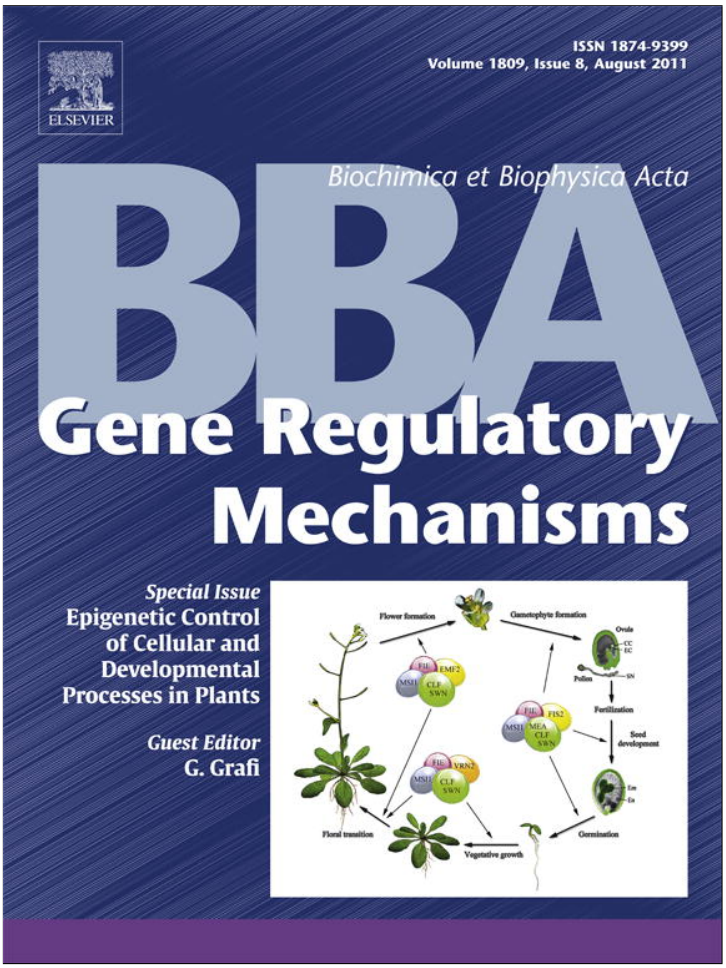

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Plant histone acetylation: In the beginning...☆

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ABSTRACT

The study of histone acetylation in plants started with protein purification and sequencing, with gel analysis and the use of radioactive tracers. In alfalfa, acid urea Triton gel electrophoresis and in vivo labeling with tritated acetate and lysine quantified dynamic acetylation of core histones and identified the replication-coupled and -independent expression patterns of the histone H3.1 and H3.2 variants. Pulse-chase analyses demonstrated protein turnover of newly synthesized histone H3.2 and thereby identified the replacement H3 histones of plants which maintain the nucleosome density of transcribed chromatin. Sequence analysis of histone H4 revealed acetylation of lysine 20, a site typically methylated in animals and yeasts. Histone deacetylase inihibitors butyrate and trichostatin A are metabolized in alfalfa, but loss of TSA is slow, allowing its use to induce transient hyperacetylation of histones H2B, H4 and H3. This article is part of a Special Issue entitled: Epigenetic Control of cellular and developmental processes in plants.

This is not a review. It is a personal historical perspective. It reflects the time before post-synthetic modifications (PTMs) were studied by specific antibodies in westerns, by immuno precipitation (IP) and by chromatin immuno precipitation (ChIP). It reflects the time before mass spectrometry (MS) provided identification of modified amino acids and before MS became a standard method to analyze histone protein sequences. It was before genomic and massive parallel sequencing became routine methods in the study of chromatin and histones, and in all the processes they are involved in. To name just a few: DNA replication and repair, gene transcription, recombination, mitosis and meiosis, and inheritance through epigenetic histone and DNA modifications.

Studies on plant histone acetylation started in the 1960's when classical Edman degradation protein sequencing was applied to purified histone H3 and H4 by James Bonner and collaborators. Histones were purified in what are now considered mass quantities from germinating peas (*Pisum sativum*) and calf thymus [1,2]. Sequencing of chromatographically purified proteolytic peptides established for the first time the primary protein sequence of histone H4 [3,4]. H4 acetylation was discovered at lysine 16 (K16ac), at lysines 5, 8 and 12, and methylation at lysine 20 (K20me) (Fig.1A). Identification of acetylated lysines in histone H3 came next [5] but confirmation that lysines 4, 9, 14, 18, 23 and 27 were all targets for acetylation in plant H3 histones (Fig. 1B) only emerged gradually [6].

