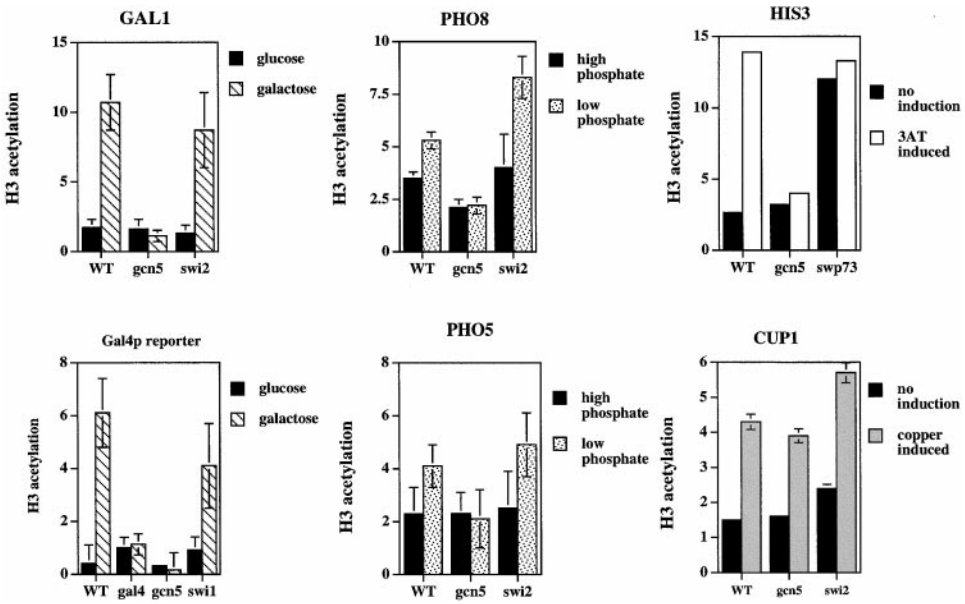

Figure 1. Histone H3 Acetylation Surrounding Diverse, Inducible Promoters Is Independent of SWI/SNF

Asynchronous yeast cultures were grown in the indicated noninducing or inducing media, and cells were processed for chromatin immunoprecipitation (ChIP) using antibodies that recognize histone H3 acetylated at positions 9 and 14. DNA obtained from either the input or immunoprecipitated material was applied to slot blots, and these blots were hybridized with probes specific to the TATA region of the indicated genes. Slot blots were quantified by phosphorimager, and the results are expressed as a ratio of the bound to the input material. Black bars indicate the level of H3 acetylation under noninducing conditions; hatched bars indicate induced levels of H3 acetylation. The Gal4p reporter is integrated at the *URA3* locus; it contains two low-affinity, nucleosomal Gal4p binding sites upstream of a *GAL1-lacZ* fusion gene (Burns and Peterson, 1997). Note that deletion of *SWI1* or *SWP73* disrupts assembly of SWI/SNF (Peterson et al., 1994; unpublished data). In the case of *HIS3*, a single experiment was performed with the strains indicated; two additional, independent experiments with a *swi2* strain yielded similar results. For other genes, results shown are representative of three independent experiments for each inducing condition.

for the subsequent DNA binding of the SBF transcription factor, which then activates *HO* expression at the G1/S boundary. The role of Swi5p in *HO* expression seems to be limited to the recruitment of chromatin remodeling enzymes since Swi5p is degraded prior to SBF recruitment and *HO* transcription (Cosma et al., 1999).

Here we have investigated whether the SWI/SNF-dependent recruitment of GCN5-dependent HAT complexes is specific to *HO* regulation or if it reflects a more general relationship between these two enzymes. We use chromatin immunoprecipitations to monitor histone H3 acetylation at inducible genes whose expression is either dependent (*HIS3*, *PHO8*) or independent (*GAL1*, *PHO5*, *CUP1*) of SWI/SNF and Gcn5p function. We also investigate cell cycle–regulated histone H3 acetylation at a group of genes activated by Swi5p in early telophase. In the case of the inducible genes, GCN5-dependent acetylation is always independent of SWI/SNF activity; in contrast, recruitment of Gcn5p to a set of genes that are expressed in late mitosis requires SWI/SNF remodeling activity. The SWI/SNF-dependent recruitment of Gcn5p activity during mitosis is not simply a function of Swi5p, as we show that recruitment of Gcn5p HAT activity to the *GAL1* promoter is also SWI/SNF dependent in mitosis. As is the case for the Swi5p activator at the *HO* locus, SWI/SNF and Gcn5p regulate *GAL1* expression during late mitosis by governing steps subsequent to Gal4p binding. We propose that SWI/SNF and Gcn5p are essential to allow activation of genes in mitotic chromatin.

Results

SWI/SNF-Independent Recruitment of Gcn5p HAT Activity

Activation of the *HO* gene depends on a specific order of events, beginning with targeting of SWI/SNF by the Swi5p activator. This targeting of SWI/SNF is in turn required for subsequent acetylation by Gcn5p, which is followed by binding and activation by the SBF activator (Cosma et al., 1999; Krebs et al., 1999). We wished to know whether SWI/SNF activity is universally required for subsequent acetylation by Gcn5p. First, we decided to examine the *GCN5*-dependent acetylation of a variety of inducible promoters in vivo. We chose promoters whose function is known to require SWI/SNF and Gcn5p (*PHO8*, Gregory et al., 1999; *HIS3*, Filetici et al., 1998; Kuo et al., 1998; Natarajan et al., 1999; and a Gal4p reporter gene, Burns and Peterson, 1997; Biggar and Crabtree, 1999) as well as promoters that function independently of both remodeling enzymes (*GAL1*, *CUP1*, and *PHO5*, Burns and Peterson, 1997; Gaudreau et al., 1997; Gregory et al., 1998). Wild-type (WT), *swi/snf*, or *gcn5* strains were grown to mid–log phase under induced or uninduced conditions, and then cells were harvested for RNA analysis and for formaldehyde cross-linking and chromatin immunoprecipitation (ChIP). In these ChIP studies, we used antibodies directed against histone H3 acetylated at lysines 9 and 14, which provides a measurement of Gcn5p activity (Kuo et al., 1996; Krebs et al., 1999). The levels of histone H3 acetylation for these target promoters are shown in Figure 1.

