SWI/SNF is required for transcriptional memory at the yeast *GAL* gene cluster

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Post-translational modification of nucleosomal histones has been suggested to contribute to epigenetic transcriptional memory. We describe a case of transcriptional memory in yeast where the rate of transcriptional induction of *GAL1* is regulated by the prior expression state. This epigenetic state is inherited by daughter cells, but does not require the histone acetyltransferase, Gcn5p, the histone ubiquitinylating enzyme, Rad6p, or the histone methylases, Dot1p, Set1p, or Set2p. In contrast, we show that the ATP-dependent chromatin remodeling enzyme, SWI/SNF, is essential for transcriptional memory at *GAL1*. Genetic studies indicate that SWI/SNF controls transcriptional memory by antagonizing ISWI-like chromatin remodeling enzymes.

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Specific patterns of gene expression are established during development, and these gene expression programs can be maintained through many cell divisions. The process of establishing and maintaining a transcriptional state that is heritable to progeny has been termed transcriptional memory. Within eukaryotic cells, chromatin structure plays a key role in establishing and maintaining ON/OFF states of gene expression. In its simplest state, chromatin is composed of long, linear arrays of nucleosomes that contain 147 base pairs (bp) of DNA wrapped about twice around an octamer of the core histones (two each of H3, H4, H2A, and H2B). Within cells, nucleosomal arrays are condensed into higher order structures, and the dynamic folding/unfolding of these structures is associated (and likely causative) with transcriptional activity.

Genetic and biochemical analyses of transcriptional regulatory mechanisms have led to the identification of two classes of highly conserved "chromatin remodeling/modification" enzyme that regulate the dynamic state of chromatin (for reviews, see Becker and Horz 2002; Peterson and Laniel 2004). One class of chromatin remodeling/modification enzymes catalyzes the covalent attachment or removal of post-translational histone modifications (e.g., lysine acetylation, serine phosphorylation, lysine and arginine methylation, and lysine ubiquitylation). These histone marks can regulate the formation of higher order chromatin structures (e.g., H4-K16Ac) (Shogren-Knaak et al. 2006), or they can serve as the nucleating event for binding of nonhistone proteins that establish active or inactive chromatin states. For example, methylation of H3-K9 provides a binding site for the HP1 protein, which nucleates formation of repressive, heterochromatic structures (Grewal and Elgin 2002). HP1 can interact with the H3-K9 methyltransferase, which suggests a means for how this chromatin structure can be re-established following DNA replication (Grewal and Elgin 2002). Likewise, methylation of histone H3 at K4 is associated with transcriptionally active loci in many eukaryotes, and it has been suggested that H3-K4me could provide a memory of previous transcriptional activity (Ng et al. 2003).

In addition to histone modifying enzymes, a distinct class of chromatin remodeling/modification enzyme uses the free energy derived from ATP hydrolysis to enhance the accessibility of nucleosomal DNA or change the histone composition of nucleosomes (Becker and Horz 2002; Smith and Peterson 2005). This family can be subdivided into at least five groups based on their biochemical properties and overall sequence similarity of their ATPase subunits: (1) SWI/SNF, (2) ISWI, (3) Mi-2/CHD, (4) Ino80/Swr1, and (5) Rad54 (Flaus et al. 2006). Whereas many members of the ISWI-like and Mi-2/CHD-like subgroups appear dedicated to transcriptional repression pathways (Kehle et al. 1998; Fazzio et al. 2001; Unhavaithaya et al. 2002), most SWI/SNF-like enzymes play roles in the activation of transcription (Peterson and Workman 2000). Notably, the *Drosophila* SWI/SNF complex harbors the Brm ATPase, which is a member of the TrX family of gene products that function as

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"memory factors" to maintain the transcriptional active state of homeotic genes during embryonic development (Tamkun et al. 1992).

Here we find that transcriptional induction of the yeast *GAL1* gene exhibits "memory" of the preceding transcriptional state. Specifically, the rate of transcriptional induction of a naïve gene is slower than for a *GAL1* gene that was previously transcribed. This ability to reinduce *GAL1* with fast kinetics survives at least one round of DNA replication and mitosis, indicating that this memory phenomenon is epigenetically inherited. Previous studies have demonstrated that nucleosomes at the *GAL1* locus are subject to a variety of histone modifications during transcription, but we find that none of these marks are required for memory. In contrast, we find that inactivation of the SWI/SNF remodeling enzyme eliminates transcriptional memory at *GAL1*, such that the rate of transcriptional induction is nearly identical between a naïve gene and a *GAL1* gene that had been previously transcribed. Surprisingly, we find that inactivation of ISWI-based chromatin remodeling enzymes restores transcriptional memory in a *swi/snf* mutant, suggesting that SWI/SNF prevents ISWI-based enzymes from erasing the memory of a previous round of transcription.

