Supporting information for Smith *et al*. (2002) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.182424999.

Supporting Materials and Methods

Mutant Histone Libraries. Libraries of histone mutants were created in three ways by using a common starting plasmid that contained the *HHT2* and *HHF2* genes on a centromere plasmid marked with a *TRP1* gene [pMP3; (1)]. In the first library, the entire plasmid was randomly mutagenized by propagating pMP3 in the *Escherichia coli* mutator strain XL1-red (Stratagene). Plasmids from cells in the fourth passage were purified (Qiagen, Chatsworth, CA) and transformed into yeast (2). This process yielded an unbiased mutagenesis of the two histones.

Based on the notion that most known modifications occur in the tails of the histones, the other libraries were directed at identifying mutations in the NH_2-terminal tails of histone H3 and H4 by using PCR-based methods. In the first of these, two independent libraries were created with PCR primers that contained on average one randomized nucleotide within the coding sequence of amino acids 1–26 or 1–18 of histone H3 or H4, respectively. PCR was performed between primer HHT2_3 [GCA AAC ACT CCA CAA TGG CCA GAA CTA AAC AAA CAG CTA GAA AAT CCA CTG GTG GTA AAG CCC CAA GAA AAC AAT TAG CCT CCA AGG CTG CCA GAA AAT CCG CCC CAT CTA CCG] and HHT2_4 [CGA TAA ATT ACA GCA TAT ATA CCC] and primer HHF2_3 [CAA TCA ATA CAA TAA AAT AAT GTC CGG TAG AGG TAA AGG TGG TAA AGG TCT AGG TAA AGG TGG TGC CAA GCG TCA CAG AAA GAT TTT AAG AGA TAA CAT TAC AGG] and HHF2_4 [GGG GAC ACG AAC GCC ACG TCG AAG C] (the mutated residues are underlined). In separate reactions, the promoter regions of histones H3 and H4 were amplified by using primers HHT2_1 [CGC ACA ATC ACG GCT ATG GCT CGG] with HHT2_2 [CAT TGT GGA GTG TTT GCT TGG ATC C] and HHF2_1 [GCC CCG CAA TTA TGT CTG TAA ACG] with HHF2_2

[CAT TAT TTT ATT GTA TTG ATT GTT GTT TTT GC]. Finally, PCR was performed between primers HHT2_1 and HHT2_4 and between primers HHF2_1 and HHF2_4. Mutant histone H3 PCR products were then transformed into yeast with *Msc*I-digested pMP3, and mutant histone H4 plasmids were transformed with *Nsi*I-digested pMP3. The last set of libraries was created with a bias toward potentially changing the ability of each lysine in the NH_2-terminal tails to be modified (i.e., changing the recognition site for a histone acetylase). Site-specific mutagenesis was used to independently randomize each residue that is adjacent to one of the tail lysines, or in some cases, two residues from a lysine within the histone H3 and H4 tails by using QuikChange Mutagenesis (Stratagene). Oligos contained 15–20 nt flanking both sides of the residue to be mutagenized. Mutated plasmids resulting from PCR were transformed directly into yeast.

MS. An HPLC (Michrom Bioresources, Auburn, CA) was modified so solvent directly exiting the mixing chamber of the HPLC instrument was split toward a variable splitter box (Michrom Bioresources) and toward silica tubing that was coupled to a capillary HPLC column via a union. The capillary HPLC column was constructed from 100 μm internal diameter by 365 μm external diameter fused silica capillary tubing (Polymetrics, Phoenix) with a tapered tip that was constructed by carefully pulling the capillary in a bunsen burner flame. The capillary column was packed by using helium pressure to a length of 8 cm with 5 μm particle size, 100-μm pore size, Monitor C18 reverse-phase material (Column Engineering, Ontario). The mobile phases for HPLC were (A) 0.2% acetic acid and 0.005% heptafluorobutyric acid in water and (B) 0.2% acetic acid and 0.005% heptafluorobutyric acid in acetonitrile. Dried samples were typically resuspended in 15 μl of solvent A and further diluted between 3- and 10-fold with solvent A. A 5-μl sample was directly loaded onto the microcolumn by helium pressurization of the sample in a pressure cell. The HPLC was operated to deliver a gradient of 5–40% solvent B in 20 min at a flow rate of 400 nl/min. A homemade ionization source was used to hold the capillary column and couple the HPLC to the mass spectrometer. Mass spectral analyses were conducted with a LCQ electrospray ionization mass spectrometer (ThermoFinnigan, San Jose, CA) at a spray voltage of 1.8 kV and a heated inlet capillary set at 200°. Tandem mass spectra were collected in the data-dependent mode for a single precursor

ion by using a collision energy of 40% (as defined by the LCQ software) and a precursor ion isolation width of 8.0 *m*/*z*, to simultaneously obtain tandem mass spectra for a doubly charged histone peptide that contained between 0 and 4 deuterated acetyl groups.

