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Yeast Histone H4 N-Terminal Sequence Is Required for Promoter Activation In Vivo

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Summary

To search for histone domains that may regulate transcription in vivo, we made deletions and amino acid substitutions in the histone N-termini of S. cerevisiae. Histone H4 N-terminal residues 4–23, which include the extremely conserved, reversibly acetylated lysines (at positions 5, 8, 12, and 16), were found to encompass a region required for the activation of the *GAL1* promoter. Deletions in the H4 N-terminus reduce *GAL1* activation 20-fold. This effect is specific to histone H4 in that large deletions in the N-termini of H2A, H2B, and H3 do not similarly decrease induction. Activation of the *PHO5* promoter is reduced approximately 4- to 5-fold by these H4 deletions. Mutations in histone H4 acetylation sites and surrounding residues can cause comparable and, in some cases, even greater effects on induction of these two promoters. We postulate that the H4 N-terminus may interact with a component of the transcription initiation complex, allowing nucleosome unfolding and subsequent initiation.

Introduction

The nucleosome consists of a histone octamer (a histone H3–H4 tetramer and two histone H2A–H2B dimers) around which 146 bp of DNA are wrapped approximately 1.8 times. Higher eukaryotic nucleosomes also contain one molecule of histone H1, which is involved in further DNA compaction. However, H1 has not yet been proven to exist in certain lower eukaryotes (e.g., Saccharomyces cerevisiae) whose chromatin appears to be less highly condensed (Grunstein, 1990a). Nucleosomal folding of DNA represses transcription initiation. A nucleosome positioned at a promoter will block initiation in vitro (Knezetic and Luse, 1986; Matsui, 1987; Workman and Roeder, 1987; Lorch et al., 1987; Simpson, 1991). Furthermore, in S. cerevisiae, changes in histone stoichiometry alter transcription patterns (Clark-Adams et al., 1988), nucleosome loss obtained by repressing histone synthesis activates initiation (Han et al., 1988; Han and Grunstein, 1988), and nucleosomes at defined positions can repress transcription (Roth et al., 1990; Straka and Horz, 1991). Therefore, the histone octamer may be envisioned as a relatively simple protein complex that condenses DNA and, in the process, blocks access to transcription factors in the living cell.

A careful examination of structural, biochemical, and genetic data, however, suggests a more complex, multifunctional nucleosome. The core histones all contain hydrophobic C-termini that are required for nucleosome assembly and stability. Largely, it is these globular regions that are visualized by nuclease digestion, electron microscopy, and X-ray crystallography (van Holde, 1988). The histone N-termini are "invisible" in these experiments and are largely dispensable for either nucleosome assembly or stability in vitro (Whitlock and Stein, 1978) or in vivo (Schuster et al., 1986; Kayne et al., 1988). Nevertheless, it is the N-termini that are likely to provide the nucleosome with its functional complexity. By a number of studies including trypsin digestion, nuclear magnetic resonance, and antibody binding, the unordered hydrophilic N-terminal tails, which make up from one-fourth to one-third of each protein, have been shown to extend from the nucleosomal core (McGhee and Felsenfeld, 1980; Schroth et al., 1990). They are positively charged and are the sites of reversible posttranslational modifications such as acetylation, phosphorylation, and methylation, which would alter their charge and possible interactions with DNA or other proteins. For example, acetylation of epsilon amino groups on lysine residues at the histone N-termini has long been correlated with increased levels of transcription (Allfrey, 1977; Allegra et al., 1987) possibly by causing chromatin unfolding, although this has not been firmly established (Allan et al., 1982; Annunziato et al., 1988). Furthermore, an antibody to acetylated H4 residues (lysines 5, 8, 12, and 16) interacts preferentially with active chromatin (Hebbes et al., 1988). Therefore, acetylation of histones may regulate greater promoter access to transcription factors. If so, one or more histone N-termini may be required for the activation of transcription in vivo.

The functional complexity of the histone N-termini is further illustrated by structural differences between the N-terminal tails. Unlike the H2A and H2B tails, the H3 and H4 tails are much more highly conserved between plants and animals. H3 and H4 acetylation (H3 is acetylated at lysine positions 9, 14, 18, and 23; Allfrey et al., 1983) is more closely correlated with transcription during the Physarum cell cycle (Waterborg and Matthews, 1984). Also, H3 and H4 acetylation has been reported to mimic prokaryotic gyrase function, generating more negative supercoils in internucleosomal DNA released from nucleosomes (Norton et al., 1989, 1990). However, these experiments have not excluded the possibility that H3 or H4 alone may be responsible for this gyrase-like activity.

The strongest evidence for histone tail-specific functions comes from genetic data obtained in S. cerevisiae. The yeast H4 N-terminus contains a repressor (R) domain consisting of four adjacent, positively charged residues (residues 16–19). Deletion of this domain (Kayne et al., 1988) or a nonconservative change in any of these amino acids (Megee et al., 1990; Johnson et al., 1990; Park and Szostak, 1990) activates the silent mating loci specifically, and blocks mating. Deletions in either H2A or H2B N-termini have little if any effect on yeast mating (Kayne et al., 1988). Histone H4 function in silencing may be explained by a

direct or indirect interaction with a repressor of the silent mating loci. We have shown that there is a genetic interaction between the histone H4 R domain and the repressor protein Sir3, since a point mutation in the H4 R domain is suppressed by either of two mutations in the N-terminus of Sir3 (Johnson et al., 1990).

In this study we have investigated whether any of the histone N-termini are required for gene activation in vivo. We have discovered that the H4 N-terminus is required for induction of *GAL1* by galactose and *PHO5* by low concentrations of inorganic phosphate. The H4 N-terminal domain necessary for promoter activation contains the invariant sites of reversible acetylation. Replacement of these sites with other amino acids causes activation to be repressed as much or in some cases even more than deletion of these residues. These effects are specific to *GAL1* and *PHO5* in that expression of the rapid response gene, *CUP1*, and two constitutive genes, *GAL4* and *PRC1*, is not much affected by most of the H4 N-terminal lesions. Since the other histone H2A, H2B, and H3 N-termini do not have similar functions, we have now defined both an activator and a repressor function unique to the H4 N-terminus.

Results

The Histone H4 N-Terminus Is Required for *GAL1* Activation In Vivo

S. cerevisiae contains two gene copies for each of the core histones (Hereford et al., 1979). We have previously shown for histone H4 that both of the copies may be disrupted and the cell rescued by a centromeric plasmid (plasmid A) containing H4 under *GAL1* control. This strain grows relatively normally on galactose-containing medium (YEPG) but arrests in G2 when H4 synthesis is repressed in glucose-containing medium (YEPD). To test for the effects of H4 deletions on cell viability, a second centromeric plasmid (plasmid B) containing the mutant H4 gene under control of its wild-type promoter was included in the genetic background. The replica plating of cells from galactose to glucose medium (the glucose shift viability test) allowed rapid quantitation of the effect of the H4 mutation on cell viability. Cells whose H4 mutations were shown to allow viability were then grown on the appropriate medium, allowing loss of plasmid A (Kayne et al., 1988). By this procedure we have shown that H4 N-terminal residues 4–28 were not required for cell viability (Kayne et al., 1988). A similar approach was used to demonstrate that the N-terminal residues 3–32 of histone H2B and 4–20 of H2A were also dispensable for cell growth (Wallis et al., 1983; Schuster et al., 1986).