Remarkably, plant histone H4 differed at only 2 residues from the animal H4 with I60 vs. V60 and R77 vs. K77, respectively. This extreme degree of sequence conservation, including functional side chain conservation, has been proven to be characteristic (Fig. 1A). The same holds true for plant and animal histone H3s (Fig. 1B), if one takes into account that in animals and in plants histone H3 developed into two distinct functional variants [7,8]. The replication-coupled (RC) H3 forms, like H3.1 and H3.2 in mammals, are produced predominantly or exclusively during the S phase of the cell cycle. These are used in assembling newly replicated DNA into nucleosomes using histone chaperones. In the same cells the replication-independent (RI) H3 variant, like H3.3 in animals, is produced constitutively and is incorporated into nucleosomes by specialized chaperones across transcribed DNA sequences. This process repairs the nucleosomal packaging of transcribed genes when transcription has caused loss of nucleosomes. The study of dynamic histone acetylation, synthesis and turnover in alfalfa (*Medicago sativa*) was instrumental in identifying the RI histone H3 function in plants, as described below [9]. This discovery preceded the recognition that this inherent instability of transcribed chromatin and the linked turnover of the 'replacement' RI histone H3 also existed in animals [10].

The high degree of sequence conservation between core histones from widely divergent species, and the complete conservation of all lysines which have by now been recognized as targets for histone acetylation and methylation, was the reason why I dared to start in the 1980's my exploration of the dynamic histone acetylation in plants. The fundamental assumption was that if dynamic acetylation in animals and other model organisms like *Physarum polycephalum* can reveal new mechanisms for gene regulation and if plant histones look so much like animal histones, it must be possible to use methods developed for animals to study gene regulation at the chromatin level in plants.

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The methods that I have used consist of in vivo labeling with radioactive tracers like tritiated acetate and lysine, purification of total histones, including chromatographic fractionation of histone species, and acid-urea-Triton gel electrophoresis for separation and quantitation of histone species, their variants and their acetylated forms. This approach has been complemented by protein sequencing to determine sites of lysine acetylation and methylation [11–15] and by quantitative fluorography. In combination, this approach is suited for the study of dynamics of histone acetylation, and of histone protein synthesis and turnover. It is not suited to look for histone acetylation across selected genes or chromosome regions. Antibody- and ChIP-based approaches, complemented by MS, are better suited for such analyses. However, in general, they do not provide information about the fast dynamics that are characteristic for histone acetylation.

1. Methods used in studying plant histone acetylation

Research in other organisms, in particular of *P. polycephalum*, had lead to the development of a new histone preparation method [16] for organisms replete with carbohydrates, oxidative polyphenols and degradative enzyme activities. It consists of a few steps. (1) One prepares nuclei or crude chromatin by an established method into a pellet. It is critical to minimize the activity of histone deacetylases (HDACs), proteases, phosphatases and other degradative enzymes. For plant materials like calli, mature leaves or whole plants, chromatin or nuclear yields are often limited and degradation can typically not be prevented completely despite the use of 50 mM butyrate as a general HDAC inhibitor in combination with protease inhibitor cocktails. It is possible to start with step 2 directly, avoiding deacetylation and degradation, provided that one can purify the histone(s) of interest sufficiently from the less pure histone extract obtained. This was done in studies of histone H3 because it elutes during reversed-phase (RP) hplc at high acetonitrile concentrations. (2) One fully disperses the plant material into chaotropic 40% guanidine.HCl (in 0.1 M potassium phosphate, pH 6.8) by sonication, which will also fragment the viscous DNA allowing its removal and any insoluble particulates by high speed centrifugation (30,000×*g*) under neutral pH and 0.25 N HCl conditions. (3) Full inhibition of degradative enzymes is maintained when the neutralized extract is diluted to 5% guanidine and Biorex-70 (Biorad) resin is added at 1 ml settled resin per mg DNA in the extract. (4) During overnight incubation under agitation the core histones bind to the resin but carbohydrates do not. Thus, letting the resin settle and discarding the typically very turbid supernatant removes non-histone contaminants into the 5% guanidine washes. (5) Histones are eluted by 8–10 column volumes of guanidine.HCl, dialyzed in 3500 MW cut-off dialysis membrane against 2.5% acetic acid, and quantitatively recovered, free of salt, by lyophilization. Histones, prepared in this manner, are free of carbohydrate contaminants and can be applied directly to SDS, acid-urea (AU) or acid-urea-Triton (AUT) polyacrylamide gels or chromatographic columns. This approach allowed us to make progress in the 1980's and early 1990's when other research groups were limited to looking at total histone profiles on low resolution gels following histone extraction from crude nuclear and chromatin preparations of plants [17–23].

This new method for histone preparation was combined with classical methods for chromatographic separation of histones, such as the Biogel (Biorad) sieving method in which partial acrylamide hydrolysis creates a weak ion exchange process under acid conditions that allows separation of core histones with similar molecular weights [16,24]. This slow method has now routinely been replaced by reversed-phase hplc. Such a high-resolution chromatographic method is especially important if histones are extracted from very crude chromatin preparations or from whole plants to obtain separation of the core histones of interest from non-histone proteins.