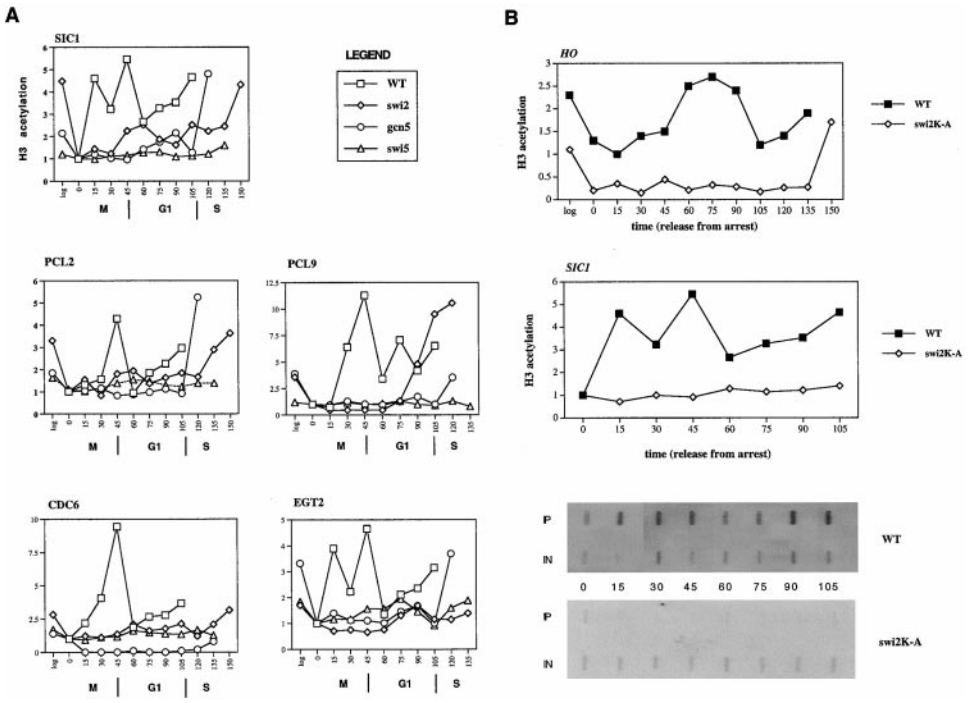

Figure 2. Cell Cycle–Regulated Acetylation at Swi5p-Regulated Genes

(A) WT (CY727), *swi2* (CY725), *gcn5* (CY724), and *swi5* (CY728) strains were synchronized with nocodazole and samples were fixed for chromatin IP after synchronous release. ChIPs were performed with the antibody to diacetylated H3. In these synchronous cultures, G1 cells (unbudded cells) appeared at 60 min, and S phase cells (small budded cells) appeared at 105–120 min. Blots were hybridized with probes for the TATA regions of each of the indicated genes. Results shown are representative of three independent ChIP time courses.

(B) ChIP analysis of histone H3 acetylation at the *HO* and *SIC1* promoters in a yeast strain (CY397) that contains an assembly competent, ATPase-defective allele of Swi2p (*swi2K798A*). Nocodazole arrest, release, and ChIP analysis were performed as in (A). Raw slot blot data for the *SIC1* gene is shown at bottom. IN, input DNA; IP, immunoprecipitated DNA.

For all six genes tested, transcriptional induction was accompanied by a large increase in histone H3 acetylation (Figure 1). Furthermore, with the exception of the *CUP1* gene (see below), these increased levels of H3 acetylation required *GCN5*. However, in contrast to what we observed previously for the *HO* gene, GCN5-dependent H3 acetylation at *GAL1*, *PHO5*, *PHO8*, *HIS3*, and the Gal4p reporter gene did not require an intact SWI/SNF complex. For example, when cells were grown in the presence of 2% galactose (left panels), we observed *GCN5*-dependent acetylation at both the endogenous *GAL1* promoter (upper left panel) and at an integrated Gal4p reporter that contains two low-affinity Gal4p binding sites upstream of a *GAL1-lacZ* fusion gene (Burns and Peterson, 1997; lower left panel). Expression of the *GAL1* gene does not require either SWI/SNF or Gcn5p, whereas transcription from the *GAL1-lacZ* reporter requires both remodeling enzymes (Burns and Peterson, 1997; Biggar and Crabtree, 1999; and data not shown). However, in neither case was SWI/SNF required for recruitment of GCN5-dependent HAT activity. Likewise, expression of *HIS3* (upper right panel) and *PHO8* (upper center panel) require both SWI/SNF and Gcn5p (Gregory et al., 1999; Natarajan et al., 1999; and data not shown), but in both cases recruitment of Gcn5p HAT activity was independent of an intact SWI/SNF complex. These results illustrate that increases in histone acetylation do not always contribute to transcriptional induction (*GAL1*, *PHO5*) and that in other cases high levels of histone acetylation are not sufficient to disrupt chromatin-mediated repression that must be further alleviated by SWI/SNF action (*GAL1* reporter, *HIS3*, *PHO8*).

Not all inducible promoters showed GCN5-dependent acetylation upon induction. When cells were exposed to 1 mM copper sulfate, we observed increased histone H3 acetylation at the *CUP1* promoter (lower right panel). However, this increase was independent of *GCN5*, indicating that a different HAT is responsible for acetylation of nucleosomes at this promoter. *CUP1* acetylation was also independent of SWI/SNF, and *CUP1* expression was robustly induced in both the *swi/snf* and *gcn5* mutants (data not shown). Thus, for many inducible genes, SWI/SNF and Gcn5p appear to serve distinct and independent functions.

GCN5-Dependent H3 Acetylation during Late Mitosis Requires SWI/SNF

SWI5 is required for the expression of a number of genes in late mitosis, including *SIC1* (Knapp et al., 1996), *CDC6* (Piatti et al., 1995), *EGT2* (Kovacech et al., 1996), *ASH1* (Bobola et al., 1996), *PCL2*, and *PCL9* (Aerne et al., 1998). To examine acetylation events at Swi5p-regulated promoters at different points in the cell cycle, we synchronized wild-type, *swi5*, *swi/snf*, and *gcn5* cells in G2/M with nocodazole, and then we washed out the nocodazole to allow the cells to progress through a synchronous cell cycle. Cell aliquots were taken every 15 min following release from nocodazole arrest, and these samples

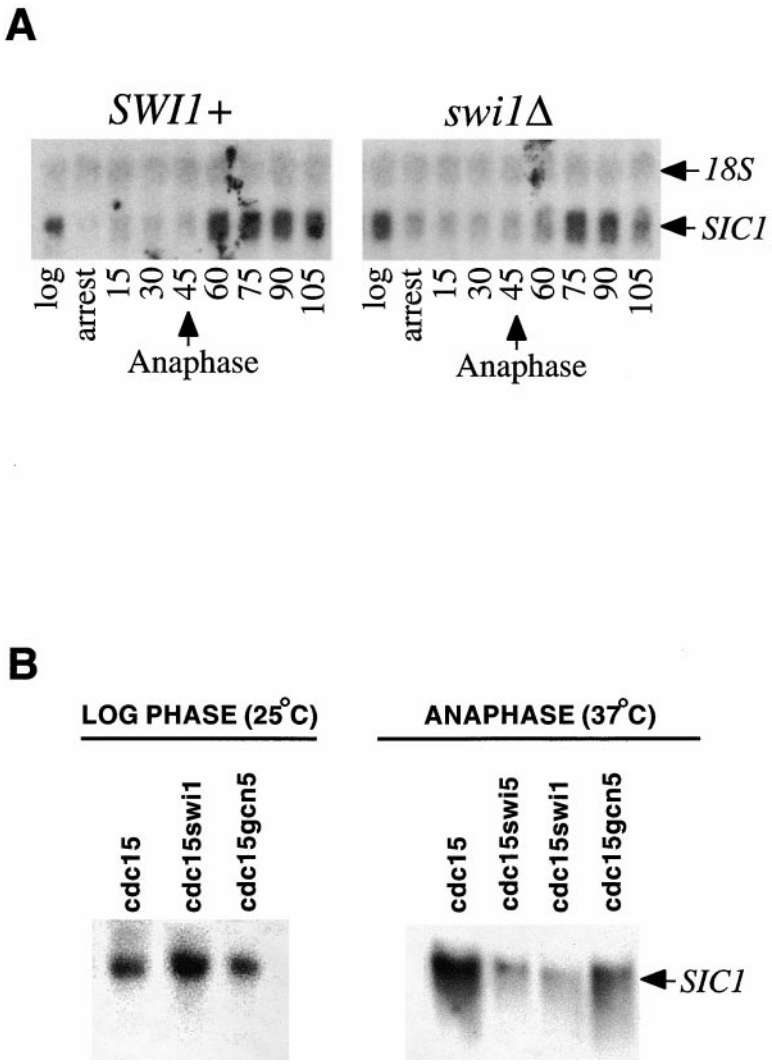

Figure 3. *SIC1* Expression during Mitosis Requires SWI/SNF and Gcn5p

(A) *SIC1* expression is delayed in a *swi/snf* mutant. Northern blot showing *SIC1* expression in synchronized wild-type and *swi/snf* strains. Cultures were synchronized by nocodazole arrest and release, and the number of cells with divided chromatin was determined by DAPI staining as previously described (Aerne et al., 1998). At least 200 cells were observed for each sample, and the onset of anaphase is defined by the first time point in which greater than 50% of the cells showed divided chromatin. Data shown is representative of two independent synchrony experiments.

