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Nucleosome Loss Activates *CUP1* and *HIS3* Promoters to Fully Induced Levels in the Yeast *Saccharomyces cerevisiae*

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We have previously shown that nucleosome loss, obtained by repressing histone H4 mRNA synthesis, activates otherwise inactive *PHO5*, *GAL1*, and *CYC1* gene promoters (fused to the bacterial β-galactosidase [*lacZ*] reporter gene) to moderate levels of activity (approximately 2 to 15% of fully induced levels). We now report that nucleosome loss activates the expression of two additional promoters that are normally induced by independent mechanisms: *CUP1* (induced by heavy-metal toxicity) and *HIS3* (induced by amino acid starvation). Surprisingly, the level of *CUP1-lacZ* and *HIS3-lacZ* activation by nucleosome loss approximates fully induced levels of transcription. These *CUP1* and *HIS3* promoter activities are increased similarly from either episomal or genomic constructs. Our results emphasize the universality of the mechanism by which nucleosome loss activates yeast promoters. Moreover, a comparison of absolute levels of activation for different promoters suggests that activation by nucleosome loss results in a relatively constant level of activation, while levels obtained by normal induction vary considerably. These data argue that nucleosome loss may play a uniquely dominant role in the regulation of certain promoters.

The fundamental component of chromatin is the nucleosome. This particle consists of a histone octamer (two molecules each of histones H2A, H2B, H3, and H4) around which 146 bp of DNA are wrapped approximately 1.8 times. The positively charged histones facilitate folding of negatively charged DNA molecules into compact structures within the nucleus. In addition to this structural role, nucleosomes have been implicated in the regulation of transcription initiation (1, 2, 8, 30). Nucleosomes located at TATA promoter elements can prevent access of basal transcription factors in vitro and thereby block transcription initiation (17, 18). However, nucleosomes will not block transcription if the basal factors, in particular TFIID (the TATA element-binding protein), are allowed to first bind the promoter (16, 22, 38). This suggests that nucleosomes and transcription factors may compete for promoter elements and that factors affecting this competition would contribute to the regulation of transcription initiation. Work from our laboratory supports this view and suggests that transcriptional repression is partially alleviated in vivo by nucleosome loss in the yeast *Saccharomyces cerevisiae* (12, 13).

To induce nucleosome loss, we placed yeast histone genes under the control of *GAL* promoters which are repressed in the presence of glucose (11–13, 15). When an episomal histone H4 gene is placed under control of the *GAL1* promoter in a strain lacking chromosomal H4 genes, glucose may be used to repress histone H4 synthesis. Cells treated in this manner lose approximately half of their nucleosomes as a round of replication occurs in the absence of H4 synthesis. The remaining nucleosomes are left less constrained, chromosomes fail to segregate, and arrest occurs in G_2 of the cell cycle (11, 15). We examined the effect of nucleosome loss on chromosomal *PHO5* transcription and on *PHO5*, *CYC1*, and *GAL1* promoters fused to the bacterial β-galactosidase (*lacZ*) reporter gene in plasmid constructs. It was shown that nucleosome loss activates otherwise inactive *PHO5*, *GAL1*, and *CYC1* gene promoters to moderate levels of activity (approximately 2 to 15% of fully induced levels). While the TATA and initiation (I) sequences are essential, this form of gene activation does not require the function of the upstream activator sequence (UAS) elements (12, 15).

In related studies (15), we have shown that the steady-state mRNA level produced by the uninduced endogenous *CUP1* gene is not increased by nucleosome loss, nor are the steady-state levels of a number of already active genes (*HIS3*, *HIS4*, *ARG4*, and *TRP1*) strongly increased. Are these genes exceptions to the generalized scheme which provides for the activation of promoters by nucleosome loss? Since steady-state mRNA levels may be regulated by posttranscriptional mechanisms and because ongoing mRNA synthesis is not expected to be altered dramatically by nucleosome loss (8), we have approached this question by examining the activation of the *CUP1* and *HIS3* promoters fused to the bacterial *lacZ* reporter gene. We report that nucleosome loss activates transcription of *CUP1-lacZ* and *HIS3-lacZ* fusion constructs to levels approximating fully induced levels whether the fusion genes are situated on episomes or are present at genomic loci. As with the other genes examined, activation of the *CUP1* and *HIS3* promoters by nucleosome depletion requires the TATA and I sequences but not the upstream regulatory elements. Finally, we find that the absolute levels of activation by nucleosome loss for five different promoters are relatively constant, while the levels obtained through normal induction vary considerably. These data are discussed from the standpoint of general mechanisms of transcriptional activation.

MATERIALS AND METHODS

Plasmids. The *CUP1-lacZ* fusion gene (pLD3Δ-241) was prepared as described previously (1). Deletions of the promoter were prepared by digesting pLD3Δ-241 with *Eco*RI (which cleaves immediately 5′ to the promoter within the vector pSEYC102 [3]) and subsequently digesting the DNA with Bal31 for various amounts of time. *Bam*HI linkers were ligated to the blunt ends, the DNA was digested with *Bam*HI (which cuts also at the *CUP1* promoter-*lacZ* gene ligation

