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Dynamics of Histone Acetylation in *Saccharomyces cerevisiae*

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ABSTRACT: Rates of turnover for the posttranslational acetylation of core histones were measured in logarithmically growing yeast cells by radioactive acetate labeling to near steady-state conditions. On average, acetylation half-lives were approximately 15 min for histone H4, 10 min for histone H3, 4 min for histone H2B, and 5 min for histone H2A. These rates were much faster than the several hours that have previously been reported for the rate of general histone acetylation and deacetylation in yeast. The current estimates are in line with changes in histone acetylation detected directly at specific chromatin locations and the speed of changes in gene expression that can be observed. These results emphasize that histone acetylation within chromatin is subject to constant flux. Detailed analysis revealed that the turnover rates for acetylation of histone H3 are the same from mono- through penta-acetylated forms. A large fraction of acetylated histone H3, including possibly all tetra- and penta-acetylated forms, appears subject to acetylation turnover. In contrast, the rate of acetylation turnover for mono- and di-acetylated forms of histones H4 and H2B, and the fraction subject to acetylation turnover, was lower than for multi-acetylated forms of these histones. This difference may reflect the difference in location of these histones within the nucleosome, a difference in the spectrum of histone-specific acetylating and deacetylating enzymes, and a difference in the role of acetylation in different histones.

Chromatin, the packaging of DNA by histones, presents a formidable obstacle to gene transcription (*1*). It is gradually becoming clearer how acetylation of histones, positively correlated with gene transcription decades ago by Allfrey (*2*), contributes to the process of initiation and elongation of transcription by RNA polymerases and provides essential and changing binding sites for transcription factors (*3*−*6*). Transcriptional coactivators localize select histone acetyltransferases (HATs)[1] to gene promoters, shifting the local balance to histones that are more highly acetylated at specific amino-terminal lysines, and allowing assembly and function of transcription-initiation complexes (*7*−*11*). Conversely, transcriptional corepressors have been shown to bring in tethered histones deacetylases (HDACs) to promoters of repressed or silenced genes (*12*−*17*). The progression of a high acetylation state across transcribed chromatin may be established through HATs associated with transcribing RNA polymerases (*1, 18*). In addition, HATs are being studied that may produce the domain-wide increases in chromatin acetylation observed for the chicken β-globin locus (*19*).

Studies in animal cells have established that the balance between acetylation and deacetylation can shift rapidly in response to stimuli that activate or repress gene transcription (*6, 11, 17, 20*−*24*). In addition, partially through the use of HDAC inhibitors such as butyrate and Trichostatin A, it has been established that histone acetylation is a very dynamic, reversible modification of histone termini. Acetyl groups are constantly added to and taken off histones, with turnover half-lives ranging from 3 min to an hour or more, when diverse chromatin fractions are studied by radioactive acetate incorporation in cells in culture (*25*−*29*). Recently, we have demonstrated through dynamic steady-state acetylation labeling without the use of HDAC inhibitors that the vast majority of acetyllysines in the chromatin of the green alga *Chlamydomonas reinhardtii* are rapidly turning over with half-lives of 2−4 min (*30*). The deduction that all acetylation of histone H3 and the majority of acetyllysines in histone H4 were subject to such a high rate of turnover suggested a reevaluation of dynamic histone acetylation in the budding yeast *Saccharomyces cerevisiae*.

Yeast has become one of the prime model systems in which eukaryotic gene expression is studied. We have just described a new method for preparing yeast histones that retains all acetylation during extraction and preparation of histones (*31*). This method demonstrated that yeast nucleosomes contain, on average, 13 acetylated lysines per nucleosome, the highest level yet reported for any species. It avoids the need for spheroplasting, a time-consuming process that prevents one from studying the dynamics of histone acetylation. The only measurement of acetylation turnover in yeast was obtained following spheroplasting (*32*). This study reported that yeast histones were neither rapidly acetylated nor rapidly deacetylated with rates on the order of hours. This is in clear contradiction to rapid activation of genes through the localized action of HATs on promoter nucleosomes (*20, 33, 34*) or to the rapid incorporation of radioactive acetate into yeast cells under pH conditions that do not limit

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[1] Abbreviations: AU, acetic acid−urea; HAT, histone acetyltransferase; HDAC, histone deacetylase; HPLC, high-performance liquid chromatography; NIB, nuclear isolation buffer; SD, synthetic defined; YPD, yeast extract/bactopeptone/dextrose.

