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Genetics

Yeast histone H3 and H4 N termini function through different *GAL1* regulatory elements to repress and activate transcription

(*Saccharomyces cerevisiae*/nucleosome/promoter/*PHO5*/β-galactosidase)

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ABSTRACT **Previous work has shown that N-terminal deletions of yeast histone H3 cause a 2- to 4-fold increase in the induction of *GAL1* and a number of other genes involved in galactose metabolism. In contrast, deletions at the H4 N terminus cause a 10- to 20-fold decrease in the induction of these same *GAL* genes. However, H3 and H4 N-terminal deletions each decrease *PHO5* induction only 2- to 4-fold. To define the *GAL1* gene regulatory elements through which the histone N termini activate or repress transcription, fusions were made between *GAL1* and *PHO5* promoter elements attached to a β-galactosidase reporter gene. We show here that *GAL1* hyperactivation caused by the H3 N-terminal deletion Δ4–15 is linked to the upstream activation sequence. Conversely, the relative decrease in *GAL1* induction caused by the H4 N-terminal deletion Δ4–28 is linked to the downstream promoter which contains the TATA element. These data indicate that the H3 N terminus is required for the repression of the *GAL1* upstream element, whereas the H4 N terminus is required for the activation of the *GAL1* downstream promoter element.**

While the nucleosomal particle is generally regarded as a repressor of transcription initiation *in vivo* (1, 2), its histones have a number of unique functions mediated by the N termini of the individual histones. These positively charged N-terminal "tails" extend from the nucleosomal core and are modified posttranslationally by charge-altering modifications such as acetylation and phosphorylation (3). The histone H3 and H4 N termini are involved to different extents in the repression of heterochromatin regions (those found at telomeres and silent mating loci) of yeast. H2A and H2B N termini do not appear to share these functions (4). In addition, H3 N-terminal deletions (removing residues 4–15) increase *GAL1, GAL7,* and *GAL10* transcription in the presence of galactose by ≈3-fold (5). In contrast, deletion of residues 4–28 at the H4 N terminus causes a 10- to 20-fold decrease in the induction of these *GAL* genes (6). To ask whether the H3 and H4 N-terminal deletions cause changes in chromatin structure at the *GAL1* promoter which may help explain these opposite effects, Fisher-Adams and Grunstein (7) recently examined the accessibility of a number of enzymes to the *GAL1* promoter *in vivo* (using ectopically produced *Escherichia coli* Dam methylase) or *in vitro* (with micrococcal nuclease). Coincident with the decrease in *GAL1* activity seen for H4 N-terminal deletions, these deletions reduced the accessibility of these enzymes to the region adjacent to the *GAL1* TATA element, converting it from an apparently "open" conformation to a "closed" one. Similar changes did not occur as a result of H3 N-terminal deletions. Therefore the H4 N terminus is important for maintaining the chromatin structure of the *GAL1* TATA promoter region. However, the H3 N terminus exerts its effect either by altering the TATA region in an imperceptible manner or by regulating a different *GAL1* promoter element.

To determine through which *GAL1* regulatory elements H3 and H4 N termini exert their effects, we pursued the following strategy. Previous work had shown that, in contrast to their effect at *GAL1*, H3 and H4 N-terminal deletions caused a similar, modest, decrease (2- to 4-fold) in *PHO5* mRNA levels (5, 6). Therefore, we made chimeric promoters containing upstream activation sequence (UAS) elements of one promoter and downstream (TATA) elements of the other. These were fused to the *E. coli* β-galactosidase (*lacZ*) reporter gene, in order to determine which of these regulatory regions mediate the differences in transcription caused by H3 or H4 N-terminal deletions. Our data suggest that the H3 N terminus functions through the *GAL1* UAS element, whereas the H4 N terminus regulates the downstream promoter region containing the TATA box.

MATERIALS AND METHODS

Yeast Media, Strains, and Plasmids. SR−Ura−Trp is yeast minimal medium containing 2% raffinose. It is supplemented with standard nutrients (5) except for uracil and L-tryptophan. Galactose was added (2%, wt/vol) to make SR/G-Ura-Trp. Phosphate-free YEPD was made as described (2).