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TABLE 1. Yeast strains

Strain	Description
UKY403	*MAT***a** *ade2-101* (ochre) *arg4-1 his3-Δ200 leu2-3 leu2-112 lys2-801* (amber) *trp1-Δ901 ura3-52 thr tyr hhf1::HIS3 hhf2::LEU2*/pUK421 (*CEN TRP1 GAL1-HHF2*)
MHY308	Isogenic to UKY403 except contains plasmid pMH310 (*CEN TRP1 HHF2*)
LDY202	Isogenic to UKY403 and contains plasmid pLD[*HIS3*] (WT[a] *HIS3*)
LDY204	Isogenic to UKY403 and contains plasmid pLDΔ35 [*his3*Δp (dA-dT)]
LDY206	Isogenic to UKY403 and contains plasmid pLD[*HIS3*](I) (WT *HIS3* integrated at *HIS3* (H4) locus)
LDY208	Isogenic to UKY403 and contains plasmid pLDΔ85 [*his3*Δp (dA-dT) and ΔUAS]
LDY210	Isogenic to UKY403 and contains plasmid pLDΔ24 (*his3*ΔT_r)
LDY212	Isogenic to UKY403 and contains plasmid pLDΔ38 (*his3*ΔT_r and ΔT_c)
LDY214	Isogenic to UKY403 and contains plasmid pLDΔ26 (*his3*ΔT_c)
LDY220	Isogenic to UKY403 and contains plasmid pLD3Δ-241 (WT *CUP1*)
LDY223	Isogenic to UKY403 and contains plasmid pLD3Δ-107 (*cup1Δ-107*)
LDY225	Isogenic to UKY403 and contains plasmid pLD3Δ-55 (*cup1Δ-55*)
LDY227	Isogenic to UKY403 and contains plasmid pLD3Δ-241(I) (WT *CUP1*, integrated at *CUP1* locus)
LDY201	Isogenic to MHY308 and contains plasmid pLD[*HIS3*] (WT *HIS3*)
LDY203	Isogenic to MHY308 and contains plasmid pLDΔ35 [*his3*Δp(dA-dT)]
LDY207	Isogenic to MHY308 and contains plasmid pLDΔ85 [*his3*Δp(dA-dT) and ΔUAS]
LDY209	Isogenic to MHY308 and contains plasmid pLDΔ24 (*his3*ΔT_r)
LDY211	Isogenic to MHY308 and contains plasmid pLDΔ38 (*his3*ΔT_r and ΔT_c)
LDY213	Isogenic to MHY308 and contains plasmid pLDΔ26 (*his3*ΔT_c)
LDY215	Isogenic to MHY308 and contains plasmid pLD[*HIS3*](I) (WT *HIS3* integrated at *HIS3* [H4] locus)
LDY221	Isogenic to MHY308 and contains plasmid pLD3Δ-241 (WT *CUP1*)
LDY224	Isogenic to MHY308 and contains plasmid pLD3Δ-107 (*cup1Δ-107*)
LDY226	Isogenic to MHY308 and contains plasmid pLD3Δ-55 (*cup1Δ-55*)
LDY228	Isogenic to MHY308 and contains plasmid pLD3Δ-241(I) (WT *CUP1*, integrated at *CUP1* locus)

[a] WT, wild type.

junction), and DNA fragments up to 300 bp long were purified from low-melting-point agarose gels. These *CUP1* promoter deletions were ligated to the *lacZ* gene again in pSEYC102, transformed into bacteria, and plated on TYE-ampicillin plates containing the chromogenic substrate for β-galactosidase, 5-bromo-4-chloro-3-indolyl-β-D-indolyl phosphate (X-Gal). The *CUP1-lacZ* fusion plasmids were isolated from blue colonies, and the *CUP1* promoters, including the 5′ ends of the *lacZ* gene, were sequenced by the method of Sanger et al. (27). pLD3Δ-107 is deleted of the UAS (sequences upstream of −107); pLD3Δ-55 is deleted of both the UAS and the TATA element (sequences upstream of −55). To integrate the wild-type *CUP1-lacZ* fusion gene into the yeast genome, the *Eco*RI-*Sal*I fragment of pLD3Δ-241 was first ligated into identical restriction sites within the integrating vector YIP5 [pLD3Δ-241(I)].

To prepare *HIS3* promoter-*lacZ* gene fusions, plasmids containing either the wild-type *HIS3* gene or the *HIS3* gene with promoter deletions (32, 33) were digested with *Dra*I, and *Bam*HI linkers were ligated to the blunt ends. After *Bam*HI digestion (or *Eco*RI-*Bam*HI digestion; Δ85) and gel purification, the *HIS3* promoters were ligated to the *lacZ* gene at the *Bam*HI (or *Eco*RI-*Bam*HI) site of pSEYC102. All constructs were sequenced to confirm the deletions and the in-frame insertion to the *lacZ* gene (27). The wild-type *HIS3-lacZ* fusion gene was integrated into the genome after first being recloned into YIP5 as described above for the *CUP1-lacZ* gene fusion {pLD[*HIS3*](I)}.

Yeast transformations. The isogenic yeast strains UKY403 and MHY308 were described previously (12, 15). Plasmids containing *CUP1-lacZ* or *HIS3-lacZ* fusion genes were transformed into these strains as previously described (28). To integrate the *CUP1-lacZ* and *HIS3-lacZ* fusion genes into the genome, the appropriate plasmids were first digested with *Xba*I [pLD3Δ-241(I)] or *Bst*BI {pLD[*HIS3*](I)}.

Yeast strains. The yeast strains used are shown in Table 1. *HHF2* is referred to as H4 in the text, figures, and figure legends. *hhf1* and *hhf2* represent gene deletions as a result of replacements by *HIS3* and *LEU2*, respectively (1).

Media, growth conditions, and β-galactosidase assays. All media have been described previously (12, 28). YEPG and SG media contains 2% galactose as the sole carbon source. Yeast cells were grown for approximately 24 h in SG minus uracil and tryptophan (SG-Ura-Trp), washed in water, and transferred to media plus or minus specific inducer. *CUP1* promoter activity was assayed after cells were grown for 6 h in YEPD or YEPG with copper sulfate (1 mM) added to the experimental media for the last 30 min of incubation. *HIS3* promoter activity was assayed after cells were grown for 6 h in SD-Ura-Trp or SG-Ura-Trp plus aminotriazole (10 mM) in the experimental media. β-Galactosidase activity was measured as described previously (23, 40). Usually 0.5 ml of culture was assayed. Units of activity were determined as described by Miller (23).

Primer extension. RNA was isolated as described previously (15) from yeast cells grown as described above for the β-galactosidase assays. RNA (30 μg) and approximately 0.3 pmol of ^{32}P-labeled primer were annealed for 3 h at 37°C in RT buffer (50 mM Tris-HCl [pH 8.3], 150 mM KCl, 0.5 mM EDTA, 1 mM dithiothreitol, 7 mM $MgCl_2$, 1 mM each dATP, dCTP, dGTP, and dTTP). Reverse transcriptase was added, and incubation was continued for 2 h at 43°C. After the reaction, RNA was digested with RNase A for 30 min at 37°C, and samples were purified by phenol-chloroform (1:1) extraction followed by ethanol precipitation. The reaction products were analyzed by electrophoresis on 6% polyacrylamide–urea gels. The oligodeoxynucleotide 5′-CAGTGAG ACGGGCAACAGCC-3′ (19) was used to prime extension of the *CUP1* and *HIS3* mRNAs.