Results

Transcriptional memory at GAL1 gene is heritable

GAL1, which encodes the enzyme galactokinase, can be transcriptionally induced by ~1000-fold when yeast cells are grown in media containing galactose. Addition of glucose leads to rapid and efficient repression of *GAL1* by multiple mechanisms, including a decrease in levels of the Gal4p activator and the galactose permease (Gal2p), and by activating several glucose repressor proteins that act at the *GAL1* promoter (Johnston et al. 1994; Carlson 1998). Further, in neutral carbon sources such as raffinose, glycerol, or lactate, *GAL1* is maintained in a poised state due to the masking of the Gal4p activation domain by the Gal80p repressor. We were specifically interested in how the *trans*-acting glucose repressors function, and therefore, we investigated the reinduction of *GAL1* following a short period of glucose repression (see Fig. 1A). Cells were first grown in raffinose media so that *GAL1* was poised for activation. Upon addition of galactose to the growth medium, *GAL1* transcription commenced and transcripts appeared by 20 min post-induction (Fig. 1A,B). However, accumulation of maximum levels of *GAL1* transcripts required >1 h of growth in galactose media. Next, *GAL1* expression was repressed by addition of 2% glucose and cells were grown for an additional hour. Surprisingly, when cells were washed into fresh media containing galactose, *GAL1* transcription resumed very rapidly (Fig. 1A,B; Supplementary Fig. S2). Reinduction of *GAL1* transcription peaked <10 min after the addition of galactose (Fig. 1A,B). Thus, these results suggest that cells "remember" that *GAL1* was previously transcribed, and consequently, they are poised to rapidly reinduce *GAL1* transcription when galactose again becomes the dominant carbon source. Similar results were found for the reinduction of the *GAL7* and *GAL10* genes, indicating that this phenomenon is a general property of the *GAL* gene cluster (data not shown).

We next tested whether the ability to reinduce *GAL1* with rapid kinetics was a transient state or whether it could survive long-term growth in glucose media. Cells were grown overnight in raffinose media and then galactose was added for 60 min to induce *GAL1* expression. Glucose was then added to repress *GAL1*, and at varying times after glucose addition, cell aliquots were transferred to galactose media and *GAL1* reinduction kinetics were monitored. Figure 1C illustrates that cells grown for 2–4 h in glucose media retained the ability to rapidly reinduce *GAL1* after subsequent addition of galactose (rapid induction defined as maximal expression at 20 min following galactose addition). In contrast, cells grown for 6–8 h in glucose reinduced *GAL1* with the slower kinetics that mirrored induction kinetics of a naïve gene (Fig. 1, cf. lanes 10–15 in C and A). Since the yeast cell cycle is ~2 h in glucose media, these results suggest that the ability to rapidly reinduce *GAL1* might survive DNA replication and/or mitosis. To test this idea definitively, we performed an elutriation experiment (Fig. 1D). Cells were grown in galactose media until mid-log phase, and then cells were arrested at the G1/S transition of the cell cycle by treatment with α factor. Arrested cells were washed into glucose-containing media and then released from the cell cycle block and allowed to undergo one synchronous cell division in glucose media. Centrifugal elutriation was then performed in glucose media to isolate small, unbudded daughter cells from this culture. The daughter cell population was then transferred to galactose media and *GAL1* reinduction kinetics were followed. The data shown in Figure 1D demonstrates that these daughter cells retained the ability to reinduce *GAL1* with rapid kinetics (i.e., peak expression at 20 min). These results indicate that memory of *GAL1* gene transcription can be epigenetically inherited.

The transcriptional machinery is disassembled during glucose repression

One possibility is that rapid reinduction kinetics involves the persistent association of one or more components of the RNAP II transcription machinery with the *GAL1* promoter during glucose repression. For instance, if TBP remains bound to the promoter during glucose repression, then reinduction might occur with faster kinetics. *GAL1* transcription is activated by Gal4p, which binds to four sites within the UAS_{GAL} between the *GAL1* and *GAL10* genes (Fig. 2A). When galactose is added to raffinose-grown cells, Gal80p-dependent inhibition of Gal4p is relieved, and Gal4p recruits a variety of transcription factors, including the SWI/SNF remodeling enzyme, the SAGA HAT complex, and the transcriptional mediator complex (Bhaumik and Green 2001; Lar-

