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Recent Transcription-induced Histone H3 Lysine 4 (H3K4) Methylation Inhibits Gene Reactivation*[S]

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Recent transcription of *GAL* genes transiently leaves an H3K4 methylation mark at their promoters, providing an epigenetic memory for the recent transcriptional activity. However, the physiological significance of this mark is enigmatic. In our study, we show that the transient H3K4 di- and trimethylation at recently transcribed *GAL1* inhibited the reinduction of *GAL1*. The H3K4 methylation functioned by recruiting the Isw1 ATPase onto *GAL1* and thereby limiting the action of RNA polymerase II during *GAL1* reactivation. Strikingly, the H3K4 methylation was also observed at the promoters of inositol- and fatty acid-responsive genes after recent transcription and played a negative role in their reinduction. Taken together, our data present a new mechanism by which H3K4 methylation regulates gene transcription.

Chromatin, the physiological template of all eukaryotic genetic information, is made of repeating nucleosomes. Each nucleosome consists of 147-bp DNA wrapping around a histone octamer, including two each of H2A, H2B, H3, and H4 (1). In the process of gene transcription, chromatin structure can be modulated at several levels, such as ATP-dependent chromatin remodeling (2), histone modifications (3), and nucleosome disassembly and reassembly (4).

Histone H3K4 methylation is one of the major histone modifications conserved in eukaryotes. Set1 is the catalytic subunit of a large complex named COMPASS (5), which is responsible for mono-, di-, and trimethylation observed in yeast (6). Set1-dependent methylation requires histone ubiquitination of lysine 123 of histone H2B via the ubiquitin-conjugating Rad6-Bre1 complex (7). Also, it is regulated by the COMPASS subunits (8). Set1-mediated H3K4 methylation positively regulates the activation of a subset of euchromatic genes, such as *RAM2*, *HAS1*, *INO1*, *PPH3*, and *MET16* (9, 10), but negatively regulates the activation of *PHO5* and *GAL1* (11). Histone H3K4 methylation, especially trimethylation, is usually associated with active transcription (12). However, our recent study shows that H3K4 trimethylation also associates with the repressed *PHO5* gene and remains essentially unchanged during *PHO5* activation and inactivation (13).

In the case of *GAL* genes, Set1-mediated H3K4 methylation is absent from their promoters under repressed conditions. During galactose induction, Set1 is co-transcriptionally recruited by the elongating RNA polymerase II (RNAPII)[2] and methylates the corresponding genes. Interestingly, hypermethylation of H3K4 persists after transcriptional inactivation for considerable time (~5 h), constituting a molecular memory within the mRNA coding region for recent transcriptional activity (14). It seems unlikely that such a robust and highly specific phenomenon occurring in living wild-type cells has no biological meaning. In this study, we confirm the post-transcriptional histone H3K4 methylation at the promoters of *GAL1* and *GAL10*. We further show that, after recent transcription, H3K4 was hypermethylated not only at the promoters but also across the ORF regions of *GAL1*/*10*. When analyzing the role of H3K4 methylation during the initial induction and reinduction of *GAL1*, we surprisingly found that elimination of H3K4 di- and trimethylation had a minor effect on the initial induction, especially within the first hour upon shifting cells from glucose to galactose culture, but greatly increased the reinduction kinetics of *GAL1*. Mechanistically, the Isw1 ATPase was absent from the *GAL1* gene after long-term (overnight) glucose repression but was present in the *GAL1* gene after a 1-h glucose repression. Isw1 functioned in the same genetic pathway as Set1-dependent H3K4 methylation to inhibit the reactivation of *GAL1*. Interestingly, we found that H3K4 methylation was also associated with many other inducible genes, such as *INO1* and *FOX2*, after recent transcription and inhibited the reinduction of the corresponding genes.

EXPERIMENTAL PROCEDURES

Strains and Antibodies—All strains originated from EUROSCARF. The strains constructed and antibodies used in this study are listed in supplemental Tables 1 and 2, respectively.

Quantitative RT-PCR—Total RNA was isolated from yeast cells with an RNeasy mini kit (Qiagen). cDNA was synthesized using a Moloney murine leukemia virus reverse transcriptase system and oligo(dT) (Promega). One microliter of the reverse transcription reaction was used in the subsequent real-time fluorescent quantitative PCR (Eppendorf).

ChIP Assay—The ChIP assay was performed as described previously (15). DNA from immunoprecipitated fractions and whole cell extracts (input) was analyzed by real-time PCR. The relative enrichment value represents the ratio of immunoprecipitated fractions to input at the indicated loci relative to the

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S4 and Tables 1 and 2.

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[2] The abbreviation used is: RNAPII, RNA polymerase II.