Of critical importance for my research of histone acetylation in plants was the discontinuous, high-resolution acid-urea-Triton (AUT) gel system developed by Bill Bonner [25–27]. This method produces much higher gel resolution and allows much larger sample loads than the continuous Panyim-and-Chalkley gel system [28] or systems based on this approach. In the Bonner gel system the characteristic differential affinity of the hydrophobic histone fold of the core histones for the non-ionic detergent Triton X-100 leads to separation of histone species and their variants. In addition, it shows a discrete reduction of gel mobility for each positively charged lysine side chain which is neutralized by acetylation. This allows one to quantitate by densitometry each distinct

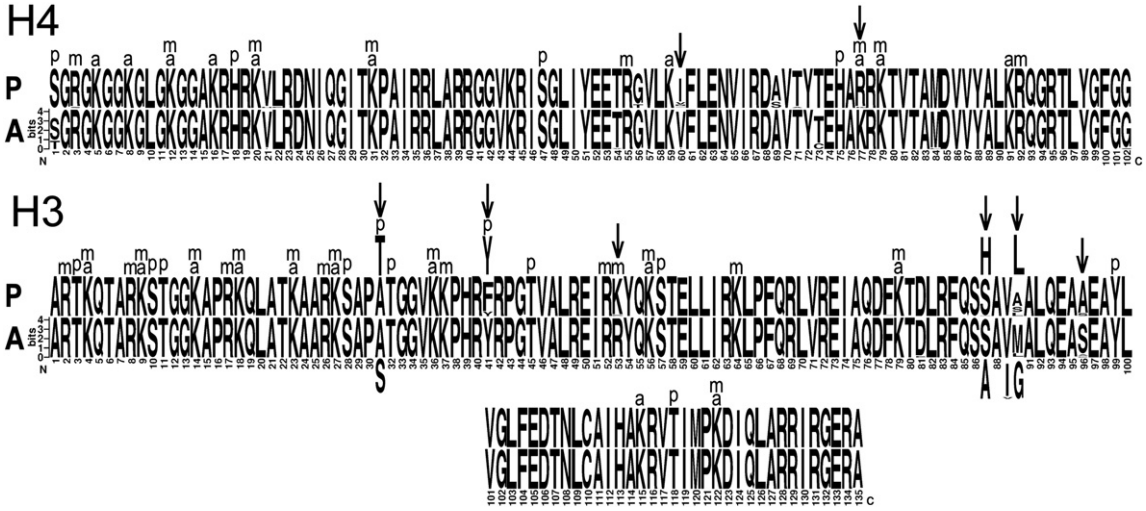

Fig. 1. Sequence conservation of core histones H4 and H3 among plants and animals, illustrated by WebLogo [59]. In the absence of any variability in the amino acid found at a sequence position, the LOGO algorithm displays the amino acid in the single letter code at full size. Any sequence variability reduces the size of the primary amino acid code(s). Top Panel. For histone H4, 24 plant (P) and 26 animal (A) species, broadly representing metazoa and viridiplantae, were used to create the upper and lower scaled representations, respectively. Vertical arrows mark the distinctive sequence differences between plant and animal H4 histones. Sites of post-translational modifications, observed within eukaryotic H4 proteins, are marked by 'a' for acetylation, by 'm' for methylation and by 'p' for phosphorylation. Bottom Panel. For histone H3, 322 animal and 179 plant species were used [57]. The amino acid sequence of the replication-coupled variants at positions 31, 41, 87, 89 and 90 is shown in line with the residues that are the same for the two H3 variant types. The amino acids in the replication-independent variants of plant and animal H3s are shown above and below the replication-coupled ones, respectively. Supplemental Tables 1 and 2 provide information on the PTMs known to exist on histone H4 and H3, respectively.