(B) Northern blot showing *SIC1* expression in *cdc15*, *cdc15 swi5*, *cdc15 swi1*, and *cdc15 gcn5* strains. The left panel shows expression of *SIC1* in these strains at the permissive temperature (25°C); the right panel shows expression of *SIC1* in cells arrested in anaphase by a shift to the restrictive temperature (37°C). Blots were also probed for actin that demonstrated that similar levels of RNA were loaded per lane (data not shown). Data shown is representative of two independent experiments.

were analyzed for position in the cell cycle (by budding pattern), transcription (by Northern analysis), and histone H3 acetylation (by chromatin immunoprecipitation), as described previously (Krebs et al., 1999). ChIPs were performed using antibodies against histone H3 acetylated at lysines 9 and 14, and the levels of H3 acetylation surrounding the promoters of *SIC1*, *CDC6*, *PCL2*, *PCL9*, and *EGT2* are shown in Figure 2A.

For every Swi5p-regulated gene tested, there is a peak of *GCN5*-dependent acetylation during late mitosis (Figure 2A, square symbols, wild type; circles, *gcn5* cells). The timing of acetylation for each gene is consistent with the timing of its expression, as acetylation occurs at or just prior to activation of transcription in late anaphase (Aerne et al., 1998; see also Figure 3A). We also examined the acetylation of these promoters in *swi5* cells (triangles), and as expected, the peak of acetylation is lost in the absence of the activator. Finally, we measured the acetylation at these promoters in a *swi2* mutant (diamonds). As is the case for *HO*, cell cycle–regulated acetylation at *SIC1*, *PCL2*, *PCL9*, *CDC6*, *EGT2*, and *ASH1* is dependent on an intact SWI/SNF complex (Figure 2A and data not shown).

The results presented in Figure 2A show that an intact SWI/SNF complex is required for cell cycle–dependent acetylation by Gcn5p at multiple Swi5p-regulated promoters. However, these studies do not indicate whether SWI/SNF merely acts as a bridge to target Gcn5p to the promoter or whether recruitment of a Gcn5p HAT complex requires the ATP-dependent chromatin remodeling activity of SWI/SNF. We addressed this question by monitoring recruitment of Gcn5p HAT activity to the *HO* and *SIC1* genes in a strain harboring a mutation in the catalytic subunit of SWI/SNF, Swi2p. This point mutation (swi2K798A) abolishes ATPase activity but does not disrupt SWI/SNF assembly (Cote et al., 1994). Wild-type and *swi2K798A* cells were synchronized by nocodazole arrest and release, and samples were processed for ChIP analysis using the antibody to diacetylated histone H3 (Figure 2B). In the wild-type strain, we observe a peak of telophase acetylation at *SIC1* (Figure 2B, lower panel); in contrast, this cell cycle–regulated acetylation is absent in the *swi2K798A* strain, similar to what we had observed previously for a *swi2* deletion strain. Similar results were also obtained for early G1 acetylation at *HO* (Figure 2B, upper panel). These results confirm that recruitment of GCN5-dependent HAT activity requires prior chromatin remodeling events by the SWI/SNF complex.

Next, we wished to determine whether SWI/SNF and

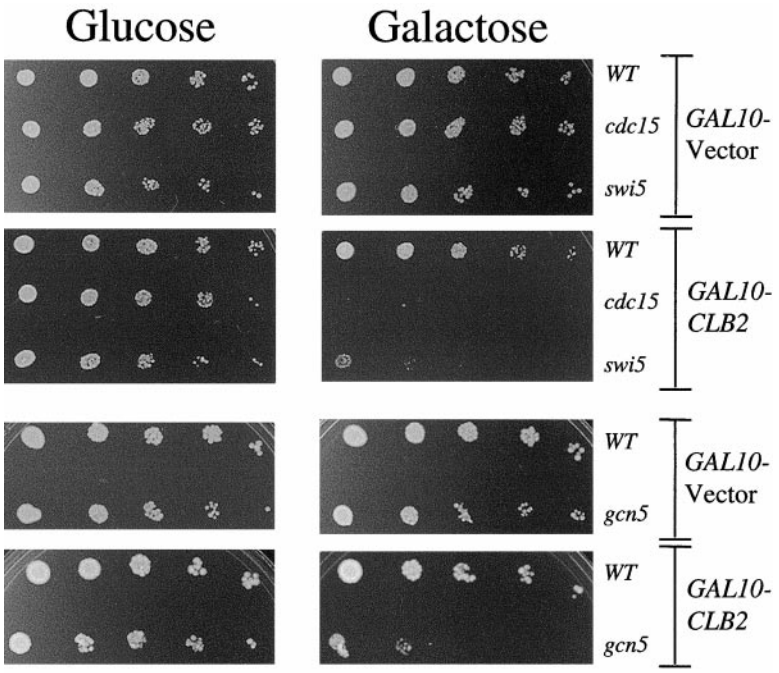

Figure 4. *swi5*Δ and *gcn5*Δ Strains Are Hypersensitive to *CLB2* Overexpression

Isogenic wild-type (WT) and mutant strains were transformed with either a *GAL10-CLB2* or empty *GAL10* expression plasmid. Transformants were grown to mid–log phase in minimal medium/2% glucose, serially diluted 5-fold, spotted onto minimal plates containing either 2% glucose or 2% galactose, and incubated for 2–3 days at 30°C. At least two transformants were tested for each strain.

Gcn5p are required for mitotic expression of Swi5p-regulated genes. Previous studies have shown that Swi5p is required for mitotic expression of *SIC1* but that Ace2p drives *SIC1* expression in early G1 (Aerne et al., 1998). Thus, in a *swi5* mutant, *SIC1* expression is only slightly decreased in RNA isolated from asynchronous cultures, and the peak of expression is delayed about 15 min in RNA isolated from synchronized cells. To examine the cell cycle timing of *SIC1* expression in *swi/snf* and wild-type strains, RNA was prepared from cells synchronized by nocodazole arrest/release and *SIC1* transcripts were measured by Northern blotting (Figure 3A). Whereas the peak of *SIC1* expression in wild-type cells occurred just after the initial appearance of divided chromatin (anaphase), expression was delayed an additional 15 min in the *swi1* mutant (Figure 3A). Thus, in the absence of an intact SWI/SNF complex *SIC1* expression is delayed, and the extent of delay is similar to what has been observed due to loss of Swi5p (Aerne et al., 1998).