The N-terminus of histone H3, like that of H4, is mostly invariant in evolution (van Holde, 1988). In experiments that will be described elsewhere (R. Mann and M. Grunstein, unpublished data), we have shown that large H3 deletions (residues 4–30) in the hydrophilic N-terminus will also allow cell viability in a genetic background lacking wild-type chromosomal H3 genes. The deleted H3 sequence contains all four invariant acetylated lysine residues (at positions 9, 14, 18, and 23). Therefore, we have now shown that the hydrophilic N-termini of all four core histones are dispensable for viability. Since acetylation of histone N-termini, especially of the H3 and H4 ends, is correlated with transcriptional activity, we wished to determine whether any of the histone N-termini are required for gene activation.

We chose to examine the *GAL1* gene due to its inactivity in glucose or raffinose and its induction, by close to 1000-fold, in galactose. Induction of new *GAL1* mRNA synthesis was measured after galactose treatment for 6 hr, at various ratios of raffinose and galactose, by hybridization to a ^{32}P-labeled, *GAL1*-specific DNA probe in a Northern blot analysis and examined in yeast strains lacking separately each of the four core histone N-terminal tails. Corresponding isogenic wild-type strains were included as controls (Figure 1). Strains containing H4 del(4–28) (Kayne et al., 1988), H3 del(4–30), H2A del(4–20) (Schuster et al., 1986), and H2B del(3–32) (Wallis et al., 1983) were analyzed. In each histone, all the potential sites of acetylation and other posttranslational modifications shown to be present in calf and conserved in other eukaryotes have been removed by the deletion in question (Figure 2). It is evident in Figure 1 that only the H4 N-terminal deletion causes an obvious, sizable decrease in *GAL1* induction. To control for possibly varying amounts of RNA loaded on the gel prior to Northern blot analysis, the RNA blot was washed and rehybridized to a carboxypeptidase Y (*PRC1*) DNA probe whose mRNA level has been shown to be relatively constant throughout the cell cycle (Xu et al., 1990; also see section below). These data demonstrate the unique effect of the H4 N-terminal deletion on repressing *GAL1* induction.

H4 N-Terminal Deletions Localize the Region Required for *GAL1* Activation between Residues 4 and 23

To determine which H4 N-terminal sequences are required for *GAL1* induction, *GAL1* mRNA levels were examined by Northern blot analysis in isogenic strains carrying nested deletions H4 del(4–14), H4 del(4–19), H4 del(4–23), and H4 del(4–28) (Kayne et al., 1988). Each of these deletions decreases the amount of *GAL1* mRNA below wild-type levels after galactose treatment. In contrast, the level of *PRC1* mRNA probed as a control on this blot is not obviously changed in the deletion strains analyzed (Figure 3). To quantitate *GAL1* promoter induction more carefully, we analyzed episomal fusions between the *GAL1* promoter and the Escherichia coli β-galactosidase (*lacZ*) gene (West et al., 1984). As shown in Figure 4, H4 del(4–14) reduces the *GAL1*-regulated β-galactosidase level approximately 5-fold compared with the wild-type level. A similar comparison shows a reduction of approximately 7-fold in H4 del(4–19) and 20-fold in both H4 del(4–23) and H4 del(4–28). Therefore, the H4 N-terminal effect on *GAL1* induction may be attributed largely to a sequence within the domain 4–23.

GAL1, *GAL7*, and *GAL10* Activation Is Dependent on H4 N-Terminal Sequences

GAL1, *GAL7*, and *GAL10* are members of a gene family whose promoters are all regulated by the constitutively synthesized activator protein *GAL4* (Johnston, 1987). Each of these genes may be induced some three orders of magni-

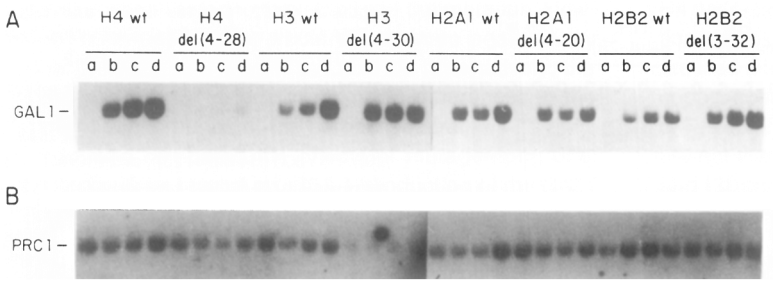

Figure 1. *GAL1* mRNA Levels in Wild-Type and Variant Yeast Strains

Yeast strains PKY501 (WT *HHF2* or H4), PKY813 (H4 del(4–28)), RMY200 (WT *HHT* or H3–2), RMY430 (H3–2 del(4–30)), TSY223 (WT *HTA1* or H2A1), TSY226 (H2A1 del(3–20)), TSY150 (WT *HTB2* or H2B2), and TSY155 (H2B2 del(2–32)) were induced 6 hr in YEP containing the following ratios of raffinose to galactose: lanes a, 2%:0%; lanes b, 1.5:0.5%; lanes c, 1%:1%; lanes d, 0%:2%. RNA (15 μg) was analyzed by Northern blot hybridization. (A) RNA was hybridized to a uniformly labeled EcoRI fragment of pNN78 (*GAL1* DNA; St. John and Davis, 1981). (B) The radioactive *GAL1* probe was washed from the blot in (A) and the blot was rehybridized to uniformly labeled *PRC1* (carboxypeptidase Y) DNA used as a loading control.

tude in the presence of galactose. To determine whether the mRNA levels of all three genes are dependent on the H4 N-terminus, a single Northern blot was probed with ^{32}P-labeled fragments of each of these genes. As shown in Figure 3, *GAL1*, *GAL7*, and *GAL10* mRNA levels are all reduced to comparable levels in the H4 deletion strains after 6 hr of induction. The activator *GAL4* gene is believed to be transcribed constitutively in glucose and galactose (Laughon and Gestelend, 1982). To determine the extent to which *GAL4* mRNA levels were affected by H4 deletions, aliquots of the same RNA preparations described above were blotted and hybridized to a *GAL4* DNA probe. As shown in this figure, there is no obvious decrease in *GAL4* mRNA levels in strains carrying the H4 N-terminal deletions. It is especially noteworthy that the *GAL4* mRNA levels are relatively constant across the entire range of H4 N-terminal deletions in contrast to the proportionately greater decrease in *GAL1*, *GAL7*, and *GAL10* mRNA levels. This was confirmed by densitometry of the exposed autoradiograph and normalizing the *GAL4* mRNA levels to those of the loading control *PRC1* levels (data not shown). Therefore, it is highly unlikely that a decrease in *GAL4* mRNA levels is responsible for the reduced *GAL1*, *GAL7*, and *GAL10* mRNA levels seen in Figure 3.

