REVIEW / SYNTHÈSE

Dynamics of histone acetylation in vivo. A function for acetylation turnover?

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Abstract: Histone acetylation, discovered more than 40 years ago, is a reversible modification of lysines within the amino-terminal domain of core histones. Amino-terminal histone domains contribute to the compaction of genes into repressed chromatin fibers. It is thought that their acetylation causes localized relaxation of chromatin as a necessary but not sufficient condition for processes that repackage DNA such as transcription, replication, repair, recombination, and sperm formation. While increased histone acetylation enhances gene transcription and loss of acetylation represses and silences genes, the function of the rapid continuous or repetitive acetylation and deacetylation reactions with half-lives of just a few minutes remains unknown. Thirty years of in vivo measurements of acetylation turnover and rates of change in histone modification levels have been reviewed to identify common chromatin characteristics measured by distinct protocols. It has now become possible to look across a wider spectrum of organisms than ever before and identify common features. The rapid turnover rates in transcriptionally active and competent chromatin are one such feature. While ubiquitously observed, we still do not know whether turnover itself is linked to chromatin transcription beyond its contribution to rapid changes towards hyper- or hypoacetylation of nucleosomes. However, recent experiments suggest that turnover may be linked directly to steps in gene transcription, interacting with nucleosome remodeling complexes.

Key words: histone, acetylation, turnover, chromatin, transcription.

Résumé : L'acétylation des histones, découverte il y a plus de 40 ans, est une modification réversible des résidus lysine dans le domaine N terminal des histones du coeur. Les domaines N terminaux des histones contribuent au compactage des gènes en fibres de chromatine réprimée. Leur acétylation produirait un relâchement localisé de la chromatine, ce qui est une condition essentielle, mais insuffisante, pour les processus où il y a empaquetage de l'ADN, telles la transcription, la réplication, la réparation, la recombinaison et la formation du spermatozoïde. Alors qu'une augmentation de l'acétylation des histones stimule la transcription de gènes et que la désacétylation réprime et rend silencieux les gènes, la fonction des réactions d'acétylation et de désacétylation continues ou répétitives rapides, ayant des demi-vies seulement de quelques minutes, demeure inconnue. Une revue de 30 ans de détermination in vivo du taux de renouvellement des groupes acétyle et des vitesses de changement des niveaux de modification des histones est faite afin d'identifier les caractéristiques communes de la chromatine mesurées par différents protocoles. Il est maintenant possible d'étudier un plus large éventail d'espèces qu'auparavant et d'identifier les caractéristiques communes. Le taux de renouvellement rapide de la chromatine compétente et active lors de la transcription est une de ces caractéristiques. Bien qu'il soit observé dans toutes les espèces, nous ne savons pas si le renouvellement lui-même est lié à la transcription de la chromatine, au-delà de sa contribution aux changements rapides entraînant l'hyper- ou l'hypoacétylation des nucléosomes. Cependant, des expériences récentes suggèrent que le renouvellement pourrait être lié directement à des étapes de la transcription des gènes, en interagissant avec les complexes de remodelage du nucléosome.

Mots clés : histone, acétylation, taux de renouvellement, chromatine, transcription.

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Introduction

Reversible acetylation of histones was discovered in 1964 (Allfrey et al. 1964). Allfrey and co-workers presented some of the early evidence for a functional correlation with gene expression (Allfrey 1970, 1977; DeLange and Smith 1971; Edwards and Allfrey 1973; Wangh et al. 1972). It has taken more than 20 years to confirm this correlation, and only re-

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cently has direct, detailed molecular evidence been obtained for a role of histone acetylation in the mechanics of gene transcription. However, the functional importance for one of the early observations, the fast rate of turnover of histone acetyllysines, remains an enigma. This review surveys more than 30 years of experimental quantitative observations on acetylation dynamics obtained by a variety of experimental approaches in distinct model systems. Early publications reporting new features are quoted in preference over reviews or later, confirmatory papers.

Histone acetylation changes the structure of chromatin as demonstrated by changes in chromatin folding (Ausio and Van Holde 1986; Fletcher and Hansen 1995; Garcia-Ramirez et al. 1995; Tse et al. 1998; Wang et al. 2001), solubility (Alonso et al. 1987; Davie and Candido 1978; Perry and Chalkley 1981; Wang et al. 2001), and susceptibility to nucleases (Litt et al. 2001; Perry and Chalkley 1981; Vidali et al. 1978). General and site-specific hypersensitivity of chromatin to fragmentation by DNase I has been well documented (Bonifer et al. 1997; Gross and Garrard 1988; Sealy and Chalkley 1978*a*). It has been correlated functionally with cellular processes ranging from transcription to replication, recombination, repair, and other processes that rearrange or repackage nucleosomes as occur, for instance, during spermatogenesis in many species (Candido and Dixon 1972; Chicoine et al. 1986; Grunstein 1997; Hazzouri et al. 2000; Kwon et al. 2000; Meistrich et al. 1992; Oliva and Mezquita 1982; Ramanathan and Smerdon 1989; Wade et al. 1997; Wu et al. 1986).

Early experiments using radioactive acetate tracers in vivo readily demonstrated that histone acetylation is a rapid and reversible process (DeLange and Smith 1971). Only in recent years has the multiplicity of enzymes involved been recognized. Histone acetyltransferase (HAT) activities have been discovered as essential features of basal transcription factors or have been identified as the transcription-stimulating activities in transcription factors that directly or indirectly are recruited into transcription initiation and elongation complexes (Marmorstein 2001; Ogryzko 2001; Roth et al. 2001). Many different HATs have been identified with distinct histone specificities, including specificities for distinct lysines within the amino-terminal domain of core histones. Removal of acetyl moieties from acetylated histones depends on multiple distinct histone deacetylase (HDAC) enzymes that are now recognized to include repressors of gene transcription and factors required for gene and chromosome silencing (Bertos et al. 2001; Graessle et al. 2001; Khochbin et al. 2001). In addition, deacetylation of new histone H4 is observed when nucleosome spacing matures in chromatin across newly replicated DNA (Ridgway and Almouzni 2001).

In recent years, antisera specific for acetyllysine in general or at specific sites of amino-terminal domains of core histones have been used to demonstrate that histone acetylation increases within nucleosomes at gene promoters and across genes when gene expression is induced (Christenson et al. 2001; Hebbes et al. 1994; Kuo et al. 1996; Suka et al. 2001; Turner and Fellows 1989; White et al. 1999). Conversely, decreased histone acetylation is seen when genes or X chromosomes are repressed or silenced (Bird and Wolffe 1999; Wolffe and Matzke 1999). Clearly, experiments using such antisera have demonstrated that acetylation levels change when the HAT/HDAC balance changes, generally through the addition of HDAC inhibitors or locally from selective enzyme recruitment, activation, dissociation, inhibition, nuclear–cytoplasmic shuttling, or proteolytic destruction (Kruhlak et al. 2001; Zhao et al. 2001; Zhou et al. 2000). Many examples are now known where changes in RNA transcription are closely related to changes in nucleosome acetylation. Some of these changes occur within an hour, but in many experimental systems, changes are measured in hours. Chromatin immunoprecipitation (ChIP) analyses are starting to allow measurement of acetylation turnover rates at selected genes and DNA domains that collectively produce the observed general changes in histone acetylation levels (Katan-Khaykovich and Struhl 2002).