The delayed expression of *SIC1* in a *swi1* mutant suggests that an intact SWI/SNF complex might only be required for mitotic expression and that Ace2p-dependent transcription of *SIC1* during G1 might be SWI/SNF independent. To further test this idea, we generated a set of double mutants with a temperature-sensitive *cdc15* mutation to arrest *swi1*, *gcn5*, or *swi5* mutants in mitosis. Figure 3B shows the levels of *SIC1* expression by Northern blot in strains grown at the permissive temperature (left panel) or in strains arrested in anaphase at the restrictive temperature (right panel). In asynchronous cells, *SIC1* levels are unaffected by *swi5*, *gcn5* or *swi/snf* mutations (Figure 3B and data not shown). At the restrictive temperature, *cdc15*^{ts} cells arrest in early anaphase, and although this arrest point is prior to the point of maximal *SIC1* activation, there is sufficient *SIC1* expression to detect by Northern blot (Figure 3B). However, *SIC1* expression is reproducibly decreased in the *gcn5 cdc15* double mutant and nearly eliminated in the *swi5 cdc15* and *swi1 cdc15* double mutants after mitotic arrest. These results indicate that SWI/SNF and Gcn5p are required for expression of *SIC1* during mitosis but not for expression of *SIC1* during G1.

Cells Lacking Gcn5p Exhibit Mitotic Exit Phenotypes

Previous studies have shown that *swi5* mutants exhibit mitotic exit phenotypes, such as hypersensitivity to overexpression of the B cyclin *CLB2* (Toyn et al., 1997). These phenotypes are due in part by the failure of *swi5* cells to express the Sic1p cdk inhibitor during mitosis. Since SWI/SNF and Gcn5p are also required for late mitotic gene expression, we predicted that *swi/snf* or *gcn5* strains might also exhibit mitotic exit phenotypes. To this end, we tested the sensitivity of our *swi5* and *gcn5* strains to overexpression of Clb2p. Strains were transformed with either a *GAL-CLB2* plasmid or the corresponding empty vector. Cell dilutions were then plated on both glucose and galactose plates, and the results of these growth studies are shown in Figure 4. Consistent with previous studies, *cdc15*^{ts} and *swi5* cells were hypersensitive to Clb2p overexpression, as indicated by their inability to grow on galactose media when they contained the *GAL-CLB2* plasmid (Figure 4). *gcn5* mutants were also sensitive to *CLB2* overexpression, confirming that loss of Gcn5p also interferes with the M to G1 transition (Figure 4). This result is consistent with the recent observation by Roth and colleagues demonstrating that asynchronous cultures of *gcn5* mutants have an increased proportion of cells with G2/M DNA content (Zhang et al., 1998). Unfortunately, we were unable to test our *swi/snf* strains for hypersensitivity to Clb2p overexpression since *swi/snf* mutants grow slowly on galactose. However, since *SIC1* expression in mitosis is more severely compromised in *swi/snf* as compared to *gcn5* cells (Figure 3B), it seems very likely *swi/snf* cells are also hindered for mitotic exit.

The Mitotic Activity Domain of Swi5p Maps to a Gcn5p Recruitment Element

Although *SWI5* and *ACE2* encode similar zinc finger proteins and they both enter the nucleus at the same time

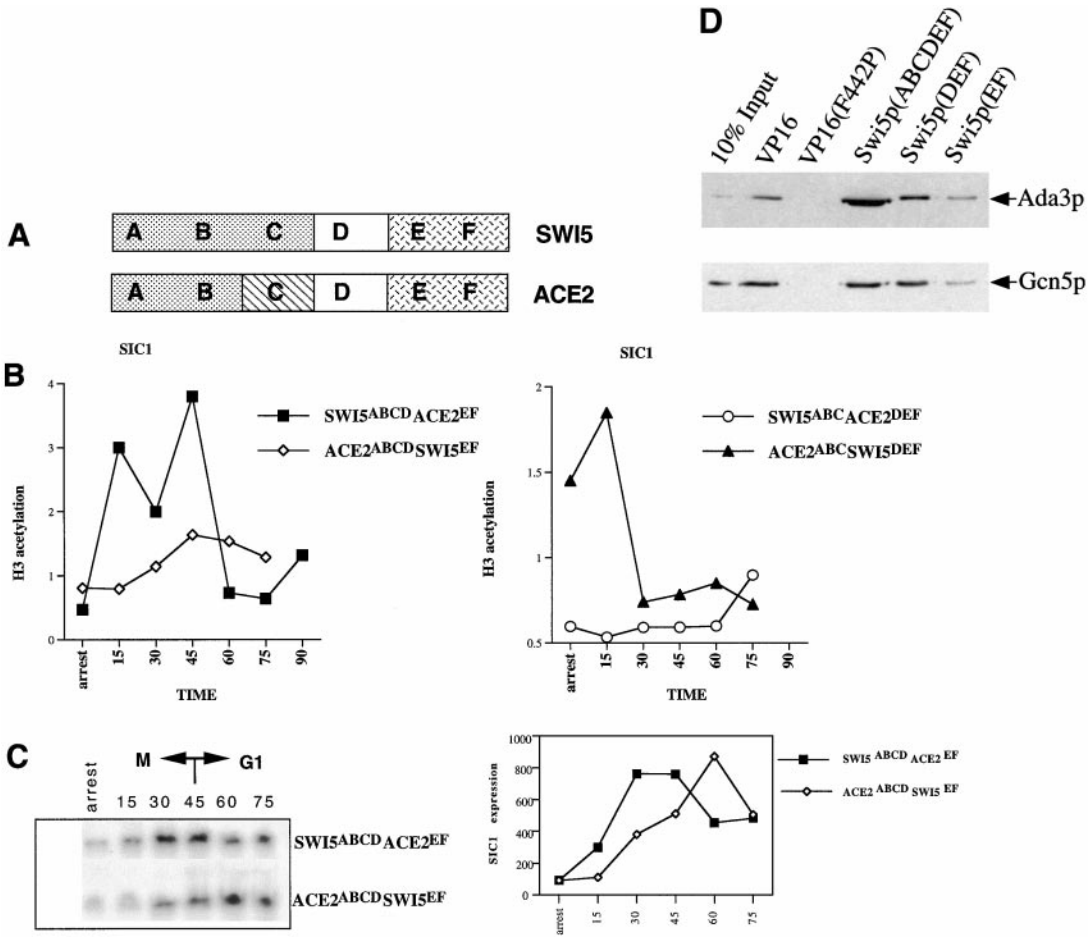

Figure 5. Identification of a Domain of Swi5 Required for Correct Timing of *SIC1* Acetylation and Expression

(A) Schematic of Swi5p and the related activator Ace2p. Region E contains the zinc finger DNA binding domain, region F contains the nuclear localization sequences. Region C of Ace2p is required for Ace2p-specific gene expression (*CTS1*), while region D of Swi5p is required for specific activation of *HO* and *SIC1* (McBride et al., 1999).

(B) ChIP analysis of cell cycle–regulated acetylation at the *SIC1* promoter in *swi5 ace2* strains that express Swi5p/Ace2p hybrids. Cells were synchronized by nocodazole arrest and release. Filled symbols denote hybrids that contain the Swi5 D region; open symbols denote hybrids that lack this region. In each time course, appearance of unbudded cells (G1) occurred between 45 and 60 min. These ChIP analyses are representative of two independent experiments.

(C) Northern analysis of *SIC1* expression in the time course experiments shown in the left graph of (B). The approximate time of the M to G1 transition is indicated, as measured by the appearance of large unbudded cells in the synchronized populations. Quantitation of the Northern (using hybridization to actin mRNA as an internal control, data not shown) is shown on the right.