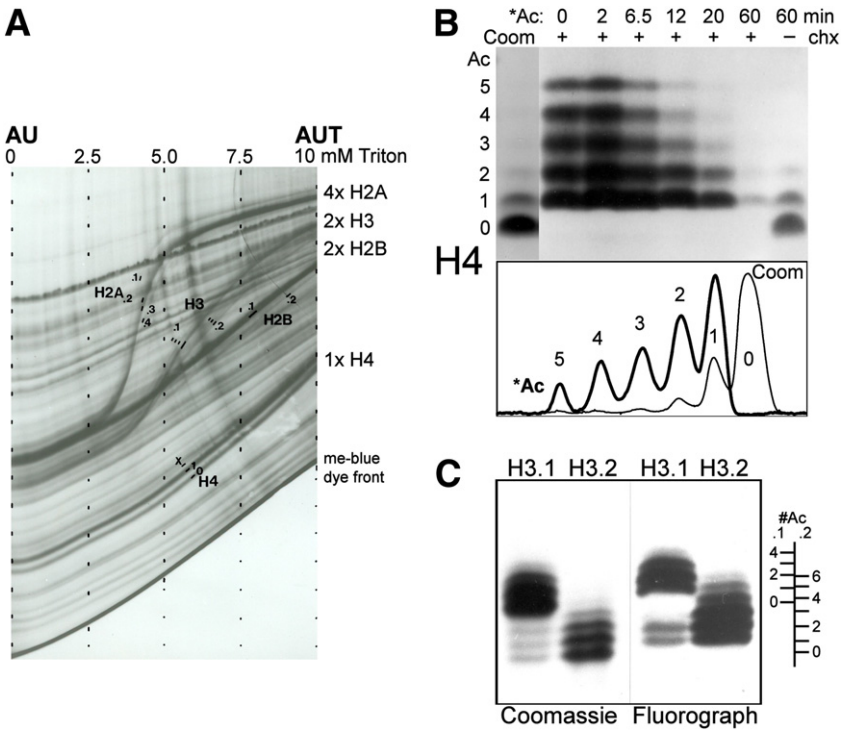

Fig. 2. AUT gel separation of acetylated histones. Panel A. Separation of total histone extract from crude nuclei of alfalfa callus cultures across an AU gel with a transverse gradient of Triton X-100. This gel methodology has been described in detail [27]. The Coomassie-stained bands for histone H4, of several H2B variants, of the two H3 variants H3.1 and H3.2, and of several H2A variants are marked. They have a characteristically reduced mobility in the presence of Triton X-100 relative to non-core histone proteins [24]. Panel B. Example of Chlamydomonas histone H4 separation in AUT gel electrophoresis into 6 discrete bands based on the presence of 0 up to 5 acetylated lysines in the N-terminal protein domain. Coomassie (Coom) staining illustrates the diminishing relative abundances of higher acetylated forms, also illustrated by the thin densitometric trace in the panel below the gel lanes. The number of acetylated lysines is marked. They represent acetylation at lysines 5, 8, 12, 16 and 20 [14,15]. The fluorography lanes show the tritiated acetylation (*Ac) after in vivo labeling for the indicated number of minutes in the presence (+) or absence (−) of the translation inhibitor cycloheximide. The fluorographic pattern at "0" min is shown in the thick densitometric trace. It illustrates the enhanced detection of dynamic acetylation by radioactive tracers of multi-acetylated histone forms. The overall fluorographic pattern also illustrates the continuous and rapid turnover of the acetylation groups which, in combination with depletion of the radioactively labeled acetylCoA pool, results in the loss of labeling over time [15]. A similar pattern can be observed for histones of alfalfa after 5 min pulse labeling [30]. Panel C. Example of separation of the alfalfa histone H3.1 and H3.2 variants in AUT gel electrophoresis, extracted from whole cells and purified by RP hplc (*e.g.* see Fig. 3). The steady state acetylation of H3.2 is ~2 times that of H3.1, as measured by Coomassie staining. Fluorography following tritiated acetate pulse labeling suggested that the 6 N-terminal lysines 4, 9, 14, 18, 23 and 27 are the targets for acetylation. This was confirmed by protein sequencing [13].

level of acetylation for each core histone species in Coomassie-stained gels if the Triton concentration is judiciously chosen to prevent overlapping histone species patterns. This concentration can be determined experimentally using crude histone preparations in a 1 M acetic acid, 8 M urea 15% polyacrylamide gel with a transverse gradient from 0 to 10 mM Triton X-100, as shown for alfalfa histones in Fig. 2A [24]. Typically long (30 cm) separating gels are used to optimize separation and quantitation of Coomassie-stained bands and in gel fluorography of radioactively labeled histone preparations [29].

The use of radioactive tracers in vivo, primarily acetate and lysine, was central in providing analysis tools for probing the dynamics of histone acetylation and synthesis. High specific activity tritiated acetate was used with growth media close to or below the pK value of acetic acid, if possible, in order to allow fast diffusion of the organic solvent acetic acid across the cell membrane [30]. Its rapid dissociation into protons and acetate ions at the neutral pH of the cytoplasm assures rapid, no-lag labeling, as illustrated for histone H4 (Fig. 2B) in the green alga *Chlamydomonas reinhardtii* [31]. Plant calli, such as alfalfa A2 cells, were incubated with tritiated acetate in the presence of the protein translation inhibitor cycloheximide (10 mg/l) for periods ranging from 5 min to optimize detection of rapid acetylation dynamics [30] up to 1–2 h [13,14] for near steady-state histone acetylation labeling [30]. Fluorography of acetate labeled histones strongly enhances the detection and quantitation of multi-acetylated forms (Fig. 2B, C). Densitometry allows the determination of specific radioactivity based on Coomassie staining and fluorographic film darkening, which is linear if X-ray films are appropriately pre-flashed and used within experimentally defined exposure periods [29]. It complements specific labeling measurements from liquid scintillation counting and protein absorbance during RP hplc chromatography for reasonably pure histone preparations. Histone protein synthesis was typically measured by incorporation of tritiated lysine for a period of 2 h into alfalfa A2 microcalli [9] or by tritiated acetate in the absence of cycloheximide, as used for *C. reinhardtii* to determine the cell cycle dependence of histone synthesis [15].

