[Frontiers in Bioscience S1, 142-153, June 1, 2009]

Mass spectrometric analysis of histone variants and post-translational modifications

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1. ABSTRACT

Mass spectrometry based proteomics has revolutionized many aspects of modern biological research. One key area where mass spectrometry continues to significantly contribute is in the analysis of histone post-translational modification (PTM) patterns. Dynamic histone PTMs are known to be intricately associated with gene regulation (both activating and silencing), and also with epigenetic processes, therefore, accurate qualitative and quantitative mapping of modification sites on these proteins is of immense value. Mass spectrometry has been utilized to confirm, discover, quantify and determine the simultaneous combination of histone PTMs from many organisms. Here the recent mass spectrometry based studies of histone variants and the characterization of their modifications is reviewed.

2. INTRODUCTION

The core histone proteins H2A, H2B, H3 and H4 complex with about 146 bp of DNA to generate the basic repeating unit of chromatin called the nucleosome (1). Nucleosomes are then linked together by DNA and another histone called linker histone H1. Histones have important roles as structural proteins forming the chromatin fibers, however, recent evidence suggests that they also have a much more dynamic role in the regulation of genes. At the heart of gene modulation are post-translational modifications (PTMs) to histones that occur mostly on the N-terminal tails that protrude from the nucleosomal surface (2, 3). PTMs to histones include modifications on lysine residues such as acetylation, ubiquitination and mono-, di- and trimethylation, mono- and asymmetric or symmetric dimethylation on arginine residues, and phosphorylation on

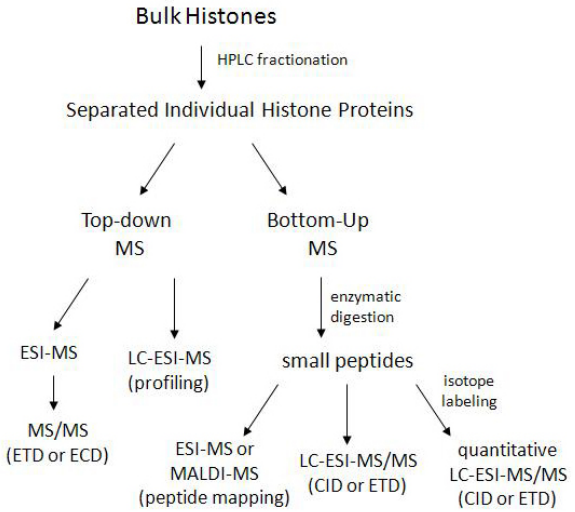

Figure 1. Flowchart describing various mass spectrometry methods that can be used to analysis histone proteins. CID (collision-induced dissociation), ECD (electron capture dissociation), ETD (electron transfer dissociation), LC (liquid chromatography), MS (mass spectrometry), MS/MS (tandem mass spectrometry), ESI (electrospray ionization), MALDI (matrix-assisted laser desorption ionization), HPLC (high performance liquid chromatography).

serine and threonine residues (2, 3). Within the past several years, reports have linked many of these modifications to many biologically important events. For example, the trimethylation of histone H3 at lysine 9 (H3K9me3) is a modification that has been associated with heterochromatin and hence gene silencing (4), while H3K4me3 is typically correlated with gene activation (5). Some histone PTMs have also been shown to be associated with other biological processes such as cell cycle or cellular differentiation (6-9). These modification sites act as recruitment platform for proteins that bind these specific modified residues leading to gene silencing or activation, or other nuclear events as postulated by the "Histone Code" hypothesis (10). As histones can be densely clustered with modifications within certain sequences, the role of coexisting modifications and what effect they might exert becomes a significant question.

Histone modification analysis has been normally accomplished by the use of Edman degradation for protein sequence determination and the use of site-specific antibodies. While Edman sequencing was primarily used in early histone PTM studies, it is a time-consuming procedure that requires high amounts of very pure starting material, thus making the method non-ideal. With the advent of site-specific antibodies, the characterization of histone modifications, both structure and function have reached high levels. Nevertheless, there are some serious problems associated with the sole use of antibodies for histone PTM analysis (11, 12). For example, antibody analysis can suffer from having the epitope the antibody recognizes blocked by PTMs on adjacent and even nearby residues, a phenomenon known as epitope occlusion (11). Additionally, cross-reactivity of the antibody on the same or a different protein can often occur, further complicating data interpretation. Mass spectrometry on the other hand is an unbiased approach that is capable of analyzing proteins containing many diverse modifications simultaneously, irregardless of the protein sequence. Unlike antibody based methods, mass spectrometry can also be used in a very quantitative manner for comparative analysis of protein expression and differential expression analysis of protein modifications. For these and other reasons, mass spectrometry has emerged as a prominent tool for histone PTM investigations, acting as a complementary technique when combined with biological methodology. In this review, modern mass spectrometry as applied to histone protein analysis will be discussed.

3. BOTTOM UP MASS SPECTROMETRY (MS) FOR HISTONE ANALYSIS

3.1. Bottom Up MS identification of histone modification sites

Histone analysis by mass spectrometry can take many different forms. For example Figure 1 shows several potential routes to histone characterization by either Bottom Up MS for analysis of small peptides, or Top Down MS for analysis of large polypeptides or intact protein mass spectrometry (13-15). Some of the first reports of MS characterization of histone proteins involved the protein profiling of bulk histone modification patterns using on and off-line liquid chromatography coupled to electrospray mass spectrometry for intact protein modification studies (16-18). Banks *et al.* examined the phosphorylation changes of histone H1 from mouse cells after treatment with the drug dexamethasone (17). Results showed that certain H1 isoforms (H1.0, H1.1 and H1.2) were non-responsive to the treatments, while dephosphorylation of H1.3, H1.4 and H1.5 were observed with the treatment. Nevertheless, exact sites of phosphorylation were not determined. Ahn and co-workers demonstrated that LC-MS profiling of modification patterns could be deduced on all core histones following dose and time-dependent experiments using the phosphatase and histone deacetylase (HDAC) inhibitors okadiac acid and trichostatin A, respectively (18). Nevertheless, these methods also did not allow for site specific modifications to be mapped.

A joint effort by the Allis and Hunt labs first demonstrated that mass spectrometry could be a useful tool for discovering novel histone PTMs, as they found that histone H4 could be monomethylated in asynchronously grown 293T cells (19). Subsequent biological experiments determined that PRMT1 was the arginine methyltransferase responsible for this modification and that this methylation site is conserved from yeast to humans. Various mass spectrometry analyses were also vital to the identification of histone H3K79 methylation as a new PTM on the core domain (20, 21). Burlingame and co-workers were the first to publish comprehensive mass spectrometric investigations of any core histone, in these cases histone H3 and H4 (22, 23). They confirmed that histones from chicken erythrocytes were abundant in methylation and acetylation modifications on histone H3 at the known

residues K4, K9, K14, K18, K23, K27, K36 and K79 after a series of MALDI-TOF and nanoLC-MS/MS experiments. Analysis of HeLa histone H4 using similar methodology resulted in the identification of a "zip" model of H4 acetylation at K5, K8, K12 and K16, where acetylation progressed from K16 down to K5, and deacetylation followed in opposite fashion. Another study using a peptide mass fingerprinting approach was used to map modification sites on all core histones from calf thymus (24). The authors report the identification of several novel modifications, but could not verify the exact modification site on many peptides as no tandem mass spectrometry experiments could be performed. Zhang *et al.* used cation-exchange HPLC to separate differently acetylated H3 species for further MS experiments (25). Results demonstrated a progressive link between H3 acetylation and K4 and K79 methylation, as the highest levels and degrees of methylation on these residues were observed on the most highly acetylated H3 forms. Zhang *et al.* and Kim *et al.* also determined that immomium ions produced from internal lysine residues on histone peptides could be used to differentiate between peptides containing either acetylated or methylated lysine residues (26, 27). More recent mass spectrometry based studies have also identified or confirmed a variety of modifications such as novel methylation and acetylation sites (28-32), as well as new or unique modifications including biotinylation, propionylation, butyrylation and formylation on many histone proteins (33-36), however, the significance of many of these PTMs during nuclear processes still remain unclear.