Southern transfer and hybridization of genomic DNA. Genomic DNA was isolated from yeast cells as described previously (24). The DNA was digested with *Eco*RI, frac-

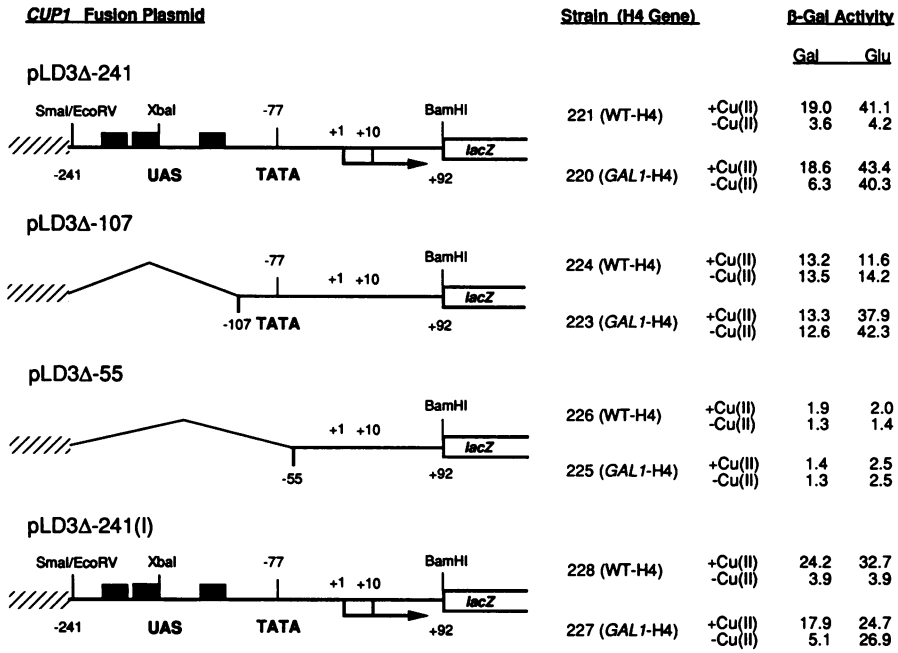

FIG. 1. Activation of *CUP1-lacZ* fusion genes by depletion of histone H4 and nucleosomes. Plasmid pLD3Δ-241 was prepared by ligating the entire *CUP1* promoter, including coding sequences through codon 8, in frame to codon 8 of the *lacZ* gene in pSEYC102 (3). Three Ace1-binding sites (UAS_{CUP1}) are shown between −105 and −210. The TATA element is at −77, and transcription start sites are +1 and +10. Plasmids pLD3Δ-107 and pLD3Δ-55 are 5′ deletions of pLD3Δ-241. pLD3Δ-241(I) contains the *CUP1-lacZ* fusion from pLD3Δ-241 in the integrating vector YIP5. All promoter constructs were transformed into UKY403(*GAL1*-H4) and MHY308(WT-H4), and yeast cells were grown under four different conditions: in galactose (Gal)- or glucose (Glu)-based medium with (+) or without (−) copper sulfate (1 mM). β-Galactosidase (β-Gal) assays were repeated at least three times, with each of two independent transformants, for each growth condition. The average β-galactosidase activity is reported. Error is less than 20%.

tionated by agarose gel electrophoresis, transferred to nitrocellulose by the method of Southern (31), and hybridized to uniformly labeled DNA probes (21).

RESULTS

Activation of the *CUP1* promoter by nucleosome loss. Copper resistance in *S. cerevisiae* is controlled by two distinct mechanisms: gene amplification and transcriptional induction of the *CUP1* locus. Transcriptional regulation is mediated by the *ACE1* gene product, which interacts with copper ions from the medium prior to binding at the *CUP1* UAS (UAS_{CUP1}) to induce gene transcription (7). The *CUP1* protein then rapidly sequesters copper ions from the medium, preventing cell toxicity. The promoter elements of the *CUP1* gene are diagrammed in Fig. 1. Multiple binding sites (UAS_{CUP1}) for the Ace1 protein lie between approximately −105 and −210 bp relative to the transcription start site. A TATA element is present at −77 bp (4, 7, 14).

We first wished to investigate whether the *CUP1* gene, like the *PHO5*, *GAL1*, and *CYC1* genes, is activated by the repression of histone H4 mRNA synthesis and nucleosome loss (12). We had previously constructed yeast strain UKY403, in which the sole episomal histone H4 gene (H4-2 or *HHF2*) is under the control of the *GAL1* promoter. In this strain, glucose treatment results in repression of histone mRNA synthesis and subsequent nucleosome depletion. The isogenic control strain MHY308 contains its sole episomal H4 (*HHF2*) gene under the control of its wild-type promoter and does not undergo nucleosome loss in glucose. The entire *CUP1* gene promoter plus sequences encoding the first eight Cup1 amino acids were fused to the *Escherichia coli lacZ* gene in a centromere-containing, autonomously replicating (*CEN ARS*) plasmid (pSEYC102 [3]) to produce plasmid pLD3Δ-241. This plasmid was introduced into UKY403, resulting in strain LDY220. Transformation of pLD3Δ-241 into MHY308 produced the control strain LDY221 (Fig. 1). For purposes of clarity, strains containing the H4 gene under the control of its own promoter will be designated WT-H4, and those containing the H4 gene under *GAL1* promoter control will be designated *GAL1*-H4.

We found that *CUP1* promoter activity is induced by copper sulfate (1 mM) approximately 5-fold in galactose-based medium (YEPG) and 10-fold in glucose-based medium (YEPD) in the control strain, LDY221(WT-H4) (Fig. 1). In the absence of Cu^{2+}, there is little activation of the *CUP1* promoter in either carbon source. In contrast, repression of histone H4 synthesis by glucose in the absence of Cu^{2+} leads to the activation of the intact *CUP1* promoter in LDY220 (*GAL1*-H4) to 98% of the Cu^{2+}-mediated fully induced level in the control strain LDY221(WT-H4) (40.3 U of β-galactosidase from nucleosome loss; 41.1 U in the copper-induced control strain). This high level of activation by nucleosome loss is surprising, since uninduced *CUP1* mRNA levels (as measured by Northern [RNA] blot analysis) are not elevated by nucleosome loss (13). This lack of apparent activation of the intact chromosomal *CUP1* gene by nucleosome loss is consistent with increased posttranscriptional degradation of *CUP1* mRNA compared with that of the *CUP1-lacZ* fusion mRNA (see Discussion).