(D) Efficient in vitro binding of SAGA HAT complex requires domain D of Swi5p. GST pull-downs were performed using partially purified SAGA complex, and the indicated GST fusions bound to glutathione-sepharose beads. After binding, the beads were washed with low salt buffer and eluted with buffer containing 1% SDS, and eluates (50%) were analyzed for SAGA by Western blot with antibodies directed against Gcn5p and Ada3p. Similar results were observed in three independent experiments.

in late anaphase, only Swi5p can activate transcription of target genes in late mitosis. Given our results shown in Figure 2, we considered the possibility that the failure of Ace2p to function in mitosis might be linked to an inability to recruit chromatin remodeling enzymes. In this model, Ace2p can function in early G1 in the absence of chromatin remodeling activities since chromatin is globally decondensed at the M/G1 boundary. To investigate this possibility, we took advantage of a series of yeast strains that express various Swi5p/Ace2p hybrid activators as the only source of Swi5p/Ace2p (Figure 5A; McBride et al., 1999). Figure 5A shows a schematic of Swi5p and Ace2p in which each protein is divided into six domains, A–F. Domains E and F contain the zinc finger DNA binding domains and the nuclear localization signals, both of which are highly conserved between Swi5p and Ace2p (83% identity between E regions, 48% identity between F regions). Previous studies have shown that region C of Ace2p confers the ability to activate the *ACE2*-regulated gene, *CTS1*, whereas hybrid activators that contain the D region of Swi5p are more potent for activation of *SIC1* and *HO* in logarithmically growing cells. One possibility is that region D of Swi5p might be the domain involved in targeting chromatin remodeling enzymes during late mitosis. To test this possibility, we first examined both the acetylation and expression of *SIC1* in the presence of different hybrid activators.

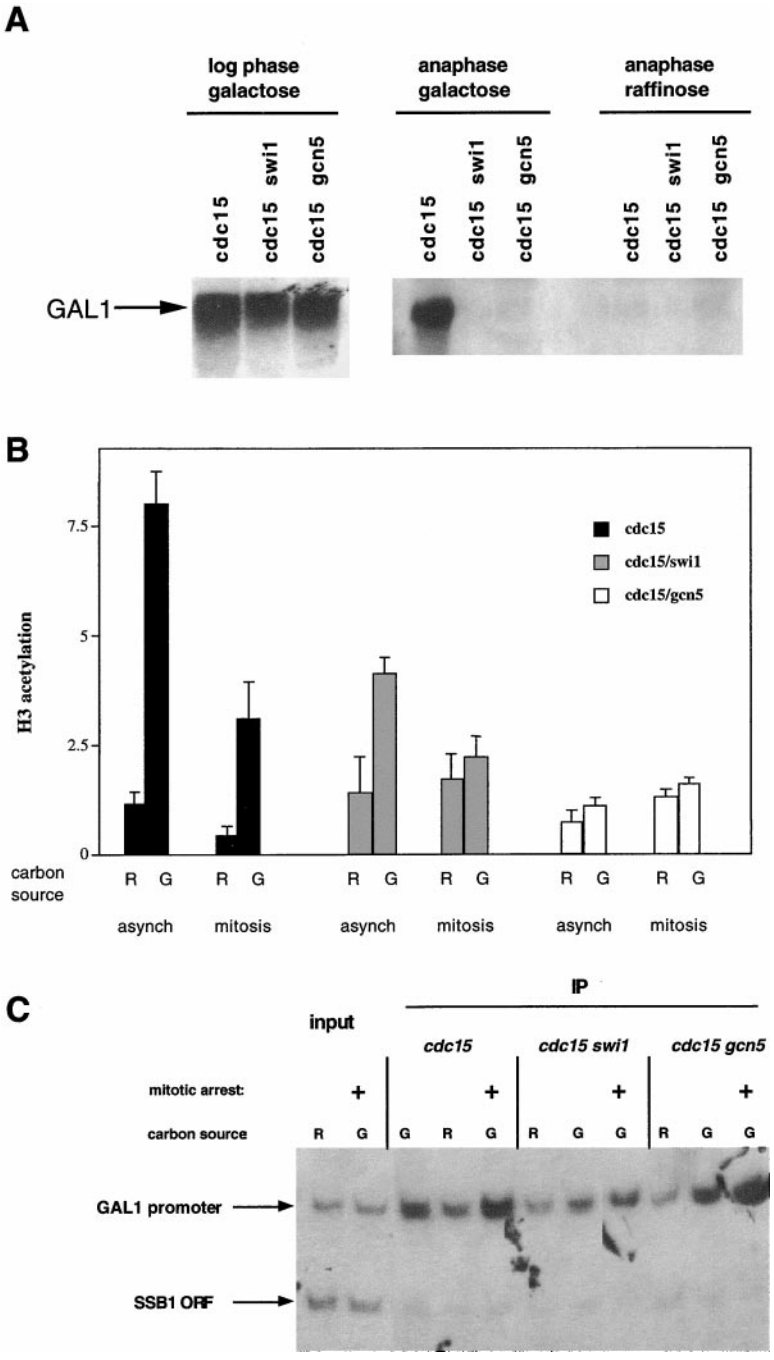

Figure 6. *GAL1* Expression and Promoter Proximal Histone Acetylation Is SWI/SNF- and GCN5-Dependent in Mitosis

(A) Northern analysis of *GAL1* induction in *cdc15*, *cdc15 swi1*, and *cdc15 gcn5* strains. Strains were grown in YEP/2% raffinose at room temperature. The left-hand panel shows the level of *GAL1* expression in strains after 2% galactose was added and cells incubated for an additional 60 min at room temperature. The far right panel shows the uninduced levels (raffinose) of *GAL1* in cells arrested in anaphase after a shift to 37°C. In the center panel, strains were shifted to 37°C to arrest cells in anaphase, and then 2% galactose was added for 60 min. These blots were subsequently reprobed for *ACT1* mRNA to ensure comparable loading (data not shown). Similar results were observed in three independent experiments.

(B) ChIP analysis of histone H3 acetylation at the *GAL1* promoter in asynchronous or mitotic cultures. Cultures from the identical aliquots as shown in (A) were processed for ChIP with antibodies to diacetylated H3. Black bars indicate acetylation levels in the *cdc15* strain, gray bars indicate acetylation levels in the *cdc15 swi1* strain, and white bars represent the *cdc15 gcn5* strain. R, raffinose; G, galactose. Similar results were observed in three independent experiments.

(C) ChIP analysis of Gal4p binding at the *GAL1* promoter in asynchronous and mitotic cultures. Cultures indicated in (A) were processed for ChIP with a polyclonal antibody to Gal4p. Input and immunoprecipitated DNAs were analyzed by quantitative PCR using primer sets for the *GAL1* promoter and the *SSB1* coding region. Note that Gal4p binds to the *GAL1* UAS in raffinose and galactose media in all strains and in both asynchronous and mitotic cultures. Data shown is representative of two independent experiments.

We performed nocodazole arrest/release time courses in *swi5 ace2* strains containing various Swi5p/Ace2p hybrids (Figure 5B). In each pair of hybrids, only the hybrid containing the Swi5p D domain (filled symbols in each panel) shows high levels of *SIC1* acetylation (Figure 5B) and expression (Figure 5C) during late mitosis. For example, *SIC1* expression in a strain containing the $Swi5^{ABCD}Ace2^{EF}$ hybrid peaks during late mitosis, while *SIC1* expression in strains containing the $Ace2^{ABCD}$ $Swi5^{EF}$ hybrid peaks 15–30 min later, in G1 (Figure 5C). Thus, the Swi5p D domain is able to confer recruitment of H3 HAT activity and mitotic transcriptional activity onto the Ace2p zinc finger domain (EF). Furthermore, these results indicate that the inability of Ace2p to function in mitosis is not due to an inactive DNA binding domain or to inappropriate timing of nuclear localization.