Yeast strains PKY501 and PKY813 (6) were described previously. RMY200U and RMY415U are isogenic and are similar to RMY200 and RMY415 (5), respectively, except that the *TRP1* gene was replaced by the *URA3* gene. Plasmids and promoters they contain are as follows: pRY131(*TRP1*), *GAL1*; pJWP400, *PHO5*; pJWG107, *PHO5*-URS-*GAL1*; pJWG113, *PHO5-GAL1*; pJWP403, *GAL1-PHO5*; pJWP402, *PHO5-GAL1**; pJWG116, *GAL1-PHO5**; pJWP405, *5GAL4-PHO5*. These plasmids carry the *TRP1* gene and the 2μ plasmid replication origin. All cloning and plasmid preparations were performed as described (8). Yeast transformations were performed with the standard lithium acetate protocol. All PCR-generated fragments for plasmid constructions were confirmed by dideoxy sequencing of the final product with Sequenase (United States Biochemical). Plasmid pRY131(*TRP1*) was described (6). pJWP400 was constructed by ligating the 3.5-kb *Eco*RI–*Sal* I fragment containing the *PHO5-lacZ* fusion from pJWP401 to the 7-kb *Eco*RI–*Sal* I fragment from the vector pYcDE-2 (*TRP1*, 2μ) (9). pJWP401 was constructed by ligating the 600-bp *Bam*HI fragment containing the *PHO5* promoter from pMH313 (2) to the *Bam*HI site on pSEYC102(*TRP1*) in the orientation which drives *lacZ* expression. pSEYC102(*TRP1*) is pSEYC102 (6) with the *URA3* gene disrupted by *TRP1*. pJWP402 is pJWP400 with the *PHO5* TATA sequence 5′-GTATATAAGCG-3′ changed to 5′-ATATATAAATG-3′ by recombinant PCR (10). The *Bam*HI sites flanking the promoter were used for cloning. pJWP403 was made by

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Abbreviations: UAS, upstream activation sequence; UAS_G, upstream activation sequence of the *GAL1* promoter; URS_G, upstream repression sequence of the *GAL1* promoter.

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ligating a 300-bp *Bam*HI fragment containing the *GAL1-PHO5* promoter from pJWP302 to the *Bam*HI site in pJWP404 in the orientation which drives *lacZ* expression. pJWP404 is pJWP400 with the 600-bp *Bam*HI fragment containing the *PHO5* promoter removed. pJWP302 was constructed as follows. A DNA fragment was synthesized by PCR using the oligonucleotides BS7 (5′-GTCGGATCCCTCGAGCCCGGGTGGTCACCTT-ACTTGGCAAGGC-3′) and BS8 (5′-GTCGCACAGCGTG-TACC-3′) and pMH313 as the template. After cleavage by *Bam*HI, this fragment contains sequences from −175 to +6 relative to the *PHO5* translational start site. Hence, the fragment is without the *PHO5* UAS. At the 5′ end of this fragment, a *GAL1* UAS also generated by PCR was cloned into the *Xho* I site. The *GAL1* UAS was made by using the oligonucleotides BS3 (5′-GTCCTCGAGGTACGGATTAGAAGCCGCCGA-3′) and BS4 (5′-GTCCTCGAGGTTCGGAGCAGTGCGGCGCGA-3′) and pRY131 (11) as the template for PCR. This 300-bp *Bam*HI fragment containing the *GAL1-PHO5* promoter was then cloned into the *Bam*HI site of pMH313(*TRP1*) (6), replacing the wild-type *PHO5* promoter to form pJWP302. pJWP405 is pJWP403 except that its *Eco*RI–*Bam*HI fragment, containing the *GAL1* UAS, is replaced by an *Eco*RI–*Bam*HI fragment which contains five synthetic GAL4 binding sites (5′-CGGAGTACT-GTCCTCCG-3′). The five GAL4 binding sites were made by PCR with oligonucleotides JW27 (5′-GTCGAATTCGGATC-CGCATGCCTGCAGGT-3′) and JW28 (5′-CCATTATATAC-CCTCTCGAGTC-3′) and $GAL4_5$/E1bCAT (see Acknowledgements) as the template. pJWG107 contains the *GAL10-GAL1* promoter fused to the *lacZ* gene as in pRY131 except that *GAL1* UAS sequence from −559 to −299 relative to the ATG translational initiation codon was replaced by the *PHO5* UAS, which was inserted by means of an *Xho* I linker. First, the *GAL1* UAS was deleted by combining the fragments from pLR1Δ2 (≈10 kb *Bam*HI), pRY121-522 (≈0.5 kb *Bam*HI–*Xho* I) and pRY123-261 (≈0.3-kb *Bam*HI–*Xho* I fragment) in a three-way ligation reaction as described in West *et al.* (11). *PHO5* UAS was made by using BS1 (5′-GTCCTCGAGCTTATGTGCGCTGCTTTA-ATG-3′), BS2 (5′-GTCCTCGAGATTTGATAATTTGGCAT-GTGCG-3′), and pMH313 as the template for PCR. Finally, the *URA3* gene on the fragment from pLR1Δ2 was replaced by a *TRP1* gene to form pJWG107. pJWG113 is identical to pJWG107 except that the *GAL1* sequence from −559 to −190 upstream of the ATG was replaced by the *PHO5* UAS (see construction of pJWG107) cloned with *Xho* I linkers. Deletion of this *GAL1* sequence was done by combining the fragments from plasmids pJWG107 (≈11-kb *Bam*HI), pLR1Δ20B (≈0.3-kb *Bam*HI–*Xho* I), and pRY123-261 (≈0.3-kb *Bam*HI–*Xho* I) in a three-way ligation reaction (11). pJWG116 is identical to pRY131 except that the *GAL1* TATA sequence (5′-ATATATAAATG-3′) has been replaced by the *PHO5* TATA sequence (5′-GTATATA-AGCG-3′) by recombinant PCR (10). The *URA3* gene in pRY131 has been replaced by the *TRP1* gene in the making of pJWG116.