3.2. Chemical derivatization for improved MS analysis

Although the enzymatic digestion of histone proteins with several different enzymes has proved to be useful for sample preparation before MS analysis, several limitations still remain. As histone proteins are very basic, containing a high number of Arg and Lys residues, the digestion of the proteins into peptides is far from ideal. Digestion of histones with enzymes that cleave at these basic residues such as trypsin or LysC result in the production of many small peptides that do not retain well on reverse phase HPLC columns. Second, many different peptides containing the same modification site are produced, and generating reproducible peptides between samples is very difficult. If other enzymes that cleave after acidic or aliphatic residues are used, then long high multiply charged peptides are created, and the MS/MS spectra produced from the fragmentation of these peptides in most standard MS instruments are incredibly challenging to interpret. Therefore, several groups have used chemical derivatization of histones peptides to facilitate downstream MS experiments. One of the first reports of chemically modifying histones used deuterated acetic anhydride followed by trypsin digestion to create reproducible 4-17 peptide fragments from histone H4 extracted from yeast mutant cells that increased telomeric silencing (37, 38). The acetic anhydride reacted with unmodified lysine residues and thus blocked cleavage by trypsin. Mass spectrometry proved that all mutants were deficient in H4K12ac, and it was suggested that the lack of this specific modification could act in repressing the state of the telomeric gene. This same derivatization procedure was also carried out to determine histone H3 PTM changes from Drosophila embryos at different stages (39). Changes in histone H3 modification patterns were noted, presumably from developmental changes of the embryos. Specifically, loss of acetylation, an increase in K79 methylation and an inverse relationship between K27 and K36 methylation were uncovered.

Instead of using deuterated acetic anhydride, three research groups independently developed chemical derivatization strategies based on using propionic anhydride (39-42). The propionylation reaction is similar to the acetylation reaction previously used (37-39), but the mass shift induced by the propionyl group (56 Da) is less likely to be confused with an endogenous modification of either trimethylation of acetylation (42 Da). This approach was used by Jenuwein and co-workers to quantify levels of histone H3 methylation from wild-type and *Suv39h* and *G9a* deficient embryonic cells as mentioned above (40). They reacted synthetic and *in vivo* generated histones with propionic anhydride before trypsin digestion, resulting predicted nested sets of peptides that could be straightforwardly identified in the mass spectrometer. In addition to using deuterated acetic anhydride to modify histones from developing *Drosophila* embryos, Bonaldi *et al.* also used the propionic anhydride reagent to better distinguish between naturally occurring acetylation or chemically induced modifications in *Drosophila* histone H3 (39). Hunt and co-workers also used the propionic anhydride methodology to yield histone peptides that could be easily analyzed by nanoLC-MS/MS (41). Recent work has used this same technique to analyze histone PTM differences across many eukaryotic species, uncover new modifications and compare the histone PTMs changes (discussed in more detail below) under several cellular conditions (31, 43-45). Some of the important discoveries from those studies included discovering that most lysine residues on histone H3 could be both acetylated and methylated. This helped, identify a K9 methylation site adjacent to S10 phosphorylation leading to the "binary switch" hypothesis discussed below. Other studies showed that lower eukaryotic species utilize different modification sites and have distinct PTM patterns as opposed to higher eukaryotes such as human. Lastly, the propionic anhydride method has been adapted to modify histones that have been previously separated by 1D-SDS-PAGE (46). This in-gel propionic anhydride reaction was utilized to improve MS characterization of the modifications across shrimp core histones possessing antimicrobial properties.

3.3. Determination of histone variants species

Mass spectrometry is a good method for identification of histone variants. Many histone family members have various isoforms or variants, differing in sequence by as little as one amino acid residue. Nevertheless, difficulties arise as the amino acid differences between the variants can occur in multiples of 14 Da mass shifts, which can be mistaken for methylation modifications. Recently, a Top Down mass spectrometry approach was employed to identify major HeLa histone H2B and H2A isoforms and their modifications (see below for more details) (47, 48). In contrast, a Bottom Up

approach was utilized to characterize histone H2A and H2B proteins from Jurkat cells (49). The main H2A variants, H2AO, H2AL and H2AC were found be modified by acetylation at K5 and S1 phosphorylation. H2BA was the main H2B variant and it was observed to be acetylated at K12, K15 and K20. Other histone H2A variants, such as H2AZ, H2AX and macroH2A have been analyzed by mass spectrometry and found to be modestly decorated with acetylations, methylation, phosphorylation and ubiquitination (50-52). A recent paper analyzing histones from the model plant system *Arabidopsis thaliana* showed that several H2B and H2A isoforms are expressed, many carrying similar modifications as seen in mammalian cell types (53). Analyses of human histone H3 variants using tandem MS have resulted in identifying PTMs that are enriched on specific variants and to find differences between the modification profiles on the variants and also before chromatin incorporation (45, 54).

3.4. Phosphorylation of histone proteins

The reversible, dynamic phosphorylation of histones has been reported to be associated with chromosome condensation during mitosis, but has also been implicated in the activation of early response genes as well (55). Several mass spectrometry studies have characterized the phosphorylated forms of histones H1, H3, H2A and H4. The combination of immobilized metal affinity chromatography (IMAC) and tandem mass spectrometry allowed the detection of 19 sites of phosphorylation across 6 major human H1 isoforms, including histone H1.X (56). These experiments also produced data on a peptide from H1.4 that was found to be both lysine methylated and adjacently phosphorylated on a serine residue, producing experimental evidence in support of the 'binary switch' hypothesis (57). This hypothesis postulated by the Allis research group states that an effector protein that recognizes and binds a PTM site such as with the binding of H3K9me3 by heterochromatin protein 1 (HP1) could be affected by the addition of another modification adjacent to the previous site. These adjacent modifications could serve as a molecular switch regulating those interactions and potentially modifying any further downstream events. A comprehensive survey of histone H3 variants from mitotically arrested HeLa cells further found other potential 'switches' on H3 including T3phK4me1, K9me1-3/S10ph and K27me1-3/S28ph and distinct modification patterns (58). The 'binary switch' hypothesis was later supported by biological evidence using site-specific antibodies raised to single and dual modified peptides showing that the phosphorylation of H3S10 by the kinase Aurora B during mitosis induced the loss of binding of HP1 to H3K9me3 (59). Therefore, the combination of mass spectrometry and follow-up validation by antibody methods proved successful in determining the role of H3 phosphorylation in modulating the binding of HP1. The first histone H3 variant specific modification on H3.3 (S31 phosphorylation) was also detected by MS experiments (60). Phosphorylation of H3.3 was observed to be mitotic specific, as other H3 phosphorylation modification sites, but H3.3S31ph differed in chromosomal localization and timing compared to the other known phosphorylation sites S10 and S28 as revealed by accompanying immunofluorescence experiments. Many of these phosphorylation modifications on H1 and H3 have been subsequently confirmed by different MS methods (36, 61). Finally, a complete proteomic analysis of phosphorylation of histone H1 from *Tetrahymena thermophila* found a unique hierarchal pattern of phosphorylation and compared phosphorylation between starved and growing cell cultures (62).

