Published in final edited form as:

Curr Mol Med. 2014 ; 14(9): 1164–1172.

Histone Cleavage as a Mechanism for Epigenetic Regulation: Current Insights and Perspectives

Peter Zhou¹, Erxi Wu², Hasan B. Alam¹, and Yongqing Li^{1,*}

¹Section of General Surgery, University of Michigan Hospital, Ann Arbor, MI 48109, USA

²Department of Pharmaceutical Sciences, North Dakota State University, Fargo, ND 58102, USA

Abstract

Discovered over a century ago, histones constitute one of the oldest families of proteins and have been remarkably conserved throughout eukaryotic evolution. However, only for the past 30 years have histones demonstrated that their influence extends far beyond packaging DNA. To create the various chromatin structures that are necessary for DNA function in higher eukaryotes, histones undergo post-translational modifications. While many such modifications are well documented, others, such as histone tail cleavage are less understood. Recent studies have discovered several proteases that cleave histones and have suggested roles for clipped histones in stem cell differentiation and aging in addition to infection and inflammation; the underlying mechanisms, however, are uncertain. One histone class in particular, histone H3, has received outstanding interest due to its numerous N-terminal modification sites and prevalence in regulating homeostatic processes. Here, with special consideration of H3, we will discuss the novel findings regarding histone proteolytic cleavage as well as their significance in the studies of immunology and epigenetics.

Keywords

Cleavage; histone; H3; post-translational modification

INTRODUCTION

Histones, among the most highly conserved eukaryotic proteins, order DNA into structural units called nucleosomes and are thus responsible for fundamental chromosome packing in cell nuclei [1]. There exist five major histone classes: H1, H2A, H2B, H3, and H4; H2A, H2B, H3, and H4 represent the core histones, while H1 is a linker histone [2]. Chromatin is formed when ~146 nucleotide pairs are wrapped 1.7 times around a histone octamer [3]. In constructing a nucleosome, H3-H4 and H2A-H2B dimers are formed from the binding of histone folds, and two H3-H4 dimers then combine to form an H3-H4 tetramer; the H3-H4 tetramer finally combines with two H2A-H2B dimers to create the octamer core around

*Corresponding author: Yongqing Li, MD, PhD., University of Michigan Medical School, Section of General Surgery, University of Michigan Hospital, Ann Arbor, MI 48109, USA; Tel: 734-763-0848; yqli@med.umich.edu.

CONFLICT OF INTEREST

The authors have no financial conflict of interest.

which DNA is wound [4, 5]. In this respect, for more than a century after their discovery, histones were largely dismissed as inert spools for DNA [6].

Recently, however, exciting studies have suggested novel roles for histones across various disciplines. For instance, histones have been found to play active roles in neurodegenerative disorders and cancer, and special interest has also been given to the relevance of histones in inflammatory conditions, such as sepsis [7-9]. Nevertheless, despite numerous investigations, the molecular mechanisms responsible for histone-mediated diseases remain unclear [2]. Covalent tail modifications of histones are carefully controlled and have been heavily implicated in the regulation of gene expression [10]. Thus, current research designates post-translational modifications (PTMs) of histones as highly promising targets of study.

Despite the exceptionally conserved nature of core histones, an array of PTMs that help form the different chromatin structures required for gene expression can be added to histone tails in nucleosomes [11, 12]. Modifications, including citrullination, acetylation, methylation, and phosphorylation, commonly occur on N-terminal histone tails and have important implications for the transcription, replication, and repair of nuclear DNA [13]. For instance, acetylation neutralizes the positively charged lysine residues of the histone N-terminus, thereby weakening the histone-DNA interactions, causing the nucleosomes to unfold and increasing access to transcription factors [14]. Such modifications have contributed to the histone code hypothesis, which dictates that the combinations and interactions of PTMs on histones determine both how and when the packaged DNA can be accessed [15]. While the roles of many PTMs are well established, the significance of histone tail proteolysis is relatively unclear [16-18]. Histone hydrolysis via proteolytic activity is an irreversible PTM made possible by an abundance of lysine and arginine residues, which make up more than one-fifth of the amino acids in each of the core histones [19]. Proteolysis, the breakdown of proteins into smaller polypeptides or amino acid residues, is typically achieved by enzymes known as proteases. Enzymes, as fundamental biological molecules, are responsible for homeostatic chemical interconversions, and proteolysis is known to play crucial roles in the regulation of countless cellular processes [20]. As such, modification through the proteolytic cleavage of histones is a highly active and rapidly developing topic of epigenetics [21, 22]. Herein, this review examines current findings regarding the cleavage of histones and discusses their respective significance.

HISTONE H3 CLIPPING AND ITS SIGNIFICANCE

While all core histones can be clipped, H3 cleavage has been regarded with outstanding interest. Histone H3 possesses numerous sites that are susceptible to proteolytic processing, and the N-terminal of H3 can be cleaved under many circumstances, including during the cell cycle, development, infection, mouse embryonic stem cell differentiation, aging, spermatogenesis, and sporulation [23].

H3 proteolytic cleavage sites

Just as there are many conditions under which histone H3 can be cleaved, numerous enzymes can proteolytically process H3 at various cleavage sites, where the peptide bond is

hydrolyzed by a protease enzyme. One such enzyme, cathepsin L, is a well-known cysteine protease that is largely associated with H3 clipping [24]. As shown in Fig. 1, cathepsin L, which also contributes to the development and function of the immune system, cleaves histone H3 in murine embryonic stem cells at numerous cleavage sites with Ala21-Thr22 and Lys27-Ser28 serving as the primary and final cleavage sites, respectively [25]. In addition to cathepsin L, a currently unidentified serine protease clips the H3 N-terminus at A21, R26, and A31 in human embryonic stem cells [26]. The tissue-specific histone H3-specific N-terminal cleavage enzyme identified as glutamate dehydrogenase (GDH) can also degrade H3 [27]. Using a chicken model, Mandal *et al.* [23] discovered that GDH unexpectedly cleaves histones at two sites: Lys23-Ala24 and Lys27-Ser28, indicating a propensity of this enzyme to cleave between after lysine residues. In *Saccharomyces cerevisiae*, another enzyme was found to proteolytically clip the H3 tail. While purified vacuolar protease B (Prb1) cleaves yeast histone H3 between Lys23 and Ala24 *in vitro*, *PRB1*-dependent serine endopeptidase generates a single H3 form via cleavage between Ala21 and Thr22 *in vivo* [28, 29]. Other enzymes have been reported to cleave H3 as well, such as foot-and-mouth disease virus (FMDV) protease 3C—with cleavage site Leu20-Ala21—, suggesting the existence of several protease classes that can clip the N-terminal of H3 [28, 30].