The reason for high rates of acetylation turnover remains unknown. Half-lives as short as a few minutes certainly would allow an organism to change patterns of gene expression. However, the turnover rates appear faster than would be needed to account for the rate of change observed in histone acetylation levels and gene transcription. Furthermore, acetylation turnover extends across whole genes, even in the absence of transcription, and across multigene domains and intergenic regions (Forsberg et al. 2000; Hebbes et al. 1994; Litt et al. 2001; Myers et al. 2001; Vogelauer et al. 2000). One can understand that general and localized accessibility for factors, detected as general and hypersensitive site-specific DNase I susceptibility or across locus control regions that define gene domain boundaries, is part of the overall control mechanism to regulate gene expression, genome replication, etcetera. What is not understood is the mechanical role that the continuous churning of acetylation could play. Inhibition of transcription has not been shown to affect histone acetylation or its turnover directly, even though partial inhibition has been observed in some systems (Arrigo 1983; Edwards and Allfrey 1973; Moore et al. 1979; Waterborg and Matthews 1984). Butyrate (Candido et al. 1978; Cousens et al. 1979; Riggs et al. 1977; Sealy and Chalkley 1978b), trichostatin A (TSA), and other natural HDAC inhibitors (Furumai et al. 2001; Graessle et al. 2001; Ransom and Walton 1997; Yoshida et al. 1995) block histone deacetylation and thus acetylation turnover, but this loss of turnover does not inhibit gene transcription. In fact, over time, the opposite effect is seen as acetylation levels tend to rise globally in chromatin and transcription of genes is facilitated when nucleosome hyperacetylation lifts some of the repressive effects of chromatin compaction. The lack of HAT inhibitors, usable in vivo, has limited experimental options to test how continuous or frequent conversion between lysine and acetyllysine states in the amino termini of core histones may be linked to the repetitive steps of the transcription process. Recently, complex in vivo experiments in genetically modified cells show promise for an in vivo dissection of processes like gene transcription and nucleosome remodeling and identification of a preferred or obligatory linkage (Reinke et al. 2001).

This paper reviews observations and measurements of the dynamics and turnover of histone acetylation reported over

the last 30 years. A variety of methods have been employed, each with their own implicit limitations. Measurements have been made under conditions of unvarying steady-state acetylation or while histone acetylation levels were rising or falling. A wide variety of organisms, tissues, and cells have been used. Experimental conditions ranged from minimally invasive under normal growth or culture conditions, including cell cycle synchronization, to systems with natural or artificial induction or repression of gene expression, often including the addition or removal of HDAC inhibitors. This review is limited to those studies that report time-course data that allow a determination or a reasonable approximation of the rate of change or turnover in histone acetylation. Excluded are papers that report changes observed in histone acetylation or acetate labeling multiple hours after the start of an experimental protocol. The attempt is made to interpret and reanalyze older reports in light of what has been learned in recent years about gene expression in chromatin, about induction, repression, and silencing of genes, about the diversity of HATs and HDACs and their regulated localization within chromatin localities, about the efficacy and indirect effects of HDAC inhibitors, and about the metabolism of acetate and acetyl-CoA.

It should be realized that site-specific histone acetylation represents only a part of the language that histones speak through multiple global and residue-specific posttranslational, often reversible, modifications, including serine phosphorylation, lysine methylation, and likely arginine methylation (Davie and Dent 2002; Jenuwein and Allis 2001; Strahl and Allis 2000). No attempt has been made to reinterpret older experimental results in the light of these very new, exciting, and partially recognized processes that clearly affect or control histone acetylation and its turnover or that are affected or controlled by histone acetylation.

Measurement of histone acetylation turnover rates

Bona fide measurements of acetylation turnover rates can be determined only when levels of histone acetylation are constant and at steady state and when strict conditions of use of radioactive tracer compounds are observed. Two distinct procedures are able to meet these conditions: pulse-chase and continuous-label protocols. Turnover values determined by these protocols are reported as "half-life" values, the time required for 50% turnover.

It should be realized that in most experimental systems, it is difficult to determine the fraction of the chromatin that is being analyzed. Radioactive acetyl labeling only occurs for those histones that are incorporating radioactive acetyl-CoA. Histones that exist as acetylated forms but that do not interact with HAT and (or) HDAC enzymes do not take part in the determination of turnover rates even though they could represent 10, 50, or 90% of the acetylated histone forms detected by polyacrylamide gel analysis and thus contribute to the measurement of specific radioactivity of a histone or histone form, the amount of radioactivity present per amount of protein (Brotherton et al. 1981). Under some experimental conditions, it is possible to estimate the fraction of histone that is responsible for the reported acetyl turnover rates.

This review is limited to rates of posttranslational, reversible lysine acetylation across the amino-terminal domains of the core histones. Radioactive acetate can be incorporated cotranslationally when histone H4, H2A, and H2B are synthesized and the amino-terminal residue is acetylated by an N-terminal acetyltransferase enzyme. In addition, radioactive acetate or acetyl-CoA can be metabolized so that the radioactivity labels new amino acids that are used in the synthesis of new histones. Such cotranslational and translational labeling of histones is frequently avoided by inclusion in experimental protocols of translation inhibitors like cycloheximide (Covault and Chalkley 1980; Duncan et al. 1983; Marzluff and McCarthy 1970; Waterborg 1998, 2001). Some experimental conditions, such as the absence of histone synthesis, allow measurement of posttranslational acetylation turnover rates in the absence of translation inhibitors.

Pulse-chase analysis of acetylation turnover

In the pulse-chase protocol, radioactive acetyl-CoA is rapidly introduced into permeabilized cells for a short period of time, the "pulse" time, during which histones are highly radioactively labeled. Subsequently, the radioactive tracer is rapidly removed by washing of the cells or by dilution of the medium with a 100- or 1000-fold excess of unlabeled acetyl-CoA to start the condition of the "chase". In view of the impermeability of cell plasma membranes for acetyl-CoA and the difficulty in establishing and maintaining homogeneously permeabilized cell cultures, the acetyl-CoA is in most in vivo experiments replaced by its precursor, radioactive acetate.

Radioactive acetate can be used provided that entry or uptake of the acetate into the cell is essentially instantaneous, i.e., not rate limiting, and that the cells tested will rapidly convert the acetate into acetyl-CoA. The latter condition is generally assumed when incorporation of label from radioactive acetate into histones is fast and does not display an appreciable lag phase (Waterborg and Matthews 1983*b*). This appears generally to be the case for animal cells in tissue culture medium (Sanders et al. 1973; Seidman et al. 1979). Acetate uptake can be rate limiting for some cell types, including the yeast *Saccharomyces cerevisiae*, when acetate entry into the cell solely depends on diffusion of the undissociated organic acetic acid, and labeling conditions are near pH 7, much above the 4.76 pK_a value for acetic acid. At pH 7, less than 1% of added acetate would be available for diffusion into the cells (Nelson 1982; Waterborg 2001). When culture conditions with a pH below the pK_a value are used, acetate uptake is generally not limiting because more than half of the acetate exists as uncharged acetic acid, which can diffuse into the cells. The neutral cytoplasmic pH of the cell will instantly dissociate the cytoplasmic acetic acid for more than 99% into protons and acetate, keeping the cytoplasmic acetic acid concentration low and diffusion effective (Waterborg 2001; Waterborg and Matthews 1983*b*). A practical limitation of this approach is that acidification of the cytoplasm should be avoided, requiring the use of low concentrations of radioactive acetate of very high specific radioactivity. In pulse-chase protocols, the accumulation of cytoplasmic tracer acetate causes problems because the high concentration of cytoplasmic radioactive

acetate or acetyl-CoA must be diluted effectively at least 100-fold to establish effective chase conditions (Zhang and Nelson 1988*b*) while cytoplasmic acidification is avoided.