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acetate flux (*31*). In this paper, we use the method for acetate labeling to steady-state, developed in *Chlamydomonas* (*30*), in combination with the new procedure to isolate yeast histones without loss of acetylation (*31*) to measure the overall turnover rate of acetylation for each of the histones in yeast. Differences in the pattern of acetate turnover for each of the histone species and differences in turnover rates of histones modified to different acetylation levels are described. The extent to which yeast chromatin is subject to rapid acetylation turnover is discussed. The results reported here are consistent with recent analyses on global dynamic acetylation surrounding several genes in yeast (*35*).

EXPERIMENTAL PROCEDURES

Histone Sample Preparation. Throughout this study, wild-type haploid yeast strain SNY28 with few auxotrophic markers has been used. Genetic information and growth in YPD at pH 7 and 4.5 and in minimal auxotrophic marker-supplemented synthetic defined SD medium, with logarithmic cell doubling times of 90 and 150 min, respectively, has been described in detail elsewhere (*31*). All experiments in YPD were performed at pH 4.5. Use of dicarboxylicsuccinic acid to adjust the pH of the growth medium prevents cytoplasmic acidification which would occur were acetic acid used (*31*). The translation inhibitor cycloheximide was used at a final concentration of 10 μg/mL growth medium, added 10 min before addition of radioactive acetate from fresh, nonsterile stock (2 mg/mL in ethanol). High specific activity [^{3}H]-NaOAc (1.5×10^{14} Bq/mol, NEN Life Science Products) was used as specified in each experiment. Tritiated lysine (1.7×10^{15} Bq/mol, NEN) was used without cycloheximide. The detailed procedure for collecting of cells, quick-freezing in methanol−dry ice, isolation of crude nuclei by glass bead homogenization in Nuclear Isolation Buffer (NIB) (0.25 M sucrose, 10 mM $MgCl_2$, 2.5 mM spermidine, 0.5 mM spermine, 20 mM HEPES, 100 mM *n*-butyric acid, 0.1% Triton X-100, 5 mM 2-mercaptoethanol, adjusted to pH 7 by KOH), and extraction of histones into chaotropic guanidine hydrochloride, pH 6.8, has been described (*31*). Histones were eluted from Bio-Rex 70 resin (Bio-Rad) as described (*30*, *31*), dialyzed into 2.5% acetic acid with 0.1 mL of 2-mercaptoethanol per liter, filtered through Amicon YM100 membranes, and lyophilized (*31*). Histones were fractionated into pools of crude histone H2B, histones H4 with H2A, and histone H2A by reversed phase HPLC on Zorbax Protein-Plus as described (*31*). The specific radioactivity of fractions was determined by liquid scintillation counting and optical density measurements at 214 nm (*31*). Following lyophilization, histones were analyzed on acetic acid−urea (AU) gels by densitometry of Coomassie staining and fluorography, as described elsewhere (*30*, *36*). For quantitative determination of the specific radioactivity of acetylated histone bands from yeast cells incubated with tritiated acetate in the presence of cycloheximide, histones extracted from not more than 10^{10} yeast cells were analyzed in single 30-cm-long AU gel lanes. This prevents nonlinear Coomassie staining of high-abundance histone forms but limits the accuracy in quantitating low-abundance bands. Multiple preflashed fluorography exposures were made from each gel to obtain data within the linear range of fluorography and X-ray film dynamics. SigmaPlot for Windows, Version 5.00 (SPSS Inc., Chicago, IL), was used for linear and nonlinear regression analyses. Data presented met all statistical tests for convergence and fit.

