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Gcn4 Activator Targets Gcn5 Histone Acetyltransferase to Specific Promoters Independently of Transcription

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Summary

Histone acetylation correlates well with transcriptional activity, and histone acetyltransferases (HATs) selectively regulate subsets of target genes by mechanisms that remain unclear. Here, we provide in vivo evidence that the yeast transcriptional activator Gcn4 recruits Gcn5 HAT complexes to selective promoters positioned in natural or ectopic locations, thereby creating local domains of histone H3 hyperacetylation and subsequent transcriptional activation. A significant portion of the Gcn4-targeted histone acetylation by Gcn5 is independent of transcriptional activity. These observations provide strong evidence for promoter-selective, targeted histone acetylation by Gcn5 that facilitates transcription in a causal fashion. In addition, Gcn5 also functions in an untargeted manner to acetylate H3 on a genome-wide scale.

Introduction

Histone acetylation is conserved through all eukaryotic species. The steady-state balance of histone acetylation is maintained by histone acetyltransferases (HATs) and deacetylases (HDACs). HATs and HDACs play key roles in gene regulation (for reviews see Brown et al., 2000; Sterner and Berger, 2000). Two poorly understood aspects of these enzymes are that multiple HATs/HDACs exist in each organism, and that each HAT/HDAC is likely important for transcriptional regulation of subsets of genes. These observations lead to popular models of specific promoter targeting via recruitment of these enzymes by transcriptional activators and repressors (Struhl, 1998). Mechanisms that account for target gene specificity and the general issue of global versus local acetylation remain important areas of current investigation.

The yeast Gcn5 is perhaps the best studied HAT, and it remains to be the prototype of transcription-associated HATs. Originally identified as a transcriptional adaptor or coactivator (Berger et al., 1992; Georgakopoulos and Thireos, 1992), Gcn5 controls expression of many inducible genes, including those involved in amino acid biosynthesis (e.g., Georgakopoulos and Thireos, 1992). Recent data document the importance of Gcn5 HAT activity in the expression of genes expressed late in mitosis (Krebs et al., 2000). In addition, microarray analyses showed that multiple genes may require Gcn5 for appropriate expression in rich medium (Holstege et al., 1998).

Gcn5 is the catalytic subunit of at least two complexes, SAGA and ADA (Grant et al., 1997). The SAGA complex contains Ada1–5, Spt3, 7, and 8, several TBP-associating factors (TAFs), and Tra1 (reviewed in Grant et al., 1998; Brown et al., 2000). Functions of the SAGA complex are pleiotropic, and deletion of different components of the SAGA complex causes phenotypes with different severity (Roberts and Winston, 1997; Sterner et al., 1999). The Spt components of the SAGA complex facilitate TBP–TATA association (Dudley et al., 1999). Compared with SAGA, the function(s) and composition of the ADA complex are less well defined (Eberharter et al., 1999). Transcription of Gcn5 target genes in vivo is critically dependent on the HAT activity of Gcn5, as mutations abolishing the catalytic function of Gcn5 significantly weaken its ability to activate transcription (Kuo et al., 1998; Wang et al., 1998).

The action of Gcn5 is largely promoter specific (Kuo et al., 1998; Krebs et al., 1999; Vignali et al., 2000). Current in vitro transcription data support a general model of targeted histone acetylation (e.g., Utley et al., 1998; Ikeda et al., 1999; Kundu et al., 2000; Vignali et al., 2000). Importantly, stimulation of transcription by HAT complexes in vitro requires acetyl CoA (Ikeda et al., 1999; Kundu et al., 2000). In mammalian cells, viral infection and hormone induction trigger histone hyperacetylation at promoters (Chen et al., 1999; Parekh and Maniatis, 1999). Similarly, Rpd3-mediated histone deacetylation is concentrated at the promoter region of genes repressed by Ume6 (Kadosh and Struhl, 1998; Rundlett et al., 1998). Collectively, these data argue for a model of promoter-specific recruitment of histone modification during transcriptional regulation.

In this work, in vivo evidence is presented that Gcn5 is targeted to specific promoter regions by a transcription activator, Gcn4. Using chromatin immunoprecipitation (ChIP) assays, we show that promoter-targeted histone acetylation is observed at Gcn4-activated genes but not general class II genes or in at least one other inducible gene, *CYC1*. Most importantly, Gcn5-mediated histone hyperacetylation can be relocated to a new region when the activator Gcn4 is redirected to that area, strongly suggesting that Gcn4 is a major factor in the recruitment of Gcn5 HAT complex to specific promoters. In addition, we also show that Gcn5 maintains substantial activity in *HIS3* gene acetylation even when *HIS3* expression is drastically reduced, indicating that histone acetylation indeed plays an upstream, causal role in transcriptional activation independent of transcription per se.

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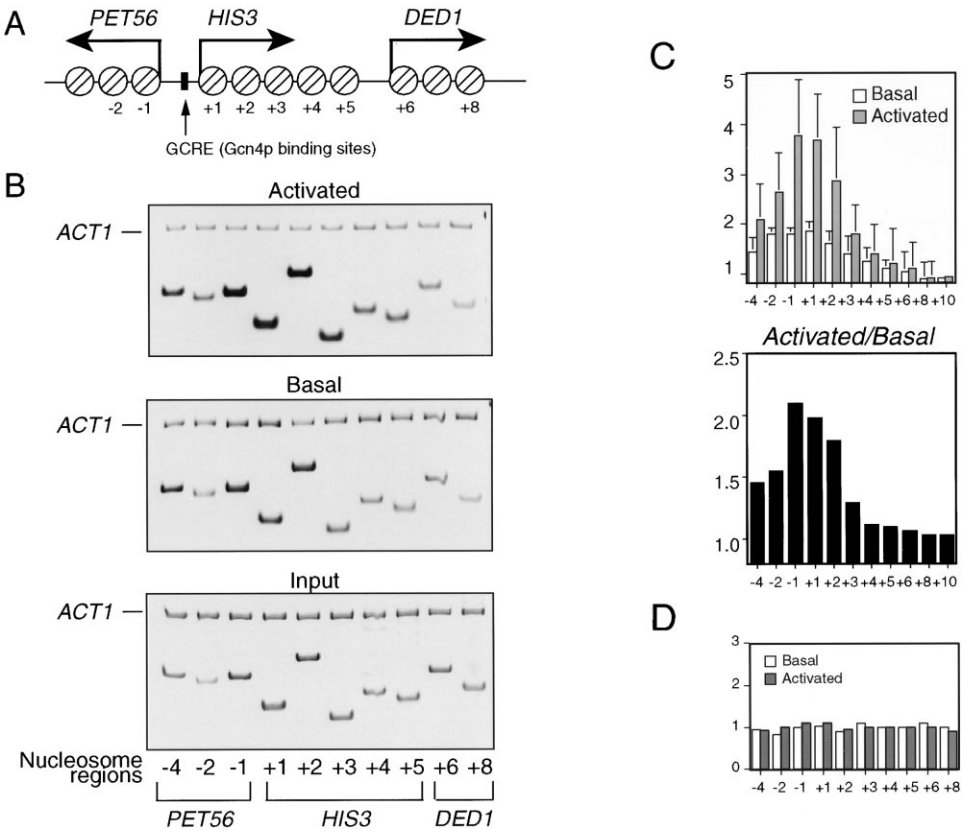

Figure 1. Gcn5-Mediated Histone Hyperacetylation Is Confined to the *HIS3* Promoter and Immediate Neighboring Areas

(A) Schematic drawing of the chromatin structure of the *PET56-HIS3-DED1* locus. Each positioned nucleosome is numbered with respect to the *HIS3* transcription unit.

(B) Quantitative PCR results of ChIP experiments using antidiacetylated H3 antibodies. Shown are inverted images from EtBr-stained gels. Note that all PCR reactions were carried out under identical conditions. Slight and reproducible variation of PCR efficiency is observed between different primers. These variations were taken into account for quantitation (see Experimental Procedures). In this figure, only nucleosomes –4 to +8 are shown. The −4 nucleosome represents a fragment from 481–621 base pairs downstream of the start codon of *PET56*.

(C and D) Quantitation of the relative acetylation levels across the *PET56-HIS3-DED1* region. ChIP and quantitative PCR results were derived from yMK839 (the parental wild-type strain, [C]) and yMK842 (*gcn5*Δ, [D]). The intensity of each PCR fragment was divided by that of the internal control *ACT1* fragment. The relative acetylation levels were expressed either as individual nucleosomes relative to the *ACT1* internal control (top panel, [C]) to show the total acetylation across the locus or as the ratio of activated-to-basal conditions (bottom panel, [C]) to show the increase in acetylation after induction over basal, noninduced conditions. The numbers were obtained from more than three independent yMK839 whole-cell extracts and seven ChIP and PCR reactions. Two independent whole-cell extracts and ChIPs were conducted from the yMK842 strain.