The specific radioactivity of each histone is determined over the period of the chase by determining radioactivity levels per amount of histone protein, either by liquid scintillation measurements during column chromatographic elution of histone species determined by UV absorbance or by autoradiography or, for tritium label, by fluorography in combination with quantitation of the Coomassie brilliant blue dye bound to histone polypeptides in fixed and stained polyacrylamide gels. In many papers, the standardization of the acetate labeling is implied, e.g., by assuming that equal amounts of histone are obtained in parallel samples. SDS (sodium dodecyl sulfate), AU (acetic acid – urea), and AUT (acetic acid – urea – Triton X-100) gel electrophoresis has been used for separating histone species and their primary sequence variants for histone acetylation analysis. A single- or multicomponent exponential decay is observed that is translated mathematically into the single or multiple half-life values that describe acetylation turnover. Experimental accuracy typically limits this analysis to the detection of one or two distinct half-life values, each responsible for a calculated subpopulation of the labeled chromatin. It is unlikely that any such approximation is completely valid, as heterogeneous mixtures of chromatin environments are likely to exist. Also, small populations of histone molecules with turnover rates that are much faster or much slower than the main observed value(s) are difficult to detect unless specific labeling conditions are chosen.

The choice of the length of pulse labeling can affect which kinetic components are detected and significantly affects the relative fraction of histone acetylation attributed to fast and slow compartments. Before relative contributions of fast and slow kinetic compartments can be evaluated, it must be established which fraction of the chromatin is represented by the histones that are seen by the acetate labeling. Likely, this will vary with labeling times, but no experimental determinations have been reported. Only recently have some educated guesses been made (Waterborg 1998, 2001). They identify fractions of the total chromatin that appear consistent with the suspected size of the combined compartments of transcriptionally active and competent chromatin, e.g., 10 or 20% in animal cells or near 100% in yeast. However, it cannot be excluded that such guesses are influenced by a circular argument, the experimental observation that rapid acetylation is observed in transcriptionally active or competent chromatin.

High-resolution pulse-chase analyses identify two rate compartments in most core histones that are assumed to represent populations of nucleosomes in which acetylation turnover for all histones is very fast with half-lives of 1–5 min or is all moderately fast with half-lives of 30–60 min (Table 1). The very fast rate compartment ranges from 10 to 50% of the acetate labeled histone pool but, remarkably, is reported to vary among core histone species. Considering data accuracy and variability in size between species and even experiments executed by different or the same investigators in the same experimental system, the validity of such differences appears to be rather doubtful. The very fast rate component was not identified in alfalfa plant cells, possibly due to dif-

Table 1. Histone acetylation turnover half-lives (min) in pulse-chase labeling protocols.

Species, cell	Histone H4	Histone H3	Histone H2B	Histone H2A	Histone average	Pulse time	Reference
Rat hepatoma	2–3, 40	3, 30	3, 40	2–3	2–3, 30–40	1	Jackson et al. 1975
Rat hepatoma	*26*	*8*				10	Sealy and Chalkley 1978*b*
Rat hepatoma	2, 35	2, 26				10	Moore et al. 1979
Rat hepatoma	7, 36	4, 58	3, 42	43		10–120	Covault and Chalkley 1980
	Ac1: *17*, 33						
	Ac4: *8*, *31*						
Human HeLa					*14*	15	Vidali et al. 1978
Human fibroblasts	5, 45	*12*, *40*	*8*, *53*	*12*, *28*		10	Duncan et al. 1983
SV40 in CV1					15	10	Chestier and Yaniv 1979
Duck erythrocytes	*17*	23	*21*			10	Sanders et al. 1973
Chicken erythrocytes					3, 150	10	Brotherton et al. 1981
Rooster testis	5±*1*				*8±1*	15	Oliva and Mezquita 1982
Drosophila					40–60	15	Arrigo 1983
Tetrahymena	1, 60					10	Moore et al. 1979
Alfalfa	42±9	53±7 (H3.1)	32±11			5	Waterborg and Kapros 2002
		38±4 (H3.2)					

Note: In a typical pulse-chase protocol for posttranslational acetylation, radioactive acetate is added to cells that do not synthesize histones naturally or whose protein translation is inhibited by cycloheximide. After a few minutes of label incorporation, the pulse time (min), the radioactive tracer is removed, replaced by fresh medium supplemented with unlabeled acetate, and the cell culture continued throughout the chase period. The exponential loss of label is the basis for calculation of turnover half-life values. Where two rate components were identified, both values are given. Values presented in italics were not explicitly reported in the paper but were calculated from data presented by exponential decay regression analysis using SigmaPlot for Windows, version 7.0, with estimates of uncertainty as described (Waterborg 2001).

Table 2. Histone acetylation turnover half-lives (min) in continuous-label protocols.

A. Histone species

Species, cell	Histone H4	Histone H3	Histone H2B	Histone H2A	Reference(s)
Duck erythrocytes	*7*	*6*	*6*		Sanders et al. 1973
Duck reticulocytes	*3*	*2*	*2*		Sanders et al. 1973
Saccharomyces	21±5	8±2	4±1	6±2	Waterborg 2001
Chlamydomonas	3.5±1.1	1.7±0.2	1.4±0.3	2±1	Waterborg 1998
Alfalfa	19±4	15±2 (H3) 22±6 (H3.1) 17±2 (H3.2)	23±3		Waterborg and Kapros 2002; Waterborg et al. 1990

B. Acetylated forms of histones

	Acetylation level						
Species, histone	Ac1	Ac2	Ac3	Ac4	Ac5	Ac6	Reference
Saccharomyces, histone H4	24±2	19±4	12±3	9±1			Waterborg 2001
Saccharomyces, histone H2B	10±2	8±1	5±1	3.6±0.2	4±1	3.0±0.3	Waterborg 2001
Chlamydomonas, histone H3	2.8±0.8	1.8±0.8	1.8±0.7	1.6±0.3	1.5±0.4		Waterborg 1998

Note: In a typical continuous-label protocol for posttranslational acetylation, radioactive acetate is added to cells, inhibited for protein translation by cycloheximide, and the exponential rise of the specific radioactivity of histones (Table 2A) or of distinct acetylated histone forms (Table 2B) to a stable maximum value determined. The half-life values were calculated by regression analysis using SigmaPlot as described (Waterborg 2001). Values presented in italics were not explicitly reported in the paper but were calculated from data presented.

ferences in acetate metabolism and acetyl-CoA pool sizes relative to animal cells in culture. However, it is more likely in light of continuous-label results that acetylation turnover rates in alfalfa are slower than in animal cells.

Experiments have shown that the fastest dynamic acetylation rate component resides in transcriptionally active chromatin and that the slower dynamic component did not correlate with transcription (Ip et al. 1988).

Clear deviation from an exponential decay pattern in pulse-chase experiments can reveal the absence of a regular HAT/HDAC-driven acetylation turnover. In developing rooster spermatocytes with a gradual rise in the steady-state level of histone H4 acetylation, fast turnover is detected. Pulse-chase analyses show a normal exponential decay of acetate-labeled H4 during this phase of spermatogenesis when transcription continues (Oliva and Mezquita 1982). Then, during the terminal phase of sperm formation when transcription has ceased, all of the hyperacetylated H4 is subject to a very fast decay (Table 1) that becomes nonexponential. This appears to be linked to the final increase in the level of hyperacetylation of histone H4 and other core histones, in parallel to the loss of histones due to displacement by protamines (Christensen and Dixon 1982; Grimes and Henderson 1984).