2. Initial exploration of histone acetylation in alfalfa

In the time before the structure of the nucleosome was determined [32], many hypotheses tried to address the function of histone acetylation, which had been correlated with gene transcription [33]. One of these was the idea that by mass action the neutralization of increasing numbers of positively charged lysines would reduce the binding of core histones to DNA. In turn, this would facilitate the process of gene transcription across more accessible DNA templates. In this light the initial choice of alfalfa was made. Salt-tolerant callus lines had been developed [34,35] which did not exclude salt from entering the plant cells. So, the hypothesis went, if the electrostatic interaction between histone lysines and DNA phosphates is partially shielded by additional salt, a reduction of histone acetylation would be expected to maintain homeostatic control of gene expression. As we now know, this premise was false.

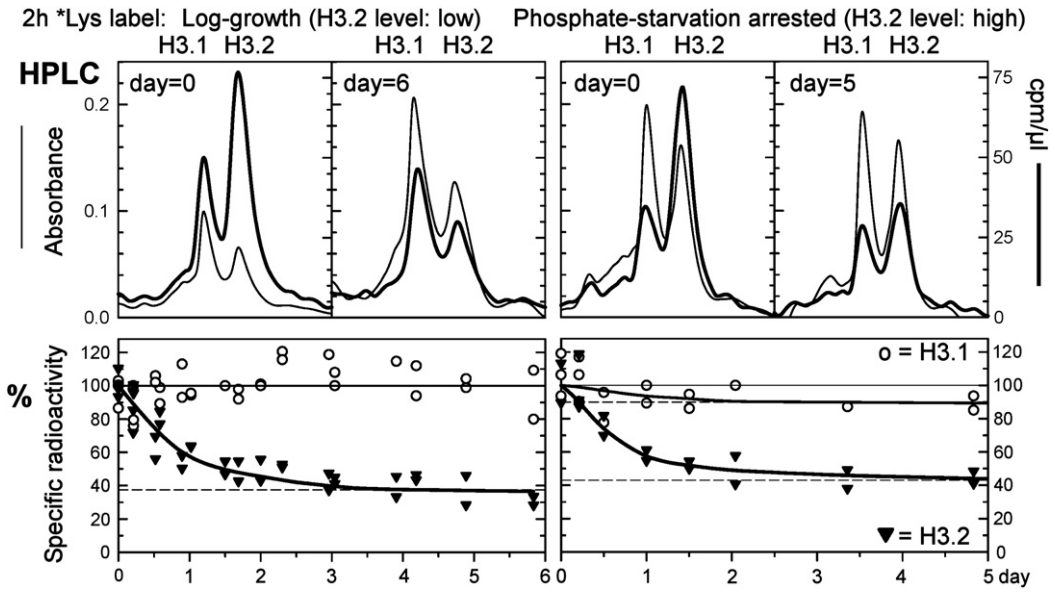

Fig. 3. Identification of alfalfa histone H3.2 as a Replacement histone variant. One of the characteristic features of a replacement histone is shown by the absorbance profile of H3 variant hplc elution. It shows the increase from a low abundance of H3.2 in cycling cells (top row, left panels) to a higher abundance under non cycling conditions such as G1-phase arrested growth caused by phosphate starvation of alfalfa A2 microcalli in suspension culture (top row, right panels). A second characteristic is the turnover of newly synthesized histone H3.2, pulse-labeled by lysine (cpm/µl during hplc elution), illustrated by the radioactivity profile shift in the top row panels during the chase period from day 0 to day 6 or to day 5 for cycling and arrested cultures, respectively. This turnover causes a decrease in the specific radioactivity of histone H3.2 for cycling (bottom row, left panel) and arrested cultures (bottom row, right panel), calculated from fluorography density over Coomassie density in AUT gels (solid symbols), setting the specific radioactivity of the pulse label to 100%. Histone H3.1 specific labeling (open symbols) was stable in cycling cells and decreased by approximately 10 % in arrested cultures (unpublished results). Approximately 60% of newly synthesized H3.2 protein is subject to turnover with an apparent half-life of 16 ± 6 h and 14 ± 4 h in cycling and arrested cultures, respectively. A similar, published analysis for slowly growing calli yielded a half-life estimate of 20 h [9].