Results

Gcn5 Functions at Promoters of Gcn4-Activated Genes

To better learn how Gcn5 might be directed to selective genes, we chose the chromosomal copy of *HIS3* as our primary model for several reasons. First, amino acid starvation induces transcription (e.g., Struhl, 1986) and histone hyperacetylation (Kuo et al., 1998) of the *HIS3* gene. Importantly, both processes require Gcn5 HAT activity (Kuo et al., 1998). Second, the *HIS3* locus is organized into a phased nucleosomal array (Losa et al., 1990) that allows for mapping histone acetylation at a single, nucleosome-by-nucleosome level. Third, the well-studied *cis*- and *trans*-acting elements (see Iyer and Struhl, 1995) contributing to *HIS3* regulation provide an excellent foundation for a detailed study of the mechanism underlying HAT targeting. Finally, *HIS3* and the neighboring genes (*PET56* and *DED1*, see Figure 1A) are closely spaced, offering a stringent test of whether histone acetylation is a more global (nontargeted) or a confined (targeted) event. *HIS3* and *PET56* are divergently transcribed from a common 191 base pair regulatory sequence, and the start codon of *DED1* is only 960 base pairs downstream of that of *HIS3*. Though closely spaced, expression of these three genes appears to be independent of each other (e.g., Struhl, 1985, 1986). Figure 1A shows a schematic drawing including the positioning of phased nucleosomes of this locus.

Relative to total DNA or to loci not regulated by Gcn5 such as *ACT1* or rDNA, we previously showed that the *HIS3* locus was hyperacetylated by Gcn5 during amino acid starvation (Kuo et al., 1998). However, it remained unclear as to how far the Gcn5-dependent acetylation signal extended on either side of the gene, nor was it clear precisely where the acetylation initiated. Thus, we

first sought to map the boundaries of transcription-related histone acetylation across *PET56-HIS3-DED1*. Wild-type yeast strains were grown in rich medium to early log phase and then transferred to minimal medium containing 10 mM 3-aminotriazole (3-AT), a competitive inhibitor of *HIS3* gene product (imidazole glycerophosphate dehydratase), to induce *HIS3* activation. Cell extracts were subjected to ChIP using an antiserum against diacetylated histone H3 that has been shown to recognize histones acetylated by Gcn5 in vivo (Kuo et al., 1998). DNA fragments recovered by ChIP, representing H3 hyperacetylated chromatin, were then amplified by quantitative PCR using primer pairs that each hybridize to within a single phased nucleosome across *PET56-HIS3-DED1*.

Figure 1B shows that a reasonably narrow region surrounding the *HIS3* promoter becomes hyperacetylated in response to amino acid starvation. DNA fragments corresponding to nucleosomes −2 to +3 are consistently enriched in the hyperacetylated fraction, with nucleosomes −1 to +2 showing the highest level (see Experimental Procedures). This acetylation requires a functional Gcn5, as neither *gcn5* null (Figure 1D) nor a catalytically defective allele (F221A; see Kuo et al., 1998; data not shown) is able to generate such hyperacetylation. In sharp contrast, the *DED1* promoter, though constitutively expressed (see Figure 4B), shows no Gcn5-dependent hyperacetylation under either growth condition. These data argue against models such as untargeted or general promoter-targeting mechanisms for Gcn5 during transcriptional induction and provide further in vivo support for the notion that Gcn5-linked acetylation is a highly promoter-specific and local process (Krebs et al., 1999, 2000).

Interestingly, the prominent acetylation peak induced under activating conditions overlaps with a moderate, preexisting acetylation domain under basal conditions (see nucleosomes −1 to +2, Figures 1B and 1C). The boundaries of these acetylation peaks overlap well; what differs is the relative magnitude of acetylation (i.e., the activation-related H3 hyperacetylation is consistently stronger than that under noninducing conditions). Gcn5 is responsible for both activated and basal hyperacetylation, as neither is detectable in a *gcn5*Δ strain (Figure 1D). In contrast, Gcn4 is not likely required for the basal acetylation because *GCN4* deletion or elimination of the apparent Gcn4 binding site (see below) in *HIS3* abrogates only the activation-related but not the basal acetylation.

The finding that the Gcn5-dependent hyperacetylation domain encompasses the upstream activating sequence (UAS) region suggests that Gcn5 may be targeted by certain transcriptional factors that bind in the vicinity (e.g., Gcn4 in the case of *HIS3* activation). To learn if this is the case, we further characterized several other amino acid biosynthesis genes. Figure 2 shows that all Gcn4 genes tested here, i.e., *TRP3*, *ARG1*, *HIS3*, *CPA1*, and *CPA2*, displayed H3 hyperacetylation similar to *HIS3*. In contrast, of those non-Gcn4-regulated genes tested (i.e., *DED1* and *PGK1*), no increase in acetylation is observed. Together, these data underscore a critical role played by the activator Gcn4 in recruiting Gcn5 to specific loci.

In vitro data have shown that the SAGA complex functionally interacts with at least two acidic activators, the herpes simplex virus activator VP16 and the yeast Gcn4 (Ikeda et al., 1999; Vignali et al., 2000). To see whether Gcn5 functions through all acidic activators, we characterized the acetylation and transcription status of the *CYC1* gene. *CYC1* encodes iso-1-cytochrome c, and its transcriptional induction requires the HAP2/3/4 acidic activator complex (e.g., Olesen and Guarente, 1990). As shown in Figure 2E, neither the basal nor the induced expression of *CYC1* depends on *GCN5*. Similarly, there is no detectable change in the level of H3 acetylation at this gene (Figure 2D). Thus, we conclude that Gcn5 functions through a selective group of transcriptional activators for targeted histone modification and gene activation in vivo.

Gcn4 Recruits Gcn5

If binding of Gcn4 to its target sequences (general control response elements, GCREs) is sufficient for initiating the cascade events of histone acetylation and transcriptional activation, several testable predictions can be made: (1) Gcn4–GCRE interaction should be upstream and independent of histone acetylation, (2) removal of GCREs from the *HIS3* UAS would prevent H3 hyperacetylation, and (3) a new hyperacetylation domain could be generated if GCREs are created ectopically at a UAS that previously possesses no such elements. To test these predictions, we again focused our attention on the *PET56-HIS3-DED1* three-gene cluster. Across this region, there is one known functional GCRE in the *HIS3* UAS (Struhl, 1985) that is essential for amino acid starvation–induced transcription. This element differs from the consensus sequence by one base (consensus, ATGA$^{C}/_{G}$TCAT; GCRE at *HIS3*, ATGACTCTT). In addition, well within the *DED1* open reading frame (ORF), another single-base mismatch of the GCRE consensus is present (984 base pairs downstream of ATG of *DED1*, ATG ACTCCT). This element appears poorly accessible to *GCN4* in vivo, as it is inefficiently cleaved by Hinf I (cleavage sequence, GANTC) in vivo compared with *GCN4* binding sites in promoter regions (Mai et al., 2000). No GCRE-like sequence is found at the *DED1* UAS region, consistent with its insensitivity to amino acid starvation. Figure 3A shows these GCREs.

To test the requirement of Gcn4 activator for targeting Gcn5 HAT activity, we first examined whether Gcn4 was able to bind its target sequences without Gcn5. ChIP assays using anti-Gcn4 serum were performed on *GCN5*$^{+}$ and *gcn5*Δ extracts. Quantitative PCR results shown in Figure 3A indicate that deleting *GCN5* has no obvious effect on Gcn4 binding at either *HIS3* or *TRP3* (not shown) UAS regions. The near-perfect GCRE found within the *DED1* ORF (fragment C) does not bind Gcn4 under either growth condition, in accord with the notion that an unknown, intrinsic feature distinguishes the chromatin structure of protein-coding and promoter regions (Mai et al., 2000). We next introduced base substitutions to *HIS3* GCRE (ATGACTCTT to GTCGACGTC) to eliminate the Gcn4 binding (Figure 3B). As expected, the activation-related H3 hyperacetylation consistently seen in wild-type *HIS3* is now abrogated (Figure 3B, right), although a low level of basal acetylation remains detectable. As a control, binding of Gcn4 to the UAS as