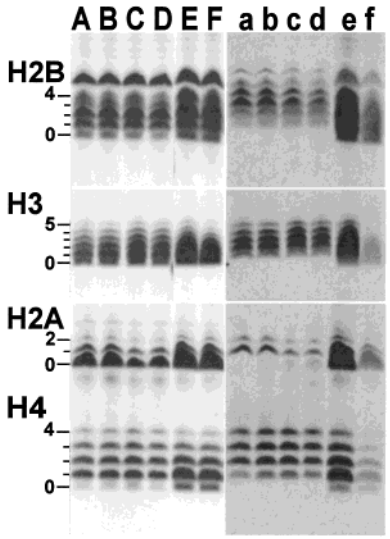

FIGURE 1: Yeast SNY28 cells [250 mL culture, 5×10^7 cells/mL of YPD medium, pH 4.5 (*31*)] were cultured with 1 mCi (37 MBq) of tritiated acetate (E) or 0.1 mCi (3.7 MBq) of tritiated lysine (F) for 2 h. Cycloheximide was added to four parallel cultures to 10 μg/mL. After 10 min, 1 mCi (37 MBq) of tritiated acetate was added. Cells were harvested after 30 (A), 60 (B), 100 (C), and 126 min (D). Histones were extracted (*31*), fractionated by HPLC into pools containing histone H2B, histone H2A with H4, and histone H3, and separated based on differences in histone acetylation levels in 30-cm-long AU gels. Histone bands were visualized by Coomassie staining (lanes A−F), and their radioactivity patterns were determined by fluorography for 54 days (lanes a−f). Acetylated bands in the Coomassie-stained gel are marked with numbers for lowest and highest visible levels of acetyllysines.

RESULTS

Posttranslational Acetylation Labeling of Yeast Histones. Acetylation of yeast histones has been demonstrated in acetic acid−urea (AU) gels by the discrete reduction in charge and thus in gel mobility when progressively an increasing number of amino-terminal lysines in core histones is acetylated (Figure 1, lanes A−F) (*31*). When histone protein synthesis is inhibited by cycloheximide, radioactive acetate is exclusively incorporated into up to six charge-modified forms of yeast histone H2B, two modified forms of H2A, four acetylated species of H4, and five acetylated forms of histone H3 (Figure 1, lanes a−d). The unmodified forms are only labeled in the absence of cycloheximide by tritiated lysine (Figure 1, lane f) or by tritiated acetate through metabolic conversion into amino acids (Figure 1, lane e).

In culture media at neutral pH, acetate labeling of histones is slow, as was observed by Nelson (*32*), because it is limited to the small fraction of acetate that can enter the cells as undissociated acetic acid through passive diffusion (*37*). Reducing the pH of the medium to below the pK_a of acetate (pH 4.7), acetate uptake and posttranslational labeling of core histones are rapid (Figure 2) without any detectable lag phase for posttranslational acetylation into histones (see below). In an attempt to study the rate of acetylation turnover, several hundredfold excess of nonradioactive acetate was added to cultures after 30 min culture with tritiated acetate. The decrease in the specific radioactivity of the histones was slow and nonexponential (results not shown), preventing use of

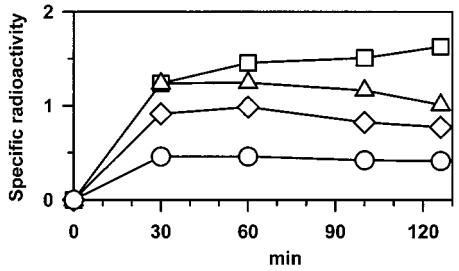

FIGURE 2: Time course of posttranslational acetate label incorporation into yeast histones. The specific radioactivity of individual histones in an arbitrary scale was calculated from fluorography band intensity (Figure 1, lanes a−d) divided by Coomassie band intensity (Figure 1, lanes A−D), determined by quantitation of optical digital densitometry recordings (*36*). Fluorography exposures ranged from 9 to 54 days. Labeling for histone H4 is shown by squares, H3 by triangles, H2B by diamonds, and H2A by circles. Nonlinear Coomassie staining was observed in this experiment for mono- and di-acetylated H2B, H3, and H4, and nonacetylated H2A (Figure 1, lanes A−D).

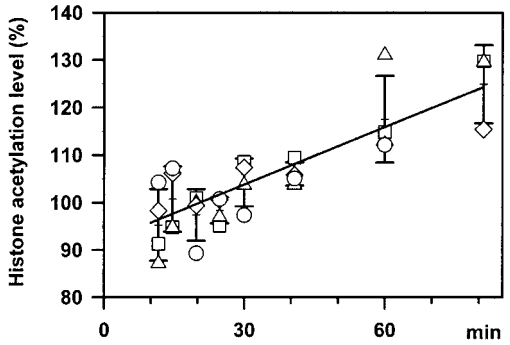

FIGURE 3: Effect of cycloheximide on the steady-state level of histone acetylation. The average number of acetyllysine groups per histone molecule were determined from the relative distribution of acetylated histone forms in the Coomassie-stained AU gel pattern. The 100% value was set by the acetylation of histones from cultures without cycloheximide. Acetylation is shown for histone H4 (squares), H3 (triangles), H2B (diamonds), and H2A (circles). The error bars indicate the standard deviation error in the relative acetylation level of all core histones at each time point. The line depicts the linear regression calculated from all values, with a standard error for the slope of 11% (SigmaPlot).

this experimental approach to measure the rate of turnover of histone acetylation.