4. COMPARATIVE MS ANALYSIS OF HISTONE POST-TRANSLATIONAL MODIFICATIONS (PTMs)

4.1. Label-free quantification of histone PTMs

As mass spectrometry has shown to be a great tool for the confirmation and discovery of histone PTM sites, studies have shifted towards the quantification of histone modifications under various biological conditions. A simple method for relative quantification of histone peptides is to use a label free approach where modification level changes can be assessed by integrating the area under the curve for each charge state of a specifically modified histone peptide and expressing it as a percentage of the total histone peptide. Nevertheless, this technique requires the use of an enzyme that will produce reproducible peptides that can be easily detected in the mass spectrometer. An example of this was published by Henikoff and co-workers in 2004 (63). The authors used an in-gel digestion procedure with the protease ArgC to cleave histone H3 variants specifically after Arg residues producing uniform peptides from histone H3 extracted from *Drosophila* cell lines undergoing differentiation by β-ecdysone treatment. *Drosophila* contains two H3 variants (H3 and H3.3), and these studies determined that H3.3 was present in abundance enough to essentially package all transcribed genes, and mass spectrometry and antibody experiments showed H3.3 to be decorated with PTMs associated with gene activation such as hyperacetylation and K4 methylation, while H3 was enriched in modifications associated with silenced chromatins such as K9me2.

As mentioned earlier, Jenuwein and co-workers were able to use chemical derivatization to generate uniform peptides from histone H3 to compare PTMs from *Suv39h* and *G9a* deficient embryonic cells (40). Fraga *et al.* combined high-performance capillary electrophoresis with LC-MS/MS analysis to interrogate histone H4 from a wide array of selected cancer cell lines (64). Marked differences were revealed from the MS analysis of leukemia cell lines in comparison to normal lymphocytes and it was determined that loss of H4K16ac and H4K20me3 were common in all human cancers. An in-depth analysis of all core histones from small cell lung cancer cells was performed by Jensen and co-workers (65). A total of 32 modifications were site-specifically mapped to the core histones, including many new modifications sites following separation of histones with SDS-PAGE, in-gel trypsin digestion and LC-MS/MS analysis. Results showed that upon treatment with the HDAC inhibitor PXD101, the abundance level of many modified peptides changed. As expected, the acetylation levels at many known

modifications sites on H3, H2B and H4 increased. However, unexpectedly the decrease of H2BK57me2 may indicate the role of this particular PTM in the PXD101 induced apoptosis in the cancer cells. Lastly, label free MS quantification methods have also been applied to measuring the level of demethylase activity that many enzymes impose on *in vitro* or *in vivo* histone peptide or protein substrates (66-68).

4.2. Chemical stable isotope labeling of histones for differential expression experiments

Chemically derivatizing proteins post-harvest for comparative analysis of PTMs between two different biological states has been achieved by incorporation of stable isotopes by a variety of methods (69). These types of quantitative methods have also been applied to proteomic histone research. Preliminary data from the first commercial linear ion trap Fourier transform mass spectrometer (LTQ-FTMS) described the use of ethanolic HCl to convert carboxylic acid functional groups on histone peptides to their corresponding ethyl esters (41). Asynchronous histone H3 peptides were converted to d_0-ethyl esters, and mitotic histone H3 peptides were converted to d_5-ethyl esters and samples equally mixed before MS analysis. This allowed for the quantification of the PTMs between the physiologically different cell types and phosphorylated peptides were found enriched on the mitotic samples. Ethyl esterification reactions were performed for differential expression analysis of histones from wild-type and methyltransferase mutant *Arabidopsis* strains and also to quantify PTMs from a mouse model of lupus (43, 44). The latter study suggested that aberrant modification patterns on histone H4 and H3 exist on lupus diseased mice that could be reset by the treatment of the mice with histone deacetylase inhibitors *in vivo* resulting in a beneficial effect in the autoimmune disease. A similar methyl esterification derivatization procedure was used to quantify the levels of modifications between all histone H3 variants (45). Like H3.3 in Drosophila, human H3.3 was identified as being enriched in PTMs associated with active chromatin such as hyperacetylation, while H3.2 was detected to have modifications linked to repressive chromatin such as K27 methylation. Interestingly, the histone H3.1 variant only found in mammals was found to be encoded with PTMs associated with both active and silenced chromatin, although the silencing modifications were different than those on H3.2. A comparative phosphoproteomic analysis of linker histone H1 from *Tetrahymena thermophila* using methyl esterification labeling, IMAC and tandem MS showed that phosphorylation levels mainly at cyclin-dependent kinase sites were decreased on cultures undergoing prolonged starvation compared to normal growing cells (62).

4.3. Stable isotope labeling by amino acids in cell culture (SILAC)

SILAC is a metabolic labeling approach that substitutes stable isotope enriched amino acids (C^{13}, N^{15}, etc.) for selected amino acids in the cell growth media to isotopically label proteins for further mass spectrometry quantification studies (70). Primarily designed for quantitative proteomic experiments, Mann and co-workers have also utilized SILAC labeling for identification of protein methylation as well (71). Cells metabolically convert methionine to the only methyl group donor, S-adenosyl methionine (AdoMet), transferred onto proteins by the S-adenosyl methyltransferase enzyme. Treating cells with ($^{13}CD_3$)-S-adenosyl methionine (termed heavy methyl SILAC) introduces a 4 Da mass shift per methyl group incorporation that can be monitored by MS. These experiments showed that methylated peptides can be identified due to the presence of doublet peaks arising from chemically identical peptides harboring isotopic labels. Many proteins were demonstrated to be methylated at either arginine or lysines residues, and histone H3K27 and H4K20 methylated peptides were among those found.

Freitas and co-workers have also used SILAC to quantify histone H4 PTMs in a number of studies (72-74). Histones are first fully labeled with D_4-lysine, H4 modified forms separated using acetic acid-urea polyacrylamide gel electrophoresis (AU-PAGE) and then characterized using mass spectrometry. This type of methodology has been applied to determine histone H4 acetylation alterations induced by histone deacetylase (HDAC) inhibitor treatment of compounds such as depsipeptide and trichostatin A. The majority of the cell lines used were from various cancer types such as leukemia, and dose-dependent changes in histone PTMs have been recorded under a variety of biological conditions, although the H4 acetylation patterns from these cells usually were found to follow the before mentioned "zipper" model. Mizzen and co-workers have also recently used a SILAC approach combined with Top Down mass spectrometry (see below) to monitor the progression of methylation on histone H4K20 throughout the cell cycle (75). They found that newly synthesized H4 is gradually methylated during G2, M and G1 stages of the cell cycle and that H4K20 methylation turnover cannot be detected by their methods. Additionally, the authors reveal that H4 is methylated regardless of prior acetylation status and that acetylation continues to occur on H4 protein that is K20 methylated, which is in contradiction to a previous notion that H4K20 methylation antagonizes H4 acetylation. Recently, Bonefant *et al.* also used SILAC labeling and mass spectrometry to quantify histone PTM changes across the cell cycle (61). Histone H4, H3, H2B and H2A acetylation were established to reach a maximum during G1/S, and some mitotically-associated modifications especially phosphorylations were detected.

5. TOP AND MIDDLE DOWN MS FOR INTERROGATION OF INTACT HISTONE CODES

5.1. Liquid chromatography MS profiling of modified histone species

Epigenetic changes in chromatin structure have been linked to several disease states, particularly several types of cancers. However, in order to access variation in histone PTMs caused as a result of disease phenotype, reproducible high-throughput MS analysis of a potentially large number of clinical samples is required. As stated previously, early MS efforts used LC-MS histone protein profiling to view changes in histone PTMs caused by external stimuli (16-18). However, one of the first modern

LC-MS analyses of modified histone forms was accomplished by Zhang *et al* (76). Chicken blood histones modified by acetylation and methylation were examined with an on-line flow-splitting scheme that allowed a small percentage of the LC eluent to pass into the mass spectrometer for intact form identification, while the majority of the eluent was fraction collected for Bottom Up localization of PTM sites. This combined approach allowed for the mapping of PTM sites on histone H3 and H4, while also producing data on the relative number of modified protein forms. Freitas and co-workers also used LC-MS profiling to detect global core histone modification pattern changes in acute myeloid leukemia cell lines treated with HDAC inhibitors and primary leukemia cells from patients undergoing a Phase I clinical trial of the HDAC inhibitor depsipeptide (77). Although specific modified sites on the core histones could not be determined with this approach, the results showed a possible correlation between histone acetylation patterns and chronic lymphocytic leukemia, particularly in the case of histone H2A. Lastly, LC-MS methodology was employed to survey the PTM profiles from histones taken from a human colon cancer cell line (78). Reproducible RP-HPLC parameters were created to separate the histones and achieve optimum MS sensitivity, and drastic changes in the modification profiles of histone H2A, H4 and H3 were noted after HDAC inhibitor treatment.