Proteolysis in embryonic stem cells

Histone H3 cleavage has been observed in both murine embryonic stem cells (ESCs) and, more recently, human ESCs. Duncan *et al.* reported an association between cathepsin L and chromatin fragments *in vivo*, which allows cathepsin L to cleave histone H3 [25]. With histone samples from differentiating murine embryonic stem cells (ESCs), a faster migrating histone species was detected with an H3 antibody against the C-terminus of H3 but not with an H3 antibody against the N-terminus, indicating that H3 was proteolytically clipped on its N-terminal. Mass spectrometry analysis revealed that the truncated form of H3 was a product of cathepsin L histone cleavage. While it is not yet clear what role cathepsin L cleavage of H3 plays in the differentiation of ESCs, it has been suggested that such cleavage enables ESCs to alter their epigenetic signature upon differentiation, implicating a significant role for cathepsin L in understanding nuclear histone H3 cleavage as a potential mode of transcriptional modulation. Moreover, cathepsin L may play roles in apoptosis and recruitment of immune cells [31]. Endotoxin treatment in rats has been shown to activate cathepsin L activity in skeletal muscle, suggesting that bacterial infection can induce the activation of cathepsin L as well [32]. Further exploring the role of histone cleavage in stem cells, Vossaert *et al.* [26] recently reported serine protease activity in human ESCs. Interestingly, the unidentified protease shares two cleavage sites with cathepsin L (A21 and R26) and incorporates an additional cleavage site at A31 [26]. Such a proteasome should be further examined and classified, however, as current understanding is lacking.

Glutamate dehydrogenase proteolytic activity

Moreover a truncated form of histone H3 was recently discovered in chicken liver. Using histones isolated from both young and old chicken liver, Mandal *et al.* [23] observed an additional protein band with a molecular weight between those of H2A and H4; the band was determined to be a proteolytically processed form of H3. Chicken liver H3 protease

(CLH3p), which behaves similarly to cysteine proteases, was found to be a completely novel histone H3-specific protease that cleaves H3 at the N-terminus. After additional extensive experimentation and characterization, CLH3p was identified as glutamate dehydrogenase (GDH) [27]. Given that clipped H3 was observed in histone samples isolated from adult chicken livers and remained absent in those from young chickens, GDH activity has potentially significant implications – histone cleavage in adult chicken livers may correspond to clipped H3-mediated gene expression in humans and could help in understanding the mechanisms behind the regulation of aging and age-associated diseases [23].

Yeast endopeptidase proteolytic activity

Using a *S. cerevisiae* model, Santos-Rosa *et al.* [28] reported a protease activity that was induced under conditions of nutrient deprivation and sporulation with a recognition site of Gln19-Leu20-Ala21. The endopeptidase activity is regulated during the activation of different pathways leading to gene expression, and as elaborated by Xue *et al.* [29], Prb1 is responsible for most H3 N-terminus cleavage events in the yeast stationary phase. Although purified protein samples of Prb1 were reported to cleave histone H3 at Lys23-Ala24 exclusively *in vitro*, *PRB1* is still required for the nuclear H3 endopeptidase activity observed *in vivo* [29]. Similar to cathepsin L activity, yeast endopeptidase cleavage of H3 occurred in a PTM-mediated manner. Trimethylation at K4 inhibited clipping, while repressive dimethylation at R2 (Fig. 1) did not, indicating that repressive protein complexes bound to the H3 tail at promoter regions are cleared to allow activator protein complexes to take over during the induction process. Therefore, histone H3 cleavage may be viewed as a histone PTM that removes repressive marks, permitting correct induction of gene expression. Thus, yeast endopeptidase histone H3 cleavage may ultimately shed light on not only transcription, but the overall homeostatic consequences of H3 tail proteolysis as well [28].

Foot-and-mouth disease virus protease 3C proteolytic activity

Among the first instances of histone H3 cleavage in infection and disease was reported by Falk *et al.* [30]. Cleavage of histone H3 by FMDV 3C protease during infection may explain the presence of picornaviral 3C protease or 3C precursor proteins in the nucleus of FMDV-infected cells [30]. In addition, previous studies have suggested that the inhibition of cellular RNA synthesis after viral infection may be the result of the general blocking of DNA expression by histones [33]. Thus, FMDV 3C protease clipping of H3 seems to be a mechanism for shutting off host cell transcription [34]. More importantly, however, FMDV-mediated histone H3 cleavage may effectively contribute to the virtually global breakdown of host cell functions during viral infection [30].

Histone H2A specific protease proteolytic activity

While analyzing total histones on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Panda *et al.* [35] observed selective degradation of histone H2A with chicken liver histones. Through bulk purification and *in vitro* reactions with other histones (H5, H2B, H3, and H4) as controls, histone H2A specific protease (H2Asp) was discovered and confirmed to be H2A-specific as well as an aspartic acid protease with a

cleavage site of Asn90-Asp91 towards the C-terminus. While the physiological role of H2Asp remains unclear, it has been suggested that H2Asp activity was age-independent and tissue-specific, as H2Asp activity was demonstrated exclusively in liver nuclear extracts of both young and adult chickens; however H2Asp activity was not restricted to only chicken but was also observed in the liver nuclear extracts from fish, frog, lizard, and mice, suggesting generality among vertebrates. For future investigations, sequencing of H2Asp and the H2A clipped products would be warranted to unravel the mechanism of H2A proteolysis by H2Asp and also to assign a concrete physiological relevance to H2Asp [35].