Replacement of Reversibly Acetylated H4 Lysine Residues at Positions 5, 8, 12, and 16 Decreases *GAL1* Activation

Since H4 amino acids 4–23 contain, at positions 5, 8, 12, and 16, lysine residues that are acetylated (Nelson, 1982)

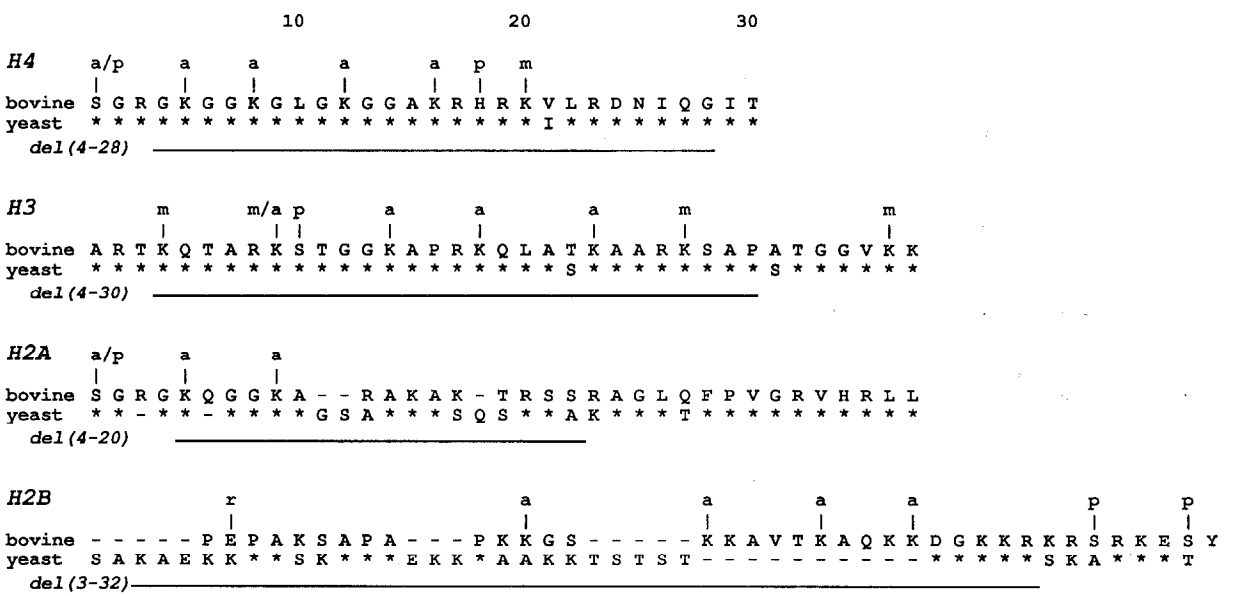

Figure 2. Histone N-Terminal Modification Sites

Amino acid sequences of bovine histone N-termini are shown, and amino acids that differ in yeast are indicated below the bovine sequences (Wu et al., 1986). A star indicates sequence conservation. A dash indicates an amino acid is missing in that sequence. In vivo posttranslational modifications are as follows: a, acetylation; m, methylation; p, phosphorylation; r, ADP-ribosylation. The underlined sequences were deleted in the relevant mutant yeast strains. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

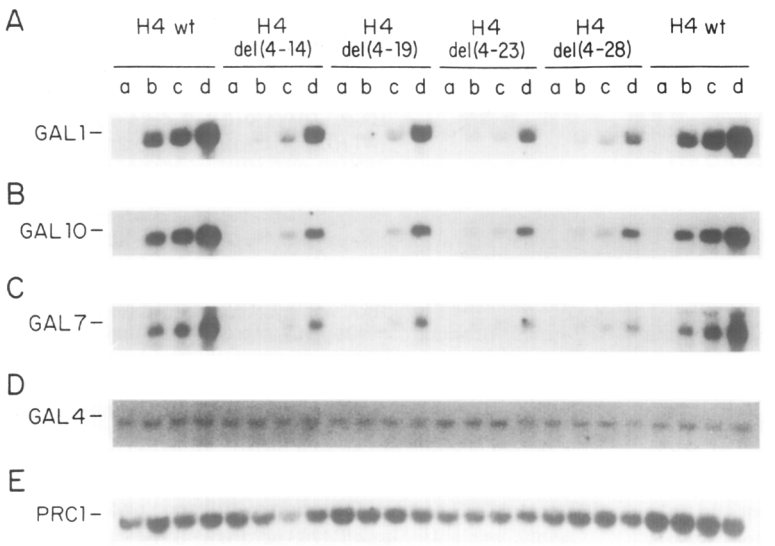

Figure 3. *GAL1*, *GAL10*, *GAL7*, and *GAL4* mRNA Levels in Yeast Strains with Wild-Type or N-Terminally Deleted Histone H4–2

PRC1 lanes are used as controls for mRNA loading. Yeast strains PKY501 (WT H4), PKY817 (H4 del(4–14)), PKY818 (H4 del(4–19)), PKY806 (H4 del(4–23)), and PKY813 (H4 del(4–28)) were induced as described in the legend to Figure 1. RNA (15 μg, or 30 μg from the same preparation for the *GAL4* blot) was analyzed by Northern blot hybridization to uniformly labeled (A) EcoRI fragment of pNN78 (see legend to Figure 1); (B) EcoRI–SalI fragment of pNN76 (*GAL10*); (C) SalI fragment of pNN75 (*GAL7*); (D) XhoI–XbaI fragment of pGR-LexGal4 (*GAL4*). (E) The blot rewashed in (A–C) was rehybridized to uniformly labeled *PRC1* DNA. The blot in (D) was also washed free of the *GAL4* probe and rehybridized to *PRC1* DNA. The levels of PRC1 mRNA were very similar to those in (E) (data not shown).

and implicated in transcriptional control, we wished to determine the effects of mutagenizing these sites on *GAL1* activation. Yeast strains, with either conservative or nonconservative changes in the histone H4 N-terminal lysine residues, were prepared as described in Experimental Procedures. Specifically, one, three, or all four of the lysine residues at positions 5, 8, 12, and 16 of histone H4 were changed to arginine (which conserves the net positive charge of lysine, but which cannot be neutralized by acetylation). Alternatively, glutamine, which resembles lysine structurally, was substituted at these four positions to mimic the charge neutralization resulting from acetylation.