To establish which of the *CUP1* promoter elements are essential for activation of the promoter by nucleosome loss, we prepared *CUP1* promoters deleted of the UAS_{CUP1} (in plasmid pLD3Δ-107) or of both the UAS_{CUP1} and the TATA

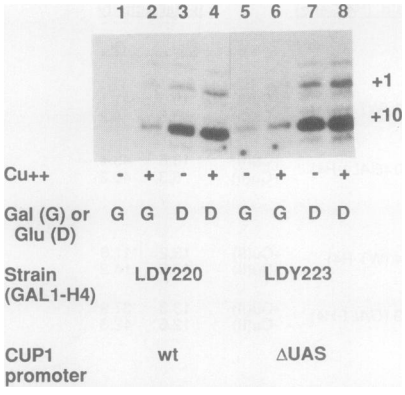

FIG. 2. Primer extension analysis of *CUP1-lacZ* fusion mRNAs. RNA was isolated from strains LDY220(*GAL1*-H4) and LDY223 (*GAL1*-H4) after growth of the strains in galactose (G)- or glucose (D)-based medium with (+) or without (−) copper sulfate (1 mM). Primer extension was done as described in Materials and Methods. The autoradiogram shows primer extension products from mRNA isolated from LDY220(*GAL1*-H4) (lanes 1 to 4) and LDY223(*GAL1*-H4) (lanes 5 to 8). Sequencing reactions of the *CUP1* promoter were electrophoresed in adjacent lanes as size standards (not shown). wt, wild type.

element (in plasmid pLD3Δ-55) (Fig. 1). These constructs were introduced into UKY403(*GAL1*-H4) and MHY308 (WT-H4) to produce strains LDY223(*GAL1*-H4), LDY224 (WT-H4), LDY225(*GAL1*-H4), and LDY226(WT-H4) (Fig. 1). The basal level of *CUP1-lacZ* transcription increased approximately fourfold after deletion of UAS_{CUP1} in LDY223(*GAL1*-H4) and LDY224(WT-H4). These results are consistent with similar results reported previously (37). Because of the absence of the UAS elements, Cu^{2+} does not induce significant further expression from the truncated promoter. In contrast, nucleosome loss in the absence of added Cu^{2+} causes increases in expression approximating those of the fully induced wild-type *CUP1* promoter. *CUP1* promoter activity after deletion of both the UAS and TATA elements in LDY225(*GAL1*-H4) and LDY226(WT-H4) is less than the basal level under all growth conditions examined. Thus, activation of the *CUP1* promoter by nucleosome loss requires the TATA and I sequences but not the UAS_{CUP1}. Interestingly, a TATA-like element at position −33 (TAT AAT) differs from the consensus TATA element (TATAAA) at position −77 by only one base but does not function for activation either through the UAS_{CUP1} element or by nucleosome loss.

To confirm that mRNA synthesis activated by nucleosome loss initiates accurately from the wild-type and UAS_{CUP1}-deleted *CUP1* promoters, we extended a primer from the *lacZ* region of the *CUP1-lacZ* fusion mRNA to the *CUP1* transcription start site (Fig. 2). *CUP1* mRNA has previously been shown to initiate predominantly at two sites, +1 and +10 (14). *CUP1* mRNA initiates from identical sites in the wild-type *CUP1-lacZ* fusion gene in LDY220(*GAL1*-H4) in the presence of copper sulfate in either galactose- or glucose-containing medium (Fig. 2, lanes 2 and 4). Additionally, in strain LDY220(*GAL1*-H4), there is a similar level of mRNA produced in glucose through normal Cu^{2+} induction and through nucleosome loss in the absence of Cu^{2+} (lanes 3 and 4). Furthermore, in LDY223(*GAL1*-H4) (which lacks the UAS_{CUP1}), there is also a similar mRNA level produced in glucose in the presence or absence of Cu^{2+} (lanes 7 and 8). Therefore, transcription appears to initiate at the correct sites in the *CUP1* promoter, and the primer extension data for each strain grown under different conditions reflect the levels of promoter activity measured by the β-galactosidase assay.

Activation of the *HIS3* promoter by nucleosome loss. The *HIS3* gene encodes the protein imidazole glycerol phosphate dehydratase, which is one of the enzymes in the pathway for histidine biosynthesis. It has been reported that a low basal level of *HIS3* transcription is mediated by a poly(dA-dT) tract located approximately 120 bp upstream of the transcription start site (35) (Fig. 3). Basal transcription initiates equally at two sites (+1 and +13) and has been reported to require a constitutive TATA element (T_c) that has been mapped between −54 and −83 (20). Upon induction by amino acid starvation (or growth in aminotriazole, a competitive inhibitor of the *HIS3* gene product), *HIS3* transcription increases three- to fivefold (36). Induced transcription is controlled by binding of GCN4 to a UAS element situated about 95 bp upstream of the transcription start site. This has been reported to require the regulated TATA element (T_r). Regulated transcription initiates predominantly at +13 (34).

We wished to determine whether conditions of nucleosome loss would activate the *HIS3* promoter and, if so, which elements (constitutive and/or regulated) were required. Promoter activity from wild-type and mutant (lacking one or more control elements) *HIS3* promoters, fused to the β-galactosidase gene, was examined in yeast strains UKY403(*GAL1*-H4) and MHY308(WT-H4) (Fig. 3). Activity of the wild-type *HIS3* promoter was induced by aminotriazole approximately threefold over basal transcription when the control strain [LDY201(WT-H4)] was grown in either glucose- or galactose-based medium. Nucleosome loss in the absence of aminotriazole activated the uninduced *HIS3* promoter of this strain to 87% of the fully induced *HIS3* promoter levels (24.3 U of β-galactosidase from nucleosome loss; 27.8 U in the aminotriazole-activated control strain). Interestingly, *HIS3* promoter activity increased to above the normal, induced levels of activity when conditions of nucleosome loss were used in the presence of aminotriazole [in LDY202(*GAL1*-H4)]. We did not observe this phenomenon with other yeast promoters examined (*PHO5*, *CYC1*, *GAL1*, and *CUP1*).

Upon deletion of the poly(dA-dT) tract (sequences −101 to −136) in LDY203(WT-H4), *HIS3* basal transcription in the absence of aminotriazole decreased about threefold. However, aminotriazole still activated transcription almost threefold over this new basal level. Therefore, as expected, deletion of the poly(dA-dT) sequence reduced basal transcription but did not alter the fold induction by aminotriazole. In LDY204(*GAL1*-H4), nucleosome loss conditions activated transcription almost fourfold in the absence of aminotriazole. Again, nucleosome loss in the presence of aminotriazole activated *HIS3* transcription more than did aminotriazole induction alone. In LDY207(WT-H4) containing the *HIS3* promoter deleted of both the poly(dA-dT) tract and the UAS (sequences upstream of −83), basal transcription was again very low [fourfold lower than the level in LDY201(WT-H4) in the absence of aminotriazole]. Evidently as a result of the deletion of the upstream promoter elements, aminotriazole treatment did not cause *HIS3* promoter activation over the basal level of transcription. However, in LDY208(*GAL1*-H4), nucleosome loss conditions activated the promoter approximately fourfold. In the absence of the upstream promoter elements, aminotriazole activation and nucleosome loss conditions applied together