To test whether the Swi5 D domain is directly involved in recruitment of a native Gcn5p-containing HAT complex, we measured the binding of SAGA (Spt/Ada/Gcn5/acetyltransferase) complex to GST-Swi5p fusion proteins (Figure 5D). As shown previously, SAGA complex bound to a GST fusion protein containing the VP16 acidic activation domain, but binding was not detected to the inactive VP16 F442A derivative (Figure 5D; Ikeda et al., 1999). SAGA also bound to GST fusions that contain either full-length Swi5p or an N-terminal truncation

of Swi5p that contains only Swi5p domains DEF (Figure 5D). When domain D was removed from the GST-Swi5p-DEF fusion, binding of SAGA was reduced considerably. Thus, an intact Swi5p D domain is important for efficient interaction with SAGA in vitro, recruitment of GCN5-dependent HAT activity in vivo, and expression of *SIC1* during mitosis.

Global Role for SWI/SNF and Gcn5p during Mitosis

Our results indicate that mitotic expression of a group of Swi5p-regulated genes requires SWI/SNF and Gcn5p. Furthermore, these Swi5p-regulated genes appear to use a unique sequential pathway of gene activation in which SWI/SNF remodeling activity is required for recruitment of a Gcn5p-containing HAT complex. A simple model posits that these properties are unique to the Swi5p activator. Alternatively, we considered the possibility that SWI/SNF and Gcn5p might play a more global role in mitotic gene expression. To address this question, we asked whether transcription of the inducible *GAL1* gene, which is normally SWI/SNF and GCN5 independent, might require SWI/SNF and GCN5 in cells arrested in late mitosis.

Wild-type, *swi1*, and *gcn5* strains that harbor a *cdc15^{ts}* mutation were grown in raffinose media at the restrictive temperature to arrest cells in late mitosis. Galactose was then added to cultures to induce *GAL1* expression, and samples were harvested for both Northern and ChIP analyses (Figure 6). When cells are grown at the permissive temperature, *GAL1* expression does not require either SWI/SNF or GCN5, as previously observed (Figure 6A, left panel; see also Burns and Peterson, 1997). However, after prearresting cells in mitosis, induction of *GAL1* expression is almost completely blocked in both the *swi1* and *gcn5* mutant (Figure 6A, center panel). Similar results were obtained if cells were incubated in galactose for either 30 or 60 min prior to harvesting RNA. Thus, *GAL1* expression requires chromatin remodeling enzymes during mitosis but not in interphase cells.

The functional relationship between SWI/SNF and Gcn5p also changes during induction of *GAL1* in mitosis. When cells are grown at the permissive temperature, galactose-dependent increases in H3 acetylation do not require an intact SWI/SNF complex (Figure 6B; see also Figure 1A). However, when cells are prearrested in mitosis prior to *GAL1* induction, GCN5-dependent acetylation is eliminated in a *swi1* mutant (Figure 6B). Thus, an obligatory, sequential order of chromatin remodeling events appears to be a property of gene expression during mitosis, rather than a feature of the Swi5p activator.

Previous studies have shown that SWI/SNF and Gcn5p are not required for binding of the Gal4p activator or TBP to the *GAL1* promoter region in asynchronous cells (Burns and Peterson, 1997; Dudley et al., 1999). In contrast, SWI/SNF does facilitate binding of Gal4p to low-affinity, nucleosomal GAL4 binding sites (Burns and Peterson, 1997). We wished to determine if chromatin remodeling enzymes facilitate the binding of the Gal4p activator during mitosis. Formaldehyde-cross-linked samples were immunoprecipitated with antibodies to Gal4p, and immunoprecipitated DNA was analyzed for *GAL1* sequences by PCR. As expected, when cells were grown at the permissive temperature (asynchronous cells), binding of Gal4p was not affected by inactivation of either SWI/SNF or Gcn5p (Figure 6C). When cells were prearrested in late mitosis by growth at the nonpermissive temperature, Gal4p binding was also independent of SWI/SNF and GCN5 (Figure 6C). These results suggest that at least one role for chromatin remodeling enzymes during mitosis is to facilitate one or more steps subsequent to activator binding.

Our results suggest that SWI/SNF and Gcn5p may play a more general role in gene activation during mitosis. DNA microarray studies by Spellman and colleagues have identified 800 yeast genes that show cell cycle–regulated expression, and of these genes, 113 show peak levels of expression in late mitosis (Spellman et al., 1998). We wished to address what proportion of these mitotically expressed genes are SWI/SNF or GCN5 dependent. DNA microarray studies of logarithmically growing cells have indicated that *swi/snf* or *gcn5* mutations lead to decreased expression of only ~3% of the all yeast genes (Holstege et al., 1998, #403; dependence defined as 2-fold or greater changes in gene expression). We reanalyzed this data and asked what proportion of mitotically expressed genes score as SWI/SNF and GCN5 dependent in the RNA samples isolated from asynchronous cultures. Surprisingly, 23%–25% of the genes expressed in late mitosis score as either SWI/SNF or GCN5 dependent even in RNA samples from log phase cultures. Furthermore, within a cluster of genes that show patterns of expression similar to *SIC1* (23 genes; Spellman et al., 1998), 59% require SWI/SNF or Gcn5p. Likewise, several mitotically expressed genes, such as *SAG1*, *YGP1*, *PHO84*, *PHO11*, and *PHO12*, are among the most highly SWI/SNF- or GCN5-dependent genes scored in DNA microarray analyses (Holstege et al., 1998; Sudarsanam et al., 2000). Thus, genes expressed in mitosis are highly enriched for SWI/SNF- and GCN5-dependent genes. Furthermore, since *swi/snf* or *gcn5* mutations can have a major effect on the timing of expression and less of an effect on the overall level of mRNA in asynchronous cultures (Figure 3), these DNA microarray results likely underestimate the importance of chromatin remodeling enzymes in mitotic gene expression.

Discussion

In this study we have investigated the functional relationship between the SWI/SNF and Gcn5p chromatin remodeling enzymes during late mitosis and interphase. We found that recruitment of Gcn5p HAT activity is associated with transcriptional induction of many genes, both in mitosis and interphase. At some loci (e.g., *PHO8*, *HIS3*) these increases in histone acetylation are required for gene expression irrespective of cell cycle position, whereas in other cases (e.g., *GAL1*), GCN5-dependent histone acetylation plays a key role only during mitosis. Likewise, we find that the functional relationship between SWI/SNF and Gcn5p changes as a function of cell cycle position. When Gcn5p is recruited to target genes during interphase, histone acetylation is independent of SWI/SNF remodeling activity. Thus, in these cases, recruitment of SWI/SNF and Gcn5p appear to be independent events. In contrast, recruitment of Gcn5p HAT activity during mitosis requires the remodeling activity of SWI/SNF. Furthermore, the differing roles of SWI/SNF and Gcn5p during mitotic or interphase stages of the cell cycle can occur at the same gene, *GAL1*, and with transcription driven by the same activator, Gal4p.

Thus, the novel, sequential recruitment of remodeling enzymes appears solely to be a function of cell cycle position.