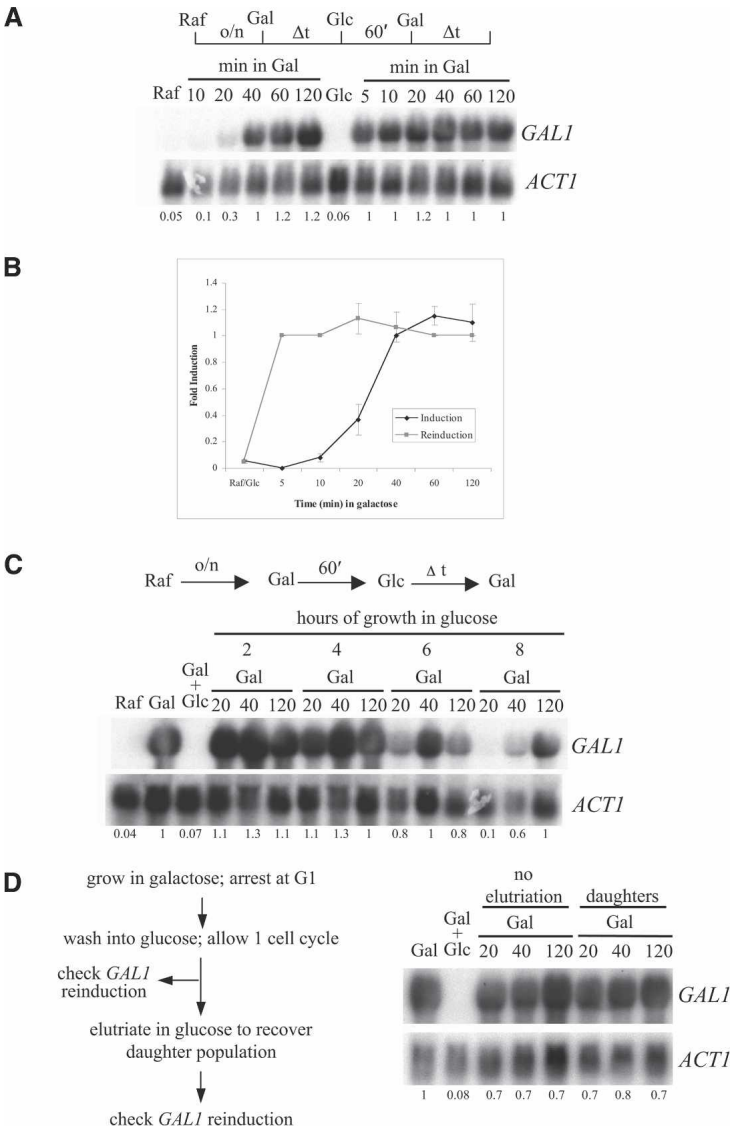

Figure 1. Transcriptional memory at the *GAL1* gene. (*A*) Northern analysis of *GAL1* RNA levels. Schematic at *top* depicts regimen of growth in different carbon sources. (Raf) 2% raffinose; (Gal) 2% galactose; (Glc) 2% glucose. Initial induction of *GAL1* occurs with slower kinetics than when *GAL1* is reinduced following glucose repression. (*B*) Graph comparing kinetics of *GAL1* induction and reinduction, averaged over three experiments performed as described in *A*. Error bars represent the standard deviation at each point. Slightly different time points were taken in different experiments, so in these cases no error bars are shown. (*C*) Cells were grown overnight in raffinose prior to addition of galactose. After 1 h, cells were transferred to glucose media. At indicated times, an aliquot of cells was washed into fresh galactose media and *GAL1* reinduction was observed. The "memory" state is maintained through at least 4 h of repression. (*D*) Glucose-grown daughter cells retain transcriptional memory. Cells were grown overnight in galactose media and then arrested at the G1/S boundary with α factor (lane labeled "Gal"). Arrested cells were then released from α factor into glucose medium to repress *GAL1* and simultaneously undergo one synchronous division (lane labeled "Gal + Glc"). An aliquot of these cells was washed into galactose media to monitor reinduction kinetics (lanes labeled "no elutriation"). The remainder of the cells were elutriated to isolate daughter cells that had undergone mitosis in glucose media. Daughter cells were washed into galactose media to follow kinetics of *GAL1* reinduction. The *bottom* panel represents an *ACT1* loading control for total RNA levels. The numbers indicate fold induction of *GAL1* transcripts normalized to *ACT1* transcripts, with the maximally induced state set to a value of 1.

schan and Winston 2001; Lemieux and Gaudreau 2004; Dhasarathy and Kladde 2005). These events are followed by assembly of the preinitiation complex (PIC) and transcription of *GAL1*. We used chromatin immunoprecipitation (ChIP) to follow the recruitment of these factors to the *GAL1* promoter during growth in galactose and during subsequent glucose repression (Fig. 2). As expected, robust levels of Gal4p were detected at UAS_{GAL} when cells were grown in raffinose or galactose media. During glucose repression, Gal4p remained at high levels for the first 1 h in glucose, and then levels decreased approximately twofold at extended times in glucose media (Fig. 2B, top). The lower level of Gal4p was also observed in long-term glucose grown cultures and likely reflects partial occupancy of the multiple Gal4p-binding sites (Ren et al. 2000). These ChIP signals are specific to Gal4p since a *gal4*Δ strain showed no enrichment of Gal4p at the UAS_{GAL} (Fig. 2B, bottom). We also found that TBP, Mediator, RNA Polymerase II, SWI/SNF, and SAGA were recruited to the *GAL1* promoter following galactose addition, but unlike Gal4p, none of these factors could be detected at *GAL1* after 1 h of transferring the cells to glucose-containing medium (Fig. 2C). Thus, components of the transcription machinery rapidly dissociate from the promoter soon after *GAL1* transcription is repressed by glucose addition. Since the ability to rapidly reinduce *GAL1* persists for 2–4 h in glucose, these results suggest that this phenomenon is not due simply to persistent association of transcription factors with the promoter.