One can determine the rate of turnover of a compound by measuring the exponential increase in specific labeling provided that a steady-state condition is reached. This methodology has been used successfully to measure histone acetylation turnover rates of 2−4 min in *Chlamydomonas* (*30*). Can cycloheximide be used for a sufficient length of time to allow measurement of acetylation turnover in yeast cells? Figure 2 suggests that an apparent steady-state condition for acetate label incorporation may be achieved within 1 h. It is clear from the absence of acetate label in nonacetylated histone forms, 30 min after the addition of tritiated acetate (Figure 1, lane a), that preincubation for 10 min with cycloheximide at 10 μg/mL is effective in preventing acetate incorporation into newly synthesized protein. The steady-state level of histone acetylation for all four core histones appeared unchanged during culture for at least 2 h (Figure 1, lanes A−D) and identical to that observed in untreated parallel cultures (Figure 1, lanes E,F). Similarly, the pattern of acetate incorporation appeared stable (Figure 1, lanes a−d).

However, inhibitor effects did exist. While cell viability remained unaffected by a treatment of yeast cells with cycloheximide for 2 h (results not shown), cell division ceased almost immediately. Cell numbers remained constant during cycloheximide treatment, as did histone levels (Figure 1, lanes A−D), while they doubled in parallel cultures without the drug (Figure 1, lanes E,F). To determine whether the balance between histone acetylation by HATs and deacetylation by HDAC activities really remained unaffected by cycloheximide treatment or cessation of cell cycle progression, additional experiments were performed. Steady-state levels of histone acetylation were determined repeatedly over the course of experiments that were performed to measure rates of histone acetylation turnover (see below). In several experiments, like the one shown in Figure 1, no statistically significant change in histone acetylation was observed. However, in some experiments a detectable increase in steady-state histone acetylation was observed, provided that data for all four core histones were combined (Figure 3). If histone acetylation values for histones were individually assessed, significant changes in steady-state acetylation were never obtained. If detectable, changes were always small, producing a 10% or maximally 20% increase in acetylation after 1 h of cycloheximide treatment (Figure 3). A significant drop in histone acetylation during culture in the presence of cycloheximide was never observed. Overall, it appeared that cycloheximide could be used for a limited period of time to study turnover of histone acetylation.

Histone Acetylation Turnover Measurements. Yeast cells were grown in rich YPD medium with cell doubling times of 90 min and in minimal defined SD medium with cell doubling times of 150 min to assess the possibility that acetylation turnover might differ under distinct metabolic conditions. Growth in rich versus minimal medium did not affect steady-state levels of histone acetylation (*31*). Cells were collected during logarithmic growth in 400−500 mL aliquots; cycloheximide was added, followed 10 min later by 2 or 3 mCi (37 or 74 MBq) of tritiated acetate. At the time of labeling, the pH of YPD medium, adjusted by succinate, was 4.5. It ranged from pH 3.2 to pH 3.9 in unbuffered SD medium. Incubation in the culture shaker at 30 °C was terminated by sedimenting cells rapidly in a cooled centrifuge, decanting the growth medium, and freezing the cell pellet immediately in methanol−dry ice. Histones were extracted, fractionated by HPLC, and electrophoresed in long AU gels. The gels were stained for densitometric analysis of the Coomassie dye pattern. Multiple fluorography exposures were made for up to 98 days to obtain results within the linear range of preflashed XAR film, and digitized. Figure 4 shows an example of a fluorography exposure from an experiment in SD medium, in parallel to histones labeled with acetate for 90 min without cycloheximide and a representative lane of the Coomassie-stained gel. Label incorporation was immediate, without a lag phase (Figure 5D). The specific radioactivity of each histone (Figure 5A−C), and of all histone bands individually, was calculated and used for nonlinear regression analysis to determine the half-life of histone acetylation (Table 1). Values obtained for acetylation turnover rates were not significantly different for experiments in YPD and SD and between duplicate experi-