Continuous-label acetylation turnover measurements

Turnover rates can be measured under steady-state conditions when one can label the precursor pool instantly to a high level and maintain a stable specific labeling of the pool. This approach has been used successfully by adding ^{32}P inorganic phosphate to cells, labeling the nucleotide pool highly, and analyzing the increase in the specific radioactivity of mRNA. The exponential rise in specific radioactivity to a stable maximum value allows one to calculate the half-life of mRNA turnover. This approach has recently been applied to *Chlamydomonas* (Waterborg 1998), yeast (Waterborg 2001), and alfalfa microcallus culture (Waterborg and Kapros 2002) for measuring acetylation turnover. Depending on system variables like the mode of acetate uptake into cells, the rate and extent of metabolic conversion into acetyl-CoA, and the metabolic use of acetyl-CoA, one can establish apparent steady-state conditions by just adding small amounts of labeled acetate to cells like yeast (Waterborg 2001) or by preincubation of cells with nonradioactive acetate followed by the addition of a small tracer amount of radioactive acetate, as was required experimentally to establish apparent steady-state conditions in *Chlamydomonas* and alfalfa cell cultures (Waterborg 1998; Waterborg and Kapros 2002). Application of this analysis method to published patterns of acetate labeling that appeared to conform to the requirement of an exponential rise in acetate labeling until a stable steady-state condition is reached (Sanders et al. 1973) yielded turnover rates for avian erythrocytes (Table 2A) that were similar to rates measured in pulse-chase protocols (Table 1).

Continuous-label experiments can determine half-life values for any histone that is detectably labeled by acetate (Table 2A). They yield in many instances at least qualitative and often quantitative measures for acetylation turnover of the individual acetylated forms of these histones. Turnover rates for histone H3 were similar for mono- through

pentaacetylated forms across yeast, algae, and plant cells. In contrast, turnover was much faster for multiacetylated forms of H4 and H2B than for lower and monoacetylated species (Waterborg 1998, 2001; Waterborg and Kapros 2002) (Table 2B). This characteristic difference had already been reported for histone H4 in 1980 when pulse-chase experiments of HTC cells showed that the fast component turnover of tetraacetylated H4 was twice as fast as that of monoacetylated H4 (Table 1) (Covault and Chalkley 1980).

The difference between H4 and H2B versus H3 has been interpreted as a difference in chromatin accessibility (Waterborg 1998, 2001; Waterborg and Kapros 2002). Histone H3 interacts with DNA entering and exiting the nucleosome (Leuba et al. 1998*a*, 1998*b*). Its accessibility to acetylating enzymes would change with the folding state of the chromatin and the presence of linker histone H1 and not directly with the acetylation level of histone H3. In contrast, the amino-terminal domains of histones H4 and H2B in their non- or monoacetylated state interact with neighboring nucleosomes in a compacted 30-nm chromatin fiber (Luger et al. 1997; White et al. 2001), while the multiacetylated forms cause the relaxed chromatin fiber where HATs and HDACs can accelerate acetylation turnover.

This interpretation is also consistent with an additional result that is derived from continuous-label protocols, i.e., the observation that most or all acetyllysines in histone H3 are subject to turnover, while the fraction of acetyllysines with dynamic acetylation in histones H4 and H2B becomes progressively smaller for more compacted chromatin environments with lower levels of acetylation (Waterborg 1998, 2001; Waterborg and Kapros 2002).

Rate measurements when histone acetylation levels change

A rate of change in the level of histone acetylation, positive or negative, can never be translated into a value of acetylation turnover because it inherently applies to a non-steady-state condition. To distinguish rates of change reported under non-steady-state conditions, the term that is consistently used throughout this review is the "half-time" value, the time required for a 50% change in acetylation during an exponential process. This is a value fundamentally distinct from the turnover rate or "half-life" value, a value that implies a steady-state condition during which turnover is measured.

Fundamentally, rates of change towards a more hyperacetylated or hypoacetylated state of the histones directly reflect a change in the local or global balance of effective HAT and HDAC activities. They do not report an intrinsic property of acetylation dynamics but rather a condition external to the chromatin that exists at the time.

Rate of histone hyperacetylation in the presence of HDAC inhibitors

When the HDAC inhibitors butyrate or TSA are added to cells, one changes rapidly the HAT/HDAC balance and this will ultimately result in increased histone acetylation. Experimentally, the change in the level of histone acetylation typically follows an exponential pattern until a new balance and steady state are attained where histones are visibly hyperacetylated. This pattern allows calculation of the half-time value for the hyperacetylation reaction for many experimental results that have, to date, only been described qualitatively. Table 3A presents these experimental results. Similar half-time values are derived when hyperacetylation of total histone protein is calculated using Coomassie staining, Western detection of acetylated histone, or steady-state labeling of histones with radioactive lysine. Pulse labeling with acetate prior to butyrate addition will preferentially label chromatin with highly dynamic acetylation. Even for this subpopulation of histones, the apparent rate of hyperacetylation (Table 3A) is clearly slower than the turnover rates detected in transcriptionally active chromatin by pulse-chase (Table 1) or continuous-label methods (Table 2A). It is possible that the chromatin compartment with the slow acetylation turnover rates (Table 1) is related to the chromatin that is slowly hyperacetylated when HDAC inhibitors are applied. If true, it would only represent a fraction of the hyperacetylating chromatin, as much of nuclear chromatin becomes hyperacetylated during long-term treatment.

Why is the fraction of the chromatin with the fast acetylation turnover (Table 1) not detected in these hyperacetylation analyses as a minor, fast-rate component? The most likely explanation is that it represents only a small fraction of the genome and that the number of data points in the hyperacetylation curves is too small to allow identification of a fast but minor component. However, its existence is suggested by the half-time values calculated for experiments where the fast dynamic fraction is a major part of the histones analyzed for hyperacetylation. This applies when histones are pulse labeled with acetate prior to the addition of butyrate. The values calculated for rat hepatoma cells and chicken erythrocytes following acetate pulse labeling are significantly lower than values obtained for bulk histone hyperacetylation (Table 3A).

In 1980, Chalkley and co-workers developed a way to quantitate a fast dynamic component in addition to a slower rate component that was like the rate of bulk hyperacetylation (Table 3B). They pulse labeled cells with acetate, added butyrate during the chase, and determined the rate by which the label disappeared from the lowest labeled, monoacetylated form of histone H4 while histone H4 became hyperacetylated (Covault and Chalkley 1980). Their method did not see the majority of histone H4, including the major, nonacetylated form. It selected by its labeling protocol to look only at monoacetylated H4 that resided within the rapidly acetylated and thus rapidly labeled chromatin. Within this select environment, only analyzing the exponential loss of acetate label that shifted to more highly modified forms, they observed two distinct kinetic components, similar in size. The fast half-time (Table 3B) is similar to the rapid half-life values measured in pulse-chase procedures (Table 1). The slow component appears to be identical to the bulk of total histone H4 in its rate of hyperacetylation (Table 3A).

Fractionation of rat hepatoma chromatin has allowed demonstration that rapid hyperacetylation of histone H4 occurred in transcriptionally active and competent chromatin complexes, while the slow bulk component was not associated with transcription (Ip et al. 1988). The same conclusion was

Table 3. Histone hyperacetylation half-time rates (min) induced by HDAC inhibitors.