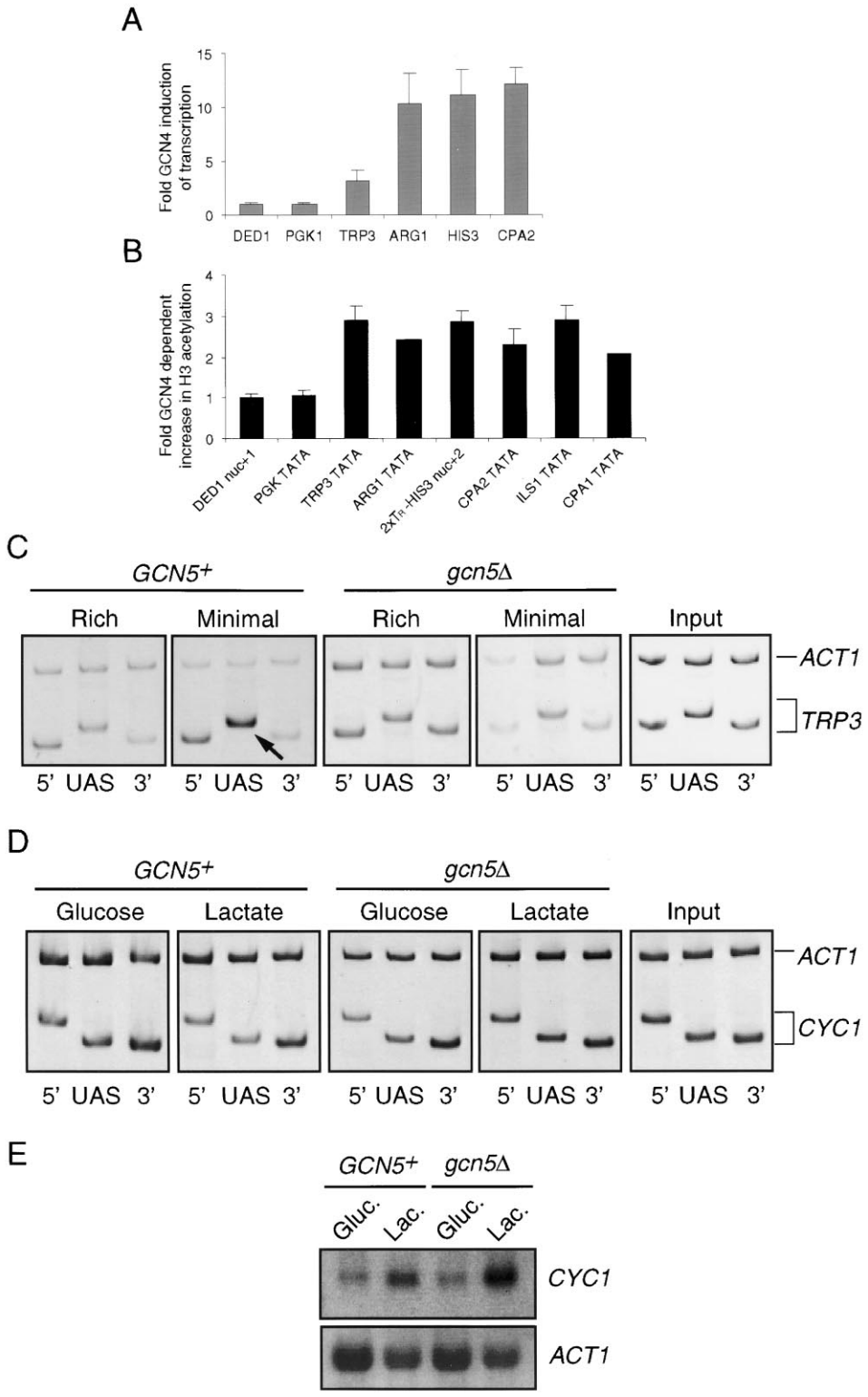

Figure 2. Gcn4-Activated Genes Are Specific Targets for Gcn5 Action

Transcriptional induction of several general control genes is activated by Gcn4, whereas *DED1* and *PGK1* expression is unaffected. Gcn4 was expressed from a Cu(II) inducible promoter in a *gcn4*Δ background (A and B), and mRNA levels were quantified by S1 mapping. (B) shows fold increase in acetylated H3 over the promoter region of the genes tested in (A) plus two more known Gcn4-induced genes, *ILS1* and *CPA1*. "TATA" indicates that the primers chosen for quantitative PCR amplify a region including the TATA element of that gene, otherwise a region comprising the indicated nucleosome. The *HIS3* gene promoter in (A) and (B) was engineered to possess an optimal Gcn4 binding site and a second T_R TATA element (2× T_R) in place of the wild-type T_C TATA element. (C) shows detailed studies of Gcn5-dependent acetylation at the *TRP3* locus. *GCN5*$^+$ and *gcn5*Δ strains (both maintained their chromosomal wild-type *GCN4*) were grown in YPD or minimal medium

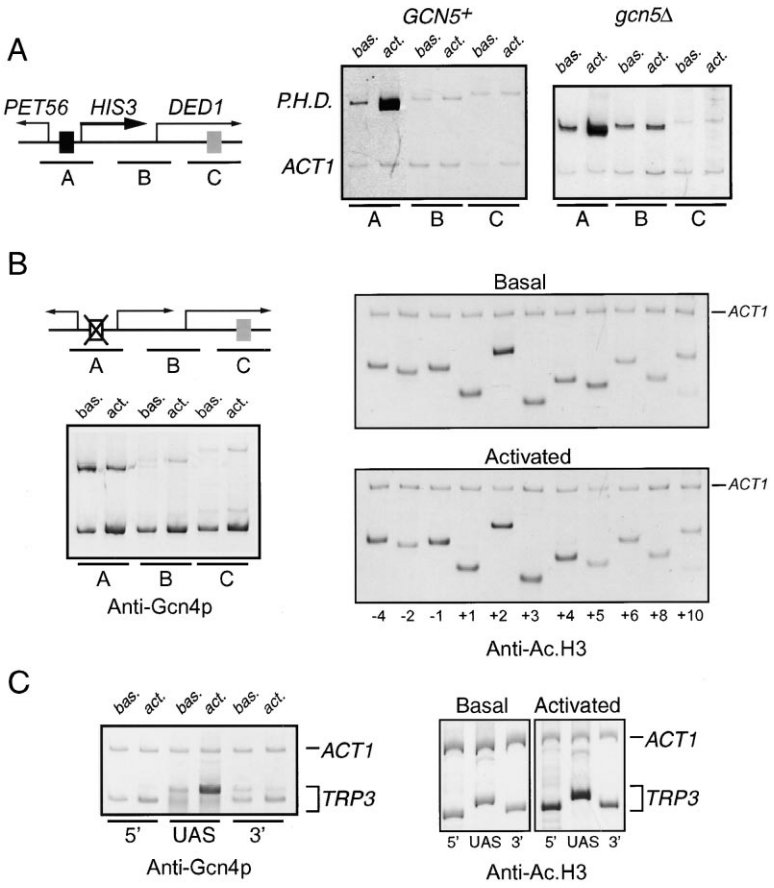

Figure 3. Gcn4–GCRE Interaction Is Upstream and Essential for Gcn5 Targeting

(A) Gcn4 binds GCREs independently of Gcn5. The left panel shows the position of GCREs (black box) and the GCRE-like element within the *DED1* coding region (gray box). The three bars labeled A, B, and C represent, respectively, the three PCR fragments corresponding to the *PET56-HIS3*, *HIS3-DED1* intergenic region, and a 549 bp fragment that is 564 bp 3′ to the *DED1* start codon including the GCRE-like element. The middle and right panels show quantitative PCR results of ChIP conducted with an anti-Gcn4 Ab under basal ("bas.") and activating ("act.") conditions in *GCN4*$^{+}$ strains.

(B) GCRE is essential for Gcn5 targeting. The crossed open box represents mutated GCRE. The lower left panel shows ChIP with the anti-Gcn4 Ab showing the loss of Gcn4 binding. The panels on the right show ChIP results using antiacetylated H3 Ab. Note that the basal acetylation domain persists without a functional GCRE.

(C) Gcn4 binding and Gcn5 action remain intact at the *TRP3* locus. Shown are quantitative PCR results of ChIP using anti-Gcn4 (left) or antidiacetylated H3 (right) antibodies. See Figure 2C for details.

well as H3 acetylation of *TRP3* were tested and found to be unaffected by mutations aimed at the *HIS3* promoter (Figure 3C). These data argue that Gcn4 binding is essential for Gcn5-medated histone acetylation at *HIS3*, whereas the function of Gcn5 is not required for Gcn4 to bind its cognate targets.