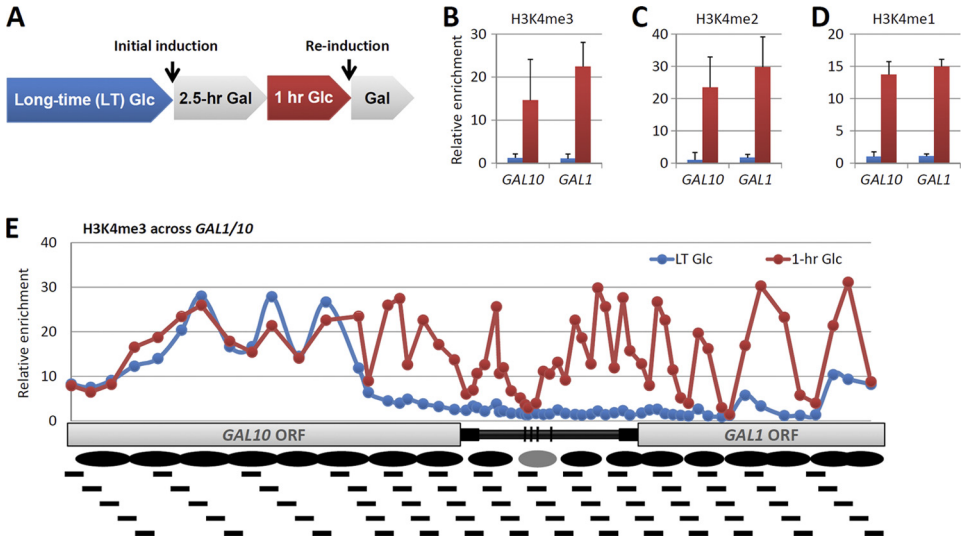

FIGURE 1. **Persistence of H3K4 methylation at the *GAL1/10* gene after transcriptional inactivation.** *A*, schematic of galactose induction and reinduction time course used for the following data sets. *B–D*, ChIP of H3K4me3, H3K4me2, and H3K4me1, respectively, at the promoters of *GAL1/10* under either long-term or 1-h glucose conditions. The *y axis* shows the abundance (immunoprecipitated fraction/input) at indicated loci relative to *TEL-VIR* (subtelomeric region of the right cerm of chromosome VI). Values that are >1 indicate more enrichment than the background. *E*, ChIP of H3K4me3 across *GAL1/10* genes. The *bottom black lines* indicate the locations of the overlapping primers. The *black ovals* are predicted nucleosomal loci.

corresponding internal control. All ChIP experiments were performed in triplicate on paired isogenic wild-type and mutant strains.

Preparation of Yeast Chromatin—Yeast chromatin was prepared as described previously (16). The chromatin pellets were resuspended in 1× SDS loading buffer, boiled, separated by 15% SDS-PAGE, and then subjected to Coomassie Blue staining or Western blotting.

RESULTS

Histone H3K4 Methylation Associates with Recently Repressed GAL1/10—In a previous study, Struhl and co-workers (14) showed that histone H3K4 methylation persisted after transcriptional repression for at least 5 h. To study the function of the persisting H3K4 methylation, we set up a similar assay detecting the level of H3K4 methylation at *GAL1*. Briefly, long-term glucose-cultured yeast cells were shifted into galactose culture for 2.5 h (initial induction). The culture was then shifted back to glucose culture for an additional 1 h. Finally, the short-term repressed cells were rechallenged by galactose (reinduction) (Fig. 1*A*). We compared the level of H3K4 methylation at the promoters of *GAL10* and *GAL1* between the long- and short-term repressed states. ChIP combined with real-time PCR was performed to detect the relative enrichment of H3K4 trimethylation (H3K4me3), dimethylation (H3K4me2), and monomethylation (H3K4me1) at the gene promoters (3). The results showed that H3K4me3, H3K4me2, and H3K4me1 were almost absent from the long-term repressed promoters but were readily apparent at the 1-h repressed promoters (Fig. 1, *B–D*). These data are consistent with the previous report showing that H3K4 methylation persisted for >5 h after transcriptional repression of *GAL10* (14). Distribution analysis of H3K4me3 with 60 overlapping primers throughout the promoter and gene coding regions of *GAL1/10* (17) showed that, in 1-h repressed cells, H3K4me3 occupied both the promoter regions and the ORF regions of *GAL1/10* genes (Fig. 1*E*). Together, these data suggest that repression-associated H3K4me3 covers the entire *GAL1/10* genes.

Elimination of Di- and Trimethylation of H3K4 Greatly Increases the Reactivation Rate of GAL1—The transcriptional induction of *GAL1* occurs with faster kinetics if it has been previously expressed, a phenomenon called transcriptional memory (18–20). Given that the H3K4 methylation was associated with recently expressed *GAL1*, we then wondered whether H3K4 methylation was involved in the formation of the transcriptional memory. To test this idea, we detected the effect of *SET1* deletion, which abolished H3K4 methylation (Fig. 2*A*), on the initial induction and reinduction of *GAL1*. A time course mRNA analysis was performed under conditions of repeated glucose repression and galactose induction as described for Fig. 1*A*. We detected the mRNA level of *GAL1* in the first hour upon galactose induction. From the results, we found that, in wild-type cells, the reactivation kinetics of *GAL1* after a 1-h repression was much more robust than the initial activation kinetics (Fig. 2*B*), supporting the transcriptional memory of *GAL1*. Previous reports showed that Set1 repressed *GAL1* transcription in galactose-containing medium plus 0.5% glucose (11) or *GAL1* induction from raffinose to galactose culture (21). We found that *SET1* deletion had a minor effect on the induction kinetics of *GAL1* from long-term glucose to galactose culture, especially within the first 3 h in galactose culture (Fig. 2, *B* and *C*, and supplemental Fig. 1*A*). In striking contrast, 1-h repressed *GAL1* was activated more rapidly in *set1*Δ cells than in wild-type cells (Fig. 2, compare *B* and *C*). We further checked if *SET1* deletion also increased the peak level of *GAL1* transcription during reactivation. The results demonstrated that the peak level in *set1*Δ cells during reactivation was comparable with that during initial activation (supplemental Fig. 1, compare *A* and *B*). Although Set1-dependent H3K4