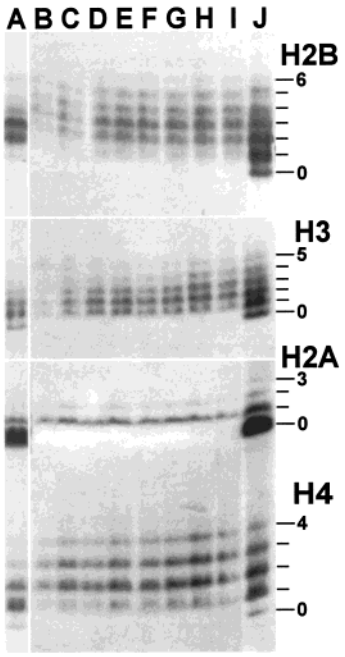

FIGURE 4: Example of AU gel fluorography for 98 days of histones from yeast cells, grown in SD medium and labeled by tritiated acetate. Labeling was for 1.7 min (lane B), 4.7 min (lane C), 9.8 min (lane D), 14.8 min (lane E), 20 min (lane F), 30.8 min (lane G), 50 min (lane H), and 71 min (lane I) in the presence cycloheximide, and for 100 min without cycloheximide (lane J). Lane A shows a representative lane from the Coomassie-stained gel that was prepared for fluorography. Acetylated bands are marked, and the lowest and highest visible levels of acetylation are numbered.

ments in the same growth medium. The pattern of label incorporation into identified histone H2B, H4/H2A, and H3 HPLC peaks (*31*) generally matched the pattern of specific radioactivity obtained from gel analyses (Figure 5). The levels of total acetate incorporation and of specific labeling of histone species were very similar in YPD and SD. Although overall acetate incorporation was almost linear with time (Figure 5D), nonlinear regression analysis suggests that exhaustion of label, a decrease over time in the specific radioactivity of acetate, did occur with a half-life of 1−1.5 h. Thus, it is likely that the specific radioactivity of the acetylCoA pool, the precursor for lysine acetylation by HATs, did not maintain strictly the steady-state condition required for a complete and accurate determination of the turnover rate of histone acetylation. The results of Figure 2 support the impression that, over time, histone acetylation labeling may start to decrease. However, the inaccuracy of specific radioactivity of the histones obtained through gel analysis (Figure 5A−C) contributes a much larger error to the estimates on turnover rates reported (Table 1) than a slowly decreasing, quasi-steady-state condition would.

Table 1: Turnover Rates of Posttranslational Acetylation of Histones in Yeast[a]

histone	half-life in YPD (min)	half-life in SD (min)
H4	20.6 ± 5.2	13.6 ± 1.4
H3	8.2 ± 1.7	11.5 ± 4.2
H2B	3.7 ± 1.4	4.1 ± 1.3
H2A	5.8 ± 2.1	5.3 ± 3.1

[a] Accuracy of the estimates for the half-life values is represented by the standard error of the estimate (SigmaPlot) for a nonlinear regression fit representing an exponential increase to a maximum level of specific radioactivity. *P* values were below 0.04 except for a poorer fit of H2A in SD ($P = 0.17$).

