Review

Regulation of histone modifying enzymes by the ubiquitin–proteasome system

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ABSTRACT

Histone post-translational modification is a key step that may result in an epigenetic mark that regulates chromatin structure and gene transcriptional activity thereby impacting many fundamental aspects of human biology. Subtypes of post-translational modification such as acetylation and methylation are executed by a variety of distinct modification enzymes. The cytoplasmic and nuclear concentrations of these enzymes are dynamically and tightly controlled at the protein level to precisely fine-tune transcriptional activity in response to environmental clues and during pathophysiological states. Recent data have emerged demonstrating that the life span of these critical nuclear enzymes involved in histone modification that impact chromatin structure and gene expression are controlled at the level of protein turnover by ubiquitin–proteasomal processing. This review focuses on the recent progress on mechanisms for ubiquitin–proteasomal degradation of histone modification enzymes and the potential pathophysiological significance of this process.

1. Introduction

Histone proteins are a group of nuclear proteins including histone H1, H2A, H2B, H3 and H4. Two sets of histone H2A, H2B, H3 and H4 form an octameric core; the core and the surrounding ~147 base pairs of genomic DNA comprise the basic structural unit referred to as the nucleosome. Notably, histone NH_2-terminal tails are free from the nucleosome core structure. The primary composition of the histone NH_2-terminus makes it prone to targeting by a variety of post-translational chemical modifications [1–5]. Well-recognized histone modifications include acetylation [6,7], methylation [8,9], ubiquitination [10], phosphorylation [11], sumoylation [12], ADP-ribosylation [13], and recently O-palmitoylation [14,15]. Histone post-translational modifications are catalyzed by enzymatic processes. These enzymes catalyze the covalent attachment of moieties to vulnerable residue(s) of histone NH_2-terminal tails [6,8–11]. For example, histone acetyltransferases specifically catalyze histone acetylation by addition of an acetyl group to the lysine residue of the histone, while histone methyltransferases add a methyl group to lysine or arginine residues of histones. The complexities of modification of histones continue to expand as are the

enzymes that catalyze these activities, yet there is little known regarding control mechanisms that govern availability of histone related enzymes.

Histone protein modifications can occur simultaneously within different acceptor residues of one histone or among different histone proteins [16]. Analyses of calf histones using peptide mass fingerprinting mass spectrometry identified numerous concurrent modifications. For example, methylation, acetylation, and ubiquitination within histone proteins have been identified revealing 13 modification sites in histone H2A, 12 modification sites in histone H2B, 21 modification sites in histone H3, and 14 modification sites in histone H4 [17,18]. Notably, the most common acceptor sites within histone are located in histone NH_2-terminal free tails, although residues residing within the core structures can be also modified. Histone modifications are also dynamic and reversible [16]. For example, lysine residues can be modified by lysine acetyltransferases (KATs) or lysine methyltransferases (KMTs), and subsequently these groups can be removed by actions of lysine deacetylases (HDACs) and lysine demethylases (KDMs). Histone modifications are recognized by other histone modifiers that contain specific recognition binding domains that elicit functional operations. In this regard, histone modification enzymes can recruit transcription factors during assembly of functional complexes that regulate chromatin structure and transcriptional activity. Therefore, histone modification enzymes can interact coordinately and synergistically.

Histone post-translational modifications impact chromatin-templated processes such as gene transcription, DNA replication, and DNA repair.

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The modification status of the histone NH_2-terminal tail often impacts chromatin architecture with regard to an active (euchromatin) or inactive (heterochromatin) state that is linked to gene transcriptional activation, or gene repression, respectively. In general, histone acetylation opens the chromatin structure that increases gene transcription, whereas histone methylation condenses chromatin that inhibits gene activation [5,6]. For example, histone acetylation at H3K14 increases gene transcription, whereas histone H3 methylation inhibits gene activation. Hence, there appears to be a delicate balance between various epigenetic modifications on histone proteins that modulates gene transcriptional activity in responding to the environmental pathophysiological stimuli. These post-translational modifications on histones regulate diverse processes including development, differentiation, cell proliferation, and innate immunity relevant to acute and chronic diseases.

Histone modification is also regulated in response to a variety of environmental clues. There appears to exist a coordinate level of alterations in degree of protein modification within a histone that reflects changes in the type or magnitude of some stimuli [5,6]. In this manner, it is reasonable that there also exists an appropriate similar pattern of expression or activities of regulated histone modifiers. The factors that control concentrations of nuclear histone modification enzymes may be critical therefore in regulating gene expression. However, limited information is available regarding molecular processes that control availability of these histone modifiers. Among various regulatory processes, the ubiquitin proteasomal system (UPS) is a universal pathway that controls steady-state protein levels in eukaryotes. Here, we will focus on the recent progress identifying the ubiquitin–proteasomal machinery as a key mechanism that governs the availability of histone modification enzymes thereby impacting gene expression (Table 1).