MS Data Analysis. The majority of b and y ions produced by fragmentation of the histone H4 tryptic peptide were easily assigned in MS/MS spectra with Excalibur software (ThermoFinnigan). The intensities of peaks corresponding to the Ac_H and Ac_D forms of various b or y ions were determined from maximum ion currents of appropriate isotope peaks in selected ion plots. The quality of the mass spectra was assessed from the intensities and shapes of isotope peaks. In most cases, measurements were made for a particular primary peak along with its two isotopic peaks. That the third isotopic peak of Ac_H occurred at the same *m*/*z* as the primary peak for the Ac_D was not significant. Only fragment ions that exhibited the expected isotopic pattern were analyzed; certain ions were excluded from the analysis if their spectra contained signals that were overlapping, erratic, or too weak. Thus, multiple b and y ions were used to determine the level of *in vivo* acetylation of each lysine. The data were inserted into a Microsoft EXCEL spreadsheet for normalization and calculation of the level of acetylation at internal lysine residues.

To determine the fraction of proton acetylation (fAc) within a particular fragment ion, the signal intensity (I) was measured for each peak and then divided by the sum of the intensities for all H and D species for that particular fragment and from this expression, the fraction of acetylation at all others sites was then subtracted. The simplest case occurred for K16 or K5. Here, the fraction of proton acetylation (fAc) was equal to the intensity caused by the protonated form divided by the intensities for both the protonated and deuterated forms of a particular fragment ion. (As an example, we present the analysis occurring from the y ions, but the same was done for b ions as well.) The equation describing the calculation for K16 is: $\mathrm{fAc(K16)} = \frac{I_H}{I_D + I_H}$.

As described in the main text, determining the fraction of proton acetylation at K12 was more complicated because the fragment ions that contain information for this site also contained information for K16. Therefore, a second equation was derived to calculate the level of acetylation at this internal site: $fAc(K12) = \frac{I_{\substack{HD \\ DH}} + 2I_{HH}}{I_{DD} + I_{\substack{HD \\ DH}} + I_{HH}} - (fAc(K16))$.

In the first part of this equation, the numerator consists of the sum of the intensity for the peak representing fragments containing a single proton acetyl (HD or DH) plus twice the intensity of the peak representing fragments containing two proton acetyl groups (HH). The denominator consists of the sum of the HH, HD, and DD peaks. The fraction of acetylation determined for K16 is then subtracted from this expression. A similar approach was used to derive equations to calculate the levels of acetylation at K8 and K5:

$$fAc(K8) = \frac{I_{\substack{DDH \\ DHD \\ HDD}} + 2I_{\substack{HHD \\ HDH \\ DHH}} + 3I_{HHH}}{I_{DDD} + I_{\substack{DDH \\ DHD \\ HDD}} + I_{\substack{HHD \\ HDH \\ DHH}} + I_{HHH}} - (fAc(K12) + fAc(K16))$$

$$fAc(K5) = \frac{I_{HDDD} + 2I_{\substack{HHDD \\ HDHD \\ HDDH \\ DHHD \\ DHDH \\ DDHH}} + 3I_{\substack{HHHD \\ HHDH \\ HDHH \\ DHHH}} + 4I_{HHHH}}{I_{DDDD} + I_{\substack{HDDD \\ DHDD \\ DDHD \\ DDDH}} + I_{\substack{HHDD \\ HDHD \\ HDDH \\ DHHD \\ DHDH \\ DDHH}} + I_{\substack{HHHD \\ HHDH \\ HDHH \\ DHHH}} + I_{HHHH}} - (fAc(K8) + fAc(K12) + fAc(K16))$$

Detailed descriptions of the derivations for each equation will be presented elsewhere.

1. Kelly, T. J., Qin, S., Gottschling, D. E. & Parthun, M. R. (2000) *Mol. Cell. Biol.* **20,** 7051–7058.

2. Wu, T. H., Clarke, C. H. & Marinus, M. G. (1990) *Gene* **87,** 1–5.