Species, cell	Histone H4	Histone H3	Histone H2B	HDAC inhibitor	Method	Reference(s)
A. Hyperacetylation of histones[a]						
Rat hepatoma	120, 480 *86±13*			50 mM butyrate	10-min pulse	Cousens et al. 1979
Rat hepatoma	*23*±5 *35±13*			50 mM butyrate	5-min pulse Coomassie	Covault and Chalkley 1980 Covault et al. 1982
Rat hepatoma	*175±21*			50 mM butyrate	Lysine	Cousens and Alberts 1982
Mouse T cells	*200*			20 ng TSA/mL	Western	Taplick et al. 1998
Hamster CHO	*161±19*	*66±12*		5 mM butyrate	Coomassie	Schröter et al. 1981
Human lymphoma	*187±39*			5 mM butyrate	Coomassie	Schröter et al. 1981
Human lymphocytes	*388±136*			5 mM butyrate	Coomassie	Schröter et al. 1981
Human lung	*191±21*			5 mM butyrate	Coomassie	Schröter et al. 1981
Human fibroblasts	*100±18*			5 mM butyrate	Coomassie	Schröter et al. 1981
Chicken erythrocytes	*44±4*			10 mM butyrate	15-min pulse	Zhang and Nelson 1988*a*
Chlamydomonas	*102±40*	*40±21*		100 ng TSA/mL	Coomassie	Waterborg 1998
Alfalfa	54±14	253±99(H3.1) 53±10 (H3.2)	71±20	100 ng TSA/mL	Coomassie	Waterborg and Kapros 2002B
B. Decrease of labeled monoacetylated histone[b]						
Rat hepatoma	7, 190			50 mM butyrate	5-min pulse	Covault and Chalkley 1980
Human fibroblasts	10–15, 140–200			50 mM butyrate	5-min pulse	Duncan et al. 1983
Human breast cancer	8, 200–350	8, 400	10, 350	10 mM butyrate	15-min pulse	Sun et al. 2001
Chicken erythrocytes	12, 300			10 mM butyrate	15-min pulse	Zhang and Nelson 1988*a*

[a]In a typical protocol to measure the rate of histone hyperacetylation, cells are transferred from medium without deacetylase inhibitors, sometimes after pulse labeling with acetate, to fresh growth medium containing butyrate or TSA at concentrations that effectively or completely inhibit HDAC activities. Histones are electrophoresed in AU or AUT gels and the acetylation distribution quantitated. The average number of lysines per histone was calculated from the percentage of each acetylated form, visualized by the method noted: fluorography for acetate pulse label, Coomassie staining, steady-state lysine label, or Western blotting with acetyllysine-specific antisera. Half-time values were derived from the exponential rise to a maximum by SigmaPlot regression analysis. Values presented in italics were not explicitly reported in the paper but were calculated from data presented.

[b]In the acetate pulse label variant of the protocol to measure the rate of histone hyperacetylation, gels are fluorographed and quantitated with correction for variability in gel loading. Plotting the exponential loss of label from the monoacetylated histone form, frequently an initial rapid exponential loss is followed by a slower exponential decrease, allowing calculation of two rate constants and thus two half-time values for hyperacetylation.

reached for maturing chicken erythrocytes where the fast dynamic acetylation component, subject to rapid butyrate hyperacetylation, was enriched for transcriptionally competent and transcriptionally active genes (Zhang and Nelson 1988*a*). Thus, rapid hyperacetylation appears to occur within accessible chromatin environments when HDAC enzymes are inhibited. The relatively rapid hyperacetylation of newly replicated and maturing chromatin (Cousens and Alberts 1982) is consistent with this conclusion.

The vast majority of chromatin responds quite slowly to the artificial increase in the HAT/HDAC activity balance when butyrate or TSA is added to cells (Table 3). In fact, progressively increasing rates of hyperacetylation have been documented, in a few instances, in mouse lymphocytes and *Chlamydomonas* cells (Taplick et al. 1998; Waterborg 1998). This suggests that the accessibility of potential acetylation targets increases over time, consistent with the general relaxation and solubility of chromatin that would follow increasing levels of nucleosome acetylation (Alonso et al. 1987; Davie and Candido 1978; Perry and Chalkley 1981; Wang et al. 2001). Thus, the large amount of slow histone hyperacetylation likely occurs in chromatin domains that under normal physiological conditions are nonacetylated or that have only low levels of acetylated histones without detectable rates of turnover.

What is the physiological importance of this slow hyperacetylating component? We can speculate that the natural transition of an inactive gene domain into a potentially active one is subject to the same forces that apply to slow hyperacetylation. If valid, it would be consistent with many of the slower processes where inactive genes are induced to become transcriptionally active or competent over a period of hours. To date, only a limited number of genes have been shown to be induced rapidly. In these examples, changes in the acetylation state of one or a few promoter nucleosomes appear to be sufficient to induce gene transcription (Christenson et al. 2001; Deckert and Struhl 2001; Erkine et al. 1998; Li et al. 2001; Sachs and Shi 2000; Sheldon et al. 2001; Sun et al. 2001; Wang et al. 2000; Wilkins and Lis 1997; Zhou et al. 2000). Conversion of a fully repressed gene with negligible histone acetylation into an actively transcribing gene with high levels of acetylation within a fraction of an hour has not been described. Half-time values of 2 or 3 h for hyperacetylation in Table 3 may apply to the physiological process of potentiating transcription for an inactive gene, likely part of facultative heterochromatin. It is likely that the slow dynamics of this acetylation, occurring at best at a limited number of chromatin sites during steady-state growth conditions, fall below the detection limit of the acetate pulse-chase labeling protocols that were used to measure acetylation turnover rates (Table 1).

The steady-state level of histone hyperacetylation achieved following application of HDAC inhibitors varies with the resulting HAT/HDAC ratio so that cells become more hyperacetylated in 50 mM than in 3 mM butyrate. However, hyperacetylation ceases long before every histone is maximally acetylated. The basis of this limitation is not known, although one can speculate that limiting HAT activity, acetylase substrate specificity, and incomplete inhibition of HDAC activity likely play a role. For instance, acetate pulse labeling at the end of long-term TSA treatment in alfalfa showed that acetylation turnover does not cease completely and varies among histone species, likely due to differential inhibition of the HDAC enzymes present (Waterborg and Kapros 2002). It was clearly shown that butyrate-insensitive HDAC enzymes limit butyrate-induced hyperacetylation (Chalkley and Shires 1985). At extreme HAT/HDAC ratios, as found in yeast, treatment by butyrate (Nelson 1982) or TSA (Sanchez del Pino et al. 1994) is without effect because the dominant HAT activities already keep the chromatin maximally acetylated. The same situation exists in condensing mouse spermatids where TSA fails to increase acetylation (Hazzouri et al. 2000). In contrast, the expressed fraction of the genome in differentiated chicken erythrocytes is so low that butyrate fails to induce visible histone hyperacetylation (Brotherton et al. 1981). The results of applying HDAC inhibitors in vivo often depend on cell or system characteristics that are unrelated to histone acetylation (Schröter et al. 1981). For instance, butyrate arrests cell cycle progression and (or) DNA synthesis quickly in some cells (Loidl et al. 1982; Murphy et al. 2000), slower in others (Cousens et al. 1979; Wintersberger et al. 1983), or not at all (Chalkley and Shires 1985; Littlefield et al. 1982) and may even stimulate replication (Staecker et al. 1987). Changes in the concentration of HDAC inhibitors during the course of experimental analysis, such as conversion of butyrate into acetyl-CoA (Waterborg et al. 1990) or the metabolic destruction of TSA in alfalfa plant cells (Waterborg and Kapros 2002) and human epithelial cells (Siavoshian et al. 2000), add further complexity and variability.