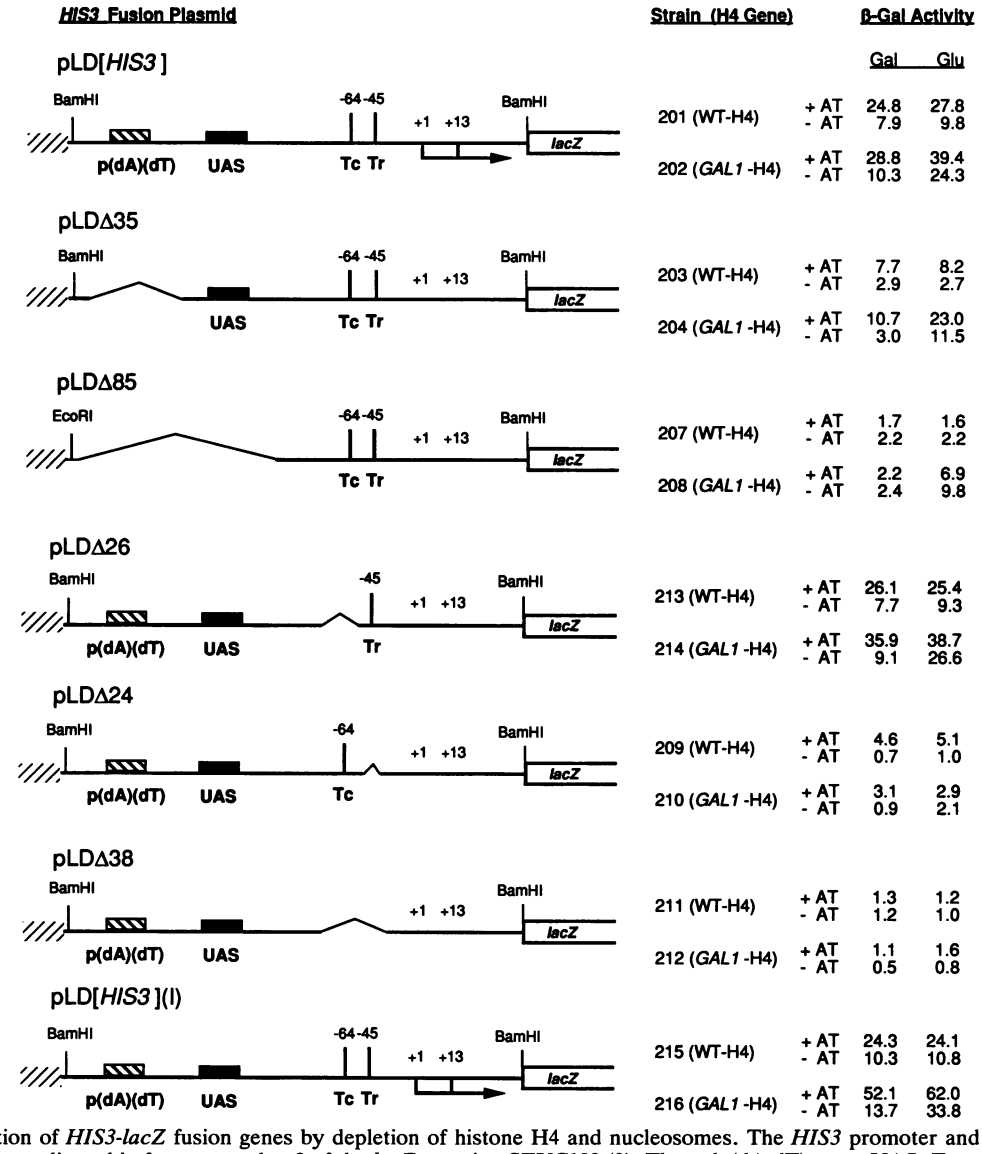

FIG. 3. Activation of *HIS3-lacZ* fusion genes by depletion of histone H4 and nucleosomes. The *HIS3* promoter and coding sequences through codon 23 were ligated in frame to codon 8 of the *lacZ* gene in pSEYC102 (3). The poly(dA-dT) tract, UAS, T_r, and T_c elements are diagrammed, and transcription start sites are at +1 and +13. pLD[*HIS3*](I) contains the wild-type *HIS3* promoter in the integrating vector YIP5. All promoter constructs were transformed into UKY403(*GAL1*-H4), and MHY308(WT-H4) and yeast cells were grown under four different conditions: in galactose (Gal)- or glucose (Glu)-based medium with (+) or without (−) aminotriazole (AT; 10 mM). β-Galactosidase (β-Gal) assays were repeated at least three times, with each of two independent transformants, for each growth condition. The average β-galactosidase activity is reported. Error is less than 20%.

did not lead to a level greater than that produced by activation by nucleosome loss alone. We conclude that activation of transcription by conditions of nucleosome loss does not require the upstream promoter elements and appears to require only the region at the TATA promoter. Moreover, in the presence of the upstream promoter elements, activation by aminotriazole and nucleosome loss produces an additive level of activity which is greater than that produced by either mechanism of activation alone.

The *HIS3* gene is unique in that it possesses both constitutive and regulated TATA elements (T_c and T_r). This fact presents an opportunity to investigate whether nucleosome loss activates constitutive as well as inducible TATA promoter elements. We thus quantified *HIS3* promoter activity by β-galactosidase assays, using mutant promoters lacking T_r, T_c, or both elements. Deletion of sequences −53 to −80 (containing the T_c element) had no significant effect on *HIS3* promoter activity compared with wild-type levels in any growth conditions tested {Fig. 3; compare LDY213(WT-H4) and LDY214(*GAL1*-H4) with the constructs containing the wild-type *HIS3* promoter [LDY201(WT-H4) and LDY202 (*GAL1*-H4)]}. Deletion of sequences −35 to −44 (T_r) in LDY209(WT-H4) greatly decreased both basal and aminotriazole-inducible levels of the *HIS3* promoter. Furthermore, in the absence of the T_r in LDY210(*GAL1*-H4), nucleosome loss activated the *HIS3* promoter only slightly (0.9 versus 2.1 U of activity). Deletion of sequences −32 to −80 (T_r and T_c) [LDY211(WT-H4) and LDY212(*GAL1*-H4)] completely eliminated basal transcription and transcription activated through aminotriazole or nucleosome loss. These results suggest that