Histone H3 PTM crosstalk

While the mechanisms required to activate histone H3 proteolysis are currently unknown, there exist several theories; of special interest is the proposal that histone PTMs can affect nearby tail modifications [36, 37]. Inherent crosstalk from multisite modifications has been observed to coordinate both intermolecular and intramolecular signaling for regulation of protein function [38]. Interestingly, H3 tail modifications can modulate cathepsin L activity [24]. Duncan and colleagues investigated effects of acetylation and methylation on cleavage activity of cathepsin L [25]. They prepared four different recombinant H3 (rH3) substrates: unmodified rH3, rH3 dimethylated at K27me2, rH3 “pan-acetylated” by treatment with acetic anhydride, and rH3 with both K27me2 and pan-acetylation. Acetylation of lysine residues greatly reduced cleavage of H3 by recombinant cathepsin L. In contrast, K27me2 increased H3 cleavage. Dimethylation at K27 and acetylation at K18 increased H3 cleavage, while acetylation at K23 reduced proteolytic activity [25]. In addition to the previously discussed crosstalk that occurs with the yeast endopeptidase, binding of heterochromatin protein 1 (HP1) to H3K9me2/3 is disrupted by phosphorylation of H3S10, and binding of spChp1 to H3K9me2/3 is inhibited by H3K4 acetylation in *Schizosaccharomyces pombe* [39]. Furthermore, in *S. cerevisiae*, phosphorylation of H3S10 promotes acetylation of H3K14, and acetylation of both H3K18 and H3K23 facilitates the methylation of H3R17 [22]. A recent study by Qian *et al.* [40] reports that the proteasome activator PA200 and its ortholog in yeast, Blm10, catalyze the polyubiquitin-independent degradation of histones during spermatogenesis. As reported, histone acetylation was required for the binding and activation of such proteases, but other uncharacterized histone modifications are likely required as well. Thus, PA200 and Blm10 specifically target the core histones for acetylation-mediated degradation by proteasomes, providing mechanisms by which acetylation regulates histone degradation, DNA repair, and spermatogenesis [40]. While Duncan *et al.* [25] discovered that K18 acetylation facilitates H3 cleavage, they also reported that K23 acetylation decreased cathepsin L proteolytic activity, and combined acetylation of both K18 and K23 resulted in further reduced histone tail clipping. However, since such observations were made *in vitro*, the exact effects of acetylation on histone cleavage have not yet been reported with *in vivo* factors considered. Therefore, the overall effect of tail acetylation on histone cleavage in histone N-terminal domains serves as a noteworthy point of discussion in regards to tail modification crosstalk. Additionally, a study conducted by Nurse *et al.* [41] reported a synergistic effect of H3 and H4 tail truncation. Namely, when H3 and H4 tails were simultaneously clipped, a more dramatic effect on the nucleosome conformation and DNA breathing motion was observed than when only H3 or H4 tails were clipped separately [41]. Such a discovery suggests that histone PTM crosstalk not only can

occur between nearby modifications on a given N-terminus, but also between modifications on neighboring histones that constitute the nucleosome. Thus, there exists an incredibly nuanced coordination between PTM crosstalk and histone cleavage, one that we are just beginning to explore.

Since the study by Allis *et al.* [6], which is regarded as the first to have observed proteolysis of micronuclear histone H3, interest in H3 clipping has increased considerably, and, over three decades later, exciting discoveries have been reported that help provide clarity on the subject of clipped histone H3-mediated gene expression in epigenetic modification and innate immunity [42]. Nevertheless, however, pressing questions remain unanswered regarding the mechanisms of histone H3 cleavage and their subsequent roles in human homeostasis.

INFLAMMATORY ROLES OF CORE HISTONE CLEAVAGE

Histones have been widely implicated in areas of research, including cell cycle, cell differentiation, and aging. For instance histone exchange of both H2A/H2B and H3/H4 occurs during transcription, significant losses of core histones have been observed during replicative aging in yeast, and the phosphorylation of H3 on Ser10 has been acknowledged as a useful epigenetic mitotic marker [43]. Nevertheless the significance of histones in inflammation is less commonly known and will thus herein be the focus of this review.

Recently, an important role for histones has been implicated in inflammatory diseases [44]. During an immune response, neutrophils can extracellularly release neutrophil extracellular traps (NETs) to entrap microbes [45, 46]. However, histones found in NETs are also cytotoxic to tissue; consequently, rapid and robust NET formation is capable of inducing a pro-inflammatory cytokine storm [47, 48]. In addition, antibodies used to block extracellular histones could rescue animals from LPS-mediated death, indicating that histones released in response to bacterial challenge mediate endothelial dysfunction, organ failure, and death during sepsis [49]. Citrullinated histone H3 has been shown to be a novel biomarker of disease progression and therapeutic target in sepsis [50]. The release of histones during the late stages of sepsis can amplify inflammation, coagulation, cell death, and multiple organ dysfunction syndrome [51]. The search for viable therapeutic interventions has led researchers to discover anti-inflammatory effects of histone deacetylase (HDAC) inhibitors (HDACIs), such as suberoylanilide hydroxamic acid (SAHA), valproic acid, and trichostatin A (TSA) [52-54]. In addition to HDACIs, proteolytic enzymes have attracted considerable attention. Some proteases can cleave or degrade histones to abrogate inflammation, while others may promote inflammation by clipping histones [55, 56].