β-Galactosidase Assays. To induce promoters containing the *GAL1* UAS, the strains were grown in SR−Ura−Trp overnight at 30°C so that the OD_{600} was ≈1.0 the next morning. The cells were then pelleted, resuspended, and diluted in SR/G-Ura-Trp to an OD_{600} of 0.2–0.4 for induction at 30°C. A sample (100 μl) from each culture was taken to assay for β-galactosidase activity as described (12). To induce promoters containing the *PHO5* UAS, the strains were grown in SD−Ura−Trp as above. The cells were then pelleted, resuspended, and diluted in YEPD to an OD_{600} of 0.2–0.4. After 2.5 hr at 30°C, the cells were pelleted, washed once with water, and suspended in phosphate-free YEPD for induction at 30°C (12). Induction of the promoter *PHO5*-URS-*GAL1* was done similarly except that glucose was replaced by galactose in all media used.

RESULTS

Hyperactivation of *GAL1* by Histone H3 N-Terminal Deletion Δ4–15 Is Linked to the *GAL1* UAS. The *GAL1* promoter is repressed in medium containing glucose. Catabolite repression of *GAL1* is mediated through both the upstream repressor sequence (URS_G) and the UAS_G (13, 14). In the presence of noninducing carbon sources such as raffinose, the activator protein GAL4 recognizes the UAS_G element; however, its activation function is prevented by association with GAL80 protein. In medium with galactose, the inhibition by GAL80 is alleviated and GAL4 protein stimulates activity of the basal transcription machinery which functions through the TATA element (15, 16). At this region, the TATA box is recognized by the TATA-binding protein (TBP), other factors of the basal transcription machinery, and RNA polymerase II (17, 18). The *PHO5* gene is regulated by the level of inorganic phosphate in the medium. In the presence of medium depleted of phosphate, the activator proteins, PHO4 and PHO2, whose activity is otherwise regulated by PHO80, function through the UAS element to activate the basal transcription machinery at the TATA region (19, 20). The UAS element of each of these genes can be activated by their inducers even when fused to heterologous TATA elements (21, 22).

Histone H3 N-terminal deletions cause hyperactivation at the *GAL1* promoter by 2- to 4-fold but an ≈2-fold decrease in *PHO5* activation (5). To determine whether this *GAL1*-specific hyperactivation is linked to its UAS- or TATA-containing elements, we made chimeric promoters (*GAL1* UAS-*PHO5* TATA and *PHO5* UAS-*GAL1* TATA, designated below as *GAL1-PHO5* and *PHO5-GAL1*, respectively) with the upstream promoter element of one gene and the downstream promoter element of the other. To construct the *GAL1-PHO5* promoter, we used sequences from −456 to −333 bases upstream of the translational start site of the *GAL1* gene as the *GAL1* UAS. These sequences contain all four GAL4 binding sites (11, 22, 23). The *PHO5* downstream promoter (TATA-containing region) contains nt −175 to +6 relative to the *PHO5* translational start site, which excludes all the UAS elements required for phosphate regulation (19, 24). These chimeric promoters were fused in frame to the *E. coli lacZ* reporter gene so that we might use β-galactosidase levels to accurately measure promoter activity.