Figure 4 shows the N-terminal amino acids of wild-type histone H4 (in strain PKY501) and the specific amino acid substitutions and deletions that were introduced into this sequence. All yeast strains containing amino acid substitutions at the H4 N-terminus were viable whether one, three, or all four lysines were changed to arginine or glutamine. These results confirm those reported previously (Megee et al., 1990; Johnson et al., 1990; Park and Szostak, 1990) with one exception. Megee et al. (1990) have previously reported the lethality of yeast containing arginines at positions 5, 8, 12, and 16. In our experiments, LDY722, which bears these same changes, is viable although slow growing (doubling in 330 min as opposed to 120 min for the wild-type control strain, PKY501). This discrepancy may be attributed to different approaches used to assay viability. In contrast to the glucose shift viability test, Megee et al. (1990) assayed viability by the absence of a particular strain after sporulation or after transformation. This ab-

Strain	Histone H4 N-terminus				Doubling Time (min)	β-galactosidase activity		
	5	8	12	16		GAL1	PHO5	CUP1
	Ac	Ac	Ac	Ac				
PKY501	S G R G K G G K G L G K G G A K R H R K				120	1.00	1.00	1.00
PKY817	del(4-14)				137	0.20	0.56	0.81
PKY818	del(4-19)				142	0.14	0.60	0.42
PKY806	del(4-23)				167	0.06	0.24	0.53
PKY813	del(4-28)				180	0.05	0.21	0.48
LDY101	R				124	1.56	1.39	0.99
LDY103		R			121	1.38	1.24	0.93
LDY105			R		119	0.87	1.25	1.31
PKY506				R	115	1.31	1.26	2.25
PKY508		R	R	R	132	0.26	0.89	0.83
PKY821	R	R	R		154	0.19	0.48	0.80
LDY722	R	R	R	R	330	0.02	0.01	0.11
LDY107	Q	Q	Q	Q	153	0.10	0.53	0.28

Figure 4. Mutational Analysis of the H4 N-Terminus

The N-terminal 20 amino acids of H4 are shown. Deletions are indicated and mutations shown schematically below the sequence. All strains are isogenic to PKY501. The β-galactosidase assays were repeated three times with each of two individual transformants for each strain. Error was less than 20%. The data are presented as a percentage of the wild-type (PKY501) activity for each of the three genes examined.

sence was attributed to the lethality of the mutation. However, such approaches would discriminate against very slow growing strains such as LDY722. Alternatively, the discrepancy may be due to unknown differences in the genetic backgrounds of the respective strains.

We then compared the effects of mutagenizing one, three, or all four lysine residues on induction of the *GAL1* promoter. Changing single lysine residues at position 5, 8, 12, or 16 to arginine does not significantly decrease the level of *GAL1–lacZ* induction (Figure 4). Nor do single substitutions altering the positively charged amino acids at position 17, 18, 19, or 20 in a nonconservative manner (L. Durrin and M. Grunstein, unpublished data). However, changing lysines 8, 12, and 16 to arginines (PKY508) or changing lysines 5, 8, and 12 to arginines (PKY821) decreased *GAL1–lacZ* induction 4- and 5-fold, respectively. Replacement of all four lysines 5, 8, 12, and 16 to arginines (LDY722) repressed *GAL1–lacZ* induction greatly, approximately 50-fold. The analogous change to four glutamine residues in LDY107 had a strong (10-fold) but lesser effect on induction of *GAL1*. These data suggest a redundancy in the requirement for the conserved acetylated lysine residues in *GAL1* activation, and suggest also the need for charge neutralization at the H4 N-terminus.

Doubling times were measured for the wild-type (PKY501) and mutant yeast strains growing in medium with glucose as the sole carbon source (YEPD) (Figure 4). In general, the more extensive the lesion at the histone H4 N-terminus, the slower the growth of the yeast strain. This is especially evident in comparing the H4 N-terminal deletions. Removal of residues 4–14 and 4–19 increases the doubling time from 120 to 137 and 142 min, respectively. Deletions 4–23 and 4–28 further increase doubling time to 167 and 180 min. While mutation of a single lysine residue to arginine at position 5, 8, 12, or 16 has a negligible effect on growth rate, mutation of three of the four lysines to arginine increases doubling time to 132–154 min, and mutating all four lysines to arginine has a considerable effect on growth rate, increasing the doubling time to 330 min. Charge neutralization at these sites may be very important since the analogous mutation of these positions to four glutamine residues, or deletion of all four lysine residues in PKY818 (H4 del(4–19)), only increases doubling time to 153 and 142 min, respectively. It is important to indicate that the effects we see on *GAL1* induction are not due simply to slower growth rate. *GAL1–lacZ* induction in control strains containing mutations in nonhistone genes and exhibiting very slow growth (doubling at 175–264 min) is decreased no more than a factor of 2 (data not shown).

The H4 N-Terminus Is Also Required for *PHO5* Promoter Activation

To investigate whether the histone H4 N-terminus is required for the activation of other regulated yeast promoters, we examined *PHO5* promoter–*lacZ* and *CUP1–lacZ* constructs in the H4 wild-type and mutant yeast strains. The *PHO5* promoter is repressed by high concentrations of inorganic phosphate in the medium and is activated by low concentrations of inorganic phosphate (Oshima, 1982). As shown in Figure 4, H4 del(4–23) in PKY806 and H4 del(4–28) in PKY813 each decrease *PHO5–lacZ* induction approximately 4- to 5-fold. These same deletions depressed *CUP1–lacZ* fusions, activated by the presence of copper sulfate (Fogel and Welch, 1982; Karin et al., 1984), only 2-fold. These decreases in *PHO5* promoter activity are not due to the slower growth of these strains (167 min and 180 min, respectively) since a control strain containing nonhistone mutations and growing with a division time of 175 min shows no decrease in *PHO5–lacZ* activity (data not shown). Single amino acid changes, at any of the acetylated lysine residues, do not decrease *PHO5* promoter activity. Even substitution of any three of the four lysines to arginine (in PKY508 or PKY821) or substituting all four lysines 5, 8, 12, and 16 to glutamine (in LDY107) results in at most a 2-fold depression in activity.

Of the amino acid substitutions, only the replacement of lysines 5, 8, 12, and 16 with arginine, in LDY722, greatly decreased *PHO5–lacZ* activity (approximately 100-fold) and *CUP1–lacZ* activity (approximately 10-fold). This mutation, which decreased *GAL1–lacZ* induction 50-fold, has a strong effect on transcription of the constitutive *PRC1* gene as well. In Figure 5 is shown a Northern blot containing induced *GAL1* mRNA and control *PRC1* mRNA in strains containing either wild-type H4 or mutant H4 genes. While the levels of *GAL1* induction are reduced the most in LDY107 (lysines 5, 8, 12, and 16 changed to glutamine) and in LDY722 (lysines 5, 8, 12, and 16 changed to arginine) (Figure 5A), the *PRC1* levels are significantly reduced only in LDY722 (Figure 5B). This is the case even though approximately equal amounts of RNA (measured by ribosomal RNA content) were loaded in each well (Figure 5C). Therefore, LDY722 shows decreased transcription of all four genes examined (*GAL1*, *PHO5*, *CUP1*, and *PRC1*), arguing that this H4 mutation has a generally repressive effect on transcription. This is the likely explanation for its abnormally slow growth.