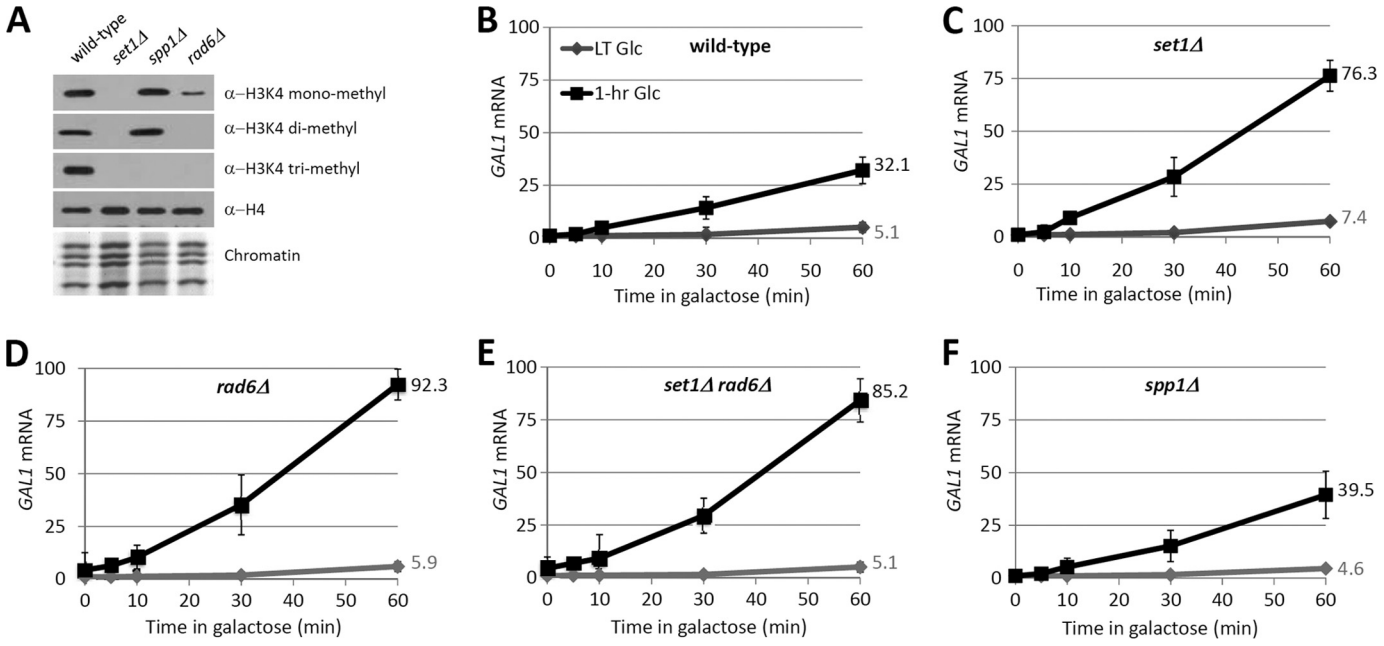

FIGURE 2. **Elimination of H3K4 di- and trimethylation accelerates the reactivation of *GAL1*.** *A*, *upper panel*, Western blot of wild-type and mutant strains. Antibodies used are indicated on the right. *Lower panel*, Coomassie Blue-stained histones (chromatin). *B–F*, induction and reinduction kinetics of *GAL1* in the genetic background as indicated. Cells were treated as depicted in Fig. 1*A* before galactose culturing. The mRNA level of *GAL1* was normalized to that of *ACT1*. The relative mRNA level in long-term (*LT*) glucose was defined as 1.

methylation slightly inhibits the initial induction of *GAL1* from glucose to galactose medium, it has a much stronger repressive effect on the reactivation of *GAL1*.