But, not knowing this at the time, a method was developed to extract histones from crude chromatin preparations, aimed at preserving histone acetylation and preventing proteolysis. AU Triton gradients gels were used to determine the number of core histone variants (Fig. 2A). All alfalfa core histones showed the distinct bands that suggested the presence of acetylated forms, although in varying amounts and to varying levels [24]. The identity of the acetylation PTM was initially confirmed by pulse labeling with tritiated acetate and fluorography. It was subsequently confirmed by automated protein sequencing of the two histone H3 variants. Because the N-terminus of histone H3 proteins is unmodified, it allowed Edman degradation of intact proteins. This analysis revealed acetylation of lysines 4, 9, 14, 18, 23 and 27 [13]. For N-terminally acetylated histone H4, proteolytic fragmentation followed by protein sequencing was required to establish that alfalfa H4 is acetylated at lysines 5, 8, 12, 16 and 20 [14]. Acetylation of lysine 20, which in animals is typically methylated, appears to be a characteristic of plants, including Arabidopsis [36]. Recent MS analysis suggests that acetylation of H4 lysine 20 may exist at low levels in other species [37].

In animal cells butyrate had been shown to induce hyperacetylation of histones in vivo and to prevent histone deacetylation during histone purification. In the alfalfa histone preparation method, 50 mM butyrate inclusion did not affect the histone acetylation levels obtained. In vivo, up to 24 h treatment with 50 mM, butyrate also appeared without effect on histone acetylation levels. The same observation has been reported for tobacco [20]. In fact, when tritiated butyrate was used to study its effects in alfalfa, it was found that butyrate was readily metabolized and through acetyl-CoA incorporated into acetylated histone forms [38]. This represents a clear difference between plants where butyrate is readily metabolized and fails to effectively inhibit HDAC activities, and animal cells where butyrate effectively inhibits HDACs and, in addition, can cause cell cycle arrest.

With these method developments completed, the comparative analysis of histone acetylation between salt-sensitive and salt-tolerized strains in the absence and presence of salt stress proved to be disappointing. Exposure to 1% NaCl lead to moderate increases in histone acetylation, an effect opposite to that postulated [39].

3. Exploring the reason why alfalfa H3.1 and H3.2 variants differ in histone acetylation

The studies of histone acetylation under salt stress, while not informative in clarifying the role of histone acetylation in gene expression, firmly established that the acetylation levels of the two alfalfa H3 histone variants were quite distinct (Fig. 2C). Histone H3.2 had a 50–100% higher level of steady-state acetylation than histone H3.1 and acetate labeling revealed a 2–3 fold higher level of dynamic acetylation [13]. This raised the following questions. Was this difference caused by differences in the primary protein sequences of the two H3 variants? Was it caused by differences in the patterns of protein synthesis? Was it caused by differences in the local chromatin environments into which the H3 variants were deposited?

The first question was addressed at two levels: the gene and the protein. The histone transcripts and genes for the H3.1 and H3.2 genes were cloned and sequenced [40,41]. They showed that these proteins differed only at positions 31, 41, 87 and 90 (Fig. 1). This result made it unlikely that primary protein sequence differences were responsible for the observed differences in acetylation. The gene sequencing result was confirmed by protein sequencing [13]. These Edman degradation analyses also established that the levels of steady state lysine acetylation were quite different in the two variant forms. Furthermore, these results demonstrated that lysines 4, 9, 14, 18, 23 and 27 could all be acetylated and that mono-, di- and tri-methylation of these lysines co-existed with acetylation to varying extents at each position [13].

Clear differences between the H3 variant genes were detected by studying transcript levels. The H3.1 genes of alfalfa – 56 per haploid genome – were expressed in a S phase dependent manner. This identified the H3.1 variant as a canonical replication-coupled (RC) variant. It is the major H3 protein in nucleosomes on newly replicated