5.2 . Electron capture dissociation (ECD) of histones

Bottom Up MS experiments have thrust mass spectrometry as an extremely valuable tool to complement biological studies of histones and have the capability of discovering new modifications and quantifying modifications from different cellular states. Nevertheless, with the more recent concentration of efforts on the effect of histone PTM combinations on cellular function, a different kind of MS methodology is needed. Bottom Up MS could not be utilized for these studies as histones must be enzymatically cleaved into small peptides ranging in length from 9-20 amino acids. Therefore, if two modification sites are not present on the same peptide, then there is no way to distinguish which peptides were generated from the same individual histone protein. As a result, the connectivity of histone PTMs or the identification of entire Histone Codes is lost in these analyses. In order to detect the combinations of histones PTMs that may occur across the whole protein sequence, a Top Down mass spectrometry approach must be used. Top Down MS using electron capture dissociation (ECD) varies from standard MS methods as polypeptides or intact proteins are ionized and trapped in the ion cyclotron resonance (ICR) cell of a Fourier transform instrument and reacted with low energy electrons (79). This fragmentation method cleaves sequences between the amide nitrogen and the α-carbon to form z·- and c-type fragment ions. Additionally, as the dissociation of the molecule occurs differently than with standard MS methods, the weakest bonds normally broken in most Bottom Up experiments are not preferentially cleaved. This results in labile PTMs such as phosphorylation to remain on the protein molecule. Additionally, this type of fragmentation also allows for the dissociation of large polypeptides and intact proteins (80).

The Kelleher research group was one of the first to apply Top Down MS to histone PTM analysis. A home-built 8.5 Tesla quadrupole Fourier transform mass spectrometer was used to examine histone H4 extracted from asynchronously grown and sodium butyrate treated HeLa cells. Several modified forms of histone H4 with multiple acetylations and mono-, di or trimethylation could be seen and these forms were dissociated using ECD. The ECD data was then searched using the Prosight PTM software against a custom-built database containing 18,750 theoretically modified forms of histone H4 and confirmed all the known modifications sites on this histone (81). Another study also utilized ECD to identify modifications on histone H4 (82). These authors compared the Top down MS data to data generated from Bottom Up MS where histone H4 was digested with various enzymes including Glu-C, pepsin and trypsin. Overall, the Top Down data was found to be in good agreement with Bottom Up produced data determining acetylation and methylation sites.

A nice advantage of being able to characterize larger peptide or intact protein sequences is the ability to discern small amino acid changes commonly found between protein isoforms. In that regard, Top Down MS has been also been recently used to identify histone protein isoforms and their PTMs. Burlingame and co-workers demonstrated that both Top Down and Bottom Up MS could be applied to analyze the PTM status of *Tetrahymena Thermophila* H2B variants (83). Top Down MS was used to detect two major forms of histone H2B having mass shifts of +42 and +84 Da, while Bottom Up MS localized the modifications as acetylation on K4 and trimethylation on the N-terminus or K3. As mentioned earlier, Hunt and co-workers also used a dual Bottom Up and Top Down proteomic tactic to comprehensively map phosphorylation sites on linker histone H1 from *Tetrahymena Thermophila (*62). While Bottom Up MS was advantageous for mapping low level phosphorylation sites, Top Down Ms proved vital for the determining the sequential order of phosphorylation on H1.

Consecutive papers reporting Top down MS of the other core human histones variants from H2B, H2A and H3 have also been published (47, 48, 84). HeLa cell histones were HPLC purified into family members and analyzed by high-resolution FTMS. Experiments showed that 14 distinct H2A species are present in HeLa cells, and the most abundant modification to these H2A variants was N-terminal acetylation (47). Only a small amount of internal acetylation was detected on these forms even after HDAC inhibitor treatment. Lastly, no major bulk modification profile differences were detected from H2A proteins extracted from various cell cycle phases, except that H2A was modified by ubiquitin only during the mitosis/G1 phase. Similarly, Siuti *et al.* reported that several H2B variants were found in HeLa cells (48), with most isoforms bearing no major PTMs. The same research group determined that histone H3 was highly modified compared to the H2A and H2B proteins (84). Intact histone H3.1 and H3.3 were probed and ECD dissociation

of all modified forms *en masse* allowed for the relative PTM occupancy of the bulk H3 proteins to be calculated, even though the modification status of any individual H3 molecule could not be determined. The authors found that H3K4 was only 5% monomethylated, while approximately 50% of H3.1 was dimethylated (H3K9me2). Analysis of histones variants is enhanced by treatment of histones with mild performic acid to convert proteins with methionine or cysteine residues to their oxidized forms (85). This allows for the determination of true methylated or variant forms (ΔM = 14 Da) versus partially oxidized methionine or cysteine residues on histone proteins which have similar mass shifts (ΔM = 16 Da).

Recent reports have also characterized all core histones isolated from rat brain tissue and yeast (86, 87). In the first study, the authors detect several H2A and H2B isoforms that are modestly modified. Intact histone H3.2 and H3.3 variants were also found to contain large number of PTMs, but tandem mass spectrometry ECD experiments could only be used to obtain a broad view of the modification complexity. After digesting H3 with the GluC protease they obtained an increased signal in the mass spectrometer and higher sequence coverage which were used to quantify the H3 PTMs from distinct rat brain subsections, a procedure termed Middle Down MS. A global view of yeast core histones was also provided via Top Down MS and revealed at least 50 unique modified forms (87). A link between histone H3 acetylation and K4 methylation was also established and the analysis of gene knockout yeast strains showed which histone H3 variants were quantitatively affected by certain histone acetyltransferases (i.e. H3 and H2B PTM profiles vastly changed in *gnc5*Δ cells).

To date, nearly all of the Top Down MS analyses have been performed on bulk histones. However, being able to generate data on the combinations of modifications on single modified histone forms would be very beneficial to the chromatin biologist. In this regard, the development of hydrophilic interaction chromatography (HILIC) method that fractionated the 1-50 polypeptide of histone H3.2 into over 30 fractions containing differently modified H3 forms that could be analyzed by MS methods was created (88). Results showed that over 150 unique modified forms of H3.2 were present from asynchronously and butyrate-treated HeLa cells, as well as uncovering certain patterns involving acetylation and K4 methylation. Lastly, one can use ion abundances from Top down fragmentation in association with the ion abundances from the parent ions for quantification of isomeric histone forms in the same mixture. Recently, it has been demonstrated that the protein/peptide precursor ion relative ratios (PIRRs) combined with fragment ion relative ratios (FIRRs) could be employed to estimate isomeric histone H4 proteins and polypeptides from asynchronous, butyrate-treated and cell cycle time-points (89), and this methodology has also been initiated on histone H3 (86, 88).