Next, we wondered which type(s) of H3K4 methylation was involved in the regulation of *GAL1* reactivation. We inactivated Spp1, one of the key subunits of COMPASS (8), and Rad6, the ubiquitin-conjugating enzyme responsible for H2BK123 ubiquitylation (supplemental Fig. 2*A*) (22). Western blot analysis showed that *SPP1* deletion specifically eliminated the trimethylation of H3K4, whereas *RAD6* deletion eliminated both di- and trimethylation of H3K4 (Fig. 2*A*). We then analyzed the roles of Rad6 and Spp1 in the initial induction and reinduction of *GAL1*. Notably, *RAD6* deletion also increased the reactivation rates of *GAL1* and *RAD6*; *SET1* double deletion resembled this phenotype (Fig. 2, *B–E*). We also found that H2BK123 ubiquitylation was associated with *GAL1/10* promoters 1 h after glucose repression (supplemental Fig. 2*B*). Therefore, H2B ubiquitylation functions in the same genetic pathway as H3K4 methylation. In contrast, *SPP1* deletion did not affect the reactivation rate of *GAL1* (Fig. 2, compare *B* and *F*), suggesting that either di- or trimethylation is sufficient for the function of H3K4. Therefore, we concluded that H2BK123 ubiquitylation-dependent H3K4 methylation after transcriptional inactivation inhibits the reactivation of *GAL1*.

Isw1 Negatively Regulates Galactose Memory in an H3K4 Methylation-dependent Manner—According to the literature, the repressive effect of H3K4me3 on transcription involves the Isw1 ATPase (23, 24). We hypothesized that the reduction of transcriptional competence of *GAL* genes during reactivation by H3K4 methylation requires Isw1. We then examined the binding of Isw1 protein at the promoter, 5′-region, and 3′-region of *GAL1* (Fig. 3*A*) after long-term or 1-h glucose culturing. As shown in Fig. 3*B*, there was little Isw1 binding at *GAL1* in the long-term repressed state. Interestingly, transcriptional inactivation recruited Isw1 to the *GAL1* gene (Fig. 3*B*). Set1, but not Spp1, was essential for the association of Isw1 with *GAL1* (Fig. 3*B*). Accordingly, *ISW1* deletion led to a subtle increase in the kinetics of the initial induction of *GAL1* but a substantial increase in mRNA levels during the reinduction of *GAL1* (Fig. 3, compare *C* and *D*). *isw1*Δ*set1*Δ double mutant cells resembled *isw1*Δ single mutant cells in the induction and reinduction kinetics of *GAL1* (Fig. 3, *C–E*). Therefore, we concluded that the Isw1 ATPase occupies the recently repressed *GAL1* gene in an H3K4 methylation-dependent fashion, and, like H3K4 methylation, the Isw1 ATPase particularly inhibits the reactivation of *GAL1*.

Histone H3K4 Methylation Suppresses the Action of RNAPII during GAL1 Reactivation by Isw1—To address how the recruitment of Isw1 affects the induction of *GAL1*, we examined the occupancy of RNAPII at the promoter and 5′- and 3′-ends of the *GAL1* ORF (Fig. 3*A*). ChIP of Rpb3, one of the subunits of RNAPII, showed that, in all strains, Rpb3 was absent from *GAL1* before induction or reinduction (Fig. 4*A*), excluding the possibility that RNAPII pre-occupied *GAL1* in the *set1*Δ or *isw1*Δ strain. As expected, when the *GAL1* gene was initially induced, little Rpb3 was recruited in the wild-type and mutant strains (Fig. 4*A*). For the wild-type strain, we observed an obvious 5′-bias for RNAPII distribution during the first hour of the reinduction procedure. In striking contrast, in the *isw1*Δ strain, Rpb3 was localized predominantly to the 3′-region of *GAL1* during *GAL1* reactivation, suggesting that Isw1 inhibits transcription elongation during *GAL1* reactivation. A similar 3′-bias for Rpb3 was also evident in the *set1*Δ strain (Fig. 4*A*), suggesting a related function.