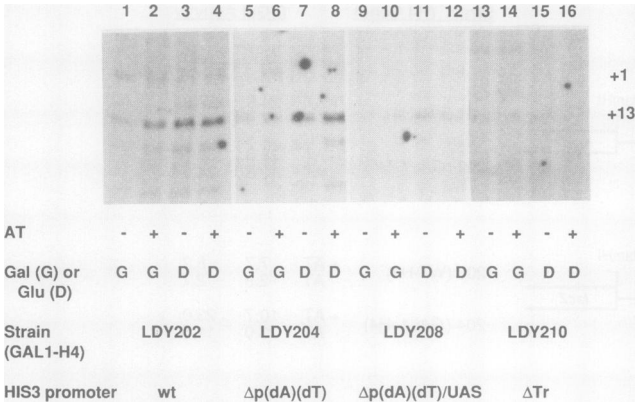

FIG. 4. Primer extension analysis of *HIS3-lacZ* fusion mRNAs. RNA was isolated from strains LDY202(*GAL1*-H4), LDY204 (*GAL1*-H4), LDY208(*GAL1*-H4), and LDY210(*GAL1*-H4) after growth of the strains in galactose (G)- or glucose (D)-based medium with (+) or without (−) aminotriazole (AT; 10 mM). Primers were extended as described in Materials and Methods. The autoradiogram shows primer extension products from RNA isolated from LDY202(*GAL1*-H4) (lanes 1 to 4), LDY204(*GAL1*-H4) (lanes 5 to 8), LDY208(*GAL1*-H4) (lanes 9 to 12), and LDY210(*GAL1*-H4) (lanes 13 to 16). Sequencing reactions of the *HIS3* promoter were electrophoresed adjacent to the primer extension products as size standards (not shown). wt, wild type.

in our yeast strains, both the poly(dA-dT) and UAS control elements drive transcription initiation through sequences located mainly at the T_r. However, the UAS element can function through another, less efficient TATA element (T_c), since promoter activity was induced (from a very low basal level) in LDY209(WT-H4) and LDY210(*GAL1*-H4) by aminotriazole or nucleosome loss. Therefore, as with the other genes examined (*PHO5*, *GAL1*, *CYC1*, and *CUP1*), nucleosome loss activation of *HIS3* gene transcription requires a functional TATA (T_r) promoter element.

To confirm that the *HIS3-lacZ* gene transcripts initiated correctly, we extended a primer from the *lacZ* region of the *HIS3-lacZ* fusion mRNA. The majority of transcripts from the wild-type *HIS3* promoter initiated from +13 when LDY202(*GAL1*-H4) was induced with aminotriazole in either glucose- or galactose-based medium (Fig. 4). In the absence of aminotriazole, lesser levels of constitutive transcription initiated equally from +1 and +13, in agreement with previous data (20). In the absence of aminotriazole and under conditions of nucleosome loss, transcription initiates predominantly from +13. Transcription from the *HIS3* promoter deleted of the poly(dA-dT) tract in LDY204(*GAL1*-H4) originates largely from +13, whether induction is by aminotriazole or by nucleosome depletion. In LDY208 (*GAL1*-H4) [deleted of both the poly(dA-dT) and UAS elements], few transcripts originate from +1 under any growth conditions. Transcription due to nucleosome loss originates largely from +13. In LDY210(*GAL1*-H4) (ΔT_r) as well as in LDY212(*GAL1*-H4) (ΔT_r and ΔT_c; not shown), transcripts are not easily detected under any growth conditions, reflecting the low promoter activity of these strains (Fig. 4). These experiments show that nucleosome loss activates initiation of transcription in a manner similar to that occurring when the UAS is utilized. They also show that levels of transcript visualized by primer extension largely reflect the levels seen by assaying β-galactosidase activity.

Integrated and episomal promoter-*lacZ* fusions are activated in a similar manner by nucleosome loss. *CUP1* steady-state mRNA levels produced through the genomic gene copies do not appear to be elevated under conditions of nucleosome loss (13, 15), yet results described above show that the episomal *CUP1-lacZ* fusion construct is strongly activated under these conditions. Is this finding due to the possible differences in topological constraints between episomal and integrated constructs? To address this question, we integrated the wild-type *CUP1-lacZ* fusion construct (pLD3Δ-241) into the *CUP1* locus of strains UKY403(*GAL1*-H4) and MHY308(WT-H4), resulting in strains LDY227 (*GAL1*-H4) and LDY228(WT-H4). The expected integration and Southern blot results from this event are shown in Fig. 5. We find that basal and copper sulfate-induced promoter activity from the integrated *CUP1-lacZ* construct is similar to activity measured from the episomal construct (Fig. 1, bottom). Furthermore, the integrated *CUP1-lacZ* construct is activated by nucleosome loss to a level comparable (5.3-fold) to that seen in the analogous episomal construct (6.4-fold). Therefore, activation of the *CUP1* promoter by nucleosome loss occurs in a similar manner on plasmids and in the genome.

We also integrated the wild-type *HIS3* promoter-*lacZ* fusion construct described in Fig. 3 (bottom) at the *HIS3* locus. The expected integration event and the Southern blot illustrating the occurrence of this event are shown in Fig. 6. The strains produced were LDY215(WT-H4) and LDY216 (*GAL1*-H4). Conditions of nucleosome loss in strain LDY216(*GAL1*-H4) led to a 2.5-fold activation of the *HIS3* promoter, whereas similar treatment of the control strain [LDY215(WT-H4)] did not result in any activation. This value compares with the 2.4-fold activation seen in the episomal construct [LDY202(*GAL1*-H4)] under similar conditions. Therefore, we conclude that the integrated and episomal *HIS3* promoters are also activated similarly by nucleosome loss.

DISCUSSION

The results presented above and those published previously (12, 13) have shown that all five yeast promoters examined (*PHO5*, *CYC1*, *GAL1*, *HIS3*, and *CUP1*) are activated by repression of histone H4 synthesis. Unpublished work from our laboratory has shown, in addition, that yeast heat shock genes *HSP26*, *HSP70* (*SSA3*), and *HSP70* (*SSA4*) are also activated under these conditions. These latter activities are not simply due to the stress of cell cycle arrest, since G_2 arrest in the presence of the tubulin inhibitor nocodazole does not elicit similar induction of these genes (14a; data not shown). Therefore, genes that are regulated by a variety of activators are all induced by conditions of nucleosome loss. We have also shown that only the TATA and I elements are required for this activation. Thus, nucleosome loss is a general activator of downstream (TATA) promoter elements.