5.3. Electron transfer dissociation (ETD) of histones

Although ECD has been applied to many research projects, there are some apparent limitations of ECD. For instance, in order to acquire an ECD MS/MS spectrum with good signal intensity, one must average several single spectra. This is because the MS/MS products from the ECD fragmentation of the protein are typically of very low intensity. Therefore, the average time to collect an ECD spectrum ranges from minutes to hours depending on the operating parameters. As a result, ECD fragmentation therefore cannot easily be combined with on-line liquid chromatography where typical elution times for peptides are seconds. ECD experiments also require much larger sample amounts, as static electrospray infusion is normally utilized, instead of on-line nanoflow HPLC where samples are concentrated. Finally, ECD can only be performed on an FTMS system, requiring the user to purchase one of the most expensive mass spectrometry instruments available. In order to create an ion/ion analogue of ECD that could be available to a larger number researchers using bench top ion trap instruments, Hunt and co-workers created electron transfer dissociation (ETD) (90). The ETD fragmentation event like ECD reacts electrons with the peptide cations. However, the main difference is that the electrons used in ETD are generated from a radical anion such as fluoranthene that is generated by a chemical ionization source before introduction into the mass spectrometer. The fluoranthene anion then transfers electrons to the peptide cations causing dissociation of the peptide generating z·- and c-type product fragments just like those in ECD reactions. A main difference between ETD and ECD is that the ETD reaction is very rapid (millisecond timescale), allowing this procedure to be coupled with on-line liquid chromatography experiments, and as a result possesses sensitivity of a Bottom up mass spectrometry experiment (90, 91). Additionally, like ECD, ETD can be used to fragment large polypeptides of intact proteins and labile modifications such as phosphorylation or sulfonation remain on the precursor ion as well (14). For example, Figure 2 shows the single-scan on-line nanoLC acquired ETD spectrum of a mixture of histone H3.1 species possessing 5 methyl groups, with the sequences H3K9me3K27me2 and H3K9me2K27me3.

Initial applications of ETD to histone analysis allowed for the on-line characterization of differentially modified histone H3 forms and H2A variants (91). After isolating the 1-50 polypeptide fragment (Middle Down MS), Hunt and co-workers used ETD and a second charge reducing reaction to identify H3 species that contained simultaneous modifications at K4, K9, and K36. As high sequence coverage can be obtained, a complete readout of modified residues on this H3 polypeptide can be achieved. In the same study, ETD experiments were also employed to discover a novel chicken histone H2A.Z protein that contained only four amino acid changes compared to the known H2A sequence. A more comprehensive analysis of *Tetrahymena Thermophila* modified histone H3 forms has been most recently completed (92). Histone H3 was first separated using cation-exchange chromatography and then digested with GluC protease to generate the 1-50 H3 polypeptide followed by ETD mass spectrometry interrogation. Over 40 differently modified forms were revealed in these studies and bivalent domain modifications

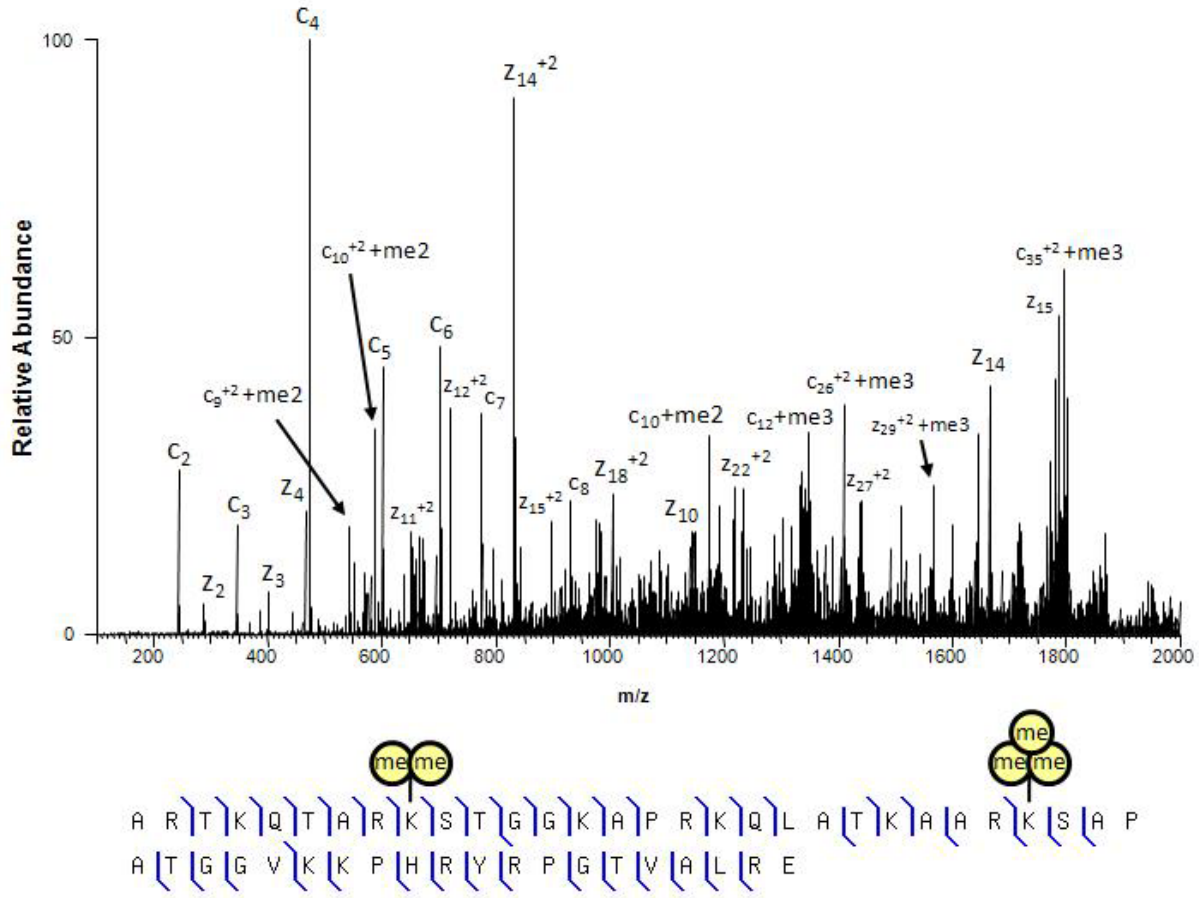

Figure 2. On-line acquired nanoLC-MS/MS ETD mass spectrum of purified HeLa histone H3.1. Two forms are present with the most abundant form having the sequence H3K9me2K27me3.

including K4 methylation and K27 methylation were detected.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

Analysis of histone modifications and modification patterns has become a central focus in many research labs worldwide. While the use of antibody based methods are still a mainstay of most biological studies, the integration of mass spectrometry in chromatin biology has been on the rise. Intrinsic properties that make mass spectrometry attractive for histone PTM analysis include that MS studies are not susceptible to epitope occlusion as are antibodies, the ability to relatively quantify modifications, and with recent advances in instrumentation, the ability to simultaneously detect combinations of modifications. Constant advances in biochemical experimentation and mass spectrometry technology will seemingly drive the chromatin biology field to new levels.

7. ACKNOWLEDGEMENTS

Financial support from Princeton University is gratefully acknowledged.

8. REFERENCES

1. K. Luger and T. J. Richmond: The histone tails of the nucleosome. Curr Opin Genet Dev, 8(2), 140-6 (1998)

2. B. D. Strahl and C. D. Allis: The language of covalent histone modifications. Nature, 403(6765), 41-5 (2000)

3. B. M. Turner: Reading signals on the nucleosome with a new nomenclature for modified histones. Nat Struct Mol Biol, 12(2), 110-2 (2005)

4. M. Lachner, D. O'Carroll, S. Rea, K. Mechtler and T. Jenuwein: Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature, 410(6824), 116-20 (2001)

5. H. Santos-Rosa, R. Schneider, A. J. Bannister, J. Sherriff, B. E. Bernstein, N. C. Emre, S. L. Schreiber, J. Mellor and T. Kouzarides: Active genes are tri-methylated at K4 of histone H3. Nature, 419(6905), 407-11 (2002)

6. W. L. Cheung, K. Ajiro, K. Samejima, M. Kloc, P. Cheung, C. A. Mizzen, A. Beeser, L. D. Etkin, J. Chernoff,

W. C. Earnshaw and C. D. Allis: Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase. Cell, 113(4), 507-17 (2003)

7. H. Goto, Y. Tomono, K. Ajiro, H. Kosako, M. Fujita, M. Sakurai, K. Okawa, A. Iwamatsu, T. Okigaki, T. Takahashi and M. Inagaki: Identification of a novel phosphorylation site on histone H3 coupled with mitotic chromosome condensation. J Biol Chem, 274(36), 25543-9 (1999)