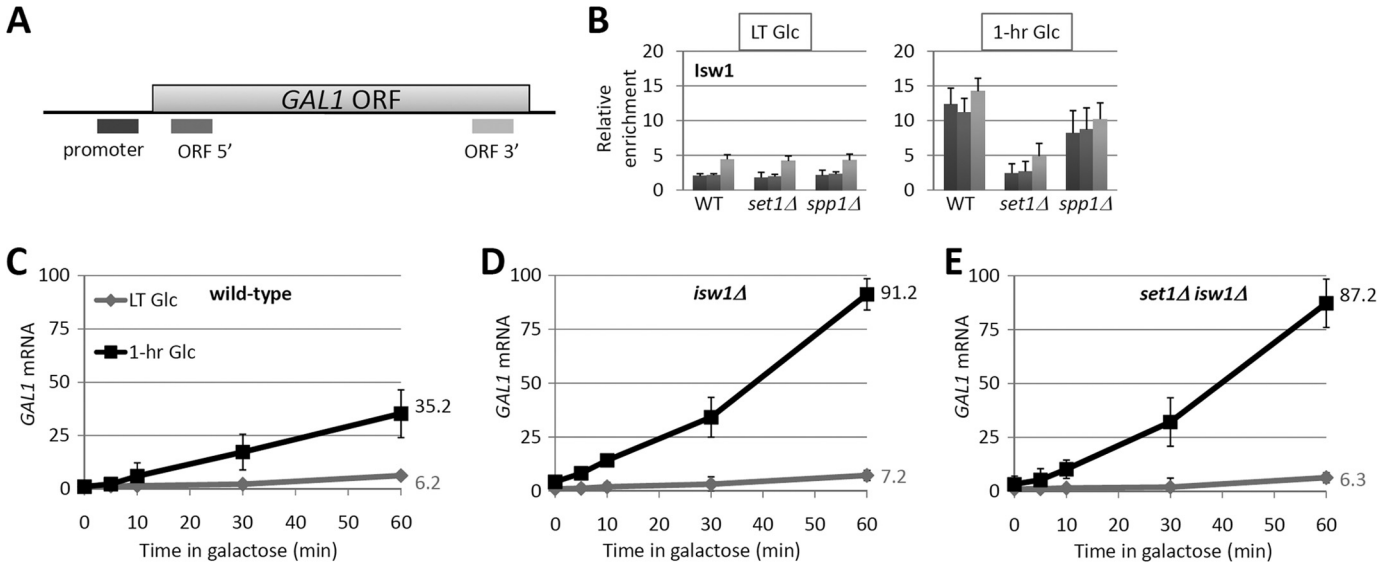

FIGURE 3. **Histone H3K4 methylation recruits the Isw1 ATPase to recently transcribed *GAL1*.** *A*, schematic of the *GAL1* gene and the location of the primers used in the following experiments. *B*, ChIP of Myc-tagged Isw1 in wild-type and mutant cells. Cells were treated as depicted in Fig. 1*A*. *C–E*, induction and reinduction kinetics of *GAL1* in wild-type and mutant cells. mRNA analysis was performed as described in the legend to Fig. 2*B*. *LT*, long-term.

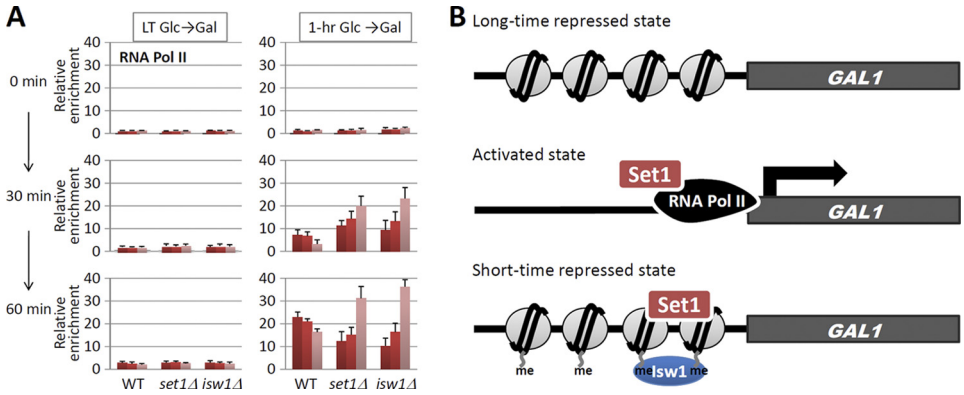

FIGURE 4. **Histone H3K4 methylation reduces the processivity of RNAPII during *GAL1* reactivation by Isw1.** *A*, ChIP of Myc-tagged Rpb3 in wild-type and mutant cells cultured in glucose for various time as depicted in Fig. 1*A*. *LT*, long-term; *RNA Pol II*, RNAPII. *B*, model for the inhibition of *GAL1* reactivation by short-term H3K4 methylation. In the long-term repressed state, histone H3K4 methylation is absent from *GAL1*. Upon gene activation, Set1 is recruited by the elongating RNAPII and persists for a short time after the shutoff of gene transcription. This short stay of Set1 is sufficient for methylating the newly incorporated histone at the corresponding gene upon transcriptional inactivation. The Isw1 ATPase is then recruited by di- and trimethylated H3K4 onto methylated *GAL1* and suppresses the action of RNAPII during gene reactivation. Because Set1 is quickly released from the gene after repression, the induced H3K4 methylation is diluted and finally lost with cell division.

Epigenetic Memory of Inositol- and Fatty Acid-responsive Genes after Recent Transcription—We were curious whether the function of H3K4 methylation in galactose metabolism could be extended to other cellular processes. Therefore, we chose five other external stimulus-responsive genes, including *INO1* (induced by inositol), *FOX2* (induced by oleic acid), *HSP26* (induced by high temperature), *MET16* (induced by methionine starvation), and *PHO5* (induced by phosphate starvation), and investigated whether these stimuli affect H3K4 methylation at the corresponding genes (Fig. 5*A*). In the long-term repressed state, H3K4me3 was absent from the promoters of *INO1*, *FOX2*, and *HSP26* but present at the promoters of *MET16* and *PHO5* (Fig. 5*B*). Interestingly, in the 1- or 3-h repressed state, the H3K4me3 level was significantly increased at the promoters of *INO1* and *FOX2* but remained unchanged at the promoters of *HSP26*, *MET16*, and *PHO5* (Fig. 5*B*), suggesting that post-nutrient stimuli are able to induce H3K4 methylation at the promoters of *INO1* and *FOX2*.