The kinetics of RNA Polymerase II recruitment to the *GAL1* promoter was also analyzed throughout an induction/reinduction time course (Fig. 2D). In the initial round of *GAL1* induction, high levels of RNAPII was detected at the *GAL1* promoter within 20–40 min (Fig. 2D), consistent with the appearance of *GAL1* mRNA (Fig. 1A). Strikingly, RNAPII was recruited much faster during reinduction of *GAL1*, with significant amounts of RNAPII detected 5 min after galactose addition. Similar results were found for recruitment of TBP (data not

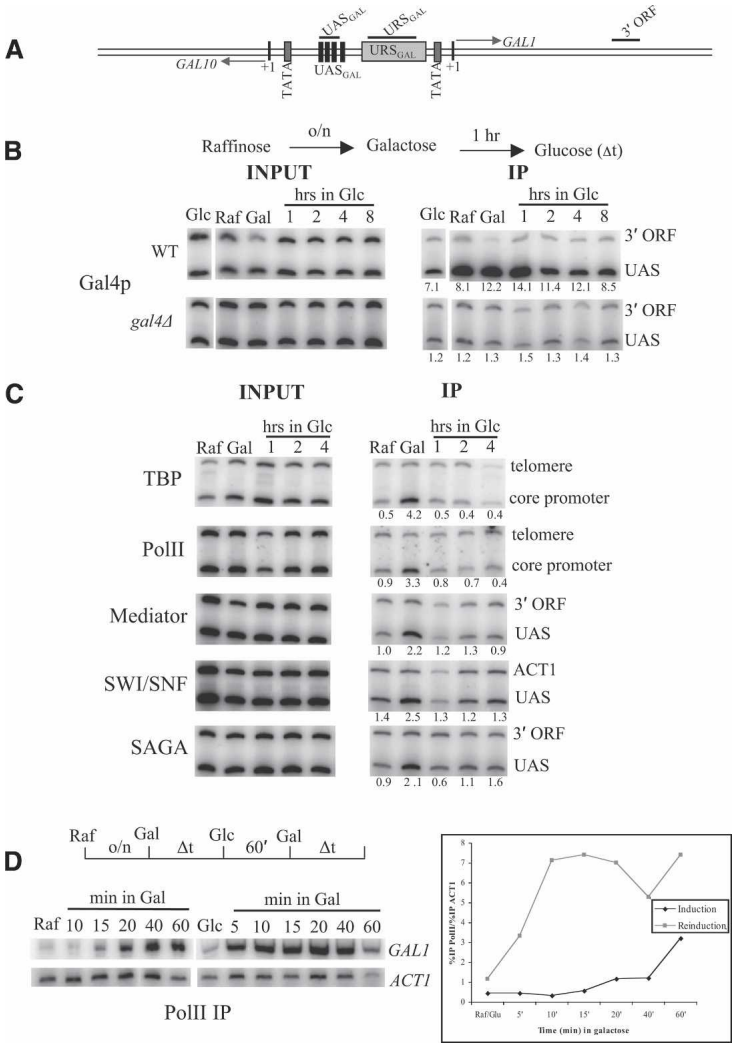

Figure 2. Rapid dissociation of the transcription machinery during glucose repression. (*A*) Schematic representation of the *GAL1–10* regulatory region. UAS_{GAL} marks the Gal4p-binding sites. URS_{GAL} contains the binding sites for the glucose repressor, Mig1p. Regions covered by primers for ChIP are shown as horizontal lines. TATA represents the TBP-binding sites and +1 represents the transcription start sites. (*B*) Gal4p ChIP in wild-type and *gal4*Δ strains. (*C*) ChIP for TBP, RNA Polymerase II, Mediator (α-Srb4-13myc), SWI/SNF (α-Snf6), and SAGA (α-Spt3-13myc). For all factors, appropriate strains were grown in raffinose media until mid-log phase, and *GAL1* was induced for 1 h by adding galactose. Cells were then washed into media with glucose to repress *GAL1* transcription for the indicated times. 3′ *GAL1* ORF, telomere (Chr VI-70 bp from the *right* end), and *ACT1* PCR primer sets were included as nonspecific controls. Numbers indicate ratio of percentage of immunoprecipitation values to corresponding nonspecific control. (*D*) RNA Polymerase II ChIP in wild-type strain grown for the reinduction regimen shown *above* the panel. *Right* panel shows quantitation of *left* panel.

shown). Thus, these results indicate that this phenomenon of *GAL1* transcriptional memory occurs at the level of transcription initiation.