Discussion

The data described above show that a yeast histone H4-specific domain (A) encompassing residues 4–23 is required for *GAL1* and *PHO5* promoter activation in vivo. Conserved acetylated lysine residues in this domain are important for activation; however, there is redundancy in their function. H4 residues 16–19 (domain R) are also required for repression of the silent mating loci *HML* and *HMR* (Kayne et al., 1988; Megee et al., 1990; Johnson et al., 1990; Park and Szostak, 1990) and nucleosome positioning adjacent to the α2 operator (S. Y. Roth, M. Shimuzu, L. Johnson, M. Grunstein, and R. T. Simpson, unpublished data). These two functions can be quite distinct from each other in that certain amino acid replacements at positions 16–19 that severely disrupt mating have no detectable effect on *GAL1* activation (see Results). Conversely, deletion or mutation of the A domain (between residues 4 and 15) can affect *GAL1* activation with little effect on repression of the silent mating loci (Kayne et al., 1988; L. Johnson and M. Grunstein, unpublished data). In contrast, the H2A, H2B, and H3 N-terminal sequences (removing residues 4–20, 2–32, and 4–30, respectively)

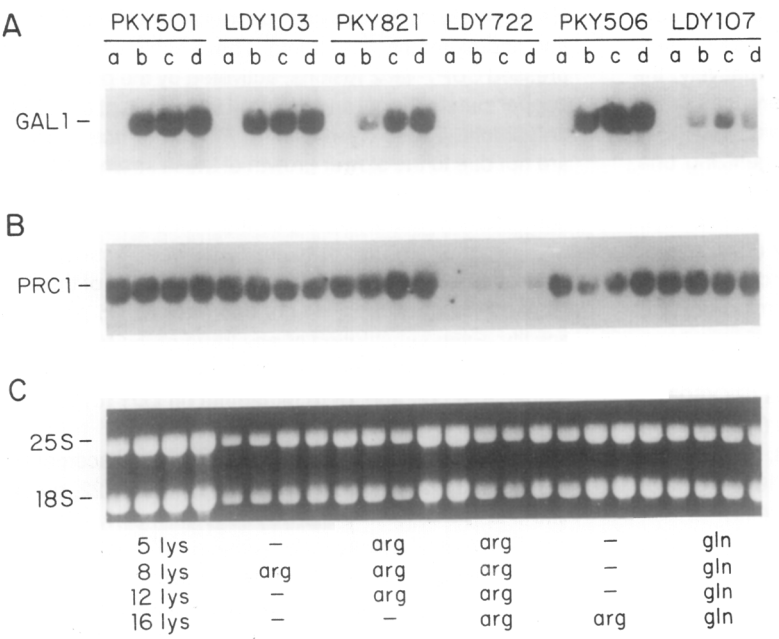

Figure 5. *GAL1* mRNA Levels in Wild-Type and Variant Yeast Strains

RNA was prepared from yeast strains PKY501, LDY103, PKY821, LDY722, PKY506, and LDY107 (see Figure 4) that had been induced with galactose as described in the legend to Figure 1. RNA (15 μg) was analyzed by gel electrophoresis and Northern blot hybridization to DNA fragments as described in the legend to Figure 1. (A) *GAL1* mRNA. (B) *PRC1* mRNA. (C) 25S and 18S ribosomal RNAs.

are not required for promoter activation, nor are they required for repression of the silent mating loci in a manner similar to that of the H4 N-terminus (Kayne et al., 1988; R. Mann and M. Grunstein, unpublished data). Therefore, we conclude that histone H4 uniquely contains domains both for promoter activation (A) and for the repression of the silent mating loci (R) (Figure 6).

These observations raise a number of questions that point to new areas of investigation. Since DNA, in the absence of histones, can serve as a template for transcription factors in vitro, why would a histone be required for gene activation in vivo? A working model we employ at this stage is shown in Figure 7. This utilizes the observation that the template in vivo is not naked DNA but chromatin. Since nucleosomes can repress transcription initiation in vivo, a direct or indirect interaction between the activation domain of the *GAL4* activator protein and the basic N-terminus of histone H4 may be necessary for the subsequent unfolding of the nucleosome. This chromatin-dependent activation step would allow access of the preinitiation complex to the TATA promoter element. Evidence for this also comes from experiments done by Workman et al. (1991), who showed that a heterologous activation domain fused to a *GAL4* derivative can prevent nucleosomal repression of initiation in vitro. Only after unfolding of the nucleosome would the interactions between the activator domain and the preinitiation complex involving possible coactivators (Lewin, 1990; Ptashne and Gann, 1990) lead to full induction. This latter step is likely to be chromatin independent.

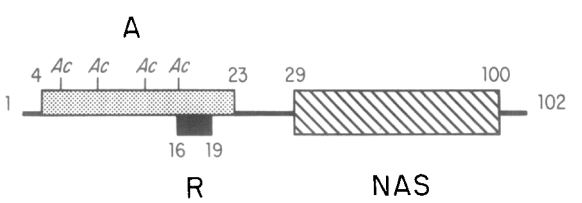

Figure 6. Diagram of Known Functional Domains of Histone H4

Domain A is the promoter activation domain located within amino acids 4–23. Domain R is the repressor domain (residues 16–19) that interacts genetically with the repressor protein Sir3 to silence *HML* and *HMR*. NAS is the hydrophobic, nucleosome assembly and stability domain that includes the C-terminal amino acids from 29–100.

The activity of the *GAL1* promoter was reduced approximately 20-fold in the presence of the more extensive H4 N-terminal deletions, while *PHO5* promoter activity was reduced 4- to 5-fold. *GAL4* and *PRC1* expression was not much affected. The reasons for these differences should be especially instructive. It is important to note that both *GAL1* and *PHO5* contain nucleosomes positioned on their promoters (Lohr, 1984; Almer et al., 1986; Fedor et al., 1988; Rainbow et al., 1989) that are likely to repress basal transcription in the absence of inducer (Han and Grunstein, 1988; Straka and Horz, 1991). Since gene activity is most often accompanied by nucleosome displacement from the promoter (Grunstein, 1990b) one might expect that the promoters of constitutive genes such as *GAL4* and *PRC1* would not be tightly folded in nucleosomes. Such differences in nucleosome positioning could be due to the underlying nucleosome positioning DNA sequence or the presence of proteins affecting nucleosome placement (Grunstein, 1990b; Simpson, 1991). For example, a protein (GRF2/protein Y) at the *GAL1* UAS_G appears to position nucleosomes adjacent to its binding site (Fedor et al., 1988). Positioned nucleosomes at the promoters of repressed genes may require the H4 N-terminus in order to allow nucleosomal displacement as a prelude to activation.