SET1 Deletion Increases the Reactivation Rates of Inositol- and Fatty Acid-responsive Genes—Next, we assessed the function of H3K4 methylation in the induction and reinduction of these genes. The mRNA analysis of *INO1* showed that, in wild-type cells, the reactivation rate of *INO1* was much slower than the initial one (Fig. 6*A*) (18, 25). In striking contrast, the reactivation kinetics of *INO1* resembled the initial activation kinetics in *set1*Δ cells (Fig. 6, *A* and *B*), suggesting that, under physiological conditions, H3K4 methylation negatively regulates the reactivation of *INO1*. Because H3K4 methylation repressed the initial induction of *INO1* (Fig. 6, *A* and *B*), we concluded that the repression-induced H3K4 methylation strengthens the repressive effect on *INO1* reinduction. Additionally, we noticed that, in *set1*Δ cells, the mRNA level of *INO1*

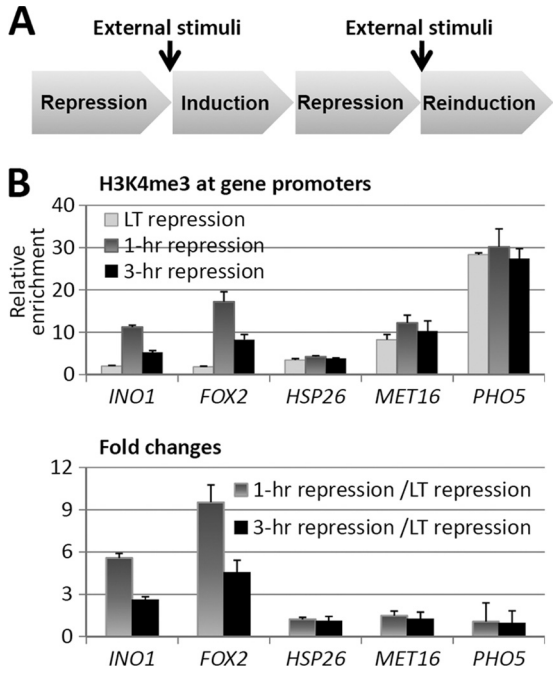

FIGURE 5. **Recent transcription of inositol- or fatty acid-responsive genes establishes H3K4 methylation at the promoters of the corresponding genes.** *A*, schematic of the induction and reinduction time course of the inducible genes used for the following data sets. *B*, ChIP of H3K4me3 at the promoter of inducible genes before and after recent induction as depicted in *A*. The *upper panel* shows the abundance of H3K4me3, and the *lower panel* shows the -fold changes after recent induction. *LT*, long-term.

after a 1-h repression was much higher than that in the long-term repressed state (Fig. 6, *A* and *B*), indicating that *SET1* also delays the transcriptional repression of *INO1*.

By analyzing the induction and reinduction kinetics of *FOX2*, we were surprised to find that *FOX2* also conferred transcriptional memory: *FOX2* was activated much more rapidly when rechallenged with oleic acid (Fig. 6*C*). However, the transcriptional memory of *FOX2* was lost within 3 h (Fig. 6*C*). This is likely a *bona fide* short-term memory because such a dramatic difference in mRNA levels is hard to be explained by dilution of the cells due to cell division. Strikingly, the Set1-mediated H3K4 methylation differentially affected the induction and reinduction of *FOX2*: in *set1*Δ cells, the initial induction rate of *FOX2* was dramatically reduced, whereas the reinduction rate of *FOX2* was restored to the wild-type level (Fig. 6, *C* and *D*). There are two possibilities for this phenomenon. First, Set1-mediated H3K4 methylation specifically regulates the initial induction of *FOX2*; second, Set1-mediated H3K4 methylation positively regulates the initial induction of *FOX2*, but, during reactivation of *FOX2*, the repression-associated H3K4 methylation overcomes the positive effect of co-transcriptional H3K4 methylation. We favor the later model because H3K4 methylation was absent in long-term repressed *FOX2* but was abundant in recently repressed *FOX2*, providing a molecular basis for an additional function of Set1-mediated H3K4 methylation in *FOX2* reactivation.

To investigate whether Set1-mediated H3K4 methylation regulates the reactivation of *INO1* and *FOX2* by the same mechanism as in *GAL1*, we detected the effect of *RAD6* or *SPP1* deletion on the induction and reinduction of *INO1* and *FOX2*. The results showed that the induction and reinduction kinetics of both genes in either *rad6*Δ or *spp1*Δ cells resembled the wild-type levels (supplemental Fig. 3, *A* and *B*), implying that mono-, di-, and trimethylation of H3K4 are all required. Moreover, *ISW1* deletion did not affect the transcription of *INO1* and *FOX2* (supplemental Fig. 3, *A* and *B*), suggesting that Set1 regulates the transcription of these genes independently of the Isw1 pathway. For the three other genes, whose H3K4 methylation was unaffected by recent stimuli, the reinduction kinetics of these genes were similar to the initial induction kinetics in both wild-type and *set1*Δ cells (Fig. 6, *E–J*), suggesting that they do not show transcriptional memory and that H3K4 methylation does not differentiate the reinduction rate from initial induction rate of these genes. Taken together, post-stimulus-dependent H3K4 methylation suppresses gene reactivation of genes.