Histone modifications are not required for transcriptional memory

Post-translational modification of the core histones is an ideal candidate for an epigenetic mechanism that could contribute to the rapid *GAL1* reinduction kinetics. Histone lysine methylation is particularly attractive, as this mark has a much longer half-life as compared with lysine acetylation (Katan-Khaykovich and Struhl 2005). Indeed, the transcription-associated methylation of histone H3-K4 (H3-K4me) has been hypothesized as a possible agent for memory of recent transcriptional activity (Ng et al. 2003). We tested whether Set1p, which is responsible for H3-K4 methylation (Briggs et al. 2001; Roguev et al. 2001), or Set2p and Dot1p, which methylate H3-K36 (Strahl et al. 2002) and H3-K79 (Feng et al. 2002; Lacoste et al. 2002; Ng et al. 2002a; van Leeuwen et al. 2002), respectively, are required for transcriptional memory at *GAL1*. Interestingly, none of these methyltransferases are required for the rapid kinetics of *GAL1* reinduction (Fig. 3A). After reinducing *GAL1* transcription, transcript levels peaked within 20 min in *set1*Δ, *set2*Δ, or *dot1*Δ strains (Fig. 3A). We also tested the reinduction kinetics of a *rad6*Δ strain, since Rad6p-dependent ubiquitinylation of histone H2B-K123 is required for H3 methylation (Dover et al. 2002; Ng et al. 2002b; Sun and Allis 2002). In this case as well, *GAL1* reinduction was rapid (Fig. 3B). Finally, we monitored reinduction kinetics in a *gcn5*Δ strain to eliminate SAGA-dependent acetylation of histone H3. In this case as well, no effect was observed on *GAL1* memory (Fig. 3C). Thus, epigenetic modifications of histones do not appear to be responsible for imparting transcriptional memory at the *GAL1* locus.

Transcriptional memory requires SWI/SNF

After determining that chromatin remodeling via histone modifications might not be required for the faster reinduction kinetics of *GAL1,* we turned to the ATP-

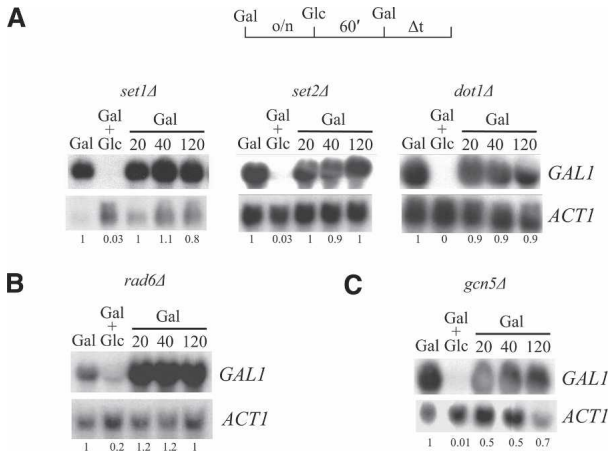

Figure 3. Histone-modifying enzymes are not essential for rapid *GAL1* reinduction. Northern blot analyses are as in Figure 1. (*A*) Schematic at *top* illustrates growth media regimen. RNA was isolated from *set1*Δ, *set2*Δ, or *dot1*Δ strains. (*B*) Identical analysis as in *A*, but with a *rad6*Δ strain. (*C*) Identical analysis as in *A*, but with a *gcn5*Δ strain. All Northerns were subsequently probed for *ACT1* as a loading control.

dependent chromatin remodeling enzymes. Previous studies have shown that SWI/SNF is recruited by the Gal4p activator to the *GAL1* locus (Lemieux and Gaudreau 2004). Several studies have also shown that inactivation of SWI/SNF does not alter steady-state *GAL1* expression (Burns and Peterson 1997; Lemieux and Gaudreau 2004). Likewise, we found that the kinetics of *GAL1* induction in a *swi2*Δ strain are nearly equivalent to those of a wild-type strain (Fig. 4A). In contrast, the *swi2*Δ strain was unable to rapidly reinduce *GAL1* following a 1-h period of glucose repression. Indeed, the kinetics of induction and reinduction were nearly identical (Fig. 4A,B). This defect in reinduction kinetics was observed irrespective of whether raffinose-grown *swi2*Δ cells were switched to galactose for 2 h (Fig. 4A) or if they were grown overnight in galactose (Supplementary Fig. S2). Thus, it appears that inactivation of SWI/SNF eliminates the memory of previous *GAL1* activation.

One possibility is that SWI/SNF is not involved in the mechanism of transcriptional memory but that SWI/SNF is generally required to antagonize glucose repression. To test this idea, wild-type and *swi2*Δ cells were grown overnight in glucose, and then *GAL1* induction kinetics were monitored after cells were transferred to galactose media. In both the wild-type and *swi2*Δ strains, *GAL1* transcripts appeared by 4 h after galactose addition, demonstrating that SWI/SNF is not required to antagonize glucose repression at *GAL1* during an initial round of transcriptional induction (Fig. 4C).