We next examined whether relocating Gcn4 binding sites to the promoter region of a previously Gcn4/Gcn5-insensitive gene would result in redistribution of the Gcn5-mediated H3 acetylation near that gene. Toward this end, we inserted two canonical GCREs at the existing XhoI site located 20 base pairs upstream of the *DED1* TATA element (Figure 4A, left). As well, the natural *HIS3* GCRE was mutated so that Gcn4 no longer binds this UAS (see Figure 3B). As expected, anti-Gcn4 ChIP assays showed that Gcn4 now is relocated to the modified *DED1* UAS (Figure 4A, right). Relocating Gcn4 also results in amino acid starvation–induced *DED1* activation (Figure 4B, lanes 3 and 6). Most importantly, a new H3 hyperacetylation domain is induced by amino acid starvation at the GCRE-containing *DED1* promoter (Figure 4C). Similar to the wild-type *HIS3* locus, the newly generated histone hyperacetylation is also sensitive to *gcn5* loss-of-function mutations (data not shown). Moreover, the naturally occurring GCRE-like sequence (residing between +8 and +10) in the *DED1* ORF that fails to bind Gcn4 does not elicit local hyperacetylation. Interestingly, the DNA fragment corresponding to the mutated *HIS3* UAS (fragment A) was modestly enriched, indicating a moderate increase in the acetylation level. While the significance of this unexpected acetylation is unknown, we suspect that the Gcn4 concentration and hence, H3 acetylation at the mutated *HIS3* UAS is increased due to the distal interaction of Gcn4 and GCREs at the *DED1* promoter (see Discussion). Overall, an excellent correlation is observed between the binding of Gcn4 and the degree of histone acetylation in the immediately adjacent region. These data indicate that Gcn5 is recruited by Gcn4 to target promoter regions even in an ectopic position.

Gcn5 Recruitment Requires the Gcn4 Activation Domain

We next asked whether the activation domain of Gcn4 is critical for Gcn5 targeting. Of the seven hydrophobic pockets within the activation domain of Gcn4, combined

containing 10 mM 3-AT to induce Gcn4 synthesis. ChIP shows that only the UAS region becomes hyperacetylated in H3 (arrow) in the presence of Gcn5. (D) and (E) show that neither induction nor H3 acetylation of *CYC1* locus requires Gcn5. Yeast cells were grown in YPD or YPlactate for Northern analyses (E) and ChIP assays using the diacetylated H3 antibody (D). The 5′ and 3′ fragments of (C) and (D) represent approximately 500 bp upstream and downstream, respectively, of the start codon, whereas the UAS fragment represents the first nucleosome including the ATG and nearby regulatory sequence.

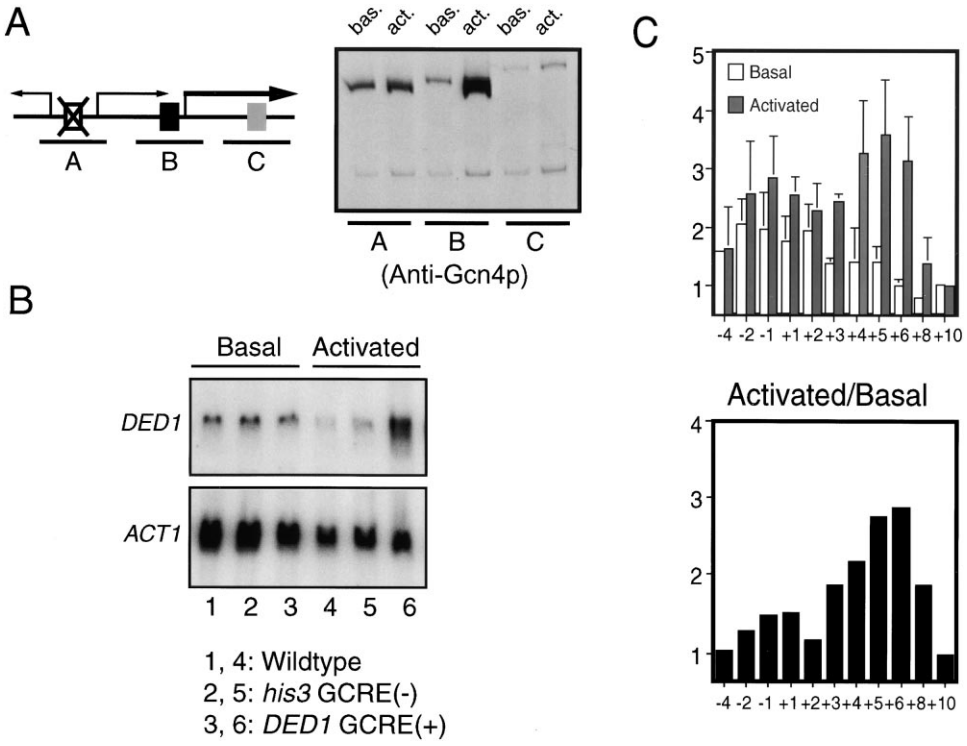

Figure 4. Relocating Gcn4 to a New GCRE Results in Ectopic Histone Hyperacetylation

(A) The left panel shows a schematic drawing of the modified *PET56-HIS3-DED1* locus. Dimeric GCREs were introduced into the *DED1* UAS region, indicated by the black box at the +5/+6 region (i.e., fragment B). The right panel shows ChIP to locate Gcn4 within the modified *PET56-HIS3-DED1* region. Amino acid starvation triggers Gcn4 binding to the new GCREs (fragment B). The GCRE-like sequence within the *DED1* ORF (fragment C) remains inaccessible to Gcn4.

(B) Northern analysis of *DED1* and *ACT1* internal control under basal and activated conditions. *DED1* transcription becomes inducible by amino acid starvation when GCREs are introduced (lanes 3 and 6).

(C) Quantitation results of ChIP measuring H3 acetylation across *PET56-HIS3-DED1*. Where the canonical GCREs are introduced, H3 hyperacetylation is induced by Gcn4 binding. Data were derived from at least four independent ChIP and PCR reactions.

mutations of the last three [(5, 6, 7)$^-$] cause nearly complete loss of Gcn4 transcriptional activation functions in vivo (Jackson et al., 1996). Such transcriptional activation defects are likely due to the inability of the mutant Gcn4 to interact with multiple key transcriptional regulators, including components within the SAGA complex in vitro (Drysdale et al., 1998; Natarajan et al., 1999). We expressed the (5, 6, 7)$^-$ mutant allele in a *gcn4*Δ background to see if targeting Gcn5 requires an intact protein–protein interaction interface of Gcn4. Figure 5A shows that in the *gcn4*Δ strain, expressing wild-type *GCN4* rescues the growth defects in 3-AT media, whereas the *gcn4* (5, 6, 7)$^-$ triple mutant barely rescues the growth. Further, ChIP assays using anti-Gcn4 or anti-Ac.H3 antibodies showed that even though the mutant Gcn4 retains its ability to bind GCREs at both *HIS3* and *TRP3*, H3 acetylation at these two loci is apparently less obvious (Figure 5B). These data strongly suggest that the Gcn4 activation domain plays an essential role in Gcn5 targeting.

Gcn5 Function Is Independent of Transcriptional Initiation

The tight correlation between Gcn5 action and the target gene expression (i.e., *HIS3* and the modified *DED1*) raises the question as to whether histone acetylation and transcriptional activation are two separable events. To address this issue, several strains possessing specific mutations at relevant promoter elements that inhibit transcriptional initiation were constructed (see Experimental Procedures). In brief, *HIS3* is normally under the control of two distinct TATA elements, T_C and T_R (Struhl, 1986; Mahadevan and Struhl, 1990; Iyer and Struhl, 1995). The T_C and T_R elements each contribute to basal and induced expression of *HIS3*, respectively. To eliminate the basal expression of *HIS3* that may interfere with the analysis, we first replaced the T_C element with a consensus TATAAA element (2× T_R). We next substituted both T_R elements with TGTAAA (2× TGTA), a point mutation that quantitatively abolishes TBP binding (Strubin and Struhl, 1992). Although this double T_R mutation eliminated transcription from the +1 and +13 transcription start sites (Chen and Struhl, 1988), *HIS3* induction still occurred in response to Gcn4 when probed with an internal *HIS3* S1 probe (Figure 6A). We reasoned that a cryptic downstream TATA element might be present that retained weak affinity for TBP and directed transcription initiation to a downstream initiation site. Close examination of the sequence indicated that a TATA-like element indeed is present at position −40 (with respect to ATG). Upon deletion of this sequence and the known TATA elements (ΔT_CT_R), *HIS3* induction decreased to near background levels (Figure 6A).