At this stage it is unclear why the *CUP1* promoter is affected the least of the regulated promoters examined, especially as we have no data regarding nucleosome positioning at the *CUP1* promoter. We speculate that the rap-

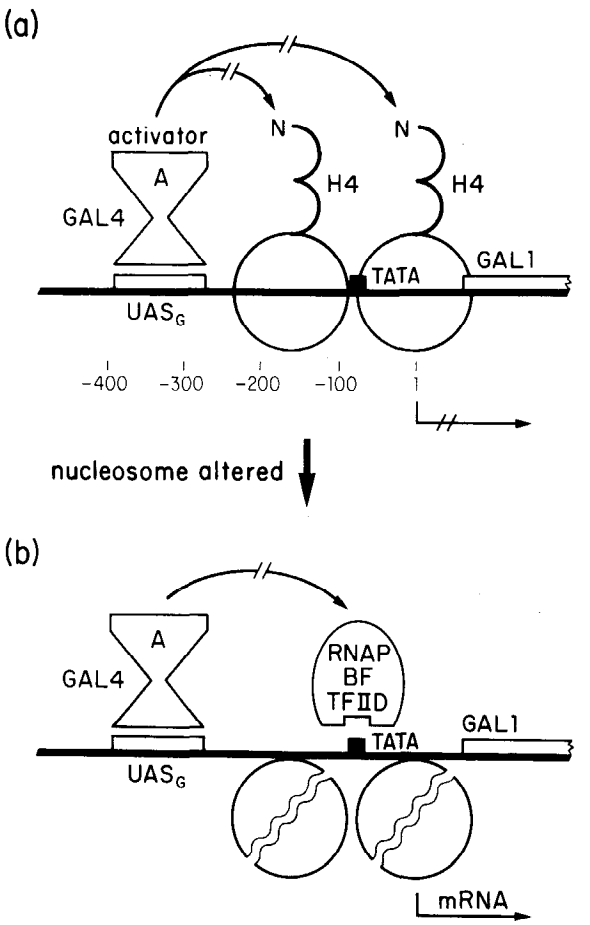

Figure 7. Model Depicting Stages in the Activation of the *GAL1* Gene

(a) A chromatin-dependent direct or indirect interaction between the activator protein (*GAL4*) and the H4 N-terminus is required for generating a structural change in one or more nucleosomes downstream of the UAS element.

(b) This structural alteration or unfolding could then allow release of nucleosomal repression of initiation and access to the TATA-binding protein (TFIID), other basal factors (BF), and RNA polymerase (RNAP). Since this preinitiation complex has partial activity in the absence of the activator (Grunstein, 1990a, 1990b) this could allow an intermediate level of activity. The activator interacting with the preinitiation complex mediated by possible coactivators in a chromatin-independent manner (Lewin, 1990; Ptashne and Gann, 1990) would then provide for full induction.

idly induced *CUP1* promoter (less than 30 min) may not have nucleosomes repressing initiation in the manner of *GAL1* and *PHO5*. As described in our results, it is unlikely that the specific effects of the H4 N-terminal deletions on *GAL1* activation are due solely to a decrease in *GAL4* expression. Also, it is unlikely that the explanation rests with H4 deletions indirectly decreasing the expression of *GAL11*, a weak coactivator of *GAL1*. Strains bearing *gal11* mutations show at most 3- to 4-fold decrease in *GAL1*, *GAL7*, and *GAL10* mRNA levels in galactose (Suzuki et al., 1988) as opposed to the 20-fold reduction in *GAL1* induction seen as a result of histone H4 deletions (Figure 4). However, we cannot exclude the possibility that there is a cumulative effect on *GAL1* activation brought on by less efficient unfolding of the nucleosome and minor effects on expression of *GAL4* activators and coactivators.

Due to the potential involvement of H4 and H3 acetylation in transcription, it is noteworthy that changing any three of the four H4 lysine residues (positions 5, 8, 12, and 16) suppresses induction of *GAL1* 4- to 5-fold, while changing all four sites to arginine (in LDY722) suppresses induction 50-fold. If histone acetylation, by neutralizing positive charges at the N-terminus, allows chromatin to unfold (Annunziato et al., 1988), then this latter mutation may make it difficult to dissociate histone H4 from DNA in order to allow transcription. Alternatively, the high charge density of this mutant H4 N-terminus may sequester acidic transcriptional activator proteins away from the promoter. These possibilities may explain why LDY722 has a much more general repressive effect on transcription leading to very slow growth (330 min doubling time versus 120 min for the isogenic wild-type strain).

It is especially noteworthy that while H3 and H4 are both almost invariant in evolution, highly acetylated, and form a tetramer in the assembly of the nucleosome (van Holde, 1988), deleting the H3 N-terminus (Figure 1) or changing all four conserved acetylated lysines at the H3 N-terminus to arginine (R. Mann and M. Grunstein, unpublished data) does not decrease *GAL1* induction. In fact it appears that there is an increase in *GAL1* induction in the presence of H3 N-terminal deletion H3 del(4–30) (Figure 1). Furthermore, unlike H4 N-terminal deletions, large H3 N-terminal deletions have little effect on repression of the silent mating loci (R. Mann and M. Grunstein, unpublished data). The involvement of H4 but not H3 in both *GAL1* induction and silencing is important in that these data conceptually separate the two histones. Therefore, it is important to reevaluate separately the effects of H3 and H4 N-terminal acetylation on transcription and on plasmid supercoiling in experiments that previously utilized a mix of acetylated H3 and H4 histones (van Holde, 1988; Norton et al., 1990).

In conclusion, both activator and repressor functions have been localized to a portion of the H4 N-terminus, functions absent from the N-termini of the other core histones. Given the extreme conservation of the rest of the H4 N-terminus and other core histone N-termini, it is likely that other functions will also emerge for different histone domains. Dissecting these mechanisms experimentally in yeast should illuminate a new view of the nucleosome in regulating chromosomal functions.

Experimental Procedures

Plasmid Construction and Strain Preparation

To prepare mutations in the N-terminus of the histone H4–2 gene (*HHF2*), sequences encoding amino acids 6–18 were deleted, creating the restriction sites BspEI and AflII (pPK626). Complementary 62 base oligodeoxynucleotides, encoding amino acids 3–22 of H4–2 and incorporating specific mutations, were synthesized by automated DNA synthesis. The annealed oligomers were ligated into pPK626 to create the H4 N-terminus. All mutant gene constructs were sequenced by the method of Sanger et al. (1977) before transforming them into the yeast strain UKY403 (*MATa ade2–101(och) arg4–1 his3–201 leu2–3 leu2–112 lys2–801(amb) trp1–901 ura3–52 thr⁻ tyr⁻ hhf1[HIS3] hhf2[LEU2]/pUK421(TRP1 GAL/HHF2)*). The plasmid pUK421 was subsequently lost by growth of the yeast on glucose-containing medium. Both

UKY403 and pUK421 have been described previously (Kim et al., 1988; Kayne et al., 1988).