Plasmids containing the chimeric or wild-type promoters were transformed into RMY415U, a strain bearing the H3 N-terminal deletion which eliminates residues 4–15 of this 135-aa acid protein (hereafter designated as the H3 Δ4–15 strain) and into an isogenic wild-type strain, RMY200U. To determine transcriptional activity, strains containing plasmids with the *GAL1* UAS were grown overnight in medium containing raffinose and then induced for 0–8 hr in medium containing both galactose and raffinose. Strains with plasmids containing the *PHO5* UAS were grown in glucose medium overnight and then induced for 0–8 hr in a phosphate-free medium containing glucose. After induction, samples from the cultures were assayed for β-galactosidase activity.

The differences in response to galactose induction between the *GAL1* and chimeric *GAL1-PHO5* constructs are shown in Fig. 1. At 6 hr of induction, activation of the *GAL1* and *GAL1-PHO5* promoters was approximately 2.2- and 1.5-fold higher respectively in the H3 Δ4–15 strain than in the wild-type strain. These data show that regardless of the TATA-containing downstream promoter used, the *GAL1* and *GAL1-PHO5* regulatory sequences responded to the H3 deletion by a similar increase in transcription. Therefore, hyperactivation caused by the H3 Δ4–15 deletion is linked to the 124-bp *GAL1* UAS. For purposes of comparison, the response of all constructs described below was determined at 6 hr of induction. We then wished to determine whether the effect of the H3 N-terminal deletion on *GAL1* hyperactivation was mediated

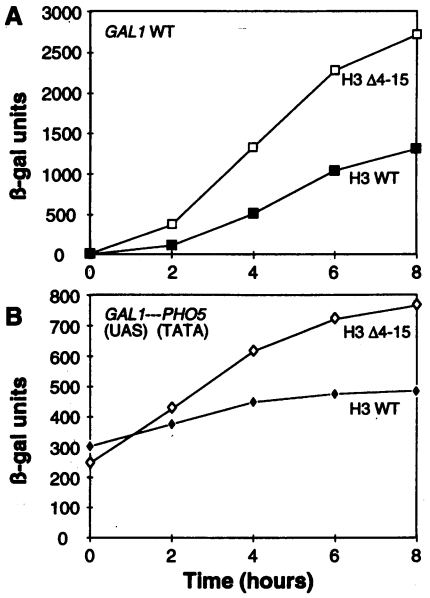

FIG. 1. Time course showing hyperactivation of the wild-type *GAL1* promoter (*A*) and the chimeric *GAL1-PHO5* promoter (*B*) by H3 Δ4–15. Time points were taken 0, 2, 4, 6, and 8 hr after induction in a 2% galactose/2% raffinose medium. The *GAL1-PHO5* promoter in *B* was constructed by fusing the 124-bp *GAL1* UAS region upstream of the 181-bp PHO5 TATA region (see text). β-gal, β-galactosidase.

solely through the GAL4 binding sites. A construct (*5GAL4-PHO5*) was made which replaces the *GAL1 UAS* in the *GAL1-PHO5* promoter with five synthetic 17-bp GAL4 binding sites (25), eliminating other elements found at the *GAL1* UAS (14, 26). After 6 hr of induction, the *5GAL4-PHO5* promoter was hyperactivated by ≈1.8-fold in the absence of the H3 N-terminal sequences, compared with ≈1.5-fold for the *GAL1–PHO5* promoter (Fig. 2). We conclude that the GAL4 binding sites mediate the hyperactivation of the *GAL1* promoter in a H3 Δ4–15 deletion genetic background.

If the effect of H3 Δ4–15 on transcription is linked to the UAS region as suggested above, then induction of promoters containing the *PHO5* UAS should all decrease in the absence of the H3 N terminus, regardless of which TATA-containing element they carry. To construct the *PHO5-GAL1* promoter,