The reasons for the different levels of activation of the various promoters by nucleosome loss may prove to be especially informative. Whereas the *PHO5*, *CYC1*, and *GAL1* promoters are activated at some 15, 4, and 2% of the fully induced levels, respectively, the levels measured from the *CUP1* and *HIS3* gene promoters, after nucleosome loss, are close to 100% of the fully induced activity (98 and 87%, respectively). The *GAL1* and *CYC1* genes may, conceivably, display such low levels of activation because of the presence of upstream repressive sequences (URS), between the UAS and TATA elements (5, 6), which mediate glucose repres-

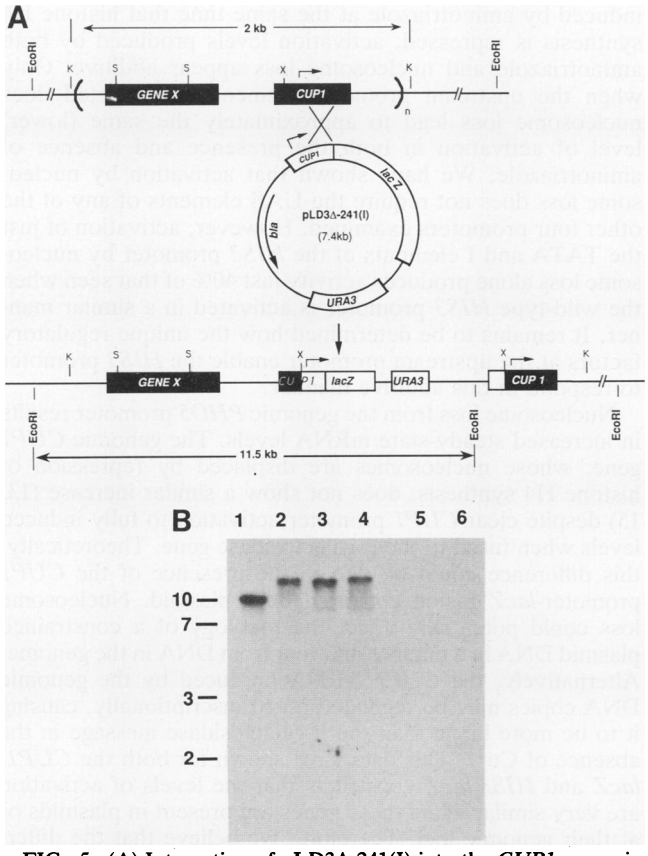

FIG. 5. (A) Integration of pLD3Δ-241(I) into the *CUP1* genomic locus. The plasmid containing the *CUP1-lacZ* fusion gene (7.4 kb) was digested with *Xba*I, which cleaves once within the *CUP1* promoter, and transformed into UKY403 and MHY308. Genomic DNA was isolated from each of two independent transformants for each strain, as well as from UKY403 and MHY308, and digested with *Eco*RI. *Eco*RI digests once within pLD3Δ-241(I) as well as 5′ and 3′ to the *CUP1* genomic locus. DNA was fractionated on a 1% agarose gel and prepared for hybridization to the *lacZ* DNA probe as described in Materials and Methods. An integrated *CUP1-lacZ* fusion gene results in a restriction enzyme fragment greater than 7.4 kb (bottom). Filled boxes represent genomic sequences; open boxes represent integrated plasmid sequences. Because of the presence of multiple *CUP1* gene copies, the *CUP1-lacZ* gene could integrate at numerous different sites. However, three of the four transformants examined [LDY227(B), LDY228(A), and LDY228(B)] appear to have integrated the *CUP1-lacZ* fusion gene at identical sites (B). UKY403 and MHY308 genomic DNAs do not hybridize to the *lacZ* DNA probe. (B) Autoradiogram of DNAs from LDY227(A) and -(B) (lanes 1 and 2), LDY228(A) and -(B) (lanes 3 and 4), MHY308 (lane 5), and UKY403 (lane 6).

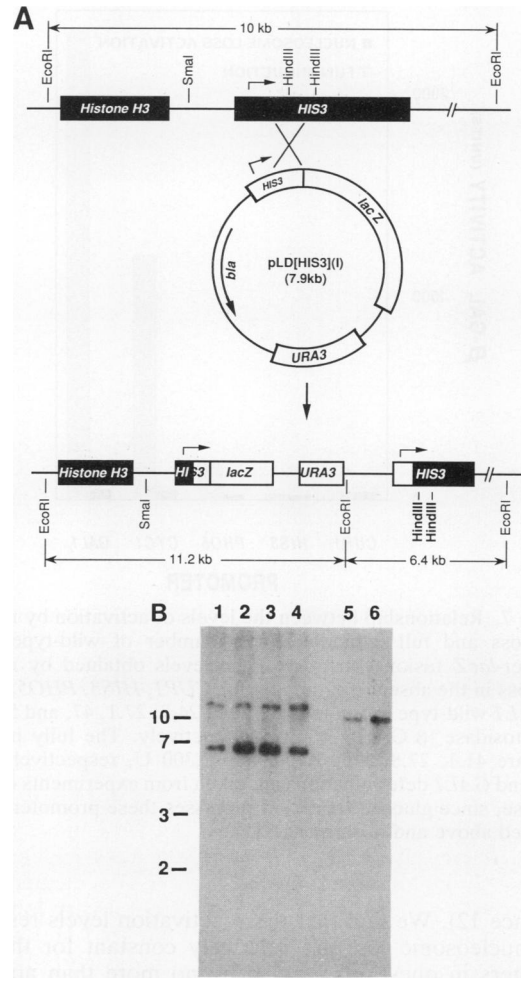

FIG. 6. (A) Integration of pLD[*HIS3*](I) into the *HIS3* genomic locus. Plasmid pLD[*HIS3*](I) containing the *HIS3-lacZ* fusion gene (7.9 kb) was digested with *Bst*BI, which cleaves the plasmid once within the *HIS3* promoter, and transformed into UKY403 and MHY308. Genomic DNA was isolated from each of two independent transformants for each strain, as well as from UKY403 and MHY308, and digested with *Eco*RI. *Eco*RI cleaves on either side of the *HIS3* gene (as shown at the top) and once within plasmid pLD[*HIS3*](I). DNA was fractionated on a 1% agarose gel and prepared for hybridization to a *HIS3* probe as described in Materials and Methods. Radioactively labeled *HIS3* DNA hybridizes to a 10-kb *Eco*RI fragment from UKY403 and MHY308 (top) and to 11.2- and 6.4-kb *Eco*RI fragments after integration of pLD[*HIS3*](I) into the genome (bottom). Filled boxes represent genomic sequences; open boxes represent integrated plasmid DNA sequences. (B) Autoradiogram of DNAs from LDY215(A) and -(B) (lanes 1 and 2), LDY216(A) and -(B) (lanes 3 and 4), MHY308 (lane 5), and UKY403 (lane 6).

sion. Therefore, the transcriptional activity measured after glucose-mediated nucleosome loss could potentially represent the net effect of induction by nucleosome loss and repression by glucose. We thus compared β-galactosidase activities for the *GAL1* and *CYC1* gene promoter-*lacZ* constructs deleted of either their UAS or both their UAS and potential URS elements. Nucleosome loss induced promoter activity similarly from either of the deletion constructs in both of these promoters (data not shown). Therefore, glucose repression cannot account for the low level of nucleosome loss activation of these two gene promoters.