8. G. M. Vidanes, C. Y. Bonilla and D. P. Toczyski: Complicated tails: histone modifications and the DNA damage response. Cell, 121(7), 973-6 (2005)

9. Y. Wei, C. A. Mizzen, R. G. Cook, M. A. Gorovsky and C. D. Allis: Phosphorylation of histone H3 at serine 10 is correlated with chromosome condensation during mitosis and meiosis in Tetrahymena. Proc Natl Acad Sci U S A, 95(13), 7480-4 (1998)

10. T. Jenuwein and C. D. Allis: Translating the histone code. Science, 293(5532), 1074-80 (2001)

11. P. Cheung: Generation and characterization of antibodies directed against di-modified histones, and comments on antibody and epitope recognition. Methods Enzymol, 376, 221-34 (2004)

12. A. L. Clayton, S. Rose, M. J. Barratt and L. C. Mahadevan: Phosphoacetylation of histone H3 on c-fos- and c-jun-associated nucleosomes upon gene activation. EMBO J, 19(14), 3714-26 (2000)

13. B. T. Chait: Chemistry. Mass spectrometry: bottom-up or top-down? Science, 314(5796), 65-6 (2006)

14. L. M. Mikesh, B. Ueberheide, A. Chi, J. J. Coon, J. E. Syka, J. Shabanowitz and D. F. Hunt: The utility of ETD mass spectrometry in proteomic analysis. Biochim Biophys Acta, 1764(12), 1811-22 (2006)

15. N. Siuti and N. L. Kelleher: Decoding protein modifications using top-down mass spectrometry. Nat Methods, 4(10), 817-21 (2007)

16. C. G. Edmonds, J. A. Loo, R. D. Smith, A. F. Fuciarelli, B. D. Thrall, J. E. Morris and D. L. Springer: Evaluation of histone sequence and modifications by electrospray ionization mass spectrometry and tandem mass spectrometry. J Toxicol Environ Health, 40(2-3), 159 (1993)

17. G. C. Banks, L. J. Deterding, K. B. Tomer and T. K. Archer: Hormone-mediated dephosphorylation of specific histone H1 isoforms. J Biol Chem, 276(39), 36467-73 (2001)

18. S. C. Galasinski, D. F. Louie, K. K. Gloor, K. A. Resing and N. G. Ahn: Global regulation of post-translational modifications on core histones. J Biol Chem, 277(4), 2579-88 (2002)

19. B. D. Strahl, S. D. Briggs, C. J. Brame, J. A. Caldwell, S. S. Koh, H. Ma, R. G. Cook, J. Shabanowitz, D. F. Hunt, M. R. Stallcup and C. D. Allis: Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. Curr Biol, 11(12), 996-1000 (2001)

20. H. H. Ng, Q. Feng, H. Wang, H. Erdjument-Bromage, P. Tempst, Y. Zhang and K. Struhl: Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. Genes Dev, 16(12), 1518-27 (2002)

21. F. van Leeuwen, P. R. Gafken and D. E. Gottschling: Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell, 109(6), 745-56 (2002)

22. K. Zhang, H. Tang, L. Huang, J. W. Blankenship, P. R. Jones, F. Xiang, P. M. Yau and A. L. Burlingame: Identification of acetylation and methylation sites of histone H3 from chicken erythrocytes by high-accuracy matrix-assisted laser desorption ionization-time-of-flight, matrix-assisted laser desorption ionization-postsource decay, and nanoelectrospray ionization tandem mass spectrometry. Anal Biochem, 306(2), 259-69 (2002)

23. K. Zhang, K. E. Williams, L. Huang, P. Yau, J. S. Siino, E. M. Bradbury, P. R. Jones, M. J. Minch and A. L. Burlingame: Histone acetylation and deacetylation: identification of acetylation and methylation sites of HeLa histone H4 by mass spectrometry. Mol Cell Proteomics, 1(7), 500-8 (2002)

24. L. Zhang, E. E. Eugeni, M. R. Parthun and M. A. Freitas: Identification of novel histone post-translational modifications by peptide mass fingerprinting. Chromosoma, 112(2), 77-86 (2003)

25. K. Zhang, J. S. Siino, P. R. Jones, P. M. Yau and E. M. Bradbury: A mass spectrometric "Western blot" to evaluate the correlations between histone methylation and histone acetylation. Proteomics, 4(12), 3765-75 (2004)

26. J. Y. Kim, K. W. Kim, H. J. Kwon, D. W. Lee and J. S. Yoo: Probing lysine acetylation with a modification-specific marker ion using high-performance liquid chromatography/electrospray-mass spectrometry with collision-induced dissociation. Anal Chem, 74(21), 5443-9 (2002)

27. K. Zhang, P. M. Yau, B. Chandrasekhar, R. New, R. Kondrat, B. S. Imai and M. E. Bradbury: Differentiation between peptides containing acetylated or tri-methylated lysines by mass spectrometry: an application for determining lysine 9 acetylation and methylation of histone H3. Proteomics, 4(1), 1-10 (2004)

28. R. R. Cocklin and M. Wang: Identification of methylation and acetylation sites on mouse histone H3 using matrix-assisted laser desorption/ionization time-of-flight and nanoelectrospray ionization tandem mass spectrometry. J Protein Chem, 22(4), 327-34 (2003)

29. F. Xu, K. Zhang and M. Grunstein: Acetylation in

histone H3 globular domain regulates gene expression in yeast. Cell, 121(3), 375-85 (2005)

30. J. Ye, X. Ai, E. E. Eugeni, L. Zhang, L. R. Carpenter, M. A. Jelinek, M. A. Freitas and M. R. Parthun: Histone H4 lysine 91 acetylation a core domain modification associated with chromatin assembly. Mol Cell, 18(1), 123-30 (2005)

31. B. A. Garcia, S. B. Hake, R. L. Diaz, M. Kauer, S. A. Morris, J. Recht, J. Shabanowitz, N. Mishra, B. D. Strahl, C. D. Allis and D. F. Hunt: Organismal differences in post-translational modifications in histones H3 and H4. J Biol Chem, 282(10), 7641-55 (2007)

32. S. A. Morris, B. Rao, B. A. Garcia, S. B. Hake, R. L. Diaz, J. Shabanowitz, D. F. Hunt, C. D. Allis, J. D. Lieb and B. D. Strahl: Identification of histone H3 lysine 36 acetylation as a highly conserved histone modification. J Biol Chem, 282(10), 7632-40 (2007)

33. G. Camporeale, E. E. Shubert, G. Sarath, R. Cerny and J. Zempleni: K8 and K12 are biotinylated in human histone H4. Eur J Biochem, 271(11), 2257-63 (2004)

34. Y. Chen, R. Sprung, Y. Tang, H. Ball, B. Sangras, S. C. Kim, J. R. Falck, J. Peng, W. Gu and Y. Zhao: Lysine propionylation and butyrylation are novel post-translational modifications in histones. Mol Cell Proteomics, 6(5), 812-9 (2007)

35. T. Jiang, X. Zhou, K. Taghizadeh, M. Dong and P. C. Dedon: N-formylation of lysine in histone proteins as a secondary modification arising from oxidative DNA damage. Proc Natl Acad Sci U S A, 104(1), 60-5 (2007)

36. J. R. Wisniewski, A. Zougman, S. Kruger and M. Mann: Mass spectrometric mapping of linker histone H1 variants reveals multiple acetylations, methylations, and phosphorylation as well as differences between cell culture and tissue. Mol Cell Proteomics, 6(1), 72-87 (2007)

37. C. M. Smith, Z. W. Haimberger, C. O. Johnson, A. J. Wolf, P. R. Gafken, Z. Zhang, M. R. Parthun and D. E. Gottschling: Heritable chromatin structure: mapping "memory" in histones H3 and H4. Proc Natl Acad Sci U S A, 99 Suppl 4, 16454-61 (2002)