DISCUSSION

In this study, we investigated the dynamic association of histone H3K4 methylation at *GAL1* in the time course of repression-initial induction-repression-reinduction. Elimination of H3K4 methylation specifically affected the reactivation rate of *GAL1* within the first hour of galactose induction. Furthermore, we showed that both di- and trimethylation of H3K4 were involved in the regulation of *GAL1* reactivation. In contrast to that under long-term glucose conditions, the Isw1 ATPase was transiently localized to *GAL1* and reduced the transcriptional competence of *GAL1* under the short-term glucose conditions (Fig. 4*B*). Interestingly, the inhibition of reinduction of genes by Set1-mediated H3K4 methylation was also observed in many other inducible genes, such as inositol- and fatty acid-responsive genes. Therefore, our data suggest that the transient H3K4 methylation induced by recent transcription reduces the transcriptional competence of the corresponding genes for reactivation.

Repressive Role of Histone H3K4 Methylation during Gene Activation—Despite the general idea that Set1 is associated with active transcription (26–28), an increasing number of arguments suggest that Set1-mediated H3K4me2/3 is also involved in gene repression. It was shown that Set1 is required for silencing of Ty1 retrotransposons *GAL1* and *PHO84* (6, 11), subsequently found to be noncoding RNA-mediated (21, 29–31). Recently, scientists (21, 32) provided evidence that H3K4me2/3 recruits the Rpd3S complex to *GAL1* as well as many other inducible genes that show cryptic transcription. In the case of *PHO5*, our recent study showed that H3K4 methylation recruits the Rpd3L complex, rather than the Rpd3S complex, to the *PHO5* promoter and suppresses the aberrant chromatin remodeling at the *PHO5* promoter (13). In this study, we have shown that H3K4 methylation recruited the Isw1 ATPase to *GAL1* (Fig. 3). It has been documented that the chromatin association of Isw1 depends on Set1 *in vivo* and *in vitro* (24). Therefore, Isw1 is an important effector of Set1 in regulating gene transcription (23, 24). Our data showed that Isw1 mediated the repressive effect of Set1 on the reactivation of *GAL1* (Figs. 3 and 4). Isw1 is also found to antagonize the positive role of the SWI/SNF complex in the reactivation of *GAL* genes (19). Therefore, it is likely that H3K4 suppresses