We next tested if the ATPase activity of SWI/SNF was required for transcriptional memory. *GAL1* reinduction kinetics were monitored in a strain harboring the *swi2K798A* allele, which encodes an ATPase-defective version of the Swi2p catalytic subunit. *GAL1* reinduction in the *swi2K798A* strain was much slower than the isogenic wild-type strain (~40 min in *swi2K798A* vs. ~5 min in wild type), indicating that the enzymatic activity of SWI/SNF is essential for the ability to rapidly reinduce *GAL1* transcription. We note that reinduction kinetics in the *swi2K798A* strain are reproducibly faster than the isogenic *swi2*Δ strain, which may indicate an additional

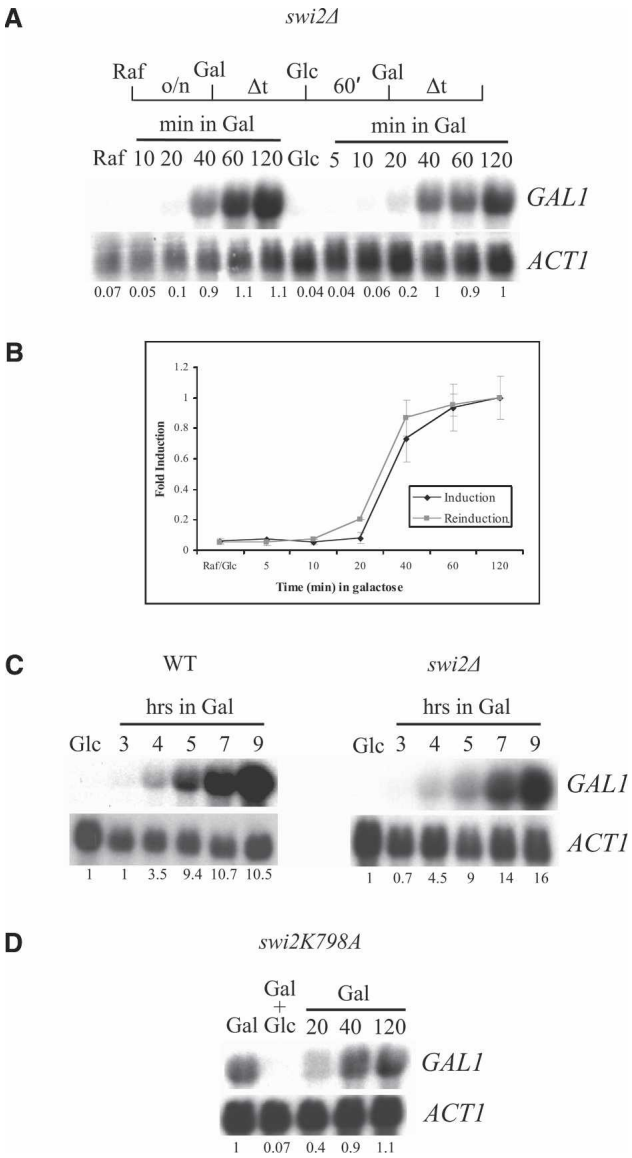

Figure 4. SWI/SNF is essential for *GAL1* memory. (*A*) Northern analyses. Schematic at *top* illustrates the growth regimen for *swi2*Δ cells. (*B*) Comparison of *GAL1* induction and reinduction kinetics, averaged over three experiments performed as in *A*. Error bars represent the standard deviation at each point. Slightly different time points were taken in different experiments, so in these cases no error bars are shown. (*C*) Isogenic wild-type and *swi2*Δ strains were grown overnight in glucose and then cells were transferred to galactose media. Both strains showed similar *GAL1* induction kinetics after overcoming long-term glucose repression. (*D*) An intact Swi2p ATPase domain is required for rapid *GAL1* reinduction. Northern analysis of RNA isolated from cells harboring a *swi2K798A* allele, which inactivates the ATPase activity of SWI/SNF.

ATP-independent role for SWI/SNF in *GAL1* memory (Fig. 4, cf. D and A).

Loss of ISWI-like enzymes restores memory at GAL1 in the absence of SWI/SNF

Our data suggest that the initial round of *GAL1* transcription establishes a heritable state that is poised for rapid reinduction. Previous studies have identified two changes in *GAL1* chromatin structure that occur during transcriptional induction and that may contribute to this SWI/SNF-dependent memory state: (1) Two nucleosomes surrounding the *GAL1* promoter are lost (Lohr and Lopez 1995), and (2) promoter-proximal nucleosomes are trimethylated at H3-K4 by the Set1p methyltransferase (Ng et al. 2003). The methylation of H3-K4 leads to the subsequent recruitment of an Isw1-containing, ATP-dependent chromatin remodeling enzyme (Santos-Rosa et al. 2003). When the transcriptionally active *GAL1* gene is repressed by glucose, the promoter proximal nucleosomes are rapidly reassembled (Lohr 1984; Lohr and Lopez 1995), and high levels of H3-K4me$_3$ persist (Ng et al. 2003; P.J. Horn and C.L. Peterson, unpubl.). One possibility is that SWI/SNF may influence the repositioning of promoter proximal nucleosomes such that subsequent PIC formation is favored. If this model is correct, then only small changes in nucleosome positioning are required, as Cavalli and Thoma (1993) have previously shown that the low resolution view (±50 bp) of *GAL1* promoter nucleosomes is identical when samples are analyzed from cells grown long term in glucose or from galactose-induced cells that are treated with glucose for <1 h. Alternatively, the first round of *GAL1* transcription may lead to a locus with a lower density of nucleosomes, even after subsequent glucose repression. Indeed, previous studies indicate that several hours of glucose repression is required before the entire *GAL1* locus is restored to a regular nucleosomal array (Cavalli and Thoma 1993). SWI/SNF action may favor this chromatin state, whereas ISWI-like enzymes may promote the reassembly of a more regular nucleosomal array.