There are a number of possible causes for these varying levels of promoter activation by nucleosome loss. Unique factors may be bound at or near the TATA elements of the *CUP1* and *HIS3* promoters in their uninduced states (29, 32). Since nucleosomes contribute to superhelical folding of DNA, nucleosome loss may have a differential effect on each TATA promoter element, altering the ease in formation of the open complex recognized by RNA polymerase (25). However, we find these possibilities less compelling than that suggested by a comparison between absolute activation levels caused by nucleosome loss and those resulting from full induction (Fig. 7) (taken from data presented above and

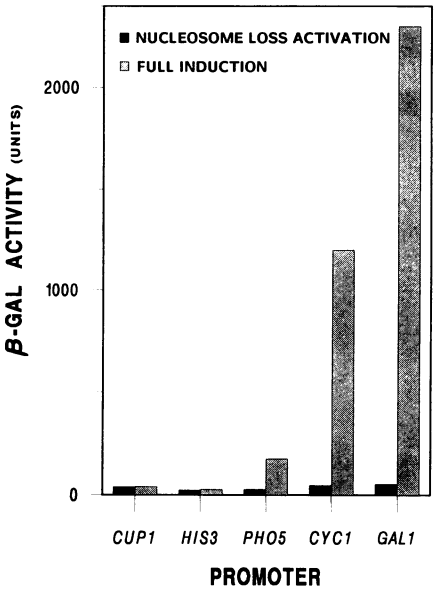

FIG. 7. Relationship between the levels of activation by nucleosome loss and full induction for a number of wild-type yeast promoter-*lacZ* fusion constructs. The levels obtained by nucleosome loss in the absence of inducer for *CUP1*, *HIS3*, *PHO5*, *CYC1* and *GAL1* wild-type promoters are 40.3, 24.3, 27.1, 47, and 54 U of β-galactosidase (β-GAL) activity, respectively. The fully induced levels are 41.1, 27.8, 175, 1,200, and 2,300 U, respectively. The *CYC1* and *GAL1* determinations are taken from experiments done in galactose, since glucose treatment represses these promoters (data described above and in reference 12).

reference 12). We find that these activation levels resulting from nucleosome loss are relatively constant for the five promoters in question, varying by no more than approximately twofold. In contrast, the fully induced levels vary approximately 40-fold. These data may be interpreted in the following theoretical context. Basal transcription may be defined as the level obtained by the recognition of the nucleosome-free TATA promoter element by basal transcription factors. This level is likely to be repressed by nucleosomes in vivo (9, 30, 39). Hence, activation by nucleosome loss mediated (directly or indirectly) by the UAS-binding activator may be viewed as an initial chromatin-dependent transcription step. However, once nucleosomes have been displaced from the site of initiation, they are unlikely to block chromatin-independent transcription resulting from (direct or indirect) interactions between activators and the preinitiation complex (9). Our data suggest that the first, chromatin-dependent activation step provides for a relatively constant, absolute level of activation. This may be due to the recognition of TATA elements by a similar preinitiation complex for all promoters. The extents to which different promoters are activated in the chromatin-independent step may then be explained by different interactions between unique activators and similar preinitiation complexes.

We have shown for *CUP1*, *PHO5*, *CYC1*, and *GAL1* promoters that nucleosome loss in combination with normal induction does not lead to activation levels greater than that seen by induction alone in the control (WT-H4) promoter strain. This finding suggests that nucleosome loss may be a component of the activation mechanism (in the case of *CUP1*, the major component). Surprisingly, when the *HIS3* promoter, containing poly(dA-dT), UAS, and T_r elements, is induced by aminotriazole at the same time that histone H4 synthesis is repressed, activation levels produced by both aminotriazole and nucleosome loss appear additive. Only when the upstream promoter elements are deleted does nucleosome loss lead to approximately the same (lower) level of activation in both the presence and absence of aminotriazole. We have shown that activation by nucleosome loss does not require the UAS elements of any of the other four promoters examined. However, activation of just the TATA and I elements of the *HIS3* promoter by nucleosome loss alone produces activity just 40% of that seen when the wild-type *HIS3* promoter is activated in a similar manner. It remains to be determined how the unique regulatory factors at the upstream promoter enable the *HIS3* promoter to respond in this additive manner.

Nucleosome loss from the genomic *PHO5* promoter results in increased steady-state mRNA levels. The genomic *CUP1* gene, whose nucleosomes are displaced by repression of histone H4 synthesis, does not show a similar increase (13, 15) despite clear *CUP1* promoter activation to fully induced levels when fused to the β-galactosidase gene. Theoretically, this difference could be due to the presence of the *CUP1* promoter-*lacZ* fusion construct on a plasmid. Nucleosome loss could potentially affect the topology of a constrained plasmid DNA in a manner different from DNA in the genome. Alternatively, the *CUP1* mRNA produced by the genomic DNA copies may be regulated posttranscriptionally, causing it to be more labile than the β-galactosidase message in the absence of Cu^{2+}. Our data have shown for both the *CUP1-lacZ* and *HIS3-lacZ* constructs that the levels of activation are very similar when these genes are present in plasmids or at their genomic loci. Therefore, we believe that the difference between steady-state mRNA levels produced by genomic and *CUP1-lacZ* constructs may be due to differences in their posttranscriptional regulation. This notion has been substantiated by mRNA stability studies in which we have evaluated the half-lives of both the *CUP1* and the *CUP1-lacZ* messages in vivo, utilizing a PolII temperature-sensitive strain (10). While the native *CUP1* message has an apparent half-life of approximately 6 to 7 min, that of the fusion message is approximately 43 min. Previous studies (26) of *lacZ* message stability in yeast cells have found a 27- to 28-min half-life, which is still four to five times higher than that found for the native *CUP1* message. These stability differences are likely to contribute considerably to the differences observed in the steady-state levels of native *CUP1* and *CUP1-lacZ* transcripts observed after nucleosome loss.

In conclusion, the repression of yeast histone H4 synthesis activates the downstream promoter elements of five different genes to approximately the same extent. In contrast, the fully induced levels for these genes vary greatly. For certain genes (*CUP1* and *HIS3*), activation by nucleosome loss nears fully induced levels, arguing that chromatin-dependent gene activation, mediated through the activator proteins, may be a major component of their induction. For other genes (*CYC1* and *GAL1*), activation by nucleosome loss represents a much smaller component of their full activation. In these cases, chromatin-independent activation appears to play the major role in their regulation. *PHO5* regulation is intermediate between these two extremes. We have little understanding at present of the mechanism by which nucleosomes are displaced in order to allow the initiation of transcription. It seems reasonable at this time to assume that this mechanism is common to most regulated yeast genes. We have recently found that the histone H4 N terminus is required for the full activation of a number of

yeast genes, including *GAL1*, *PHO5*, and *CUP1* (1). Determining which transcriptional components interact with the H4 N terminus may lead to a better understanding of the means by which nucleosomes and nucleosome displacement are involved in transcriptional initiation.

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