Activated protein C histone cleavage

In a breakthrough study by Xu *et al.* [55], activated protein C (APC) was reported to effectively cleave histones in a dose-dependent fashion and reduce their cytotoxicity. In the presence of APC, three new protein bands of 10kDa, 13kDa, and 15kDa were discovered after SDS-PAGE. Sequencing of the bands revealed that the 10-kDa protein was mouse histone H4 with internal sequence methyl-Lys20-Ile34. Furthermore, the 13-kDa protein was identified as mouse H3 internal sequence Lys27-Lys36, and the 15-kDa protein was matched

with three peptide sequences, Ala21-Arg29, His82-Arg88, and Val100-Lys118 of mouse histone H2A, after in-gel tryptic digestion. As a result, infusion of APC after baboons were challenged with a lethal dose of *Escherichia coli* rescued the baboons, further indicating the cytoprotective nature of APC. A recent study using *Plasmodium falciparum* also studied the proteolytic properties of APC. Recombinant human APC was found to cleave both *P. falciparum* histones extracted from merozoites (HeH) and recombinant *P. falciparum* H3 (PfH3), abrogating their pro-inflammatory effects [57]. Consequently, APC is currently in clinical trial for the treatment of sepsis and may develop into a potential therapeutic intervention for inflammatory diseases [58].

Factor VII activating protease histone cleavage

Factor VII activating protease (FSAP), also known as plasma hyaluronan-binding protein (PHBP), can activate coagulation factor VII [59]. Pro-PHBP, which is the 70 KD proenzyme form of FSAP, is activated *in vivo* under inflammatory conditions [60]. In addition, extracellular histones have been described as endogenous factors that promote pro-PHBP activation in plasma, thereby inducing FSAP activation. However, more importantly, pro-PHBP activation triggers histone degradation, suggesting that histones serve as both effectors for pro-PHBP activation and substrates in plasma [61]. The significance of pro-PHBP activation should be carefully considered, as proteolytic activity of PHBP is suggested to play roles in the regulation of hemostasis, vascular function, cell proliferation, and inflammation [62]. Cleavage via pro-PHBP may abrogate the detrimental effects of histones, and histone-mediated pro-PHBP activation results in bradykinin formation, which has also been implicated in septic shock [63].

Neutrophil elastase histone cleavage

Neutrophil elastase (NE) is a neutrophil-specific protease that is required for NET formation [64]. In resting neutrophils, NE and myeloperoxidase (MPO) are located in azurophilic granules, but, upon reactive oxygen species production, NE is activated and escapes the granules by translocating to the nucleus, where it cleaves histones and facilitates chromatin decondensation. MPO then interacts with chromatin in the late stages of the process and promotes further decondensation. NE and MPO thus synergistically enhance chromatin decondensation, prompting cell rupture and NET release [56]. In fact, a peak in histone degradation was detected during NET formation, and NE was found to degrade histone H4 completely, while H2A, H2B, and H3 were partially processed. Papayannopoulos *et al.* [56] also observed several shifts in the mobility of histone H3 on the western blot and indicated that histone citrullination may be involved. Citrullination of H3 has been previously suggested to increase mobility of the H3 protein bands, so citrullination might be necessary for NE activity [65]. Inhibition of peptidylarginine deiminase 4 (PAD4) activity has been reported to decrease the formation of NETs, while PAD4 treatment facilitated NET formation and chromatin decondensation, suggesting a potentially critical role for PAD4 in mediating NE activity [66]. While the role of PAD4 in NET formation has not been fully addressed, it is an interesting possibility nonetheless that histone PTMs may play a role in regulating NET formation by modulating the ability of NE to cleave histones [56]. Thus clipped histones are extracellularly released with NETs via NE proteolytic activity that may potentially be mediated by PTM crosstalk. Recent study by Li *et al.* [50] showed that

bacterial endotoxin stimulates expression and secretion of citrullinated H3 and increases cleavage of H3 in neutrophilic HL-60 cells. However, acetylation of histones by SAHA treatment attenuates such alterations. Currently, the role of cleaved H3 in the pathogenesis of sepsis remains uncertain.

Potential mechanisms for clipped histone-mediated inflammation

While the cleavage of histones has been found to reduce cytotoxicity against the host during sepsis, it may also exacerbate histone cytotoxicity. It has been previously reported that NE cleaves histones during NET formation, but it is also known that NETs are capable of inducing thrombus formation *in vitro* and tissue damage [48, 51, 56]. From such evidence, there exist two potential modes that may explain the pro-inflammatory effects of cleaved histones. One explanation is that histone tail proteolysis occurring in the nucleus promotes the decondensation of chromatin, thereby facilitating pro-inflammatory gene transcription (Fig. 2A). Proteolytic tail cleavage has been reported to increase the accessibility of selected transcription factors to nucleosomal DNA *in vitro* [18, 67, 68]. A previous study first reported that nucleosomal DNA is less accessible to transcription factors in the presence of histone tails and observed an enhanced GAL4-AH binding affinity following the removal of amino-terminal tails [67]. Furthermore, using western blot analysis, Godde *et al.* [68] revealed that TATA binding protein (TBP), when accompanied by transcription factor IIA (TFIIA), only binds to the TATA box in the nucleosome when N-terminal tails have been cleaved. Vitolo *et al.* [18] also reported, through western blot, that cleavage of H3-H4 N-terminal domains results in high-affinity binding of transcription factor IIIA (TFIIIA) to DNA. Therefore, it is possible that histone tail clipping facilitates pro-inflammatory gene expression. Another supported proposal is that, when neutrophils create and release NETs, NE cleaves histones, and the clipped histone products are subsequently released into extracellular medium. The circulating histone fragments may then bind to toll like receptor 4 (TLR4) found on other cells upon contact, initiating a hyper-inflammatory response via signaling cascade (Fig. 2B). Therefore, there are two proposals—one intracellular and one extracellular—that may effectively describe the mechanisms responsible for inflammation mediated by histone cleavage. Since APC activity abrogates histone cytotoxicity, while NE cleavage may enhance it, there seem to be distinct differences between clipped histones cleaved by various enzymes, possibly due to inherent differences in cleavage sites among the proteases. Thus, cleaved histones, which are capable of both increasing and decreasing inflammation, seem to possess a double-edged effect that is not well understood and warrants further clarification [47].