Although our data suggest that the *SET1*-dependent recruitment of Isw1p is not essential for *GAL1* reinduction kinetics (Fig. 3A), we tested the possibility that a functional relationship exists between SWI/SNF and ISWI-like remodeling enzymes in transcriptional memory at *GAL1*. Budding yeast contain two distinct ISWI-like enzymes, Isw1 and Isw2 (Vary et al. 2003; Mellor and Morillon 2004), and each functions as the catalytic subunit of distinct multisubunit remodeling complexes. We created isogenic *isw1*Δ, *isw2*Δ, *isw1*Δ *swi2*Δ, and *isw2*Δ *swi2*Δ strains and monitored *GAL1* reinduction kinetics. Whereas the *isw1* or *isw2* single mutants had no effect on *GAL1* induction or reinduction kinetics (Supplementary Fig. S1), we found that deletion of either *ISW1* or *ISW2* restored rapid reinduction kinetics in the *swi2*Δ strain (Fig. 5A,B). Furthermore, deletion of *ISW2* allowed a *swi2*Δ strain to grow on galactose/anti-mycin solid media (data not shown). In contrast, inactivation of Set1p did not restore rapid *GAL1* reinduction kinetics in a *swi2* mutant (Fig. 5C). Taken together, these results suggest that SWI/SNF controls memory of recent *GAL1* transcription by antagonizing the repressive role of ISWI remodeling complexes. Such functional antagonism between SWI/SNF and ISWI-like enzymes is not unique to the *GAL1* gene, as deletion of the *ISW2* gene also alleviates the transcriptional requirement for SWI/SNF in the induction of the *INO1* gene when cells are grown in low levels of inositol (Fig. 5D).

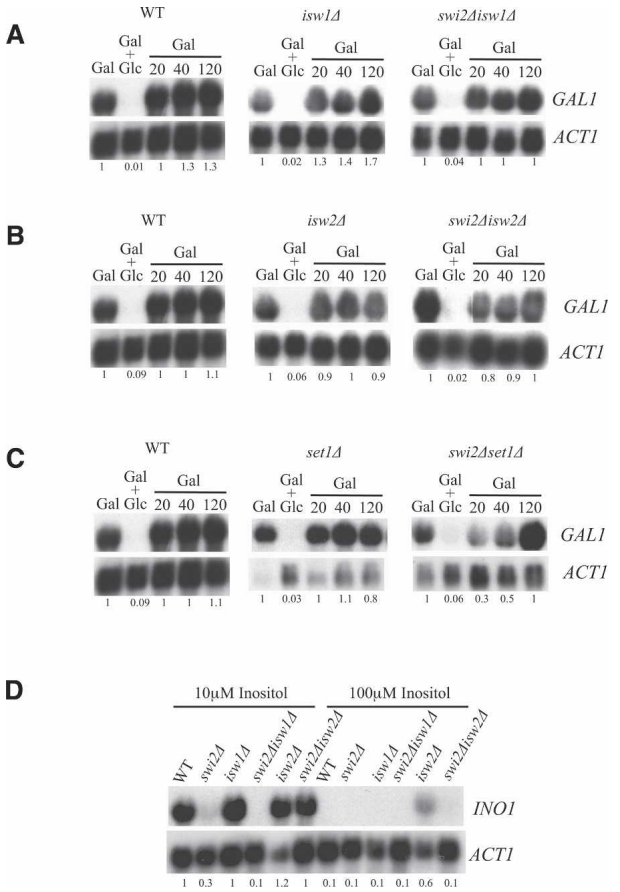

Figure 5. SWI/SNF antagonizes ISWI-like remodeling complexes. (*A*) Northern blot analysis for RNA isolated from wild-type (WT), *isw1*Δ, and *isw1*Δ *swi2*Δ strains. (*B*) Northern analysis for RNA isolated from *isw2*Δ and *isw2*Δ *swi2*Δ strains. (*C*) Inactivation of Set1p does not restore rapid reinduction kinetics in the absence of SWI/SNF. (*D*) Analysis of *INO1* expression. The indicated isogenic strains were grown in minimal media containing high (100 μM) or low (10 μM) concentrations of myo-inositol. Blots were reprobed for *ACT1* as a control.

Discussion

How do SWI/SNF and ISWI-like complexes function at GAL1?