To prepare the *CUP1–lacZ* gene fusion (pLD3), the promoter and the first eight codons of the *CUP1* gene (derived from pJW6; Fogel and Welch, 1982) were ligated to the E. coli β-galactosidase gene in the centromeric vector pSEYC102 (Emr et al., 1986). The 5′ end of the *lacZ* gene and the entire *CUP1* promoter were sequenced.

Plasmids containing the *GAL1* promoter–*lacZ* gene (pRY131; West et al., 1984), the *PHO5* promoter–*lacZ* gene (pMH313; Han et al., 1988), and the *CUP1* promoter–*lacZ* gene (pLD3) contain the selectable marker gene *URA3*. Before introduction of these constructs into yeast with wild-type or mutant *HHF2* genes, the *URA3* gene was disrupted by ligating the *TRP1* gene into the ScaI site. The resulting plasmids were designated pRY131(*TRP1*), pMH313(*TRP1*), and pLD3(*TRP1*).

Growth Rates

Yeast doubling times were measured after diluting log phase cultures into fresh YEPD medium in Erlenmeyer flasks. Cultures were shaken vigorously at 30°C, and absorbance at 600 nm (A_{600}) was measured during the logarithmic phase of growth in a Beckman model 25 spectrophotometer, using quartz cuvettes. All doubling times represent the average of two experiments, using different isolates of the yeast strain.

Media, Gene Induction Methods, and β-Galactosidase Assays

All media used have been described (Sherman et al., 1986) except YEPR contains 2% raffinose and YEPG contains 2% galactose as the sole carbon sources. YEP medium lacking phosphate and sugar was prepared as described (Han et al., 1988). Yeast strains were grown approximately 24 hr in YEPR until cells were confluent. Yeast were washed in water and aliquoted into control and inducing media and incubated 6 hr. *GAL1* promoter activity was quantified after growing yeast in 2% YEPR (control) or 2% YEPG (experimental) media. Additionally, *GAL1* mRNA was prepared and analyzed from yeast grown in YEP containing the following ratios of raffinose to galactose: 2%:0%, 1.5%:0.5%, 1%:1%, 0%:2%. *PHO5* promoter activity was measured in yeast grown in no-phosphate YEPR with 7.5 mM KCl added (experimental) or with 7.5 mM KPO_4 (pH 7.0) added (control). The *CUP1* promoter was induced during only the final 30 min of the 6 hr incubation by addition of copper sulfate (1 mM) to YEPR medium. β-galactosidase activity was measured as described (Miller, 1972; Han et al., 1988).

RNA and Northern Blot Analysis

RNA was prepared as described (Kim et al., 1988) from 10 ml yeast cultures. Northern blot analysis and hybridization were performed as described (Maniatis et al., 1982).

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References

Allan, J., Harborne, N., Rau, D. C., and Gould, H. (1982). Participation of core histone "tails" in the stabilization of the chromatin solenoid. J. Cell Biol. *93*, 285–297.

Allegra, P., Sterner, R., Clayton, D. F., and Allfrey, V. G. (1987). Affinity chromatographic purification of nucleosomes containing transcriptionally active DNA sequences. J. Mol. Biol. *196*, 379–388.

Allfrey, V. G. (1977). Post-synthetic modifications of histone structure: a mechanism for the control of chromosome structure by the modulation of histone-DNA interactions. In Chromatin and Chromosome Structure, H. J. Li and R. Eckhardt, eds. (New York: Academic Press), pp. 167–191.

Allfrey, V. G., Sterner, R., and Sun, I. Y. C. (1983). Affinity Probes for Protein Acetylation and Phosphorylation in Posttranslational Covalent Modifications of Proteins, B. C. Johnson, ed. (New York: Academic Press).

Almer, A., Rudolph, H., Hinnen, A., and Horz, W. (1986). Removal of positioned nucleosomes from the yeast *PHO5* promoter upon *PHO5* induction releases additional upstream activating DNA elements. EMBO J. *5*, 2689–2696.

Annunziato, A. T., Frado, L.-L. Y., Seale, R. L., and Woodcock, C. L. F. (1988). Treatment with sodium butyrate inhibits the complete condensation of interphase chromatin. Chromosoma *96*, 132–138.

Clark-Adams, C. D., Norris, D., Osley, M. A., Fassler, J. S., and Winston, F. (1988). Changes in histone gene dosage alter transcription in yeast. Genes Dev. *2*, 150–159.

Emr, S. D., Vassarotti, A., Garretti, J., Geller, B. L., Takeda, M., and Douglas, M. G. (1986). The amino terminus of the yeast F1-ATPase β-subunit precursor functions as a mitochondrial import signal. J. Cell Biol. *102*, 523–533.

Fedor, M. J., Lue, N. F., and Kornberg, R. D. (1988). Statistical positioning of nucleosomes by specific protein-binding to an upstream activating sequence in yeast. J. Mol. Biol. *204*, 109–127.

Fogel, S., and Welch, J. W. (1982). Tandem gene amplification mediates copper resistance in yeast. Proc. Natl. Acad. Sci. USA *79*, 5342–5346.

Gill, G., and Ptashne, M. (1987). Mutants of GAL4 protein altered in an activation function. Cell *51*, 121–126.

Grunstein, M. (1990a). Histone function in transcription. Annu. Rev. Cell Biol. *6*, 643–678.

Grunstein, M. (1990b). Nucleosomes: regulators of transcription. Trends Genet. *6*, 395–400.

Han, M., and Grunstein, M. (1988). Nucleosome loss activates yeast downstream promoters in vivo. Cell *55*, 1137–1145.

Han, M., Kim, U.-J., Kayne, P. S., and Grunstein, M. (1988). Depletion of histone H4 and nucleosomes activates the *PHO5* gene in *Saccharomyces cerevisiae*. EMBO J. *7*, 2221–2228.

Hebbes, T. R., Thorne, A. W., and Crane-Robinson, C. (1988). A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J. *7*, 1395–1402.

Hereford, L., Fahrner, K., Woolford, J., Jr., Rosbash, M., and Kaback, D. B. (1979). Isolation of yeast histone genes H2A and H2B. Cell *18*, 1261–1271.

Johnson, L. M., Kayne, P. S., Kahn, E. S., and Grunstein, M. (1990). Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating loci in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *87*, 6286–6290.

Johnston, M. (1987). A model fungal gene regulatory mechanism: the GAL genes of *Saccharomyces cerevisiae*. Microbiol. Rev. *51*, 458–476.