Nevertheless, therapeutic interventions centered on histone cleavage have promising potential. APC is a well-known therapeutic agent and became the first drug approved by the FDA to treat severe sepsis [69]. However the observed anti-inflammatory effects of APC seem to be limited to patients exhibiting the most severe symptoms [70]. Interestingly, citrullinated histone H3 has been observed in high levels in serum of mice challenged with a high dose of LPS, whereas it was undetectable in serum of mice given a low dose of LPS [50]. Therefore, there seems to be an undetermined connection between APC histone degradation and citrullinated H3 in the severe stages of sepsis. In addition to the previously proposed role of histone citrullination in NE activity, it is possible that this relationship

between APC activity and citrullinated H3 is yet another example of the potential role of arginine citrullination in mediating histone cleavage. Thus, there is need for additional studies investigating APC as a therapeutic agent. Similarly, development of NE and pro-PHBP antagonists might have therapeutic consequences and deserves additional consideration [60, 71, 72].

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The relevance of processed histones to gene expression is a highly intriguing topic, and the presented review discusses the established novel findings pertinent to histone cleavage. Such developments create a foundation on which future investigations can work to more precisely detail the complex mechanisms by which histone proteolysis regulates gene transcription; these mechanisms can be further examined to gain critical insight into epigenetic regulation and possibly create effective therapeutics for various diseases [73].

On the whole it may be important to note that many of the discussed studies were performed *in vitro*, so the true *in vivo* function of histone cleavage has yet to be fully revealed. Additionally, as many proteases have been associated with histone clipping, it is possible that such proteasomes may also possess an outside function independent of histone cleavage that could have contributed, in part, to the observed findings; thus more direct evidence accounting for such a variable may be needed. Nevertheless, outstanding discoveries have set the basis for exploring the newly-acknowledged influence of histone cleavage on epigenetic regulation.

Histone degradation, especially that of H3, has been found to play a critical role in a variety of life processes, including aging, cell differentiation, and infection [23]. Histone H3 clipping is a key factor in the regulation of gene expression, and histone cleavage seems to reduce cytotoxicity in some cases, while facilitating it in others [28, 55]. As a result, versatile and effective therapeutic agents centered on the degradation of histones may be designed in the future to improve survival in inflammatory conditions, such as sepsis [74].

However, despite the increased interest surrounding histone cleavage, there is still much that remains unknown. Additional proteases may be found for histone cleavage, and new functions of clipped histones may be uncovered [22]. Moreover, the detailed molecular mechanisms of histone clipping and their direct implications to epigenetics can be further elucidated [75].

Recent discoveries have collectively made substantial progress in exploring the exciting possibilities surrounding histone cleavage. As a result, several novel questions have been raised. How does complex interplay between acetylation, methylation, citrullination, and cleavage modifications translate the “histone code” to effectively regulate gene expression? How can the therapeutic properties of some histone-clipping proteases, such as APC, be harnessed, while the cytotoxic effects of other enzymes, such as NE, can be targeted? Can the seemingly inherent double-edged nature of clipped histones be reconciled? Nevertheless, with such dramatic discoveries already made that investigate the influence of histone PTMs, the future of epigenetics shows incredible promise.

ACKNOWLEDGEMENTS

This work was supported by NIH R01 GM084127 (to HBA) and USAMRAA W81XWH-09-2-0001 (to YL).

ABBREVIATIONS

APC	Activated protein C
ESCs	Embryonic stem cells
FSAP	Factor VII activating protease
FMDV	Foot-and-mouth disease virus
GDH	Glutamate dehydrogenase
H2Asp	H2A specific protease
HP1	Heterochromatin protein 1
HDAC	Histone deacetylase
HDACIs	Histone deacetylase inhibitors
PHBP	Hyaluronan-binding protein
MPO	Myeloperoxidase
NE	Neutrophil elastase
NETs	Neutrophil extracellular traps
PAD4	Peptidylarginine deiminase 4
PTMs	Post-translational modifications
rH3	Recombinant H3
SDS-PAG	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SAHA	Suberoylanilide hydroxamic acid
TBP	TATA binding protein
TLR4	Toll like receptor 4
TFIIA	Transcription factor IIA
TFIIIA	Transcription factor IIIA
TSA	Trichostatin A
Prb1	Vacuolar protease B

REFERENCES

- [1]. Morgan BA, Mittman BA, Smith MM. The highly conserved N-terminal domains of histones H3 and H4 are required for normal cell cycle progression. *Mol Cell Biol.* 1991; 11:4111–20. [PubMed: 2072911]
- [2]. Parseghian MH, Luhrs KA. Beyond the walls of the nucleus: the role of histones in cellular signaling and innate immunity. *Biochem Cell Biol.* 2006; 84:589–604. [PubMed: 16936831]