Our data suggest that the SWI/SNF that is recruited during the first round of *GAL1* transcription potentiates the reinduction of *GAL1* following a period of glucose repression. Furthermore, we found that this requirement

for SWI/SNF can be bypassed by removal of the Isw1- or Isw2-based remodeling enzymes. Based on these results, we speculate that these two types of chromatin remodeling enzymes compete with each other to establish a *GAL1* chromatin structure that is permissive for rapid reinduction of *GAL1*. As cells divide, this chromatin state can be inherited epigenetically but is eventually lost. In cells lacking SWI/SNF, the abundant Isw1 and Isw2 enzymes establish an alternative, repressive chromatin state. In this situation, memory of the recent transcriptional activity is erased and slow reinduction kinetics result. Since SWI/SNF and ISWI-like enzymes tend to have opposing effects on the transcription of several yeast genes (including *GAL1* and *INO1*), it is tempting to consider that such direct antagonism may be a more general phenomenon.

Recently, van Oudenaarden and colleagues (Acar et al. 2005) have described a distinct example of *GAL1* transcriptional memory in which cells "remember" whether they were previously exposed to high or low concentrations of galactose. This particular memory phenomenon requires the *GAL3* and *GAL80* regulatory loops and likely involves the cytoplasmic inheritance of the positive regulator, Gal3p. Cytoplasmic inheritance of the Gal3p that is expressed during the first round of induction may contribute to the rapid induction kinetics that follows glucose repression. However, SWI/SNF plays a dominant role in transcriptional memory that is independent of Gal3p or the Gal2p permease since SWI/SNF does not affect *GAL3* or *GAL2* expression during the first round of galactose induction (Supplementary Fig. S3). Thus, transcriptional memory at *GAL1* appears to involve a cytoplasmic mechanism that generally controls *GAL1* expression levels, and a chromatin-based mechanism that specifically regulates the rate of transcriptional reinduction following transient glucose repression.

Materials and methods

Yeast strains, media, and growth conditions

Strains used in this study are isogenic derivatives of the S288c background. Genotypes are provided in Supplementary Table S1. *Saccharomyces cerevisiae* liquid cultures were grown at 30°C in YEP (1% yeast extract, 2% bacto-peptone) media supplemented with 2% glucose, 2% galactose, or 2% raffinose + 0.2% sucrose depending on whether *GAL1* activation or repression was required. For reinduction studies, aliquots of glucose-grown cultures were centrifuged for 5 min at room temperature, and cell pellets were washed once with YEP, centrifuged for 5 min, and resuspended in prewarmed YEPGal. To activate *INO1* expression, cells were grown to mid-log phase in SD medium complete with amino acids. *INO1* expression was repressed by adding 100 µM myo-inositol. Deleted strains were made by a PCR-based method using kanamycin resistance cassette. Deletions were confirmed by PCR from genomic DNA with primers designed in the ORF of the individual deleted gene.

Centrifugal elutriation

Centrifugal elutriation of wild-type cells was performed as described (Johnston and Johnson 1997).

RNA isolation and analysis

Total RNA was isolated from yeast grown to logarithmic phase in appropriate media by hot phenol extraction method. Concentration of RNA was determined by measuring A260 after dissolving in diethyl pyrocarbonate-treated water. Ten micrograms (for *GAL1* expression) or 25 µg (for *INO1* expression) of total RNA from each sample were electrophoresed on 1% formaldehyde agarose gels and Northern blotting was done. The housekeeping gene *ACT1* was used as loading control. Radioactively labeled probes for hybridization were generated by PCR amplification of the complete *GAL1*, *INO1*, or *ACT1* ORFs from genomic DNA.

ChIP

Mouse monoclonal antibody to the Gal4-DBD (RK5C1) was obtained from Santa Cruz Biotechnology. Anti-TBP and anti-Snf6 (for SWI/SNF) antibodies were kind gifts from M.R. Green (University of Massachusetts Medical School, Worcester, MA) and J. Reese (Pennsylvania State University). Mouse monoclonal antibody to RNA Polymerase II (CTD4H8) was obtained from Covance Research Products. SAGA (13-myc tagged Spt3) and Mediator (13-myc tagged Srb4) were immunoprecipitated with mouse monoclonal anti-Myc (9E10) antibody (Santa Cruz Biotechnology). ChIP assays were performed as described (Li et al. 2000). The immunoprecipitated DNA was amplified using quantitative PCR performed with α-^{32}P-dCTP and then electrophoresed on 5% acrylamide gels. Reactions were visualized and quantified by PhosphorImager.

RT–PCR

Wild-type and *swi2*Δ cells were grown to mid-log phase in YEP medium with 2% glucose, 2% galactose, or 2% raffinose + 0.2% sucrose at 30°C. Ten milliliters of cells were harvested and total RNA was extracted as described above. First-strand cDNA was synthesized using 2.5 µg of RNA, SuperScript II RNase H$^-$ reverse transcriptase (Invitrogen), and 2 pmol each of *GAL2*, *GAL3*, or *ACT1* downstream primers, following the manufacturer's instructions. Subsequently, ^{32}P-labeled PCR was performed using 2 µL of the first-strand cDNA reaction, and gene-specific primer sets to determine the relative levels of *GAL2*, *GAL3*, and *ACT1* mRNA for each strain. After 14 cycles (for *ACT1*) or 25 cycles (for *GAL2* and *GAL3*) of amplification, PCR products were electrophoresed on 10% acrylamide gels.

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