38. C. M. Smith, P. R. Gafken, Z. Zhang, D. E. Gottschling, J. B. Smith and D. L. Smith: Mass spectrometric quantification of acetylation at specific lysines within the amino-terminal tail of histone H4. Anal Biochem, 316(1), 23-33 (2003)

39. T. Bonaldi, A. Imhof and J. T. Regula: A combination of different mass spectroscopic techniques for the analysis of dynamic changes of histone modifications. Proteomics, 4(5), 1382-96 (2004)

40. A. H. Peters, S. Kubicek, K. Mechtler, R. J. O'Sullivan, A. A. Derijck, L. Perez-Burgos, A. Kohlmaier, S. Opravil, M. Tachibana, Y. Shinkai, J. H. Martens and T. Jenuwein: Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol Cell, 12(6), 1577-89 (2003)

41. J. E. Syka, J. A. Marto, D. L. Bai, S. Horning, M. W. Senko, J. C. Schwartz, B. Ueberheide, B. Garcia, S. Busby, T. Muratore, J. Shabanowitz and D. F. Hunt: Novel linear quadrupole ion trap/FT mass spectrometer: performance characterization and use in the comparative analysis of histone H3 post-translational modifications. J Proteome Res, 3(3), 621-6 (2004)

42. B. A. Garcia, S. Mollah, B. M. Ueberheide, S. A. Busby, T. L. Muratore, J. Shabanowitz and D. F. Hunt: Chemical derivatization of histones for facilitated analysis by mass spectrometry. Nat Protoc, 2(4), 933-8 (2007)

43. L. Johnson, S. Mollah, B. A. Garcia, T. L. Muratore, J. Shabanowitz, D. F. Hunt and S. E. Jacobsen: Mass spectrometry analysis of Arabidopsis histone H3 reveals distinct combinations of post-translational modifications. Nucleic Acids Res, 32(22), 6511-8 (2004)

44. B. A. Garcia, S. A. Busby, J. Shabanowitz, D. F. Hunt and N. Mishra: Resetting the epigenetic histone code in the MRL-lpr/lpr mouse model of lupus by histone deacetylase inhibition. J Proteome Res, 4(6), 2032-42 (2005)

45. S. B. Hake, B. A. Garcia, E. M. Duncan, M. Kauer, G. Dellaire, J. Shabanowitz, D. P. Bazett-Jones, C. D. Allis and D. F. Hunt: Expression patterns and post-translational modifications associated with mammalian histone H3 variants. J Biol Chem, 281(1), 559-68 (2006)

46. S. A. Ouvry-Patat and K. L. Schey: Characterization of antimicrobial histone sequences and posttranslational modifications by mass spectrometry. J Mass Spectrom, 42(5), 664-74 (2007)

47. M. T. Boyne, 2nd, J. J. Pesavento, C. A. Mizzen and N. L. Kelleher: Precise characterization of human histones in the H2A gene family by top down mass spectrometry. J Proteome Res, 5(2), 248-53 (2006)

48. N. Siuti, M. J. Roth, C. A. Mizzen, N. L. Kelleher and J. J. Pesavento: Gene-specific characterization of human histone H2B by electron capture dissociation. J Proteome Res, 5(2), 233-9 (2006)

49. D. Bonenfant, M. Coulot, H. Towbin, P. Schindler and J. van Oostrum: Characterization of histone H2A and H2B variants and their post-translational modifications by mass spectrometry. Mol Cell Proteomics, 5(3), 541-52 (2006)

50. F. Chu, D. A. Nusinow, R. J. Chalkley, K. Plath, B. Panning and A. L. Burlingame: Mapping post-translational modifications of the histone variant MacroH2A1 using tandem mass spectrometry. Mol Cell Proteomics, 5(1), 194-203 (2006)

51. C. B. Millar, F. Xu, K. Zhang and M. Grunstein: Acetylation of H2AZ Lys 14 is associated with genome-wide gene activity in yeast. Genes Dev, 20(6), 711-22

(2006)

52. H. Zhu, T. C. Hunter, S. Pan, P. M. Yau, E. M. Bradbury and X. Chen: Residue-specific mass signatures for the efficient detection of protein modifications by mass spectrometry. Anal Chem, 74(7), 1687-94 (2002)

53. E. Bergmuller, P. M. Gehrig and W. Gruissem: Characterization of post-translational modifications of histone H2B-variants isolated from Arabidopsis thaliana. J Proteome Res, 6(9), 3655-68 (2007)

54. A. Loyola, T. Bonaldi, D. Roche, A. Imhof and G. Almouzni: PTMs on H3 variants before chromatin assembly potentiate their final epigenetic state. Mol Cell, 24(2), 309-16 (2006)

55. K. M. Johansen and J. Johansen: Regulation of chromatin structure by histone H3S10 phosphorylation. Chromosome Res, 14(4), 393-404 (2006)

56. B. A. Garcia, S. A. Busby, C. M. Barber, J. Shabanowitz, C. D. Allis and D. F. Hunt: Characterization of phosphorylation sites on histone H1 isoforms by tandem mass spectrometry. J Proteome Res, 3(6), 1219-27 (2004)

57. W. Fischle, Y. Wang and C. D. Allis: Binary switches and modification cassettes in histone biology and beyond. Nature, 425(6957), 475-9 (2003)

58. B. A. Garcia, C. M. Barber, S. B. Hake, C. Ptak, F. B. Turner, S. A. Busby, J. Shabanowitz, R. G. Moran, C. D. Allis and D. F. Hunt: Modifications of human histone H3 variants during mitosis. Biochemistry, 44(39), 13202-13 (2005)

59. W. Fischle, B. S. Tseng, H. L. Dormann, B. M. Ueberheide, B. A. Garcia, J. Shabanowitz, D. F. Hunt, H. Funabiki and C. D. Allis: Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature, 438(7071), 1116-22 (2005)

60. S. B. Hake, B. A. Garcia, M. Kauer, S. P. Baker, J. Shabanowitz, D. F. Hunt and C. D. Allis: Serine 31 phosphorylation of histone variant H3.3 is specific to regions bordering centromeres in metaphase chromosomes. Proc Natl Acad Sci U S A, 102(18), 6344-9

61. D. Bonenfant, H. Towbin, M. Coulot, P. Schindler, D. R. Mueller and J. van Oostrum: Analysis of dynamic changes in post-translational modifications of human histones during cell cycle by mass spectrometry. Mol Cell Proteomics, 6(11), 1917-32 (2007)

62. B. A. Garcia, S. Joshi, C. E. Thomas, R. K. Chitta, R. L. Diaz, S. A. Busby, P. C. Andrews, R. R. Ogorzalek Loo, J. Shabanowitz, N. L. Kelleher, C. A. Mizzen, C. D. Allis and D. F. Hunt: Comprehensive phosphoprotein analysis of linker histone H1 from Tetrahymena thermophila. Mol Cell Proteomics, 5(9), 1593-609 (2006)

63. E. McKittrick, P. R. Gafken, K. Ahmad and S. Henikoff: Histone H3.3 is enriched in covalent modifications associated with active chromatin. Proc Natl Acad Sci U S A, 101(6), 1525-30 (2004)

64. M. F. Fraga, E. Ballestar, A. Villar-Garea, M. Boix-Chornet, J. Espada, G. Schotta, T. Bonaldi, C. Haydon, S. Ropero, K. Petrie, N. G. Iyer, A. Perez-Rosado, E. Calvo, J. A. Lopez, A. Cano, M. J. Calasanz, D. Colomer, M. A. Piris, N. Ahn, A. Imhof, C. Caldas, T. Jenuwein and M. Esteller: Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet, 37(4), 391-400 (2005)