- [3]. Davey CA, Sargent DF, Luger K, Maeder AW, Richmond TJ. Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution. *J Mol Biol.* 2002; 319:1097–113. [PubMed: 12079350]
- [4]. Bohm V, Hieb AR, Andrews AJ, et al. Nucleosome accessibility governed by the dimer/tetramer interface. *Nucleic Acids Res.* 2011; 39:3093–102. [PubMed: 21177647]
- [5]. Giri S, Prasanth SG. Replicating and transcribing on twisted roads of chromatin. *Brief Funct Genomics.* 2012; 11:188–204. [PubMed: 22267489]
- [6]. Allis CD, Bowen JK, Abraham GN, Glover CV, Gorovsky MA. Proteolytic processing of histone H3 in chromatin: a physiologically regulated event in *Tetrahymena* micronuclei. *Cell.* 1980; 20:55–64. [PubMed: 6993010]
- [7]. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet.* 2007; 8:286–98. [PubMed: 17339880]
- [8]. Soragni E, Xu C, Cooper A, Plasterer HL, Rusche JR, Gottesfeld JM. Evaluation of histone deacetylase inhibitors as therapeutics for neurodegenerative diseases. *Methods Mol Biol.* 2011; 793:495–508. [PubMed: 21913121]
- [9]. Allam R, Scherbaum CR, Darisipudi MN, et al. Histones from dying renal cells aggravate kidney injury via TLR2 and TLR4. *J Am Soc Nephrol.* 2012; 23:1375–88. [PubMed: 22677551]
- [10]. Anderson DC, Green GR, Smith K, Selker EU. Extensive and varied modifications in histone H2B of wild-type and histone deacetylase 1 mutant *Neurospora crassa*. *Biochemistry.* 2010; 49:5244–57. [PubMed: 20462202]
- [11]. Kouzarides T. Chromatin modifications and their function. *Cell.* 2007; 128:693–705. [PubMed: 17320507]
- [12]. Zhang K, Sridhar VV, Zhu J, Kapoor A, Zhu JK. Distinctive core histone post-translational modification patterns in *Arabidopsis thaliana*. *PLoS One.* 2007; 2:e1210. [PubMed: 18030344]
- [13]. Bhaumik SR, Smith E, Shilatfard A. Covalent modifications of histones during development and disease pathogenesis. *Nat Struct Mol Biol.* 2007; 14:1008–16. [PubMed: 17984963]
- [14]. Grunstein M. Histone acetylation in chromatin structure and transcription. *Nature.* 1997; 389:349–52. [PubMed: 9311776]
- [15]. Cosgrove MS, Wolberger C. How does the histone code work? *Biochem Cell Biol.* 2005; 83:468–76. [PubMed: 16094450]
- [16]. Allis CD, Wiggins JC. Proteolytic processing of micronuclear H3 and histone phosphorylation during conjugation in *Tetrahymena thermophila*. *Exp Cell Res.* 1984; 153:287–98. [PubMed: 6734746]
- [17]. Lin R, Cook RG, Allis CD. Proteolytic removal of core histone amino termini and dephosphorylation of histone H1 correlate with the formation of condensed chromatin and transcriptional silencing during *Tetrahymena* macronuclear development. *Genes Dev.* 1991; 5:1601–10. [PubMed: 1885002]
- [18]. Vitolo JM, Thiriet C, Hayes JJ. The H3-H4 N-terminal tail domains are the primary mediators of transcription factor IIIA access to 5S DNA within a nucleosome. *Mol Cell Biol.* 2000; 20:2167–75. [PubMed: 10688663]
- [19]. Smith BC, Denu JM. Chemical mechanisms of histone lysine and arginine modifications. *Biochim Biophys Acta.* 2009; 1789:45–57. [PubMed: 18603028]
- [20]. Gunjan A, Paik J, Verreault A. The emergence of regulated histone proteolysis. *Curr Opin Genet Dev.* 2006; 16:112–8. [PubMed: 16510276]
- [21]. Bertin A, Renouard M, Pedersen JS, Livolant F, Durand D. H3 and H4 histone tails play a central role in the interactions of recombinant NCPs. *Biophys J.* 2007; 92:2633–45. [PubMed: 17237203]
- [22]. Suganuma T, Workman JL. Crosstalk among Histone Modifications. *Cell.* 2008; 135:604–7. [PubMed: 19013272]
- [23]. Mandal P, Azad GK, Tomar RS. Identification of a novel histone H3 specific protease activity in nuclei of chicken liver. *Biochem Biophys Res Commun.* 2012; 421:261–7. [PubMed: 22503985]
- [24]. Adams-Cioaba MA, Krupa JC, Xu C, Mort JS, Min J. Structural basis for the recognition and cleavage of histone H3 by cathepsin L. *Nat Commun.* 2011; 2:197. [PubMed: 21326229]