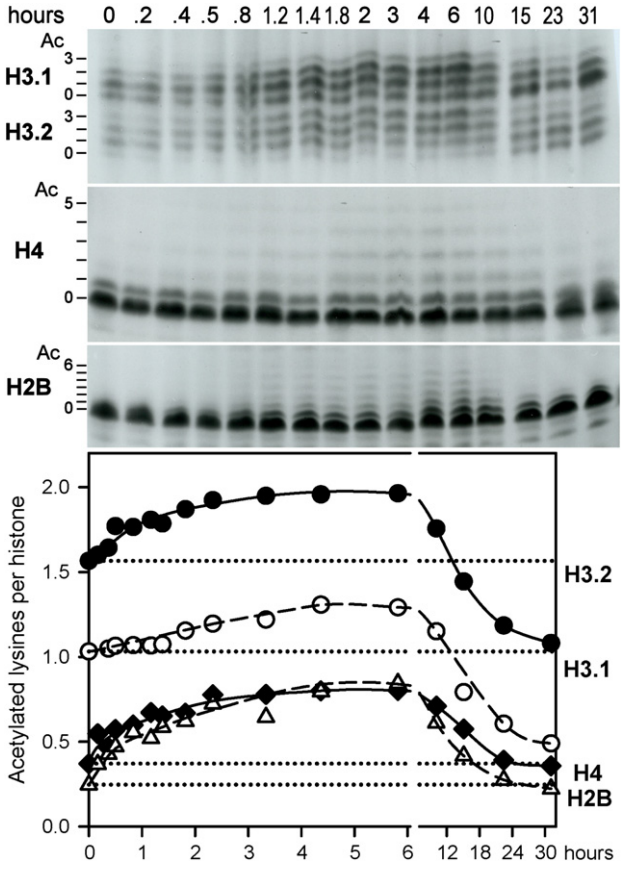

Fig. 4. The effects of trichostatin A (TSA) on alfalfa histone acetylation levels. Addition of 100 ng/ml TSA to alfalfa A2 suspension cultures induced core histone hyperacetylation. Top Panels. Coomassie-stained AUT gel analysis of histone H3.1 and H3.2 variants, histone H4 and histone H2B. Sampling at various times during the experiment is shown in hours along the top. Numbers identify the levels of acetylated lysines (Ac) per histone protein. Bottom Panels. Average number of acetylated lysines per histone protein for histone H4 (solid diamonds), H2B (open triangles), H3.1 (open circles) and H3.2 (solid circles) measured by Coomassie densitometry. Maximum levels of hyperacetylation were reached 4 to 6 h after addition of TSA. Over time, effective TSA levels drop resulting in loss of steady-state acetylation by 30 h, with hypoacetylation below the starting levels (dotted lines) for the H3 variants [30].

DNA [42]. In contrast, the 3 H3.2 genes were highly expressed in a constitutive manner, identifying this variant as a replication-independent (RI) variant [40,42]. The constitutive manner of expression is one of the central characteristics of the replacement H3.3 histone in animals. The basis for this expression pattern has been explored by transgene expression analysis in Arabidopsis [43] and alfalfa [44].

When a replacement H3 protein is synthesized during S phase, it is assembled into nucleosomes by S phase chaperones in parallel to the RC H3 variant. Because the majority of the genome is not transcribed and exists in compact chromatin with low levels of acetylation, the level of acetylation that is acquired by S phase deposited H3 histones is low. In contrast, deposition of newly synthesized replacement H3 outside of S phase is limited to those chromatin locals where nucleosomes are lost. The major process that displaces nucleosomes in cells outside of S phase is transcription. It occurs in loosened, highly acetylated chromatin fibers. Assembly of new nucleosomes into this chromatin employs specialized histone chaperones like HIRA. HIRA and related transcriptional histone chaperones selectively interact with the residues at positions 87, 89 and 90 that are characteristic for replacement H3 variants (Fig. 1) [45,46]. Assembly of the available H3.2 proteins into the transcribed chromatin environment confers the high level of acetylation observed. Thus the characteristic high level of H3.2 acetylation arises from the combination of the constitutive pattern of gene expression and the primary protein sequence that allows participation in the assembly of replacement nucleosomes.

Inherent in this process is the continued transcription of genes through chromatin with replacement nucleosomes. Thus newly assembled replacement nucleosomes are subject to the same processes of nucleosome loss that are experienced by all nucleosomes in transcribed chromatin. This was experimentally confirmed as the turnover of newly synthesized and highly acetylated histone H3.2 protein [9]. The half-life of this turnover was 15 to 20 h and involved approximately 60% of newly synthesized H3.2, *i.e.* the fraction that was produced outside of S phase (Fig. 3) [9].

4. Highly acetylated replacement histone H3.2 in other plants

Genomic, gene and transcript sequencing has demonstrated that histone H3.2 variant sequences are found in all plants [47,48], are likely to exist in Glaucophytes (blue-green algae) (Waterborg, unpublished) but are absent in Chlorophytes (green algae) such as *C. reinhardtii* [15]. The H3.2 protein in the monocots wheat, barley, rice and maize is in every case the most highly acetylated H3 protein variant [47]. In eudicots like Arabidopsis, tobacco, soybean and carrot, only two H3 variants exist, as in alfalfa, and in every case the H3.2 variant form is most highly acetylated [48]. The abundance of the replacement histone H3.2 variant protein, and the steady state level of its acetylation, were found to be inversely proportional to the size of the plant genome [47,48]. Thus in Arabidopsis with the smallest genome among the plant species analyzed, the highly acetylated replacement H3.2 variant makes up more than 50% of total H3 and is most highly acetylated [48]. Whereas replacement histone H3 levels in plants and animals are typically the lower abundance forms, even in terminally differentiated tissues, in Arabidopsis the replacement H3.2 variant is the most abundant H3 species, even in actively proliferating cell cultures. This is the result of the combination of the high proportion of the Arabidopsis genome that is transcribed and the high rate of nucleosome loss when plant chromatin is transcribed. Published reports on replacement histone accumulation in animals suggest that the frequency at which nucleosomes are lost from transcribed chromatin is lower in animals than in plants [9,10,48,49].