We next characterized the acetylation status of these

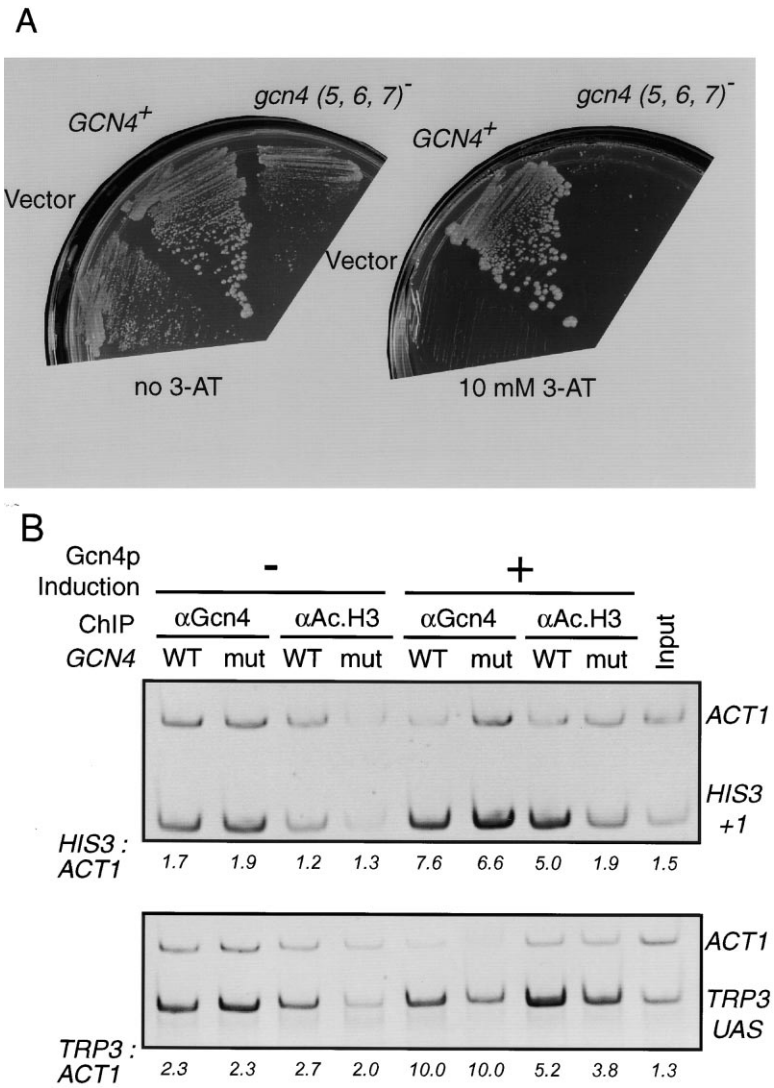

Figure 5. The Gcn4 Activation Domain Plays a Critical Role in Directing Gcn5 HAT Activity to the *HIS3* UAS

(A) Growth test in the presence of 3-AT. Yeast strains with the endogenous *GCN4* allele deleted were transformed with a Cu(II) inducible plasmid expressing either wild-type *GCN4* or a mutant allele, *gcn4* (5, 6, 7)$^{-}$, defective in protein–protein interactions. Cells were streaked on $CuSO_4$-containing medium and incubated at 30°C for 4 days.

(B) ChIP results show that while maintaining normal DNA binding ability, the *gcn4* (5, 6, 7)$^{-}$ mutant is defective in recruiting Gcn5 HAT activity to both *HIS3* and *TRP3* loci. The amount of each PCR product was measured and normalized to the *ACT1* internal control. Numbers below each lane represent the results of a typical experiment.

alleles. While the efficiency of *HIS3* induction was progressively reduced in our promoter mutants, the differential H3 acetylation level (acetylation in the presence of Gcn4 minus that in the absence of Gcn4) only decreased by 25% to 40% across the *HIS3* locus even in the most severe mutant allele, ΔTcTR (Figure 6B). In this strain, H3 acetylation at the +1 nucleosome region is approximately 70% higher than the *PGK1* internal control. In contrast, when *GCN5* is deleted in the 2× TR strain (2× TR/Δ*gcn5*), H3 acetylation of the entire region reduces to background levels irrespective of induction by Gcn4. Importantly, while Gcn4-induced transcription in the ΔTcTR mutant strain is significantly lower than that of the 2× TR/*gcn5*Δ strain, H3 acetylation levels remain considerably higher and Gcn4 dependent. Thus, a substantial portion of Gcn5-mediated histone H3 hyperacetylation is separable from *HIS3* transcription and does not depend on the *HIS3* core promoter activity.

Gcn5 Plays a Role in Global, Genome-Wide Acetylation

In contrast to the promoter-proximal hyperacetylation, deletion of *GCN5* appears to cause widespread decrease in H3 acetylation (see Figure 4 in Kuo et al., 1998; Krebs et al., 1999). These findings point to a potential role of Gcn5 in genome-wide histone acetylation that may differ from the activator-targeted promoter acetylation demonstrated in this study. To better understand the global histone acetylation by Gcn5, we quantified acetylation of H3 and H4 at 30 different loci in the presence or absence of Gcn5. *GCN5*$^{+}$ and *gcn5*Δ cells were harvested from rich media (i.e., under noninducing conditions with respect to many inducible genes) for ChIP analyses. Figure 7 shows that one half of the tested loci display 3- to 10-fold reduction in H3 acetylation in the absence of Gcn5. In contrast, H4 acetylation is only moderately affected and not coincident with the changes of H3 acetylation. In addition, we looked into H3 and H4 acetylation status throughout *PET56-HIS3-DED1* in detail and found that H3 acetylation is lost completely in the entire *HIS3* locus, whereas H4 acetylation remains essentially unchanged (data not shown). It appears that the global and targeted acetylation by Gcn5 are uncoupled, as there is no obvious correlation between the changes in H3 acetylation and the underlying transcriptional induction requirement for Gcn5. For example, al-

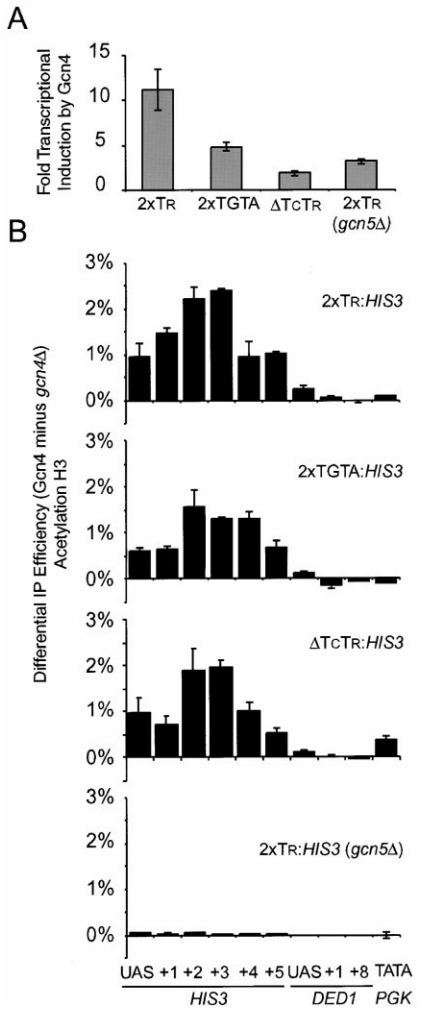

Figure 6. Activation-Dependent Histone H3 Hyperacetylation at *HIS3* Is Independent of Transcription

(A) Fold increase in transcription levels of *HIS3* with different promoter mutations upon *GCN4* induction. Mutations of the TATA elements were introduced into the *HIS3* promoter to abolish TBP binding and thus transcription but not *GCN4* binding. 2× TR, optimal Gcn4 binding site plus two optimal TATA elements; 2× TGTA, as TR but optimal TATA elements replaced with a non-TBP binding mutant element TGTAAAG (Strubin and Struhl, 1992); ΔTCTR, position –106 to –34 (with respect to ATG) deleted in the wild-type *HIS3* promoter that eliminated TC, TR, and a cryptic TATA element (−40); 2× TR/*gcn5*Δ, as 2× TR, but *GCN5* was deleted. RNA was purified from the tested strains after inducing wild-type Gcn4 by Cu(II) followed by S1 mapping to quantify the *HIS3* transcripts. Isogenic strains with vector only (i.e., no *GCN4*) were tested in parallel for noninducing conditions.

(B) The same strains as in (A) were tested by ChIP for H3 acetylation at the indicated positions of *HIS3* and adjacent *DED1* genes in the absence or presence of *GCN4*. Values shown are percent IP efficiency of strains expressing Gcn4 minus strains lacking Gcn4.

though the *HIS3* +2 nucleosome region shows a 6-fold reduction in H3 acetylation, comparable loss of acetylation is also seen in the *DED1* promoter region. Our results are most consistent with the idea that genome-wide histone acetylation is not directly linked to activation of inducible target genes but is more tied to the general transcriptional competency of a particular locus or larger chromosomal domains (see Schübeler et al., 2000).