- [25]. Duncan EM, Muratore-Schroeder TL, Cook RG, et al. Cathepsin L proteolytically processes histone H3 during mouse embryonic stem cell differentiation. *Cell*. 2008; 135:284–94. [PubMed: 18957203]
- [26]. Vossaert L, Meert P, Scheerlinck E, et al. Identification of histone H3 clipping activity in human embryonic stem cells. *Stem Cell Res*. 2014; 13:123–34. [PubMed: 24874291]
- [27]. Mandal P, Verma N, Chauhan S, Tomar RS. Unexpected histone H3 tail-clipping activity of glutamate dehydrogenase. *J Biol Chem*. 2013; 288:18743–57. [PubMed: 23673664]
- [28]. Santos-Rosa H, Kirmizis A, Nelson C, et al. Histone H3 tail clipping regulates gene expression. *Nat Struct Mol Biol*. 2009; 16:17–22. [PubMed: 19079264]
- [29]. Xue Y, Vashisht AA, Tan Y, Su T, Wohlschlegel JA. PRB1 is required for clipping of the histone H3 N terminal tail in *Saccharomyces cerevisiae*. *PLoS One*. 2014; 9:e90496. [PubMed: 24587380]
- [30]. Falk MM, Grigera PR, Bergmann IE, Zibert A, Multhaupt G, Beck E. Foot-and-mouth disease virus protease 3C induces specific proteolytic cleavage of host cell histone H3. *J Virol*. 1990; 64:748–56. [PubMed: 2153239]
- [31]. Sriraman V, Richards JS. Cathepsin L gene expression and promoter activation in rodent granulosa cells. *Endocrinology*. 2004; 145:582–91. [PubMed: 14563703]
- [32]. Kovarik M, Muthny T, Sispera L, Holecek M. Effects of beta-hydroxy-beta-methylbutyrate treatment in different types of skeletal muscle of intact and septic rats. *J Physiol Biochem*. 2010; 66:311–9. [PubMed: 20725872]
- [33]. Brown F, Martin SJ, Underwood B. A study of the kinetics of protein and RNA synthesis induced by foot-and-mouth disease virus. *Biochim Biophys Acta*. 1966; 129:166–77. [PubMed: 4291133]
- [34]. Tesar M, Marquardt O. Foot-and-mouth disease virus protease 3C inhibits cellular transcription and mediates cleavage of histone H3. *Virology*. 1990; 174:364–74. [PubMed: 2154880]
- [35]. Panda P, Chaturvedi MM, Panda AK, Suar M, Purohit JS. Purification and characterization of a novel histone H2A specific protease (H2Asp) from chicken liver nuclear extract. *Gene*. 2013; 512:47–54. [PubMed: 23041126]
- [36]. Nightingale KP, Gendreizig S, White DA, Bradbury C, Hollfelder F, Turner BM. Cross-talk between histone modifications in response to histone deacetylase inhibitors: MLL4 links histone H3 acetylation and histone H3K4 methylation. *J Biol Chem*. 2007; 282:4408–16. [PubMed: 17166833]
- [37]. Dieker J, Muller S. Epigenetic histone code and autoimmunity. *Clin Rev Allergy Immunol*. 2010; 39:78–84. [PubMed: 19662539]
- [38]. Yang XJ. Multisite protein modification and intramolecular signaling. *Oncogene*. 2005; 24:1653–62. [PubMed: 15744326]
- [39]. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res*. 2011; 21:381–95. [PubMed: 21321607]
- [40]. Qian MX, Pang Y, Liu CH, et al. Acetylation-mediated proteasomal degradation of core histones during DNA repair and spermatogenesis. *Cell*. 2013; 153:1012–24. [PubMed: 23706739]
- [41]. Nurse NP, Jimenez-Useche I, Smith IT, Yuan C. Clipping of flexible tails of histones H3 and H4 affects the structure and dynamics of the nucleosome. *Biophys J*. 2013; 104:1081–8. [PubMed: 23473491]
- [42]. Portela A, Esteller M. Epigenetic modifications and human disease. *Nat Biotechnol*. 2010; 28:1057–68. [PubMed: 20944598]
- [43]. Das C, Tyler JK. Histone exchange and histone modifications during transcription and aging. *Biochim Biophys Acta*. 2013; 1819:332–42.
- [44]. Xu J, Lupu F, Esmon CT. Inflammation, innate immunity and blood coagulation. *Hamostaseologie*. 2010; 30:5–6. 8–9. [PubMed: 20162248]
- [45]. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004; 303:1532–5. [PubMed: 15001782]
- [46]. Urban CF, Ermer D, Schmid M, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathog*. 2009; 5:e1000639. [PubMed: 19876394]

- [47]. Fuchs TA, Bhandari AA, Wagner DD. Histones induce rapid and profound thrombocytopenia in mice. *Blood*. 2011; 118:3708–14. [PubMed: 21700775]
- [48]. Saffarzadeh M, Juenemann C, Queisser MA, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. *PLoS One*. 2012; 7:e32366. [PubMed: 22389696]
- [49]. Xu J, Zhang X, Monestier M, Esmon NL, Esmon CT. Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury. *J Immunol*. 2011; 187:2626–31. [PubMed: 21784973]
- [50]. Li Y, Liu B, Fukudome EY, et al. Identification of citrullinated histone H3 as a potential serum protein biomarker in a lethal model of lipopolysaccharide-induced shock. *Surgery*. 2011; 150:442–51. [PubMed: 21878229]
- [51]. Semeraro N, Ammollo CT, Semeraro F, Colucci M. Sepsis, thrombosis and organ dysfunction. *Thromb Res*. 2012; 129:290–5. [PubMed: 22061311]
- [52]. Li Y, Alam HB. Modulation of acetylation: creating a pro-survival and anti-inflammatory phenotype in lethal hemorrhagic and septic shock. *J Biomed Biotechnol*. 2011; 2011:523481. [PubMed: 21403879]
- [53]. Chong W, Li Y, Liu B, et al. Anti-inflammatory properties of histone deacetylase inhibitors: a mechanistic study. *J Trauma Acute Care Surg*. 2012; 72:347–53. discussion 353-4. [PubMed: 22327976]
- [54]. Licciardi PV, Karagiannis TC. Regulation of immune responses by histone deacetylase inhibitors. *ISRN Hematol*. 2012; 2012:690901. [PubMed: 22461998]
- [55]. Xu J, Zhang X, Pelayo R, et al. Extracellular histones are major mediators of death in sepsis. *Nat Med*. 2009; 15:1318–21. [PubMed: 19855397]
- [56]. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol*. 2010; 191:677–91. [PubMed: 20974816]
- [57]. Gillrie MR, Lee K, Gowda DC, et al. Plasmodium falciparum histones induce endothelial proinflammatory response and barrier dysfunction. *Am J Pathol*. 2012; 180:1028–39. [PubMed: 22260922]
- [58]. Shimaoka M, Park EJ. Advances in understanding sepsis. *Eur J Anaesthesiol Suppl*. 2008; 42:146–53. [PubMed: 18289433]
- [59]. Romisch J. Factor VII activating protease (FSAP): a novel protease in hemostasis. *Biol Chem*. 2002; 383:1119–24. [PubMed: 12437095]
- [60]. Wygrecka M, Morty RE, Markart P, et al. Plasminogen activator inhibitor-1 is an inhibitor of factor VII-activating protease in patients with acute respiratory distress syndrome. *J Biol Chem*. 2007; 282:21671–82. [PubMed: 17540775]
- [61]. Yamamichi S, Fujiwara Y, Kikuchi T, Nishitani M, Matsushita Y, Hasumi K. Extracellular histone induces plasma hyaluronan-binding protein (factor VII activating protease) activation in vivo. *Biochem Biophys Res Commun*. 2011; 409:483–8. [PubMed: 21600885]
- [62]. Stephan F, Aarden LA, Zeerleder S. FSAP, a new player in inflammation? *Hamostaseologie*. 2012; 32:51–5. [PubMed: 22252569]
- [63]. Golias C, Charalabopoulos A, Stagikas D, Charalabopoulos K, Batistatou A. The kinin system--bradykinin: biological effects and clinical implications. Multiple role of the kinin system--bradykinin. *Hippokratia*. 2007; 11:124–8. [PubMed: 19582206]
- [64]. Papayannopoulos V, Staab D, Zychlinsky A. Neutrophil elastase enhances sputum solubilization in cystic fibrosis patients receiving DNase therapy. *PLoS One*. 2011; 6:e28526. [PubMed: 22174830]
- [65]. Wang Y, Wysocka J, Sayegh J, et al. Human PAD4 regulates histone arginine methylation levels via demethylation. *Science*. 2004; 306:279–83. [PubMed: 15345777]
- [66]. Wang Y, Li M, Stadler S, et al. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J Cell Biol*. 2009; 184:205–13. [PubMed: 19153223]
- [67]. Vettese-Dadey M, Walter P, Chen H, Juan LJ, Workman JL. Role of the histone amino termini in facilitated binding of a transcription factor, GAL4-AH, to nucleosome cores. *Mol Cell Biol*. 1994; 14:970–81. [PubMed: 8289837]