5. Dynamics of plant histone acetylation

The rate of turnover of lysine acetylation through the action of histone acetyltransferases (HATs) and deacetylases (HDACs) is highest, with half-lives of just a few minutes, during the process of chromatin transcription [50]. The direct physical link between progress in RNA synthesis and nucleosome remodeling appears to extend even to the linked RNA processing steps [51]. Very short pulse labeling with tritiated acetate will emphasize this highest dynamic fraction of acetylated histones (Fig. 2B) [30]. Whether effective short pulse labeling of plant cells is achievable often depends on the use of large amounts of high specific radioactivity acetate in combination with the pH of the culture medium near or below the pK_a value of acetic acid [30].

Routinely, exposure of plant cells like alfalfa for 1 or 2 h to tritiated acetate labeled the major fraction of acetylated histones with acetylation half-lives of approximately 30 min. The fluorography patterns, with signal amplification for the multi-acetylated histone forms (Fig. 2), reflect the steady-state levels of histone acetylation [13,30]. These represent largely the transcriptionally competent and active forms of euchromatin where the repressive 30 nm chromatin fiber packing is relaxed. Histone acetylation is considered one of the major factors in this relaxation that facilitates chromatin transcription.

This dynamic fraction of acetylated histones, as well as slower acetylation, is often studied as the accumulation of steady-state acetylation when HDACs are inhibited. In plant cells one cannot use butyrate, which is frequently used in animal studies, because it is rapidly metabolized [20,38]. The fungal metabolite Trichostatin A

(TSA) has been used successfully in alfalfa to induce the increased histone acetylation that has become known as hyperacetylation [30]. As illustrated for the acetylated core histones of alfalfa (Fig. 4), the rate and pattern of hyperacetylation varies by histone species. H2B and H4 acetylation levels rise quickly and dramatically from relatively low steady-state levels to detectable hexa- and penta-acetylated forms. Highly acetylated H3.2 shows a similar response while H3.1 acquires additional acetylated lysines much more slowly. These differences likely reflect the local chromatin environment of the majority of each form. When additional TSA is added at later times, transiently higher hyperacetylation levels can be attained [30]. This observation supports the notion that TSA is metabolized and that effective TSA concentrations decrease over time. Without adding extra TSA, maximal hyperacetylation levels are reached after 6 h, followed by loss of histone acetylation (Fig. 4). While histone H2B and H4 acetylation levels return to pre-treatment conditions, transient hypoacetylation is observed for the H3 variants [30].

6. Overview of histone PTMs expected for plants

Based on published studies in a limited number of plant species, the N-terminal post-translationally acetylated lysines in histones H3 and H4 are the same ones that are targets for acetylation in animals (Fig. 1). In the absence of analyses of the lysine residues that are acetylated in plant H2B and H2A histones, we do not know whether this conclusion is also valid for these core histones. While qualitatively the same between plants and animals, examples of quantitative differences have been found. Lysine methylation of pea histone H4 at lysine 20 was initially reported [52], and subsequent analyses have confirmed that this is the typical modification found on H4K20. Subsequent analyses in alfalfa [14] and Arabidopsis [36] have only detected lysine acetylation at this site. Low levels of H4K20ac have now also been detected in animal and yeast H4 [37,53].

Acetylation of H3K56 has been recognized as an important modification in yeast and animals. Histone acetyltransferase Rtt-109 acetylates the lysine 56 side chain in new H3 histones and this allows these proteins to participate in the assembly of nucleosomes during S phase, DNA repair and transcription [54–56]. H3K56ac has not been detected in plant H3, *e.g.* in MS PTM analysis of Arabidopsis histone H3 [36]. This study could also not detect H3K79ac, a modification linked in yeast to transcription.

Recent MS analyses of the PTMs detectable in the core histones of a variety of species have demonstrated that, in general, one should expect to find the presence of PTM types and sites that have been detected in other species. This expectation is based on the extreme conservation of H3 and H4 histone sequences (Fig. 1) and has been bolstered by the observed invariability of PTM sites across the broad swatch of the eukaryotic clade [57]. While some modifications may function in ways different from those described for animals or yeast, as discovered for H3K4 mono-methylation in green algae [58], one should expect to find in plants all known post-translational acetylations and other known PTMs (Supplemental Tables 1 and 2).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bbagrm.2011.02.005.

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