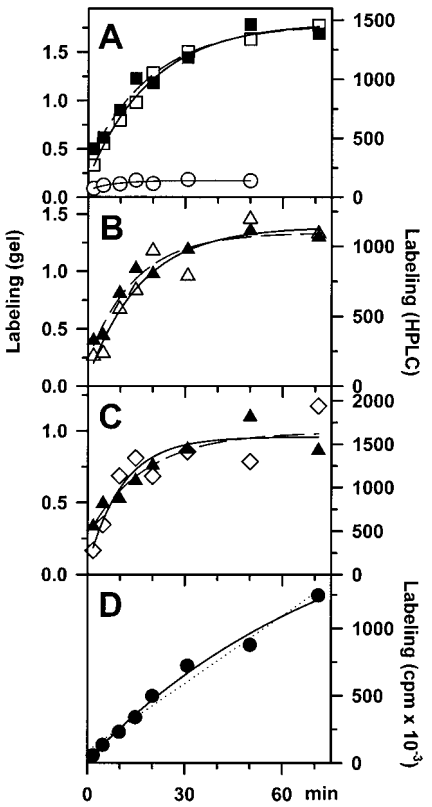

FIGURE 5: Nonlinear regression analysis of histone acetylation labeling in yeast cells, cultured in SD. The specific radioactivity, measured by densitometry of a Coomassie-stained AU gel and its fluorograph, was plotted as "Labeling(gel)" by open symbols for (A) histone H4 (squares) and H2A (circles), (B) histone H3 (triangles), and (C) histone H2B (diamonds). The data were fitted by a nonlinear regression analysis, assuming an exponential rise in the labeling to a steady-state maximum (continuous line). The specific radioactivity incorporated is also shown for histones fractionated by HPLC as cpm per peak ["Labeling(HPLC)"], and data (solid symbols) were fitted (dashed line). Note the similarity between gel- and HPLC-fitted curves despite the presence of nonhistone proteins in the HPLC fractions and the coelution of histone H2A with histone H4 (*31*). Panel D shows the total amount of radioactivity incorporated into the crude histone preparations that were subjected to HPLC fractionation. The line represents the nonlinear regression curve as shown for the histones, representing a half-life value for exhaustion of tritiated acetate incorporation of approximately 1 h. The congruency between the data and this curve was better than for a linear regression fit (dotted line).

Are Rates of Acetylation Turnover Dependent on Acetylation Levels? In *Chlamydomonas*, the rates of acetylation turnover for mono- through penta-acetylated forms in histone H3 were identical. In contrast, multi-acetylated forms of histone H4 turned over faster than low and mono-acetylated H4 (*30*). In alfalfa, turnover rates of multi-acetylated forms of histone H4 and H2B were also higher than for mono-acetylated histones, and turnover rates for histone H3 acetylation were the same for all levels of modification (unpublished results). Experiments described here suggested that such differences between histone species might not exist in yeast. It appears that the rate of increase in fluorography intensity over time could be the same for each modification level in each core histone (Figure 4, lanes B−I). Figure 6 presents the compiled data of histone acetylation turnover

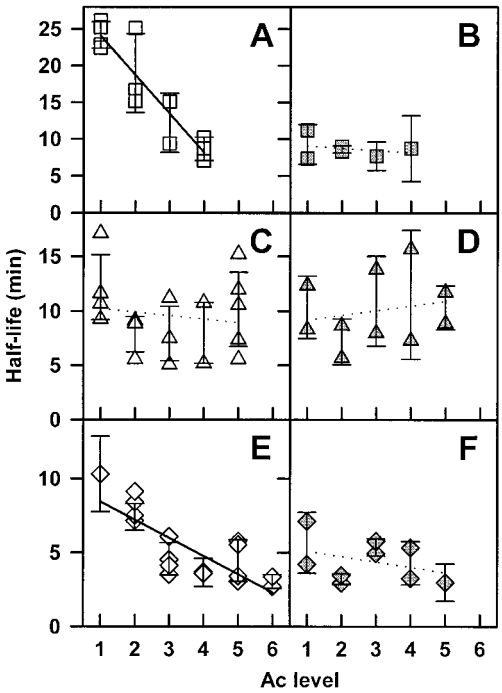

FIGURE 6: Dependency of histone acetylation turnover rates on the acetylation level of histones. Compilation of acetylation half-life values (symbols) calculated from nonlinear regression analysis of the specific radioactivity of each acetylation level for histone H4 (A,B; squares), H3 (C,D; triangles), and H2B (E,F; diamonds) of yeast cells labeled by acetate in YPD (A,C,E; open symbols) and SD medium (B,D,F; filled symbols). The error bars at each acetylation level mark the standard deviation error over all data collected. Linear regression analysis was performed for the set of data within each panel and plotted by a line if the slope was significant (standard error of estimate of 14% for panel A and 19% for panel E), and by a dotted line if the standard error of estimate exceeded the slope value calculated (SigmaPlot).

rates for each level of acetylation for histones H4, H3, and H2B for which specific radioactivity measurements could be obtained in YPD and/or SD experiments. In YPD experiments, turnover rates increased 2-fold from mono- to tetra-acetylated H4 (Figure 6A) and H2B (Figure 6E). However, no significant increase in acetylation turnover rates could be detected for either histone in SD (Figure 6B,F). Turnover rates for histone H3, subject to significant experimental error which was partially caused by the limited gel separation of acetylated forms (Figure 1), did not vary with acetylation levels in YPD (Figure 6C) or SD experiments (Figure 6D). The same appeared true for histone H2A in SD with similar rates for mono- and di-acetylated forms (results not shown).