- [68]. Godde JS, Nakatani Y, Wolffe AP. The amino-terminal tails of the core histones and the translational position of the TATA box determine TBP/TFIIA association with nucleosomal DNA. *Nucleic Acids Res.* 1995; 23:4557–64. [PubMed: 8524642]
- [69]. Sarangi PP, Lee HW, Kim M. Activated protein C action in inflammation. *Br J Haematol.* 2010; 148:817–33. [PubMed: 19995397]
- [70]. Toltl LJ, Austin RC, Liaw PC. Activated protein C modulates inflammation, apoptosis and tissue factor procoagulant activity by regulating endoplasmic reticulum calcium depletion in blood monocytes. *J Thromb Haemost.* 2011; 9:582–92. [PubMed: 21166994]
- [71]. Hayakawa M, Katabami K, Wada T, et al. Sivelestat (selective neutrophil elastase inhibitor) improves the mortality rate of sepsis associated with both acute respiratory distress syndrome and disseminated intravascular coagulation patients. *Shock.* 2010; 33:14–8. [PubMed: 19487982]
- [72]. Suda K, Takeuchi H, Hagiwara T, et al. Neutrophil elastase inhibitor improves survival of rats with clinically relevant sepsis. *Shock.* 2010; 33:526–31. [PubMed: 19953005]
- [73]. Ullrich O, Reinheckel T, Sitte N, Hass R, Grune T, Davies KJ. Poly-ADP ribose polymerase activates nuclear proteasome to degrade oxidatively damaged histones. *Proc Natl Acad Sci U S A.* 1999; 96:6223–8. [PubMed: 10339569]
- [74]. Zhang D, Pasternack MS, Beresford PJ, Wagner L, Greenberg AH, Lieberman J. Induction of rapid histone degradation by the cytotoxic T lymphocyte protease Granzyme A. *J Biol Chem.* 2001; 276:3683–90. [PubMed: 11060286]
- [75]. Berger SL. The complex language of chromatin regulation during transcription. *Nature.* 2007; 447:407–12. [PubMed: 17522673]

Figure 1. Histone H3 tail cleavage sites for different proteases and crosstalk between histone PTMs

N-terminal sequence of histone H3 displaying sites of citrullination, acetylation, methylation, and cleavage with the respective proteasomes involved. Neighboring modifications may disrupt protein binding to a particular modification. hESCp, human embryonic stem cell protease; GDH, glutamate dehydrogenase; Prb1, vacuolar protease B; FMDVp 3C, Foot-and-mouth disease virus protease 3C.

Figure 2. Clipped histones may induce hyper-inflammatory response

(A) Proteolytic tail cleavage induces chromatin decondensation, which allows for increased transcription factor activity, thereby promoting the transcription of pro-inflammatory regulators (TNF- α , IL-1, IL-6). (B) Schematic representation illustrating a potential pro-inflammatory mechanism that is mediated by histone cleavage. Upon inflammatory stimulation, neutrophils release NETs to combat infection. During NET formation, histones are cleaved by NE and are extracellularly released. Circulating clipped histones then migrate and bind to toll-like receptor 4 (TLR4) on other cells, initiating a pro-inflammatory signaling cascade.

Table 1
Characterization of the various observed histone proteases

An overview of the class of each protease as well as the respective cleavage site(s), the target histone class, and the biological model in which proteolytic activity has been reported. The specific physiological implications of histone clipping are largely undetermined.

Proteinase	Biological Significance of Activity	Cleavage Site(s)	Protease Class	Histone Specificity	Model (Study)
Cathepsin L	Embryonic stem cell (ESC) differentiation	Ala21-Thr22, Thr22-Lys23, Lys23-Ala24, Ala24-Ala25, Arg26-Lys27, Lys27-Ser28	Cysteine	H3	Mouse ESCs [25]
Human ESC protease	ESC differentiation	Ala21-Thr22, Arg26-Lys27, Ala31-Thr32	Serine	H3	Human ESCs [26]
Glutamate dehydrogenase (GDH)	Unclear chromatin modifier	Lys23-Ala24, Lys27-Ser28	Cysteine	H3	Chicken liver extracts [27]
Yeast endopeptidase	Induced under nutrient deprivation and sporulation	Ala21-Thr22	Serine	H3	<i>S. cerevisiae</i> [28]
Vacuolar protease B (Prb1)	Required for <i>in vivo</i> nuclear protease activity in yeast	Lys23-Ala24	Serine	H3	<i>S. cerevisiae</i> [29]
Foot-and-mouth disease virus 3C protease (FMDV 3C protease)	Host cell transcription shutoff	Leu20-Ala21	Serine	H3	Infected BHK-21 cells [30]
Histone H2A specific protease (H2A _{sp})	Unclear liver-specific regulation	Asn90-Asp91	Aspartic	H2A	Chicken liver extracts [35]