Rate of loss of hyperacetylation upon removal of HDAC inhibitors

In an attempt to measure histone deacetylation rates, chromatin was artificially hyperacetylated by addition of HDAC inhibitors, typically butyrate, and the level of histone acetylation was quantitated upon removal of the inhibitor. In some experiments, histones were labeled with acetate while histone hyperacetylation became established and deacetylation rates were determined by loss of radioactivity upon removal of butyrate and radioactive acetate (Table 4). Thus, the fundamental protocol resembled a pulse-chase experiment that did not take into account that the level of histone acetylation dropped continuously while the chase was being performed. The exponential decay with half-time values of 10–20 min is remarkably similar to the rate of fast acetylation turnover measured by pulse-chase (Table 1) and continuous-label protocols (Table 2). They reveal the ready accessibility of acetylated lysines within hyperacetylated chromatin and the availability of abundant HDAC activity.

The rate and extent of deacetylation of hyperacetylated chromatin upon HDAC inhibitor removal (Covault et al. 1982; Schröter et al. 1981) or metabolic destruction (Waterborg and Kapros 2002) depend on the resulting HAT/HDAC balance. Hypoacetylation may result upon release of prolonged HDAC inhibition if HDAC levels have increased (DiRenzo et al. 2000; Waterborg and Kapros 2002) or HAT levels have decreased (Covault et al. 1982; Lechner et al. 2000) in the attempt of the cell to reestablish a more normal equilibrium. In some cells, one observes persistence of limited histone hyperacetylation upon inhibitor removal

Table 4. Histone deacetylation half-time rates (min) upon release from HDAC inhibition.

Species, cell	Histone H4	Histone H3	Histone H2B	HDAC inhibitor	Method	Reference
Rat hepatoma	*70±15*			6 mM butyrate, 20 h	Coomassie	Covault et al. 1982
Mouse T cells	*5–10*			40 ng TSA/mL, 4 h	Western	Taplick et al. 1998
Hamster CHO	*15±2*	*15±2*		5 mM butyrate, 24 h	Coomassie	Schröter et al. 1981
Human lymphoma	*28±4*			5 mM butyrate, 24 h	Coomassie	Schröter et al. 1981
Human lymphocytes	*24±5*			5 mM butyrate, 24 h	Coomassie	Schröter et al. 1981
Human lung	*18±1*			5 mM butyrate, 24 h	Coomassie	Schröter et al. 1981
Human fibroblasts	*15±3*			5 mM butyrate, 24 h	Coomassie	Schröter et al. 1981
Human breast cancer	*12±2* Ac4: 3	3		10 mM butyrate, 2 h	120-min label	Sun et al. 2001
Human breast cancer with estradiol	*13±2* Ac4: 8	6	*3±0.3*	10 mM butyrate, 2 h	120-min label	Sun et al. 2001
Chicken erythrocytes, immature	*12±1* Ac4: *4±0.6*		*10*	10 mM butyrate, 3 h	180-min label	Zhang and Nelson 1988*b*
Chicken erythrocytes, mature	*30±7* Ac4: *10±2*		*25±2*	10 mM butyrate, 3 h	180-min label	Zhang and Nelson 1988*b*

Note: In a typical protocol for measuring deacetylation rates, cells are treated with effective concentrations of an HDAC inhibitor for the period of time listed and released into growth medium without HDAC inhibitor. Histones are electrophoresed in AU or AUT gels and the acetylation distribution quantitated. The decrease in the average number of lysines per histone was calculated from the percentage of each acetylated form, visualized by the method noted: fluorography for acetate labeling during pretreatment, Coomassie staining, or Western blotting with acetyllysine-specific antisera. Alternatively, loss of label from tetraacetylated H4, noted as "Ac4", or from total histone H3 was measured (Sun et al. 2001). Half-time values were derived from the exponential decay pattern by SigmaPlot regression analysis. Values presented in italics were not explicitly reported in the paper but were calculated from data presented.

(Schröter et al. 1981), suggesting that opposite metabolic adjustments may also occur.

Analysis of the loss of acetate label from tetra- and monoacetylated histone H4 revealed that the deacetylation rate for tetraacetylated H4 was faster than that for mono- or total acetylated H4 (Table 4). Relative rates were similar to those observed in pulse-chase (Table 1) and continuous-label protocols (Table 2B). Davie and co-workers have defined the chromatin with fast tetraacetylated H4 deacetylation in maturing chicken erythrocytes as Class 1 chromatin. It contains the actively transcribed β-globin genes and the other silent but transcriptionally competent globin loci (Spencer and Davie 2001; Sun et al. 2001). In contrast, they defined the slow hyperacetylating chromatin, identified by the slow loss of acetate label in the presence of butyrate (Table 3B), as Class 2 chromatin (Spencer and Davie 2001). In most cells, this chromatin would likely consist of facultative heterochromatin with visible hyperacetylation in butyrate. In the terminally differentiated chicken erythrocytes, this chromatin fraction contains less than 2% of the chromatin (Spencer and Davie 2001; Zhang and Nelson 1988*b*) and does not become visibly hyperacetylated in butyrate. The vast majority of the genome is permanently inactivated and thus represents constitutive heterochromatin with undetectable acetylation even when HDAC enzymes are inhibited.

Relating acetylation dynamics to cellular processes

Considering the range of organisms and cell systems analyzed, it is remarkable that rates of acetylation turnover under steady-state conditions, measured by pulse-chase (Table 1) and continuous-label protocols (Table 2), as well as acetylation half-time rates during histone hyperacetylation (Table 3) or loss of hyperacetylation (Table 4) give such consistent results. They have been linked in the review sections above with various chromatin conformations and states of transcriptional competence.

For the vast majority, data are limited to observations of histone H4, due to the relative ease of separation from other histones and the clear separation of the acetylated forms in AU and AUT gel electrophoresis. Fractionation of histones prior to gel analysis, typically by reversed-phase high-perfomance liquid chromatography, has allowed analysis of all core histones across a wider range of organisms (Tables 2 and 3A). Some differences between core histone species were observed, in particular between histone H4 and H2B versus histone H3 (Waterborg 1998, 2001; Waterborg and Kapros 2002). However, in general, the rates and characteristics of dynamic histone acetylation appear to be remarkably similar across histone species, cell types, and species. They have largely been interpreted as chromatin states and processes that involve gene transcription, gene induction, or gene repression.

Many other processes occur in chromatin that involve changes in nucleosome packaging. Although many of the experimental protocols reviewed were designed with gene transcription in mind, they reveal sometimes involvement of histone acetylation in other processes. Additional experiments have confirmed the role of histone acetylation in many chromatin changes, but temporal analyses typically have not been done. Thus, it is generally unknown whether acetylation turnover, in contrast with the presence or absence of histone acetylation, is required.