Why does recruitment of Gcn5p HAT activity during mitosis require the ATP-dependent remodeling activity of SWI/SNF? We and others have shown that the Gcn5p-containing HAT complex, SAGA, can directly interact with numerous activators, including Swi5p and Gal4p (Drysdale et al., 1998; Utley et al., 1998; Ikeda et al., 1999; Vignali et al., 2000; see also Figure 5), and thus, one might have envisioned that a gene-specific activator would be sufficient to recruit this HAT complex. Indeed, our data presented here suggests that activators can directly recruit Gcn5p HAT complexes to many inducible genes during interphase (see Figure 1). We propose a model in which the stable recruitment of Gcn5p-containing HAT complexes requires an interaction with both a gene-specific activator and with its substrate, the histone H3 N-terminal domain. In this view, the histone N-terminal domains may be freely available for interaction with a recruited HAT complex during interphase, but the additional condensation of chromatin during mitosis occludes the histone tails. Consequently, activators such as Gal4p or Swi5p may only be able to transiently recruit Gcn5-containing HAT complexes during mitosis. In this model, we propose that the role of ATP-dependent remodeling is to locally disrupt the condensation of mitotic chromatin, leading to more accessible histone N-terminal tails and stable Gcn5p HAT complex recruitment. This mitotic activity of SWI/SNF may be due to its well-characterized nucleosome remodeling activity, or it might reflect a novel property of SWI/SNF, such as ATP-dependent displacement of histone H1 or other nonhistone chromatin proteins that might mask the histone N-terminal domains during mitosis.

Role for Chromatin Remodeling Enzymes in Controlling Mitotic Exit

Regulation of the mitosis to G1 transition has recently emerged as a key regulatory step in the cell cycle. Dissolution of chromosome cohesion and movement of chromosomes to opposite poles must be coordinated with subsequent cytokinesis and chromosome decondensation. A key event that controls mitotic exit is the inactivation and destruction of mitotic B cyclins. The primary mechanism for inactivation of B cyclins is the anaphase promoting complex that promotes degradation of cyclin B at the end of anaphase. In addition, the CDK inhibitor, Sic1p, also contributes to inactivation of CDK/cyclin B complexes. Although Sic1p is not essential for mitotic exit, *sic1* mutants show a delay in the M/G1 transition and such mutants are also hypersensitive to overexpression of B cyclin. Likewise, *swi5* mutants show similar mitotic exit defects—slowed mitotic exit and hypersensitivity to B cyclin overexpression. In the case of *swi5*, the mitotic exit defect is due presumably to decreased mitotic expression of *SIC1* and other target genes.

Since SWI/SNF and Gcn5p are required for Swi5p to activate transcription of target genes in late mitosis, we investigated whether *swi/snf* or *gcn5* mutants might also show mitotic exit phenotypes. Previous studies have shown that asynchronous populations of *gcn5* mutant cells are overrepresented for G2/M DNA content (Zhang et al., 1998). This increased proportion of cells with G2/M DNA content probably represents a mitotic exit delay since we found that *gcn5* mutants also show the characteristic hypersensitivity to B cyclin overproduction (Figure 4). Since *swi/snf* mutations have an even larger effect on Swi5p-dependent gene expression (Figure 3), it is likely that they also exhibit delays in mitotic exit. If this mitotic exit defect is accentuated in the absence of both SWI/SNF and Gcn5p, then this may explain why *gcn5 swi/snf* double mutants grow extremely slowly in some strain backgrounds and are inviable in others. Accumulation of *swi/snf gcn5* double mutants in late mitosis may also explain why these strains have an increased spectrum of transcriptional defects as compared to *swi/snf* or *gcn5* single mutants (Roberts and Winston, 1997; Biggar and Crabtree, 1999).

What Determines whether Expression Requires SWI/SNF or Gcn5p?

Genome-wide expression screening has indicated that SWI/SNF and Gcn5p are required for expression of only a small subset of genes ($<5\%$) in asynchronous cell cultures (Holstege et al., 1998; Sudarsanam et al., 2000). Likewise, previous studies of individual genes have shown that SWI/SNF and Gcn5p are required for expression of some but not all inducible genes. What makes a gene SWI/SNF and/or GCN5 dependent? In several cases presented here, SWI/SNF and GCN5 dependence correlates directly with cell cycle position. For example, in asynchronous cells, expression of GAL1 does not require SWI/SNF and Gcn5p; however, we find that if cells are prearrested in mitosis, then GAL1 requires SWI/SNF and Gcn5p. Likewise, expression of SIC1 during late mitosis requires both SWI/SNF and Gcn5p, but expression during G1 does not require either remodeling activity. Thus, depending on cell cycle position, a gene can be a member of either the SWI/SNF- and GCN5-dependent or -independent groups. Furthermore, in contrast to our previous studies with asynchronous cells and artificial reporter genes (Burns and Peterson, 1997), SWI/SNF does not control the binding of Gal4p to the GAL1 UAS in mitosis, but rather it facilitates one or more steps subsequent to binding of the activator. These results provide the first in vivo evidence that SWI/SNF and GCN5 control steps in transcription initiation following the binding of gene-specific activators.

The fact that cell cycle position can influence the SWI/SNF and GCN5 dependence of gene expression also provides an explanation for the seemly promiscuous recruitment of chromatin remodeling enzymes by acidic activators. Previous studies have shown that SWI/SNF- and GCN5-containing HAT complexes (SAGA) can be recruited in vitro by every acidic activator tested, including artificial activators, Gal4-AH, and Gal4-VP16 (Utley et al., 1998; Ikeda et al., 1999; Massari et al., 1999; Natarajan et al., 1999; Neely et al., 1999; Yudkovsky et al., 1999). Since it is generally believed that all yeast activator proteins are members of the "acidic" class, these in vitro results suggested that chromatin remodeling enzymes might be recruited in vivo by all activators. However, this hypothesis did not seem consistent with the idea that SWI/SNF and GCN5 control expression of only a subset of yeast genes. Our studies suggest that mitosis may represent a novel cell cycle window where removing chromatin-mediated repression is globally required for transcription. For this reason, many gene-specific activators may retain the capacity to recruit remodeling enzymes even if chromatin-mediated repression does not influence expression of their target

genes throughout much of the cell cycle. Although we find that the ability to recruit remodeling enzymes can be key for mitotic expression (Figure 5), it can also lead to "wasteful" recruitment of remodeling activities during interphase that in many cases does not contribute to the levels of gene expression (Figure 1).

Role of Chromatin Remodeling Enzymes during Mitosis in More Complex Eukaryotes

Do chromatin remodeling enzymes like SWI/SNF play key roles during mitosis in more complex eukaryotes? Recently, mutations in two different SWI/SNF subunits were identified as mutations that disrupt early asymmetric cell divisions in *C. elegans* (H. Sawa et al., 2000). Furthermore, analysis of conditional alleles indicated that SWI/SNF function was required in late mitosis to facilitate the ensuing asymmetric division. In mammalian cells, transcription directed by all three RNA polymerases is repressed as cells enter mitosis, and this silencing is maintained until early telophase (reviewed in Gottesfeld and Forbes, 1997; Sirri et al., 2000). Mitotic repression correlates with Cdc2/cyclin B–dependent phosphorylation of several key components of the Pol I, II, and III transcription machinery, and the inactivation of Cdc2/cyclin B at the end of anaphase triggers the resumption of RNA synthesis. Likewise, subunits of human SWI/SNF complexes are phosphorylated as cells enter mitosis (Muchardt et al., 1996; Sif et al., 1998), and this phosphorylation is associated with inactivation of remodeling activity and removal from condensed mitotic chromosomes (Muchardt et al., 1996; Sif et al., 1998). However, human SWI/SNF subunits are also dephosphorylated in early telophase, and SWI/SNF rebinds to a small number of chromosomal foci (Muchardt et al., 1996; Sif et al., 1998). The timing of reactivation and the novel pattern of chromosome relocalization are consistent with a role for human SWI/SNF in activating expression of a subset of genes at the M/G1 boundary. Given that several recent studies have shown that human SWI/SNF is required for exit from G1 and S phases (Zhang et al., 2000), it should not be surprising that chromatin remodeling enzymes might control the key M/G1 transition in mammalian cells as well.