65. H. C. Beck, E. C. Nielsen, R. Matthiesen, L. H. Jensen, M. Sehested, P. Finn, M. Grauslund, A. M. Hansen and O. N. Jensen: Quantitative proteomic analysis of post-translational modifications of human histones. Mol Cell Proteomics, 5(7), 1314-25 (2006)

66. S. Tu, E. M. Bulloch, L. Yang, C. Ren, W. C. Huang, P. H. Hsu, C. H. Chen, C. L. Liao, H. M. Yu, W. S. Lo, M. A. Freitas and M. D. Tsai: Identification of histone demethylases in Saccharomyces cerevisiae. J Biol Chem, 282(19), 14262-71 (2007)

67. M. Huarte, F. Lan, T. Kim, M. W. Vaughn, M. Zaratiegui, R. A. Martienssen, S. Buratowski and Y. Shi: The fission yeast Jmj2 reverses histone H3 Lysine 4 trimethylation. J Biol Chem, 282(30), 21662-70 (2007)

68. B. Chang, Y. Chen, Y. Zhao and R. K. Bruick: JMJD6 is a histone arginine demethylase. Science, 318(5849), 444-7 (2007)

69. M. Bantscheff, M. Schirle, G. Sweetman, J. Rick and B. Kuster: Quantitative mass spectrometry in proteomics: a critical review. Anal Bioanal Chem, 389(4), 1017-31 (2007)

70. S. E. Ong, L. J. Foster and M. Mann: Mass spectrometric-based approaches in quantitative proteomics. Methods, 29(2), 124-30 (2003)

71. S. E. Ong, G. Mittler and M. Mann: Identifying and quantifying in vivo methylation sites by heavy methyl SILAC. Nat Methods, 1(2), 119-26 (2004)

72. A. R. Knapp, C. Ren, X. Su, D. M. Lucas, J. C. Byrd, M. A. Freitas and M. R. Parthun: Quantitative profiling of histone post-translational modifications by stable isotope labeling. Methods, 41(3), 312-9 (2007)

73. X. Su, L. Zhang, D. M. Lucas, M. E. Davis, A. R. Knapp, K. B. Green-Church, G. Marcucci, M. R. Parthun, J. C. Byrd and M. A. Freitas: Histone H4 acetylation dynamics determined by stable isotope labeling with amino acids in cell culture and mass spectrometry. Anal Biochem, 363(1), 22-34 (2007)

74. L. Zhang, X. Su, S. Liu, A. R. Knapp, M. R. Parthun, G. Marcucci and M. A. Freitas: Histone H4 N-terminal acetylation in Kasumi-1 cells treated with depsipeptide determined by acetic acid-urea polyacrylamide gel

electrophoresis, amino acid coded mass tagging, and mass spectrometry. J Proteome Res, 6(1), 81-8 (2007)

75. J. J. Pesavento, H. Yang, N. L. Kelleher and C. A. Mizzen: Certain and progressive methylation of histone H4 at lysine 20 during the cell cycle. Mol Cell Biol, 28(1), 468-86 (2008)

76. K. Zhang and H. Tang: Analysis of core histones by liquid chromatography-mass spectrometry and peptide mapping. J Chromatogr B Analyt Technol Biomed Life Sci, 783(1), 173-9 (2003)

77. L. Zhang, M. A. Freitas, J. Wickham, M. R. Parthun, M. I. Klisovic, G. Marcucci and J. C. Byrd: Differential expression of histone post-translational modifications in acute myeloid and chronic lymphocytic leukemia determined by high-pressure liquid chromatography and mass spectrometry. J Am Soc Mass Spectrom, 15(1), 77-86 (2004)

78. M. Naldi, V. Andrisano, J. Fiori, N. Calonghi, E. Pagnotta, C. Parolin, G. Pieraccini and L. Masotti: Histone proteins determined in a human colon cancer by high-performance liquid chromatography and mass spectrometry. J Chromatogr A, 1129(1), 73-81 (2006)

79. R. A. Zubarev, D. M. Horn, E. K. Fridriksson, N. L. Kelleher, N. A. Kruger, M. A. Lewis, B. K. Carpenter and F. W. McLafferty: Electron capture dissociation for structural characterization of multiply charged protein cations. Anal Chem, 72(3), 563-73 (2000)

80. Y. Ge, B. G. Lawhorn, M. ElNaggar, E. Strauss, J. H. Park, T. P. Begley and F. W. McLafferty: Top down characterization of larger proteins (45 kDa) by electron capture dissociation mass spectrometry. J Am Chem Soc, 124(4), 672-8 (2002)

81. J. J. Pesavento, Y. B. Kim, G. K. Taylor and N. L. Kelleher: Shotgun annotation of histone modifications: a new approach for streamlined characterization of proteins by top down mass spectrometry. J Am Chem Soc, 126(11), 3386-7 (2004)

82. L. Zhang and M. A. Freitas: Comparison of peptide mass mapping and electron capture dissociation as assays for histone posttranslational modifications. Intl J Mass Spec, 234(1-3), 213-235 (2004)

83. K. F. Medzihradszky, X. Zhang, R. J. Chalkley, S. Guan, M. A. McFarland, M. J. Chalmers, A. G. Marshall, R. L. Diaz, C. D. Allis and A. L. Burlingame: Characterization of Tetrahymena histone H2B variants and posttranslational populations by electron capture dissociation (ECD) Fourier transform ion cyclotron mass spectrometry (FT-ICR MS). Mol Cell Proteomics, 3(9), 872-86 (2004)

84. C. E. Thomas, N. L. Kelleher and C. A. Mizzen: Mass spectrometric characterization of human histone H3: a bird's eye view. J Proteome Res, 5(2), 240-7 (2006)

85. J. J. Pesavento, B. A. Garcia, J. A. Streeky, N. L. Kelleher and C. A. Mizzen: Mild performic acid oxidation enhances chromatographic and top down mass spectrometric analyses of histones. Mol Cell Proteomics, 6(9), 1510-26 (2007)

86. B. A. Garcia, N. Siuti, C.E. Thomas, C. A. Mizzen and N. L. Kelleher: Characterization of neurohistone variants and post-translational modifications by electron capture dissociation mass spectrometry. Intl J Mass Spec, 259(1-3), 184-196 (2007)

87. L. Jiang, J. N. Smith, S. L. Anderson, P. Ma, C. A. Mizzen and N. L. Kelleher: Global assessment of combinatorial post-translational modification of core histones in yeast using contemporary mass spectrometry. LYS4 trimethylation correlates with degree of acetylation on the same H3 tail. J Biol Chem, 282(38), 27923-34 (2007)

88. B. A. Garcia, J. J. Pesavento, C. A. Mizzen and N. L. Kelleher: Pervasive combinatorial modification of histone H3 in human cells. Nat Methods, 4(6), 487-9 (2007)

89. J. J. Pesavento, C. A. Mizzen and N. L. Kelleher: Quantitative analysis of modified proteins and their positional isomers by tandem mass spectrometry: human histone H4. Anal Chem, 78(13), 4271-80 (2006)

90. J. E. Syka, J. J. Coon, M. J. Schroeder, J. Shabanowitz and D. F. Hunt: Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. Proc Natl Acad Sci U S A, 101(26), 9528-33 (2004)

91. J. J. Coon, B. Ueberheide, J. E. Syka, D. D. Dryhurst, J. Ausio, J. Shabanowitz and D. F. Hunt: Protein identification using sequential ion/ion reactions and tandem mass spectrometry. Proc Natl Acad Sci U S A, 102(27), 9463-8 (2005)

92. S. D. Taverna, B. M. Ueberheide, Y. Liu, A. J. Tackett, R. L. Diaz, J. Shabanowitz, B. T. Chait, D. F. Hunt and C. D. Allis: Long-distance combinatorial linkage between methylation and acetylation on histone H3 N termini. Proc Natl Acad Sci U S A, 104(7), 2086-91 (2007)

Key Words: Proteomic, Histone, Mass Spectrometry, Post-Translational Modification, Chromatin, Review

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