Discussion

Promoter-Selective, Targeted Histone Acetylation

In general, our data show that levels of histone acetylation at the *HIS3* UAS correlate well with the overall rate of transcriptional induction from this gene. For example, compared with the modified *DED1* containing two GCREs (Figure 4), introducing five tandem repeats of GCREs to the *DED1* promoter evokes a further increase of both H3 acetylation and *DED1* induction during amino acid starvation (data not shown). This suggests that the level of transcription of certain yeast genes may be accounted for by the degree of local histone acetylation. On the other hand, considering the presence of multiple HATs in most, if not all, eukaryotes characterized (Sterner and Berger, 2000), whether there are qualitative differences in histone acetylation at promoters that respond divergently to transcriptional inducing signals (e.g., *HIS3* and *CYC1*) remains an important yet poorly understood aspect of gene regulation.

The domain of basal acetylation observed at the wild-type *HIS3* promoter under repressive conditions is noteworthy (Figures 1–3). This is not a property of all Gcn4-controlled genes, as we did not observe similar basal acetylation in *TRP3* promoter (Figure 2C). As well, mutagenesis of the obvious GCRE at the *HIS3* UAS leads to complete elimination of the activated acetylation domain but imposes no discernible effects on the basal acetylation (Figure 3B). We hypothesize that a yet unidentified transcriptional regulator may recruit a Gcn5 HAT complex(es) to the *HIS3* UAS region under noninducing conditions. Since deleting *GCN5* results in further reduction of the *HIS3* basal expression (Georgakopoulos and Thireos, 1992; Kuo et al., 1998; Sterner et al., 1999), it is possible that the basal acetylation at *HIS3* may play a positive role in basal transcription. Thus far, no sequence-specific positive regulators have been implicated in *HIS3* basal expression. On the contrary, genetic studies revealed several Not proteins negatively regulating *HIS3* basal expression (Collart and Struhl, 1993, 1994). Whether basal acetylation mediated by Gcn5 antagonizes negative regulators such as these Not proteins awaits further investigation.

Unexpectedly, in the strain where GCREs have been "relocated" from the *HIS3* to the *DED1* promoter, the level of *HIS3* acetylation still increases slightly upon amino acid starvation (Figure 4C). This enrichment, though less significant than that of the GCRE-containing *DED1*, appears to coincide with a moderate increase of Gcn4 occupancy at the GCRE-lacking *HIS3* promoter (Figure 4A, fragment A). Because *HIS3* GCRE mutation alone results in complete loss in the activation-associated H3 acetylation (Figure 3B), introducing functional GCREs to *DED1* is likely responsible for this mild elevation of H3 acetylation at the mutant *HIS3* promoter. Since the modified *DED1* promoter is less than 1 kb from the *HIS3* promoter, we suggest that the high concentration of Gcn4 at the *DED1* UAS may force Gcn4 surplus or turnover molecules to bind low-affinity, GCRE-like elements at *HIS3* UAS. For example, several half GCREs

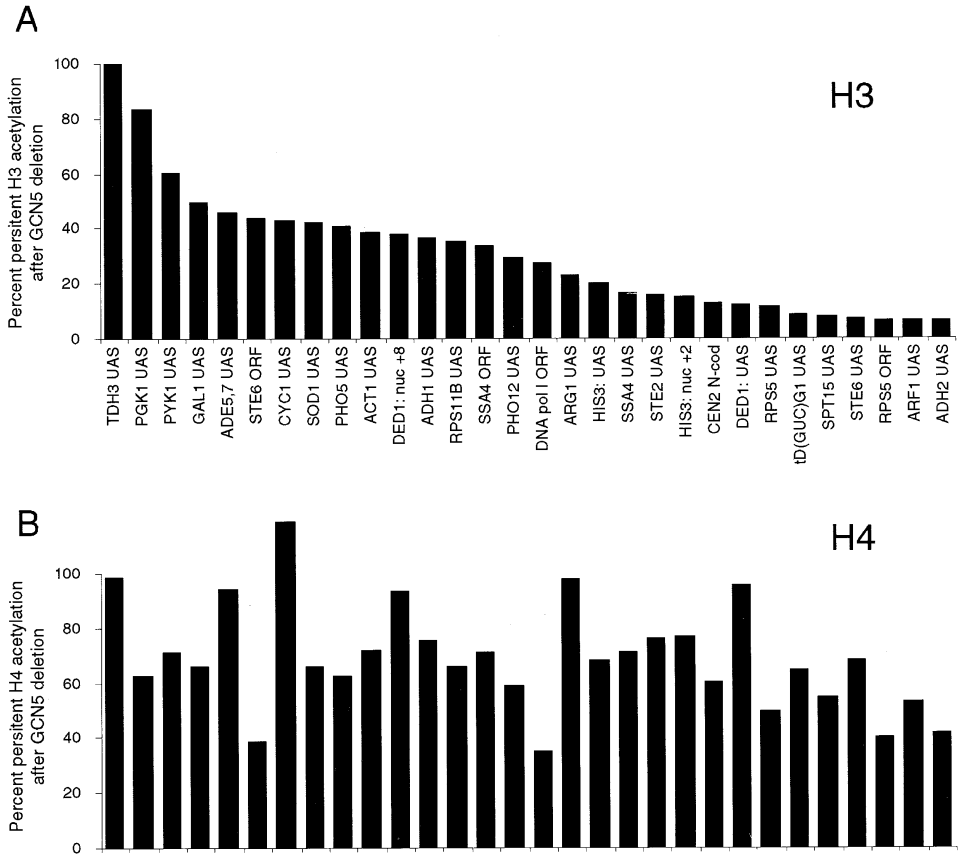

Figure 7. Gcn5 Mediates Untargeted Histone H3 Acetylation on a Genome-Wide Scale under Noninducing Conditions

GCN5$^+$ and *gcn5*Δ strains were grown in CAA medium and harvested for ChIP. Diacetylated H3 (A) and tetraacetylated H4 (B) antibodies were used to characterize the changes in acetylation level resulting from the deletion of *GCN5*. For each indicated locus (PCR fragment), the immunoprecipitation efficiency of *gcn5*Δ relative to *GCN5*$^+$ (100%) is shown, i.e., percent persistent histone H3 or H4 acetylation.

(ATGA$^{C}/_{G}$) are present in the *HIS3* UAS that may bind Gcn4 only when the local concentration of Gcn4 elevates drastically.

HIS3 versus *HO*, Gcn5 versus Swi/Snf

Using primarily ChIP assays, an elegant series of experiments in yeast has dissected the kinetic steps and interdependency of various chromatin modulating activities operating at *HO* and several other late mitotic genes (Cosma et al., 1999; Krebs et al., 1999, 2000). *HO* is a highly specialized gene characterized by an unusually large and complex promoter (see Cosma et al., 1999 for references). Multiple transcriptional regulators and chromatin modulating activities coordinately establish a cell cycle–dependent, haploid- and mother cell–specific expression pattern of *HO*. The key activator, Swi5, is essential for Swi/Snf complex recruitment to the *HO* promoter (Cosma et al., 1999; Krebs et al., 1999), whereby the enzymatic action of Swi/Snf, in turn, is crucial for Gcn5 HAT complex targeting (Krebs et al., 2000). Gcn5 then acetylates both H3 and H4 prior to *HO* transcription (Krebs et al., 1999). In all the Swi5-controlled late mitotic genes tested, Gcn5 action also depends on a functional Swi/Snf complex (Krebs et al., 2000).

In contrast, Gcn5 functions at *HIS3* and several other inducible promoters largely independently of Swi/Snf (Krebs et al., 2000), although the integrity of the Swi/Snf complex is important for *HIS3* induction (Natarajan et al., 1999). These results strongly suggest that histone acetylation and chromatin remodeling at these promoters act in parallel rather than as interdependent, stepwise control mechanisms. Further, deletion of *GCN5* leads to significant attenuation of H4 acetylation at the *HO* locus during G1 phase (Krebs et al., 1999), whereas *HIS3* and many other loci show little, if any, change in H4 acetylation in a nonsynchronous log phase culture (Figure 7), providing another piece of evidence that distinguishes *HIS3* and *HO* in their transcriptional regulatory mechanisms. We suggest that Swi/Snf-independent recruitment of Gcn5 by activators such as Gcn4 may be fairly common among yeast inducible genes. The extent and reasons for which Gcn5 function depends on Swi/Snf at other promoters such as *HO* remains an open question for future investigations.