Assessment of the Fraction of Histones Which Is Subject to Acetylation Turnover. The nonlinear regression fit of acetylation labeling to steady-state levels produces a specific radioactivity value for each level of histone acetylation. If every acetyllysine group would be subject to the same rate of turnover as acetylation of the histone overall, the specific radioactivity value derived would increase linearly with the level of histone acetylation. Applied in *Chlamydomonas*, this analysis revealed that all acetyl groups in histone H3, irrespective of the level of H3 acetylation, did turn over with a half-life of approximately 2 min. In contrast, even if more than 75% of multi-acetylated H4 appeared dynamic, less than half of mono-acetylated H4 turned over (*30*). Without a direct measurement of the specific radioactivity of the acetylCoA pool, one only derives maximum levels for the dynamic fraction of histone acetylation. Figure 7 presents this type of analysis for yeast histones H4, H3, and H2B for cultures in YPD and SD medium.

Steady-state labeling of histone H3 increased linearly with the level of acetylation in all analyses (Figure 7B). However, the labeling of mono- and di-acetylated forms was clearly below unity values. Thus, the fraction of dynamic acetyl groups at low levels of acetylation is less than half that observed for histone H3 molecules with four or five acetyllysine groups. Based on similar rates for acetylation turnover at all modification levels of H3 (Figure 6C,D), this suggests that part of mono- and di-acetylated H3 is subject to turnover while another part is not. One could speculate that this difference might represent different chromatin domains, in some of which transcription might be active while in others it would not.

Interpretation of the dynamic fraction of acetylated histone H4 (Figure 7A) and H2B (Figure 7C) is less straightforward. It is clear that in YPD experiments the fraction of histone with acetyl groups that turn over rapidly drops toward lower modification levels. This appears to parallel the observed decrease in the rate of acetylation turnover in H4 (Figure 6A) and H2B (Figure 6E). In contrast, in the SD experiments where the rate of turnover for H4 was fast at all levels of acetylation (Figure 6B), the dynamic portion of acetyl groups at all acetylation levels appears constant (Figure 7A). The size of this dynamic portion cannot be estimated in these experiments. Considering that during active growth in YPD only a fraction of low level acetylated histone H4 molecules is subject to turnover, it is more likely that in minimal SD medium only a fraction of every level of acetylated H4 molecules is dynamic than that every molecule of acetylated H4 would be subject to acetylation turnover. Similar conclusions would apply to histone H2B where a fairly constant rate of acetylation turnover in SD (Figure 6F) corresponds with a more linear response in maximum steady-state labeling (Figure 7C).

DISCUSSION

Rates of histone acetylation and deacetylation in yeast have been reported just once before (*32*) as changing over a period of hours. Changes in gene expression in response to external signals occur in yeast much more rapidly (*34*, *38*, *39*). Overall, the faster rates of histone acetylation turnover for yeast reported here correlate much better with the rates of changes in local histone acetylation and gene expression than previous estimates. It is in line with fast acetylation in other organisms, from animal cells (*25*, *28*, *29*) to algae (*30*). As in *Chlamydomonas*, the turnover rate of 15 min for histone H4 acetylation in yeast was slower than for the other core histones with, on average, acetylation half-lives of 10, 4, and 5 min for histone H3, H2B, and H2A, respectively (Table 1). Also, as in *Chlamydomonas*, the turnover rate for higher modified forms of histone H4 was higher, while rates for acetylation turnover in histone H3 were the same for all levels of acetylation (Figure 6).

The acetylation turnover measurements reported here were collected in the presence of the translation inhibitor cycloheximide and thus may be affected by the drug. No