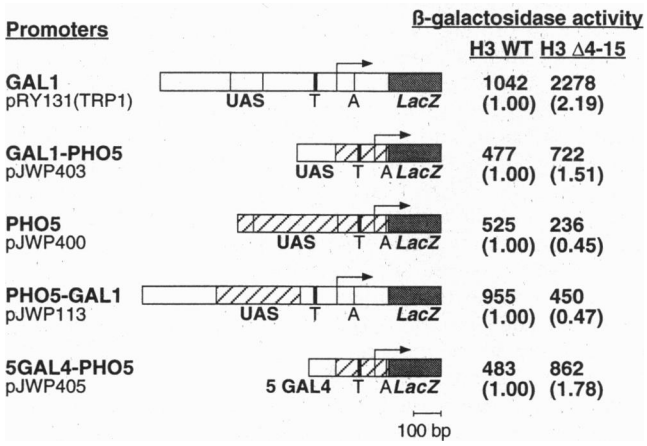

FIG. 2. Linkage of *GAL1* promoter hyperactivation in the presence of H3 Δ4–15 to the *GAL1* UAS_G. All wild-type and chimeric promoters were induced for 6 hr. Changes in β-galactosidase activity relative to the wild-type strains are shown in parentheses. *GAL1*, *PHO5*, and *lacZ* sequences are represented by the open, hatched, and solid boxes respectively. The TATA boxes and the translational start sites are marked underneath with the letters T and A, respectively. The transcriptional start sites are indicated by the arrows.

we used the sequence from −483 to −176 bases upstream of the *PHO5* translational start site as the *PHO5* upstream promoter (UAS). These sequences contain all UAS elements required for phosphate regulation (19, 24). The *GAL1* TATA-containing region was taken from position −190 to +88 relative to the translational start site of the *GAL1* promoter. Induction of both the *PHO5* and *PHO5-GAL1* promoters was decreased similarly (2.2 and 2.1 fold, respectively) by H3 Δ4–15 (Fig. 2). Therefore, the effect of the H3 N-terminal deletion on the *PHO5* promoter is also linked to its UAS domain.

Reduced *GAL1* Transcription Resulting from the H4 N-Terminal Deletion Δ4–28 Is Linked to the Downstream Promoter Region. We used the chimeric promoters (above) to determine *GAL1* regulatory sequence linkage upon H4 N-terminal deletion (Δ4–28) (Fig. 3*A*). The activation levels of the *GAL1* and *PHO5-GAL1* promoters in the H4 wild-type strain are similar in this case (833 and 1061 units, respectively). At 6 hr of induction, activation of the wild-type *GAL1* promoter is reduced 18.1-fold in the H4 Δ4–28 strain. However, despite the presence of the *PHO5* UAS in the hybrid *PHO5–GAL1* promoter, its activity is similarly reduced (19.3-fold). Since both promoters contain only the *GAL1* downstream promoter region in common, the large decrease in *GAL1* expression caused by the H4 Δ4–28 deletion is linked to the 278-bp *GAL1* TATA region and not the *GAL1* UAS. Moreover, we found that *PHO5* and *GAL1-PHO5* promoters, which have only the 181-bp *PHO5* TATA-containing region in common, responded by similar decreases in activity (2.7- and 1.9-fold, respectively) to H4 Δ4–28. Therefore, the extent of decreased activation caused by deletion of the H4 N-terminal residues 4–28 is linked to the TATA-containing downstream promoter region.

In the experiments above, the URS_G was not included in the construction of the chimeric promoters. However, we have also constructed a hybrid promoter, *PHO5*-URS-*GAL1*, which contains the URS_G, by fusing the *PHO5* UAS to the *GAL1*