Global, Nontargeted Histone Acetylation

In contrast to promoter targeting, several studies suggest that histone acetylation may also spread across relatively large chromosomal domains (e.g., Hebbes et

al., 1992; Madisen et al., 1998). Recent work on human β-globin gene activation revealed a two-step control circuit; liberating the β-globin locus from the centromeric heterochromatin and general H3/H4 acetylation together establish transcriptional competency, whereas subsequent promoter-specific H3 hyperacetylation triggers transcriptional activation (Schübeler et al., 2000). Since the *S. cerevisiae* genome is exceptionally hyperacetylated (e.g., Waterborg, 2000), it seems reasonable that most loci in yeast are constitutively poised for transcription. If correct, Gcn5's ability to enhance genome-wide acetylation implies a more general role in establishing/maintaining transcriptional competency. However, *GCN5* deletion does not cause detrimental effects on growth in rich media, arguing against an essential function(s) of Gcn5 alone under such conditions. It is likely that redundant and even synergistic functions exerted by multiple chromatin modulating activities contribute to the constitutively poised state of yeast genome. We favor the view that under noninducing conditions, Gcn5 and perhaps other HATs acetylate the yeast genome in a relatively random, less efficient fashion and that such global acetylation is not sufficient for swift, high-level transcriptional activation. When HATs are recruited by specific activators in response to selective inducing agents, maximal transcription is then triggered by promoter-selective, targeted H3 hyperacetylation.

Experimental Procedures

Strains and Media

Yeast strains used in Figures 1–4, except 2A and 2B, are derived from EJ66 (*MATa trp1*$^-$ *leu2*$^-$ *ura3*$^-$ *his4*$^-$) (see Kuo et al., 1998). To rescue the *his*$^-$ phenotype, a PstI fragment containing the entire *HIS4* gene [from plasmid FB541 pHIS4(*Pst*) provided by Fred Winston, Harvard University] was transformed into EJ66 to create yMK839. *GCN5* was deleted from yMK839 to create yMK842 using a *gcn5* knockout construct provided by Shelley Berger (Wistar Institute). To eliminate the apparent GCRE from *HIS3*, pMK219 was linearized by Eco47 III for integrative transformation into yMK839. *URA*$^+$ transformants were grown overnight in YPD and plated onto 5-FOA plates. Genomic PCR and restriction digestion were used to screen for the *ura3*$^-$ clones that maintain the GCRE(−) version of *HIS3* gene. This strain is designated yMK869 and was further transformed with a BalI-linearized plasmid, pMK223, which contained a GCRE dimer at the XhoI site at the *DED1* promoter. 5-FOA selection, genomic PCR, and restriction digestion were used to obtain yMK886, the GCRE-relocation strain.

Yeast strains used for *HIS3* TATA element mutations, acetylation of other Gcn4-regulated genes, and the global Gcn5 effect (Figures 2A, 2B, and 5–7) are based on KY320 (2× TR:HIS3 and 2× TGTA:*HIS3*, both with optimal GCRE; *ade2-101oc leu2::PET56 lys2-801am trp1-Δ1 ura3-52*) (Chen and Struhl, 1988; Iyer and Struhl, 1995) and FY833 (ΔTR-Tc:*HIS3* [Δ-106 > −34 with respect to ATG] with optimal GCRE; *ade8*$^-$ *leu2::PET56 lys2-Δ202 trp1-Δ161 ura3-52* [Struhl, 1998; a gift from S. Chou]). All strains are *gcn4*Δ, and Gcn4 is expressed without the 5′ regulatory ORF sequences from a plasmid under Cu(II) control (a gift from M. John). From each strain, an isogenic *gcn5*Δ mutant was generated by homologous recombination with a Δ*gcn5::URA3* integration plasmid.

Standard yeast manipulation techniques were employed throughout this work. YPD or CAA medium (synthetic medium supplemented with 0.5% casamino acids) was used to grow cells in repressive conditions. In *HIS3* and *TRP3* induction, synthetic minimal medium supplemented with essential amino acids and 10 mM 3-AT was used. For *CYC1* induction, YP lactate (2% bacto peptone, 1% yeast extract, and 3% lactate) was used. Typical induction time was 2–5 hr at 30°C. Alternatively, Δ*gcn4* strains were grown in synthetic complete medium (without leucine [Figures 6 and 7] or uracil [Figure 5]), and $CuSO_4$ was added to 500 μM 45 min prior to harvesting at approximately 2 ×10^7 cells/ml to induce *GCN4* expression.

Plasmid Construction

pMK197 was generated by inserting the NheI-SalI fragment of pJJ217 (Jones and Prakash, 1990), including the *HIS3-PET56* intergenic UAS region, into the XbaI-XhoI sites of pRS306 (Sikorski and Hieter, 1989). The apparent GCRE of *HIS3* was mutated by site-directed mutagenesis (pMK219). To introduce canonical GCREs at *DED1* promoter, pJJ217 was first digested with XhoI and ligated to a self-annealed primer (TCGAATGASTCAT, S = C + G) (pMK221). The BalI-SacI fragment of pMK221 containing the new GCREs was inserted into the same sites of pMK219 to create pMK223.

To create Cu(II)-inducible *GCN4* wild type and (5,6,7)$^-$ mutant constructs (pMK295 and pMK296, respectively), PCR primers were designed such that the *GCN4* open reading frame without the 5′ regulatory ORFs was flanked by the multicloning sequence of pMK120 (Kuo et al., 1998). Plasmids p2044 and pLD350, which contain the wild-type *GCN4* or the *gcn4* (5, 6, 7)$^-$ allele (Drysdale et al., 1998), respectively, were amplified by PCR and cotransformed with the BamHI/EcoRI-linearized pMK120 into yeast yMK839. Yeast DNA isolated from the *URA*$^+$ transformants were propagated in *E. coli* to obtain pMK295 and pMK296. These plasmids were thus transformed into KY97 (2× TR:*HIS3 gcn4*Δ) (Iyer and Struhl, 1995) for ChIP.

Chromatin Immunoprecipitation and Quantitative PCR

Procedures for ChIP were practically identical to those previously described (Kuo and Allis, 1999). We also noticed that the extent of sonication significantly affected the boundaries and sometimes the center of the hyperacetylation domains. In all of the experiments reported here, the typical length of the bulk chromatin fragments following sonication was between 300 to 1000 base pairs. Whole-cell extracts equivalent to 2 × 10^8 yeast cells were immunoprecipitated with 0.5 μl of crude antisera against diacetylated H3 or Gcn4 (a gift from A. Hinnebusch, NIH). PCR reactions contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0) at 25°C, 1% Triton X-100, 2 mM $MgCl_2$, 0.1 mM each dNTP, 0.5 μM each primer, and 1.25 U Taq DNA polymerase (Promega), and appropriately diluted DNA templates. PCR parameters were (94°C, 4 min; 50°C, 4 min; 72°C, 12–30 s) for 1 cycle; (94°C, 30 s; 50°C, 30 s; 72°C, 12–30 s) for 24 or 25 cycles; and 72°C, 2 min. PCR products were resolved in polyacrylamide gels. EtBr images were photographed with an AlphaImager digital camera and quantified by the AlphaEase program (Alpha Innotech). The ratio of the experimental to internal control *ACT1* PCR product was calculated, divided by the ratio obtained from their input counterpart. The variation in PCR efficiency seen in different nucleosomes was thus normalized. To assess the net change of acetylation due to transcriptional induction, the experiment-to-internal control ratio of activated culture samples was divided by the corresponding basal samples. Alternatively, for *HIS3* TATA element mutations, acetylation of other Gcn4-regulated genes, and the global Gcn5 effect (Figures 2A, 2B, 6, and 7), the antidiacetylated H3-Ab (protein A purified) and an antitetraacetylated H4-Ab were from Upstate Biotechnology Inc. PCR reactions were done in the presence of [α-^{32}P]dATP. PCR products were resolved and quantified by Fujix BSA 2040 PhosphorImager. The percentage of the assayed genomic DNA fragments being immunoprecipitated was calculated by comparing the quantity of PCR products from the input materials to the immunoprecipitated products. Otherwise, the protocol employed for cross-linking and DNA isolation was as described earlier (Kuras and Struhl, 1999).

Quantitative PCR Oligonucleotides

Sequences of the oligonucleotide primers used for quantitative PCR are available upon request.

Quantitative S1 Nuclease Protection Assay

RNA levels were determined by quantitative S1 analysis as described elsewhere (Iyer and Struhl, 1995).

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References

Berger, S.L., Pina, B., Silverman, N., Marcus, G.A., Agapite, J., Regier, J.L., Triezenberg, S.J., and Guarente, L. (1992). Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. Cell *70*, 251–265.

Brown, C.E., Lechner, T., Howe, L., and Workman, J.L. (2000). The many HATs of transcription coactivators. Trends Biochem. Sci. *25*, 15–19.

Chen, W., and Struhl, K. (1988). Saturation mutagenesis of a yeast his3 "TATA element": genetic evidence for a specific TATA-binding protein. Proc. Natl. Acad. Sci. USA *85*, 2691–2695.

Chen, H., Lin, R.J., Xie, W., Wilpitz, D., and Evans, R.M. (1999). Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. Cell *98*, 675–686.

Collart, M.A., and Struhl, K. (1993). CDC39, an essential nuclear protein that negatively regulates transcription and differentially affects the constitutive and inducible HIS3 promoters. EMBO J. *12*, 177–186.