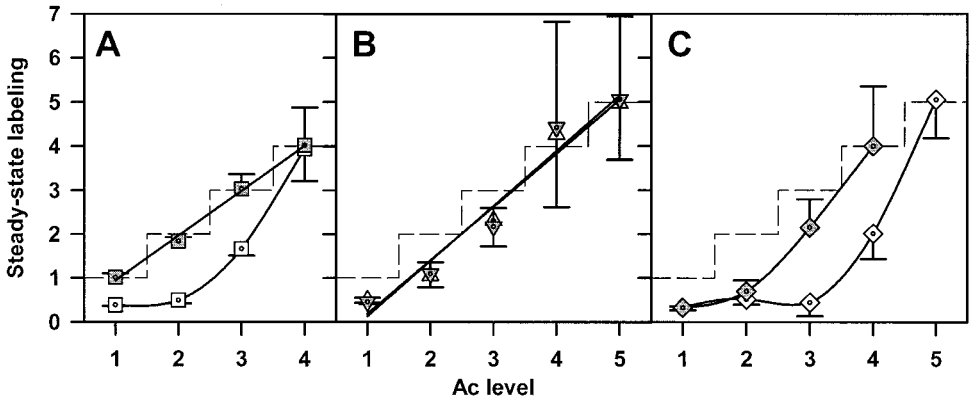

FIGURE 7: Dependency of steady-state specific radioactivity levels on the acetylation level of histones. The maximum, steady-state labeling of histones at each level of modification was derived from nonlinear regression analyses for histone H4 (A), H3 (B), and H2B (C). Results obtained in YPD experiments (open symbols, error bars below data points) and SD experiments (filled symbols, error bars above data points) were subjected separatedly to linear regression analysis. Linear correlations between histone acetylation levels and maximum specific radioactivity were obtained for histone H4 in YPD (A) and for H3 in YPD and SD experiments, with standard error of estimates of 5, 11, and 12%, respectively (SigmaPlot). Each plot was scaled to the theoretical maximum where all acetyl groups are subject to turnover at the rates plotted in Figure 6. These theoretical unity values are depicted by the dashed line plot.

methodology exists that can measure turnover of posttranslational acetylation without the presence of drugs at some time during the experiment. In pulse−chase protocols, pulse-labeling must be performed under conditions of inhibited protein synthesis (*25*, *40*), as was used in the continuous labeling experiments reported here. Based on the minimal detectable effects of cycloheximide on histone acetylation levels (Figure 3) and data collection for half-life determination largely restricted to the first 30 min after drug addition (Figure 5), the experimental measurements of histone acetylation turnover rates are likely as good as can be obtained. The close match in *Chlamydomonas* of maximal turnover rates measured by pulse−chase protocols with rates measured by labeling to steady-state (*30*) suggests that the rates reported here reflect closely the reality within the yeast cell.

An attempt was made to estimate the fraction of the yeast genome that is subject to dynamic acetylation. We have reported that most histones in yeast are highly acetylated (*31*). On average, two acetyllysines exist in every histone H4, H3, and H2B molecule, and the fraction of unmodified forms for each is quite low. Only in histone H2A with 0.2 acetyl group per molecule is the nonacetylated histone form dominant. With an average acetylation of 13 acetyllysines per nucleosome, yeast chromatin appears to exist, on average, in a transcriptionally competent and accessible state (*31*). Recent analyses of acetylation and deacetylation in the chromatin surrounding several yeast genes have clearly provided specific examples for the general conclusion that dynamic acetylation in yeast is global (*35*). This global acetylation of yeast chromatin is likely punctuated and interrupted by repressed gene promoters and silenced gene sequences (*41*−*43*).

The analysis of maximum acetylation labeling of histone molecules suggests that the vast majority of multi-acetylated histones are subject to rapid acetylation and deacetylation at all modification sites, four in histone H4 and five in histones H3 and H2B (Figure 7). We saw no indication for stable acetylation in these histones when they are highly acetylated. Based on these observations, one can calculate from the results of Figure 7 that in yeast half of all chromatin (51 ± 20%) is subject to rapid acetylation and deacetylation. On average, histones H4, H3, and H2B in this chromatin are acetylated higher (120 ± 11%) than the level revealed by Coomassie staining (Figure 1), reported previously (*31*). Relatively stable acetylation likely exists in nucleosomes with lower levels of histone acetylation [56 ± 21% of the level seen by Coomassie staining (*31*)] with a preponderance of mono- and non-acetylated histones. This is true in particular for histone H4 (Figure 7A) and H2B (Figure 7C). Over longer periods of time than used for the experiments described, slower acetylation and deacetylation processes may well exist. In addition, changes in gene activation and repression will certainly cause chromatin domains to enter or exit the global environment of dynamic acetylation.

Thus, even though only global differences in the dynamics of histone acetylation were analyzed in this paper, the existence of different chromatin environments was deduced: a major compartment with high levels of steady-state acetylation which all appear to participate in rapid turnover of acetyllysines, and a minor compartment with lower levels of acetylation where much of the lysine acetylation is more stable.

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