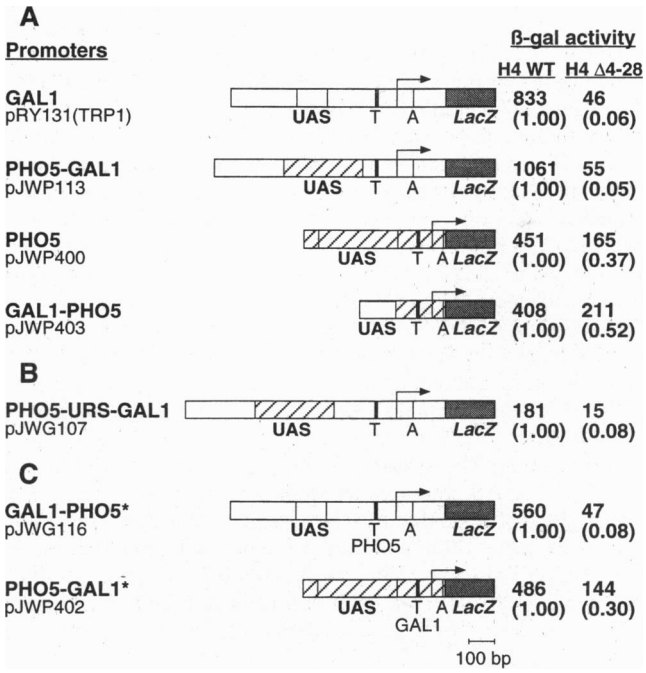

FIG. 3. Linkage of reduced *GAL1* transcription in the presence of H4 Δ4–28 to the *GAL1* downstream TATA-containing promoter. All wild-type and chimeric promoters were induced for 6 hr. (*A*) *GAL1*, *PHO5-GAL1*, *PHO5* and *GAL1-PHO5* promoters. (*B*) *PHO5*-URS-*GAL1* promoters, which contains the URS_G element. (*C*) The *GAL-PHO5** and *PHO5-GAL** promoters, whose TATA box sequences have been exchanged from their wild-type counterparts.

sequences from positions −298 to +88. Since the URS_G fused to a heterologous promoter can cause that promoter to be repressible by glucose (13, 26), induction of the *PHO5*-URS-*GAL1* promoter was performed in phosphate-free medium containing galactose. It was found that the activity from the *PHO5*-URS-*GAL1* promoter also decreased markedly (12.1-fold) in the absence of the H4 N-terminal tail, producing 181 β-galactosidase units in the wild-type strain and 15 units in the H4 Δ4–28 strain (Fig. 3*B*). The activity from this promoter is much lower than that of the promoters described above, because full induction of the *PHO5* UAS requires the presence of glucose (27). Moreover, the wild-type *PHO5* promoter is also poorly induced in galactose medium (2). Nevertheless, since the wild-type *GAL1* promoter, the *PHO5-GAL1* promoter lacking the URS_G sequence, and the *PHO5*-URS-*GAL1* promoter all respond in a similar manner to the absence of the H4 N terminus, these data suggest that the URS_G is not important in determining the extent of promoter response to H4 deletion Δ4–28.

The *GAL1* TATA-Box Sequence Alone Does Not Mediate Reduced Transcription Resulting from H4 Deletion Δ4–28. Since the effects of H4 Δ4–28 on transcription of the *GAL1* and *PHO5* promoters are linked to the TATA-containing region, we asked whether the *GAL1* TATA sequence itself is important in determining the magnitude of promoter response to the H4 Δ4–28 deletion. The *GAL1* sequence 5′-ATA-**TATAAA**TG-3′ contains the consensus yeast TATA sequence TATAAA (28), and differs from the *PHO5* TATA sequence 5′-GT**ATATAAG**CG-3′ by one base pair within the consensus TATA (in boldface type) and by two base pairs immediately outside the box. Two promoters, *GAL1-PHO5** and *PHO5-GAL1**, were created which are identical to the *GAL1* and *PHO5* promoters, respectively, except that the sequences 5′-ATATATAAATG-3′ from *GAL1* and 5′-GTATATAAGCG-3′ from *PHO5* were exchanged. The *GAL1-PHO5** promoter activity was decreased by ≈12.0-fold, from 560 to 47 β-galactosidase units in the presence of the H4 N-terminal deletion (Fig. 3*C*). This decrease is comparable to that (18.1-fold) seen for the wild-type *GAL1* promoter (Fig. 3*A*). In contrast, the *PHO5-GAL1** promoter activity was decreased by only 3.4-fold, from 486 to 144 units, which compares with a 2.7-fold decrease for the wild-type *PHO5* promoter (Fig. 3*A*). Therefore, the sequences including and immediately surrounding the TATA sequence do not by themselves determine the large decrease in promoter activity resulting from the H4 N-terminal deletion.