Collart, M.A., and Struhl, K. (1994). NOT1(CDC39), NOT2(CDC36), NOT3, and NOT4 encode a global-negative regulator of transcription that differentially affects TATA-element utilization. Genes Dev. *8*, 525–537.

Cosma, M.P., Tanaka, T., and Nasmyth, K. (1999). Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. Cell *97*, 299–311.

Drysdale, C.M., Jackson, B.M., McVeigh, R., Klebanow, E.R., Bai, Y., Kokubo, T., Swanson, M., Nakatani, Y., Weil, P.A., and Hinnebusch, A.G. (1998). The Gcn4 activation domain interacts specifically in vitro with RNA polymerase II holoenzyme, TFIID, and the Adap-Gcn5 coactivator complex. Mol. Cell. Biol. *18*, 1711–1724.

Dudley, A.M., Rougeulle, C., and Winston, F. (1999). The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. Genes Dev. *13*, 2940–2945.

Eberharter, A., Sterner, D.E., Schieltz, D., Hassan, A., Yates, J.R., III, Berger, S.L., and Workman, J.L. (1999). The ADA complex is a distinct histone acetyltransferase complex in Saccharomyces cerevisiae. Mol. Cell. Biol. *19*, 6621–6631.

Georgakopoulos, T., and Thireos, G. (1992). Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. EMBO J. *11*, 4145–4152.

Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., and Winston, F. (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev. *11*, 1640–1650.

Grant, P.A., Sterner, D.E., Duggan, L.J., Workman, J.L., and Berger, S.L. (1998). The SAGA unfolds: convergence of transcription regulators in chromatin- modifying complexes. Trends Cell Biol. *8*, 193–197.

Hebbes, T.R., Thorne, A.W., Clayton, A.L., and Crane-Robinson, C. (1992). Histone acetylation and globin gene switching. Nucleic Acids Res. *20*, 1017–1022.

Holstege, F.C., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S., and Young, R.A. (1998). Dissecting the regulatory circuitry of a eukaryotic genome. Cell *95*, 717–728.

Ikeda, K., Steger, D.J., Eberharter, A., and Workman, J.L. (1999). Activation domain-specific and general transcription stimulation by native histone acetyltransferase complexes. Mol. Cell. Biol. *19*, 855–863.

Iyer, V., and Struhl, K. (1995). Mechanism of differential utilization of the his3 TR and TC TATA elements. Mol. Cell. Biol. *15*, 7059–7066.

Jackson, B.M., Drysdale, C.M., Natarajan, K., and Hinnebusch, A.G. (1996). Identification of seven hydrophobic clusters in GCN4 making redundant contributions to transcriptional activation. Mol. Cell. Biol. *16*, 5557–5571.

Jones, J.S., and Prakash, L. (1990). Yeast Saccharomyces cerevisiae selectable markers in pUC18 polylinkers. Yeast *6*, 363–366.

Kadosh, D., and Struhl, K. (1998). Targeted recruitment of the Sin3-Rpd3 histone deacetylase complex generates a highly localized domain of repressed chromatin in vivo. Mol. Cell. Biol. *18*, 5121–5127.

Krebs, J.E., Kuo, M.H., Allis, C.D., and Peterson, C.L. (1999). Cell cycle-regulated histone acetylation required for expression of the yeast HO gene. Genes Dev. *13*, 1412–1421.

Krebs, J.E., Fry, C.J., Samuels, M.L., and Peterson, C.L. (2000). Global role for chromatin remodeling enzymes in mitotic gene expression. Cell *102*, 587–598.

Kundu, T.K., Palhan, V.B., Wang, Z., An, W., Cole, P.A., and Roeder, R.G. (2000). Acitvator-dependent transcription from chromatin in vitro involving targeted histone acetylation by p300. Mol. Cell *6*, 551–561.

Kuo, M.-H., and Allis, C.D. (1999). Formaldehyde crosslinking to study dynamic structure of chromatin. Methods *19*, 425–433.

Kuo, M.H., Zhou, J., Jambeck, P., Churchill, M.E., and Allis, C.D. (1998). Histone acetyltransferase activity of yeast Gcn5 is required for the activation of target genes in vivo. Genes Dev. *12*, 627–639.

Kuras, L., and Struhl, K. (1999). Binding of TBP to promoters in vivo is stimulated by activators and requires Pol II holoenzyme. Nature *399*, 609–613.

Losa, R., Omari, S., and Thoma, F. (1990). Poly(dA)poly(dT) rich sequences are not sufficient to exclude nucleosome formation in a constitutive yeast promoter. Nucleic Acids Res. *18*, 3495–3502.

Madisen, L., Krumm, A., Hebbes, T.R., and Groudine, M. (1998). The immunoglobulin heavy chain locus control region increases histone acetylation along linked c-myc genes. Mol. Cell. Biol. *18*, 6281–6292.

Mahadevan, S., and Struhl, K. (1990). Tc, an unusual promoter element required for constitutive transcription of the yeast HIS3 gene. Mol. Cell. Biol. *10*, 4447–4455.

Mai, X., Chou, S., and Struhl, K. (2000). Preferential accessibility of the yeast his3 promoter is determined by a general property of the DNA sequence, not by specific elements. Mol. Cell. Biol. *20*, 6668–6676.

Natarajan, K., Jackson, B.M., Zhou, H., Winston, F., and Hinnebusch, A.G. (1999). Transcriptional activation by Gcn4 involves independent interactions with the SWI/SNF complex and the SRB/mediator. Mol. Cell *4*, 657–664.

Olesen, J.T., and Guarente, L. (1990). The HAP2 subunit of yeast CCAAT transcriptional activator contains adjacent domains for subunit association and DNA recognition: model for the HAP2/3/4 complex. Genes Dev. *4*, 1714–1729.

Parekh, B.S., and Maniatis, T. (1999). Virus infection leads to localized hyperacetylation of histones H3 and H4 at the IFN-beta promoter. Mol. Cell *3*, 125–129.

Roberts, S.M., and Winston, F. (1997). Essential functional interactions of SAGA, a Saccharomyces cerevisiae complex of Spt, Ada, and Gcn5 proteins, with the Snf/Swi and Srb/mediator complexes. Genetics *147*, 451–465.

Rundlett, S.E., Carmen, A.A., Suka, N., Turner, B.M., and Grunstein, M. (1998). Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3. Nature *392*, 831–835.

Schübeler, D., Francastel, C., Cimbora, D.M., Reik, A., Martin, D.I., and Groudine, M. (2000). Nuclear localization and histone acetyla-

tion: a pathway for chromatin opening and transcriptional activation of the human beta-globin locus. Genes Dev. *14*, 940–950.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics *122*, 19–27.

Sterner, D.E., and Berger, S.L. (2000). Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. *64*, 435–459.

Sterner, D.E., Grant, P.A., Roberts, S.M., Duggan, L.J., Belotserkovskaya, R., Pacella, L.A., Winston, F., Workman, J.L., and Berger, S.L. (1999). Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Mol. Cell. Biol. *19*, 86–98.

Strubin, M., and Struhl, K. (1992). Yeast and human TFIID with altered DNA-binding specificity for TATA elements. Cell *68*, 721–730.

Struhl, K. (1985). Nucleotide sequence and transcriptional mapping of the yeast pet56-his3-ded1 gene region. Nucleic Acids Res. *13*, 8587–8601.

Struhl, K. (1986). Constitutive and inducible Saccharomyces cerevisiae promoters: evidence for two distinct molecular mechanisms. Mol. Cell. Biol. *6*, 3847–3853.

Struhl, K. (1998). Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. *12*, 599–606.

Utley, R.T., Ikeda, K., Grant, P.A., Cote, J., Steger, D.J., Eberharter, A., John, S., and Workman, J.L. (1998). Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. Nature *394*, 498–502.

Vignali, M., Steger, D.J., Neely, K.E., and Workman, J.L. (2000). Distribution of acetylated histones resulting from Gal4–VP16 recruitment of SAGA and NuA4 complexes. EMBO J. *19*, 2629–2640.

Wang, L., Liu, L., and Berger, S.L. (1998). Critical residues for histone acetylation by Gcn5, functioning in Ada and SAGA complexes, are also required for transcriptional function in vivo. Genes Dev. *12*, 640–653.

Waterborg, J.H. (2000). Steady-state levels of histone acetylation in Saccharomyces cerevisiae. J. Biol. Chem. *275*, 13007–13011.

Note Added in Proof

The recent work by Grunstein and coworkers demonstrated a role of Gcn5 in global acetylation under noninducing conditions.

Vogelauer, M., Wu, J., Suka, N., and Grunstein, M. (2000). Global histone acetylation and deacetylation in yeast. Nature *408*, 495–498.

Berger, S.L. (2000). Gene regulation. Local or global? Nature *408*, 412–414.