Experimental Procedures

Strains and Media

Strains CY727 (*ash1*), CY724 (*gcn5 ash1*), CY728 (*swi5 ash1*), and CY725 (*swi2 ash1*) are described in Krebs et al., 1999; strain CY397 (*swi2K798A*) in Cote et al., 1994; strains that contain the Gal4p reporter gene (CY532 [WT], CY533 [*gal4*], CY534 [*swi1*]) in Burns and Peterson, 1997; strains KNY61 (WT), KNY118 (*gcn5*), and KNY174 (*swp73*) in Natarajan et al., 1999 and were used for *HIS3* inductions shown in Figure 1. *swi5 ace2* strains that harbor *SWI5*/*ACE2* fusion genes are described in McBride et al., 1999. The set of congenic strains used to test *GAL1* induction in mitosis versus log phase cells includes the following relevant genotypes: *cdc15-2* (CY809); *cdc15-2 swi1* (CY877); *cdc15-2 swi5* (CY875); and *cdc15-2 gcn5* (CY879). Two sets of isogenic strains were used to test *CLB2* sensitivity. The first set includes the following relevant genotypes: *SWI5 CDC15* (CY211); *SWI5 cdc15-2* (CY809); *swi5*Δ *CDC15* (CY865). The second set includes relevant genotypes *GCN5* (CY448) and *gcn5*Δ (CY451).

Strains were grown in YEP (2% yeast extract, 1% bactopeptone) containing 2% glucose, 2% galactose, or 2% raffinose. For *PHO5* and *PHO8* inductions, cells were grown 12–24 hr in low-phosphate YEPD (Han et al., 1988). To induce *HIS3*, cells were grown in YEPD, pelleted, resuspended in S-minimal medium [6.7 g/l yeast nitrogen base without amino acids (Difco laboratories)] supplemented with amino acids as described (Stern et al., 1984) and containing 10 mM 3-amino-1,2,4-triazole (3AT, Calbiochem), and incubated at 30°C for 4 hr. *CUP1* was induced by addition of 1 mM $CuSO_4$ and incubation at 30°C for 30 min. *GAL1* and Gal4p reporters were induced in log cells by growth for 12–24 hr in 2% galactose. *Cdc15* mutants were arrested in mitosis by shifting cultures to 37°C for 3.5–4 hr until ~90% of the cells arrested with large buds.

Chromatin Immunoprecipitations

Nocodazole arrests and chromatin immunoprecipitations were performed as described previously (Krebs et al., 1999). Input and immunoprecipitated DNAs were analyzed either by slot blotting (Krebs et al., 1999) or by PCR. For PCR analysis, either 1/1000 (input) or 1/100 (IP) of the DNA from IPs was amplified using 50 pmol of both *GAL1* and *SSB1* primers in 25 μl reactions containing 200 μM dNTPs, 3 mM $MgCl_2$, 2.5 μCi of [α-^{32}P]dCTP, and 0.25 units of AmpliTaq Gold polymerase (Perkin Elmer). After 10 min at 95°C, 25 cycles of 30 s 95°C, 30 s 55°C, 1 min 72°C were performed. PCR products were electrophoresed on 6% polyacrylamide gels, dried, and exposed to film.

Primers and Probes

Most DNA probes used in this study were PCR products labeled by random priming. The primers used are as follows (5′ to 3′): *SIC1*, AATGAGAAAAGATGCCTCCG and TGGAAGGAGTCATTTTC GTG; *EGT2*, CAGGCTGACAAGGGACTAATT and TGCTTGTTTATGT TGATTCT; *CDC6*, TGGCTTGCGATTTGTTGTGT and TGGTATAGCT GACATGAACG; *PCL2*, TAGGCCTGGCTAGTTACCTAT and AAGGC TTCGTAGTTTGACATC; *PCL9*, TGCTGCACTAAAAAAAAACCA and CAGAAATCATCTCTTTGTGCG; *GAL1*, AAAATTGGCAGTAACCTGGC and CCTTTGCGCTAGAATTGAACT; *PHO5*, AAATTAGCACGTTTTC GCA and GGTAATCTCGAATTTGCTTGCTC; *PHO8*, CCACGTGCAG CGATCACTT and TGCGCGTTCAAATAATGTCC; *CUP1*, CCACCCT TTATTTCAGGCTGAT and GTGATGATTGATTGATTGATTGTACAG; *SIC1* coding, ACTCCTTCCACCCCACCAA and TCCCTAGATTGA AACAATGCC; *GAL1* coding, CAGAAGAAGTGATTGTACCTGAGTTC and TTTGTTAACCGTTCGATGCC; *ACT1* coding, ACAACGAATTGA GAGTTGCCCCAG and AATGGCGTGAGGTAGAGAGAAACC; and *SSB1* coding, CCATTTTTAGACTTTTTCTTAACTAGAATGC and CAC AATTAAAATATTTGTTTTTCTTTCGCG.

RNA Analysis

Total RNA was prepared using glass bead lysis in 0.5 M NaCl, 0.2 M Tris (pH 7.5), 10 mM EDTA, and 1% SDS, followed by phenol/chloroform extraction. Northerns were performed either using glyoxal-denatured RNA and phosphate electrophoresis buffers or standard formaldehyde gels.

GST-SWI5 Binding

The GST-Swi5pABCDEF (amino acids [aa] 1–709) and GST-Swi5pDEF (aa 496–709) expression constructs were gifts from Brenda J. Andrews (University of Toronto). GST-Swi5pEF (aa 543–709) was made by PCR using the following primers with GST-Swi5p (aa 1–709) as template 5′-GCCCGGGATCCAAGGAAATGCCTGATAAAAC ATTC-3′ and 5′-GCCGGGAATTCTACCTTTGATTAGTTTTCATTGG-3′. The PCR fragment was digested with BamHI and EcoRI and cloned into pGEX-2T (Amersham Pharmacia Biotech). GST-VP16 (aa 413–490) and GST-VP16 (aa 413–455; F442P) were gifts from Michael Green (University of Massachusetts).

For purification of SAGA, whole-cell extracts were prepared from 20 liter cultures of strain CY396 (Cote et al., 1994). Extracts were fractionated on Ni^{2+}-nitrilotriacetic acid agarose, DNA cellulose, and FPLC Mono Q as described previously (Quinn et al., 1996). Mono Q fractions containing SAGA were identified by Western blot with α-Gcn5p (Santa Cruz), α-ADA3 (Santa Cruz), and α-TAFII68 (M. Green) antibodies, in addition to assaying for histone acetyltransferase activity as described (Pollard and Peterson, 1997). GST fusion proteins were expressed and purified as described (Fry et al., 1999). For the GST pulldown assay, 20 μg of each GST fusion was incubated with 5 μl of SAGA complex in 400 μl Mono Q buffer (50 mM Tris HCl [pH 8.0], 50 mM NaCl, 10% glycerol, 0.1% Tween, 0.5 mM DTT, 0.1 mM PMSF) for 2 hr at 4°C. The GST beads were then washed 3 times with Mono Q buffer and eluted in Mono Q buffer

(+1% SDS). Equal volumes of eluted material were assayed for SAGA by Western blot.

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