DISCUSSION

We have found that *GAL1* hyperactivation caused by deletion of the H3 N terminus is linked to the upstream UAS regulatory element, whereas the large decrease in *GAL1* induction caused by the absence of the H4 N terminus is linked to the downstream promoter sequence near the TATA element. The *GAL1* promoter is regulated by both positive (UAS_G) and negative (URS_G) elements which are bound by the activator protein GAL4 and the repressor protein MIG1, respectively (15, 29). MIG1 is responsible for at least part of the catabolite repression activity of the URS_G (29). In addition, GAL4 activation is physically inhibited by the binding of GAL80 under noninducing conditions (16). Thus, the H3 N terminus may act either as an antagonist to GAL4 activation or as a component of the repression system independent of GAL4. Both of these possibilities would lead to *GAL1* hyperactivation upon removal of the H3 N terminus. Since the URS_G element, containing the MIG1 binding site, was completely removed in the *GAL1-PHO5* promoter construct and since we did not find any cryptic MIG1 sites through sequence analysis, it is unlikely that H3 hyperactivation is mediated through MIG1, because this chimeric promoter is still hyperactivated by H3 Δ4–15. Within the *GAL1* UAS, negative elements (O_2 and O_3) which overlap with the GAL4 binding sites have also been identified (14, 26). To exclude the possibility that these negative elements are involved in hyperactivation, we constructed the *5GAL4-PHO5* promoter, using synthetic GAL4 binding sites (25). We found that this promoter remained hyperactivated by H3 Δ4–15. Therefore, the H3 N terminus may function through GAL4 or another protein bound at the GAL4 sites. Xu and Johnston (30) have recently shown that the GAL6 protein can bind *in vitro* to the GAL4 binding sites and that disruption of *GAL6* also causes hyperactivation of the *GAL1* gene. They suggest that GAL6 may inhibit *GAL1* transcription by competing with GAL4 for binding to the UAS. However, we found that the *GAL6* mRNA level was unaltered in the H3 Δ4–15 strain compared with the wild-type strain in all growth conditions. Moreover, *in vitro* synthesized GAL6 proteins did not interact *in vitro* with the H3 N terminus (where residues 1–46 of histone H3 were fused to the C-terminal end of glutathione *S*-transferase) (G Fisher-Adams and M.G., unpublished data). This suggests that H3 deletions do not decrease GAL6 synthesis and that the H3 tail does not interact physically with GAL6, arguing against an indirect or direct involvement of GAL6 in H3 Δ4–15-induced hyperactivation. It is also unlikely that H3-mediated hyperactivation of *GAL1* occurs by increasing *GAL4* mRNA synthesis (5) or by decreasing *GAL80* mRNA levels. For example, *GAL80* deletion causes high constitutive expression of the *GAL1* gene under nonrepressing and noninducing conditions (15, 29, 31). However, expression of the *GAL1* promoter in strains carrying the H3 N-terminal deletion remains at low levels under these same (nonrepressing, noninducing) conditions (Fig. 1) (5). Therefore, the H3 N terminus may repress GAL4 by interfering with GAL4 or cooperating with GAL80 directly. It has been suggested that the repressive function of GAL80 on GAL4 is not completely removed upon induction (31, 32). In fact, disruption of *GAL80* also causes hyperactivation of *GAL1* in galactose medium (31, 32). If GAL80 and the H3 N terminus interact directly to repress GAL4 function, deletion of the H3 N terminus could cause GAL80 to become a less effective repressor.

In contrast to the H3/UAS linkage, the relative decrease in *GAL1* transcription caused by the H4 N-terminal deletion is linked to the 278-bp *GAL1* TATA-containing region. Although we have made attempts to delimit this region further by deleting sequences both upstream (removing the first 42 bp of this 278-bp sequence) and downstream (removing the last 88 bp of this 278-bp sequence) of the TATA element, both deletions seem to reduce the repressive effect of the H4 Δ4–28 deletion on this *GAL1* TATA-containing region (J.S.W. and M.G., unpublished data). It appears that much of the 278-bp downstream promoter fragment (but not the TATA box itself) is required to mediate the large decrease in *GAL1* transcription caused by the H4 N-terminal deletion. Therefore, the large *GAL1*-specific decrease in transcription is unlikely to be due to the action of a single protein binding site. An alternative explanation is that this region is unusually sensitive to changes in chromatin structure. The H4 N-terminal tail is important for nucleosome positioning adjacent to the yeast α2 operator (33). Chromatin structure immediately (15 bp) upstream of the *GAL1* TATA box sequence becomes less accessible to the *E. coli* Dam methylase, and nucleosome positioning at the *GAL1* promoter appears to be shifted in the absence of the H4 N-terminal tail (7). Since the *GAL1* TATA sequence is normally present in the linker region between two adjacent nucleosomes, nucleosomal positioning changes near the *GAL1* TATA sequence may prevent basal factors from accessing the TATA promoter to activate transcription. Also, because the nucleosome represses transcription initiation, the H4 N terminus may not only be required for maintaining correct nucleosome positioning but could also serve as the target for the mechanism which derepresses the downstream promoter.

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