Radioactive lysine labeling of newly synthesized histones allows one to assess by AU gel electrophoresis and fluorography the state of histone acetylation prior to, during, and following formation of nucleosomes and throughout the process of chromatin maturation (Allis et al. 1985; Annunziato 1995; Chicoine et al. 1986; Cousens and Alberts 1982; Jackson et al. 1976). For instance, newly synthesized histone H4 is typically rapidly diacetylated at lysines 5 and 12 in the cytoplasm by a cytoplasmic HAT and subsequently

deacetylated with a half-time rate of 5 min as part of the nucleosome assembly and chromatin maturation process following DNA replication (Jackson et al. 1976). The initial cytoplasmic acetylation reaction appears unidirectional, not subject to turnover, and the subsequent deacetylation reaction may also be a unidirectional change. In parallel to this replication- and histone-H4-specific process, the dynamic acetylation that has been correlated with gene transcription is established rapidly in newly replicated chromatin, as judged by acetyllysine turnover rates measured in radioactive lysine label experiments in the presence and absence of histone synthesis (Cousens and Alberts 1982). In recent years, more details have become known about such aspects as replication-specific lysine acetylation sites and the apparent dominance of histone H4 over histone H3 acetylation (Jasencakova et al. 2001; Ridgway and Almouzni 2001), but no new evidence has been reported on linking acetylation turnover with the process of chromatin replication (Annunziato and Hansen 2000).

Histone hyperacetylation during spermatogenesis, especially of histone H4, has been clearly demonstrated through acetate labeling and histone protein gel analysis (Christensen and Dixon 1982; Meistrich et al. 1992; Oliva and Mezquita 1982). However, the published data are insufficient to assess rates of acetylation turnover during the transition to hyperacetylated histone H4 or during terminal sperm differentiation. During the last phase of sperm formation in mice, hyperacetylated histones are displaced by transition proteins and protamines without apparent involvement of HDACs (Hazzouri et al. 2000). This may be the basis for the nonexponential decay of acetate label in rat (Grimes and Henderson 1984) or the apparent persistence of H4 acetylation in trout (Christensen and Dixon 1982).

The involvement of acetylation turnover in other chromatin processes is even more tenuous. Repair in chromatin is faster in acetylated locations, a reflection of accessibility to repair enzyme complexes that results from acetylation-induced changes in chromatin compaction (Brand et al. 2001; Martinez-Lopez et al. 2001; Ramanathan and Smerdon 1989). A requirement for acetylation turnover has not been demonstrated. In the same way, transcription-related chromatin acetylation provides access to recombination enzymes in developing lymphocytes (Kwon et al. 2000; McMurry and Krangel 2000) as does apoptosis-induced hyperacetylation for nucleases that are responsible for apoptotic DNA fragmentation in thymocytes (Lee et al. 1996).

Dynamic histone acetylation and gene transcription

Based on dynamic characteristics of histone acetylation, three major functional chromatin compartments can be defined. The first and most static compartment is chromatin that fails to become hyperacetylated even after extensive treatment with HDAC inhibitors. This chromatin acquires its low level of histone acetylation when newly synthesized histones, including cytoplasmically diacetylated histone H4, enter the nucleus during replication and can interact for a short time with nuclear HAT and HDAC enzymes. This interaction stops, freezing the existing state of histone acetylation, once the new nucleosomes are assembled into repressed, compacted heterochromatin (Waterborg and Matthews 1983*a*). The heterochromatic compaction of chromatin limits accessibility to acetylating enzymes, even when HDAC activities are inhibited by butyrate or TSA. This chromatin compartment contains those histones that remain non- or monoacetylated after long-term treatment. It appears likely that this chromatin with an undetectable rate of acetylation turnover consists of constitutive heterochromatin and possibly other fully repressed and compacted forms of chromatin-like centromeres and telomeres. A comparative analysis of the extent of histone hyperacetylation by butyrate or TSA across organisms with a wide range of constitutive heterochromatin levels would be able to test this deduction. Terminally differentiated chicken erythrocytes likely represent an extreme case of a genome that is fully repressed except for a few remaining active genes. These few sequences, selectively pulse labeled with acetate, respond to butyrate by hyperacetylation, but the rest of the genome is so compacted that bulk histone acetylation does not rise (Zhang and Nelson 1988*a*).

The second chromatin compartment, defined by a slow rate of acetylation dynamics, represents a large fraction of the chromatin in most cells. When HDAC enzymes are inhibited by butyrate or TSA, this chromatin will become increasingly acetylated with a half-time rate of 100–400 min (Table 3). As discussed above, the accessibility of this chromatin for HAT action increases over time as acetylation increases. One could compare this process with the slow activation of a repressed gene located within facultative heterochromatin or compacted euchromatin. Butyrate-induced changes in gene expression have been described where silent genes become activated slowly, over a period of hours (D'Anna et al. 1980; Khochbin and Wolffe 1993). TSA and the irreversible HDAC inhibitor trapoxin may induce slowly the expression of some 2% of cellular genes (Van Lint et al. 1996). Quinidine-induced loss of HDAC1 in human breast tumor cells mimics TSA and trapoxin effects by a slow parallel induction of histone acetylation and gene expression (Zhou et al. 2000). Trapoxin induces hyperacetylation of histone H3 and $p21^{waf1}$ expression (Sambucetti et al. 1999).

It is likely that this chromatin fraction contains the chromatin that is characterized by acetylation turnover with half-life values ranging from 30 to 60 min in pulse-chase protocols (Table 1) and that has been named Class 2 chromatin in chicken (Spencer and Davie 2001). Analysis of gene expression showed that this fraction did not contain genes that were actively engaged in transcription or that were judged to be transcriptionally competent (Ip et al. 1988). Direct measurement of acetylation levels by acetylation-specific antisera across long stretches of chromatin has shown that transcriptionally regulated genes are surrounded by chromatin with detectable but low levels of acetylation (Hebbes et al. 1994; Litt et al. 2001; Myers et al. 2001). Histone acetylation of this type, not associated directly with expressed gene sequences, has been called "global acetylation" (Kruhlak et al. 2001; Vogelauer et al. 2000). While this type of global acetylation and other background acetylation across transcriptionally inactive euchromatin may be characterized by acetylation half-life values of 30–60 min, this may not be true for every organism. In *S. cerevisiae*, an organism lacking linker histone

driven chromatin compaction and heterochromatin, continuous-label experiments did not reveal a kinetic component with turnover half-lives as slow as 30–60 min (Table 2). However, it is quite likely that this surrounding chromatin is enriched with histone forms with lower levels of acetylation that, on average, have slower turnover rates (Table 2B) or that appear, for a significant fraction, not to be subject to detectable acetylation turnover (Vogelauer et al. 2000; Waterborg 2001).

Most information has been accumulated about the third and most dynamic chromatin fraction, even though it represents in most cells only 10 or 20% of the total genome. Pulse-chase protocols reveal turnover half-life values ranging from 1 to 20 min with relatively high turnover rates reported at least for the multiacetylated forms of histone H4 (Table 1). It is possible that these turnover rates are two- or threefold slower in plants. This may also be true for *Drosophila* (Table 1). However, it should be noted that the *Drosophila* assessment is based on a single study of pulse-chase labeling (Arrigo 1983). No other time-course data for assessing dynamic histone acetylation in *Drosophila* have been published.

Continuous-label protocols showed a very similar range of half-life values of 1 to 20 min, again with rates in alfalfa as the representative plant relatively low (Table 2). Half-time analyses in butyrate show that the accessibility of this chromatin to HAT enzymes leads to rapid hyperacetylation with half-time values of 5–15 min when HDAC enzyme activities are inhibited (Table 3B). As expected for hyperacetylated chromatin, lifting HDAC inhibition leads to loss of acetylation at a similar rate, with the most highly acetylated forms of histone H4 most rapidly deacetylated (Table 4).