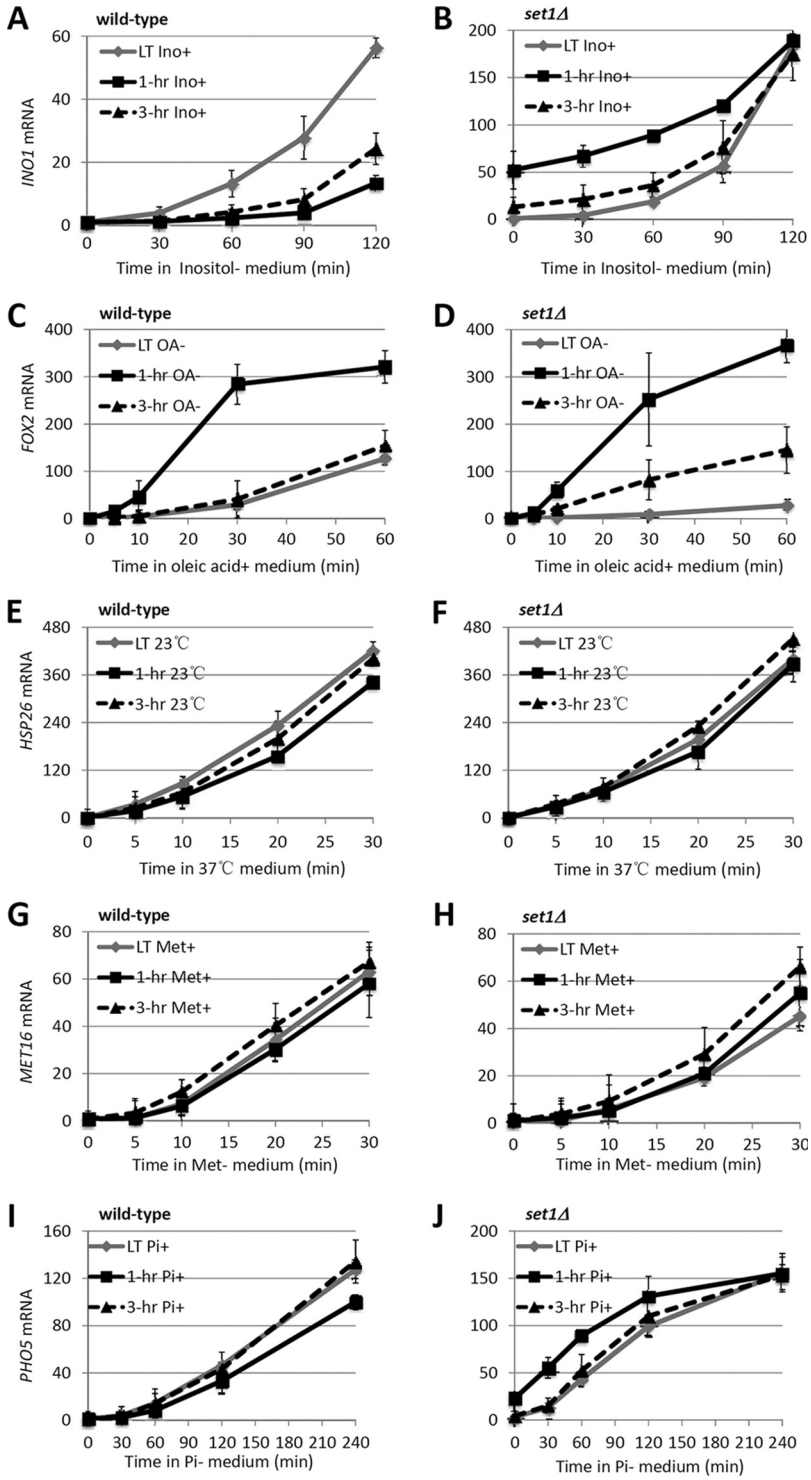

FIGURE 6. **H3K4 methylation after transcriptional inactivation negatively regulates the reactivation of *INO1* and *FOX2*.** The Induction and reinduction kinetics are shown for *INO1* (*A* and *B*), *FOX2* (*C* and *D*), *HSP26* (*E* and *F*), *MET16* (*G* and *H*), and *PHO5* (*I* and *J*) in wild-type and *set1*Δ cells. *LT*, long-term; *Ino*, inositol; *OA*, oleic acid.

GAL1 reactivation through the chromatin remodeling pathway. However, Isw1 seems dispensable for the function of Set1 in the transcription of *INO1* and *FOX2* (supplemental Fig. 3, *A* and *B*). This is not unexpected because the effectors of Set1 usually vary at different genes. It will be of interest to determine the effectors of Set1 in the transcription of *INO1* and *FOX2*.

It was recently reported that Set1 does not preferentially affect *GAL1* reactivation (19, 33), whereas our data argue that Set1 inhibits *GAL1* reactivation. The discrepancy could be attributed to different time course settings for studying *GAL1* induction and reinduction. Although *SET1* deletion increased *GAL1* reactivation in all three systems, the relative

differences in previous systems were modest (supplemental Fig. 4). In addition, we noticed that either the PCR signal of *GAL10* on the agarose gel (see Fig. 3*B* in Ref. 33) or the signal of *GAL1* on the Northern blot (see Fig. 3*A* in Ref. 19) during reactivation in wild-type cells had already been saturated and was therefore hard to distinguish from that in *set1*Δ cells. In our study, we monitored the mRNA level of *GAL1* in a more quantitative manner by using real-time PCR and thereby observed an obvious increase in the *GAL1* reactivation rate by *SET1* deletion.

Regulation of Gene Reactivation by Short-term H3K4 Methylation Represents a Distinct Function of H3K4 Methylation in Gene Transcription—The function of H3K4 methylation after transcriptional inactivation can be either in concert with or contrary to that of co-transcriptional H3K4 methylation. In the case of galactose- or inositol-responsive genes, co-transcriptional H3K4 methylation negatively regulated the expression of the corresponding genes during the activation processes (Fig. 2, *B* and *C*, and Fig. 6, *A* and *B*). Transient H3K4 methylation after transcriptional activation further reduced the transcriptional competence of these genes for reactivation. Hence, the function of H3K4 methylation in gene reactivation is a combined effect of both the recent transcription-induced one and the co-transcription one. In the case of fatty acid-responsive genes, the situation is different. Co-transcriptional H3K4 methylation positively regulated the initial induction of *FOX2* (Fig. 6, *C* and *D*). However, *SET1* deletion had little effect on the reinduction of *FOX2* compared with the wild type (Fig. 6, *C* and *D*), suggesting that H3K4 methylation after transcriptional inactivation plays a negative role in *FOX2* transcription, which counteracts the positive effect of co-transcriptional H3K4 methylation during *FOX2* reinduction.

Possible Physiology of the Epigenetic Memory after Recent Transcription—The inhibition of stimulus-responsive genes by H3K4 methylation represents a mechanism for the yeast cells to delay the second-round transcriptional response to external stimuli. Why do the yeast cells do that? We propose that yeast cells undergo a series of cellular changes after recent stimuli, and, regarding the changes to a certain stimulus, some are beneficial, some are dispensable, whereas some are even harmful. Irreversible cellular changes may result in either evolution or diseases. Therefore, cells must possess an ability to quickly reset their inherited status after "adaptive" changes (34). Recent studies by others also suggest mechanisms for the yeast cells to accelerate the second-round transcriptional response to galactose stimuli (18–20). Therefore, we propose that there is a balance between different mechanisms when the yeast cells adapt to the environment.

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