Karin, M., Najarian, R., Haslinger, A., Valenzuela, P., Welch, J., and Fogel, S. (1984). Primary structure and transcription of an amplified genetic locus: the *CUP1* locus of yeast. Proc. Natl. Acad. Sci. USA *81*, 337–341.

Kayne, P. S., Kim, U.-J., Han, M., Mullen, J. R., Yoshizaki, F., and Grunstein, M. (1988). Extremely conserved histone H4 N terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. Cell *55*, 27–39.

Kim, U.-J., Han, M., Kayne, P., and Grunstein, M. (1988). Effects of histone H4 depletion on the cell cycle and transcription of *Saccharomyces cerevisiae*. EMBO J. *7*, 2211–2219.

Knezetic, J. A., and Luse, D. S. (1986). The presence of nucleosomes on a DNA template prevents initiation by RNA polymerase II in vitro. Cell *45*, 95–104.

Laughon, A., and Gestelend, R. F. (1982). Isolation and preliminary characterization of the *GAL4* gene, a positive regulator of transcription in yeast. Proc. Natl. Acad. Sci. USA *79*, 6827–6831.

Lewin, B. (1990). Commitment and activation at pol II promoters: a tail of protein–protein interactions. Cell *61*, 1161–1164.

Lohr, D. (1984). Organization of the GAL1-GAL10 intergenic control region chromatin. Nucl. Acids Res. *12*, 8457–8474.

Lorch, Y., LaPointe, J. W., and Kornberg, R. D. (1987). Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. Cell *49*, 203–210.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Matsui, T. (1987). Transcription of adenovirus 2 major late and peptide IX genes under conditions of in vitro nucleosome assembly. Mol. Cell. Biol. *7*, 1401–1408.

McGhee, J. D., and Felsenfeld, G. (1980). Nucleosome structure. Annu. Rev. Biochem. *49*, 1115–1156.

Megee, P. C., Morgan, B. A., Mittman, B. A., and Smith, M. M. (1990). Genetic analysis of histone H4: essential role of lysines subject to reversible acetylation. Science *247*, 841–845.

Miller, J. (1972). Experiments in Molecular Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Nelson, D. A. (1982). Histone acetylation in baker's yeast: maintenance of the hyperacetylated configuration in log phase protoplasts. J. Biol. Chem. *257*, 1565–1568.

Norton, V. G., Imai, B. S., Yau, P., and Bradbury, E. M. (1989). Histone acetylation reduces nucleosome core particle linking number change. Cell *57*, 449–457.

Norton, V. G., Marvin, K. W., Yau, P., and Bradbury, E. M. (1990). Nucleosome linking number change controlled by acetylation of histones H3 and H4. J. Biol. Chem. *265*, 19848–19852.

Oshima, Y. (1982). Regulatory circuits for gene expression: the metabolism of galactose and phosphate. In The Molecular Biology of the Yeast *Saccharomyces cerevisiae*: Metabolism and Gene Expression, L. N. Strathern, E. W. Jones, and J. R. Broach, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 159–180.

Park, E. C., and Szostak, J. W. (1990). Point mutations in the yeast histone H4 gene prevent silencing of the silent mating locus HML. Mol. Cell. Biol. *10*, 4932–4934.

Ptashne, M., and Gann, A. A. F. (1990). Activators and targets. Nature *346*, 329–331.

Rainbow, M., Lopez, J., and Lohr, D. (1989). The yeast GAL1–10 UAS region readily accepts nucleosomes in vitro. Biochemistry *28*, 7486–7490.

Roth, S. Y., Dean, A., and Simpson, R. T. (1990). Yeast α2 repressor positions nucleosomes in *TRP1*/ARS1 chromatin. Mol. Cell. Biol. *10*, 2247–2260.

St. John, T. P., and Davis, R. W. (1981). The organization and transcription of the galactose gene cluster of *Saccharomyces*. J. Mol. Biol. *152*, 285–315.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA *74*, 5463–5467.

Schroth, G. P., Yau, P., Imai, B. S., Gatewood, J. M., and Bradbury, E. M. (1990). An NMR study of mobility in the histone octamer. FEBS Lett. *268*, 117–120.

Schuster, T., Han, M., and Grunstein, M. (1986). Yeast histone H2A and H2B amino termini have interchangeable functions. Cell *45*, 445–451.

Sherman, F., Fink, G. R., and Hicks, J. B. (1986). Methods in Yeast Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Simpson, R. T. (1990). Nucleosome positioning can affect the function of a cis-acting DNA element in vivo. Nature *343*, 387–389.

Simpson, R. T. (1991). Nucleosome positioning: occurrence, mechanisms and functional consequences. Prog. Nucl. Acids Res. Mol. Biol. *40*, 143–184.

Straka, C., and Horz, W. (1991). A functional role for nucleosomes in the repression of a yeast promoter. EMBO J. *10*, in press.

Suzuki, Y., Nogi, Y., Abe, A., and Fukasawa, T. (1988). *GAL11* protein, an auxiliary transcription activator for genes encoding galactose metabolizing enzymes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *8*, 4991–4999.

van Holde, K. E. (1988). Chromatin, A. Rich, ed. (New York: Springer-Verlag).

Wallis, J. W., Rykowski, M., and Grunstein, M. (1983). Yeast histone H2B containing large amino terminus deletions can function in vivo. Cell *35*, 711–719.

Waterborg, J. H., and Matthews, H. R. (1984). Patterns of histone acetylation in *Physarum polycephalum*: H2A and H2B acetylation is functionally distinct from H3 and H4 acetylation. Eur. J. Biochem. *142*, 329–335.

West, R. W., Jr., Yocum, R. R., and Ptashne, M. (1984). *Saccharomyces cerevisiae GAL1-GAL10* divergent promoter region: location and function of the upstream activating sequence UAS_G. Mol. Cell. Biol. *4*, 2467–2478.

Whitlock, J. P., Jr., and Stein, A. (1978). Folding of DNA by histones which lack their NH_2–terminal regions. J. Biol. Chem. *253*, 3857–3861.

Workman, J. L., and Roeder, R. G. (1987). Binding of transcription factor TFIID to the major late promoter during in vitro nucleosome assembly potentiates subsequent initiation by RNA polymerase II. Cell *51*, 613–622.

Workman, J. L., Taylor, C. A., and Kingston, R. E. (1991). Activation domains of stably bound GAL4 derivatives alleviate repression of promoters by nucleosomes. Cell *64*, 533–544.

Wu, R. S., Panusz, H. T., Hatch, C. L., and Bonner, W. M. (1986). Histones and their modifications. CRC Crit. Rev. Biochem. *20*, 201–263.

Xu, H., Johnson, L., and Grunstein, M. (1990). Coding and non-coding sequences at the 3′-end of yeast histone H2B mRNA confer cell cycle regulation. Mol. Cell. Biol. *10*, 2687–2694.