This chromatin fraction, defined in chicken as Class 1 chromatin, contains gene sequences that are actively transcribed and genes that exist in a transcriptionally competent or poised state, capable of transcription if and when a specific activating signal is provided (Hebbes et al. 1994; Ip et al. 1988; Spencer and Davie 2001; Zhang and Nelson 1988*a*). As deduced from the high rates of acetylation turnover and from recent direct measurements of HAT and HDAC activity levels, it is clear that this chromatin is not only enriched in HAT activity but that it is enriched in HDAC enzymes as well. Recently, acetylation-specific antisera and acetylation-specific chromatin immunoprecipitation analyses have demonstrated rapid local changes in histone acetylation on nucleosomes at or near promoters at the time of activation of transcription from poised genes. Steroid hormone induces within 15 min a fourfold increase in histone H3 acetylation in proximal StAR promoter nucleosomes, resulting in a 12-fold increase in primary gene transcripts (Christenson et al. 2001). Histone acetylation for H3 and H4 changes with an apparent half-time rate of 10 min, leading to MMTV-LTR transcription in response to a steroid hormone signal (Sheldon et al. 2001). Stress-induced promoter H3 acetylation accompanied by regulatory H3 phosphorylation induces the MAP kinase phosphatase-1 gene in mice (Li et al. 2001). In contrast with these histone H3 acetylation examples, some gene promoter activation is linked with preferential H4 hyperacetylation (Mizzen and Allis 1998; Sachs and Shi 2000; Strahl and Allis 2000), while H3 and H4 are both hyperacetylated in viral gene transcription (Parekh and Maniatis 1999). The rate of change in histone acetylation and gene expression in these examples is consistent with the fast half-life turnover rates observed in this dynamic chromatin fraction. Most specifically, ChIP analyses have measured that less than 2 and 8 min suffice for gene-specific reversal of acetylation and of deacetylation, respectively, following in vivo dissociation of HAT or HDAC enzymes recruited to a well-regulated yeast promoter (Katan-Khaykovich and Struhl 2002). Experimental half-time rates measured were 5 ± 1 min for acetylation of tetraacetylated histone H4 and diacetylated histone H3 and 2 ± 1 min for rapid deacetylation of diacetylated histone H3. These rates match the global acetylation turnover rates measured under steady-state conditions for tetraacetylated histone H4 in yeast (Table 2B) and the twofold higher rate of H3 acetylation turnover relative to histone H4 (Table 2A).

Analysis using acetylation-specific antisera across long stretches of chromatin-containing genes in various states of expression has clearly shown that, over time, histone acetylation levels and patterns vary dramatically with changes in cell differentiation and gene expression (Forsberg et al. 2000; Litt et al. 2001; Myers et al. 2001). In addition to high levels of acetylation across promoter nucleosomes, constitutive high levels of histone acetylation were found in locus control regions, sequences that appear to act as gene domain boundaries and regulators of gene expression, for instance for globin genes. Due to the way these antisera were used, it is unclear whether these highly acetylated nucleosomes are subject to rapid acetylation turnover.

In addition, these studies revealed moderately high acetylation along the length of transcribing genes. Several examples are now known that support the deduction that transcriptional elongation is directly linked with rapid acetylation turnover. The *Drosophila* MSL complex, involved in dosage compensation, produces a pattern of global H4 acetylation on the male X chromosome that directly facilitates transcriptional elongation (Smith et al. 2001). This is consistent with the observation that a HAT activity is an integral part of the elongating RNA polymerase II holoenzyme (Wittschieben et al. 1999).

Does acetylation turnover have a direct function?

The early recognition of the apparent correlation between histone acetylation and gene transcription initially prompted experiments that tried to demonstrate a direct link between the process of gene transcription and turnover of histone acetylation. No clear interdependence between transcription and acetylation turnover was demonstrated. Blocking transcriptional elongation mechanically by incorporation of actinomycin into DNA inhibited acetate incorporation in rat liver by 50% in some experiments (Jiakuntorn and Mathias 1981) but had no effect in experiments by others (Edwards and Allfrey 1973) or in *Drosophila* cells (Arrigo 1983) and histone deacetylation appeared to be unaffected in rat hepatoma cells (Moore et al. 1979). Inhibition of elongation by the nucleotide analog cordycepin inhibited acetate labeling of histones in *Physarum* (Waterborg and Matthews 1984)

and rat liver (Jiakuntorn and Mathias 1981) to a major extent but this effect was not further analyzed.

Subsequently, it was realized that the structure of nucleosomes changes during the process of chromatin transcription as detected by increased thiol reactivity of the histone H3 cysteine, which is normally buried within the histone octamer (Allfrey and Chen 1991; Boffa et al. 1990; Lee and Garrard 1991; Sterner et al. 1987). These thioreactive nucleosomes are highly acetylated and subject to fast acetylation turnover (Boffa et al. 1990; Sterner et al. 1987). Butyrate-induced hyperacetylation does not increase the fraction of reactive nucleosomes, demonstrating that the thioreactivity is not a direct result of high levels of histone acetylation (Boffa et al. 1990). Direct inhibition of transcriptional elongation by actinomycin or DRB and indirect interference with DNA supercoiling have demonstrated that transcription elongation is required to observe the thioreactive state (Chen et al. 1991), and inhibition of RNA polymerase II by α-amanitin leads to selective loss of transcripts from thioreactive nucleosomes (Boffa et al. 1990).

When elongation is stopped by actinomycin, the activated state of the nucleosome persists (Boffa et al. 1990; Chen et al. 1991), and it was recently reported that complete inhibition of deacetylation in chicken erythrocytes by butyrate or TSA preserves and stabilizes the activated state of nucleosomes involved in transcription elongation. When deacetylation was allowed to proceed, nucleosomes returned to their basal, nonreactive state (Walia et al. 1998). These observations, made in vivo, support the notion that acetylation of histones is a requirement for transcriptional elongation through a nucleosome and that return of the transient, thioreactive state of the nucleosome to a basal configuration requires deacetylation. This links the cycle of histone acetylation and deacetylation directly to steps in the process of transcription elongation through nucleosomes. We do not know at this point whether this link exists with one, a few, or all acetylated lysines within a highly acetylated nucleosome. It is likely that linked acetylation turnover is limited to specific lysines within histones H3 and (or) H4. To date, it has been impossible to recreate this process in vitro by reconstitution using purified components (Imai et al. 1986; Walia et al. 1998).

Nucleosome remodeling complexes like SWI/SNF, NURF, and SAGA, containing many polypeptides including recognized transcription factors, appear to be responsible for the transient changes in nucleosome structure that allow transcription to proceed along DNA packaged by nucleosomes. The HAT Gcn5 will accelerate chromatin remodeling when it can acetylate lysines within the N-terminal domain of histone H4 (Barbaric et al. 2001). It has now been observed that Gcn5 in the SAGA complex of yeast establishes activator-dependent hyperacetylation of those nucleosomes of the *PHO8* promoter that become remodeled upon activation. If this activated state is arrested, histone hyperacetylation is preserved. If nucleosome remodeling is permitted to go to completion, hyperacetylation is lost (Reinke et al. 2001). This experiment clearly demonstrates that a direct mechanical link exists between the cycle of histone acetylation–deacetylation and the remodeling process of nucleosomes that is required for initiation of chromatin transcription.

In conclusion, recent in vivo experiments have demonstrated that the cycle of histone acetylation and deacetylation, measured as rapid acetylation turnover, is physically tied to the processes of nucleosome remodeling for transcriptional activation (Reinke et al. 2001) and transcription elongation (Walia et al. 1998).

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