2. The protein degradation *via* the ubiquitin–proteasome

Protein concentrations are tightly controlled within living cells, with the UPS degrading the bulk of intracellular proteins (Fig. 1). The target protein destined for degradation is usually covalently labeled with monoubiquitin or polyubiquitin moieties [19,20]. Ubiquitin is a 76 amino acid (aa) protein that is highly conserved among all eukaryotes and is expressed in almost all cell types. Ubiquitination of a targeted protein involves several energy consuming enzymatic reactions [21,22]. First, an E1 ubiquitin-activating enzyme recruits and transfers ubiquitin to an E2 ubiquitin-conjugating enzyme. The terminal reaction involves interaction between an E2 ubiquitin-conjugating enzyme with a specific E3 ubiquitin ligase that coordinates ligation of the ubiquitin moiety to a target substrate *via* an isopeptide linkage between the COOH group of the carboxyl terminus in ubiquitin and the ε-amino group of a lysine residue of the targeted protein. Among the human genome, there is only one E1, but fourteen E2s, and more than one thousand E3s. The E3 ubiquitin ligase is substrate-specific, and yet one E3 ligase may control the degradation of numerous substrate proteins [23]. On the other hand, one protein could be ubiquitinated by more than one E3 ubiquitin ligase [24]. The precise targets for the majority of the E3 ubiquitin ligases remain largely unknown. Nevertheless, research into the molecular behavior of components within ubiquitin E3 ligases remain an area of high interest as they appear to regulate histone modification enzymes.

Molecular recognition signals exist in many proteins that recruit ubiquitin E3 ligase complexes. The targeted protein for degradation is usually modified before binding to an E3 ubiquitin ligase, and these modifications often involve phosphorylation, dephosphorylation, acetylation, or methylation [23,25–27]. Cellular compartmentalization of proteins is a critical issue with regard to function, and both cytoplasmic or nuclear localization of the targeted protein can be directed through ubiquitination by distinct E3 ubiquitin ligases. The E3 ubiquitin ligases distribute in both the cytoplasmic and nuclear compartments. For example, one ubiquitin E3 ligase component, termed F-box protein FbxW1 (β-transducin repeat-containing protein, β-TrCP), exists as two isoforms: FbxW1-1 and FbxW1-2. These isoforms differ in localization in the cells, with the FbxW1-2 isoform present in the cytoplasm and FbxW1-1 mainly identified in the nucleus [27,28]. Ubiquitination is also a reversible enzymatic reaction as this moiety can be removed from a protein by deubiquitination enzymes [29].

Polyubiquitinated proteins are degraded by a large proteasome complex (2.5 MDa), that consists of a 20S core particle and one or two 19S regulatory particles [19,20]. The 20S core particle contains 28 proteins containing α and β subunits forming an α–β–β–α-four-layer cylindrical structure. Each layer is comprised of 7 subunits, the beta subunits of the 20S core particle display protease activity, hydrolyzing the targeted proteins into 5–11 aa pieces in the lumen of the 20S core particle [30]. The 19S regulatory particle "caps" the two sides of the 20S core particle, which is easily dissociated from the 20S core structure. It is believed that several subunits in the lid of the 19S regulatory particle specifically bind to the ubiquitin chain, functioning to recognize and capture ubiquitinated proteins. The base subunits unfold, direct, and pass the protein into the proteolytic lumen of the 20S core. The UPS degrades proteins both in the cytoplasm and in the nucleus. It appears that the proteasome is located in close proximity to subcellular sites of massive protein degradation. Indeed, proteasomal structures are identified in both the cytoplasm near the endoplasmic reticulum and within the nucleus in the centrosome [31,32]. In neuronal exons, proteasomal localization is determined by a 19S lid subunit that specifically binds

Table 1
Histone modification enzymes that are degraded by the ubiquitin–proteasomal system.

| Enzyme	Histone substrates	Cellular process involved	E3 ligase	Disease relevance	Reference
p300/ CBP	H2A K (5); H2B K (5, 12, 15, 20);	Chromatin regulation	FbxO3, Mdm2	Epithelial cancers, myeloid leukemias	[43,68,69]
	H3K (14, 18, 23, 56); H4K (5, 8, 12)	Transcription activation	BRMS1	Rubinstein–Taybi syndrome 2	
PCAF	H3K (14), H4 (nucleosome)	Transcription activation	MDM2		[34]
GCN5	H2B K (11,16), H3K (9, 14, 18, 23, 27, 36), H4K (8,16), H2A–Z	Transcription activation chromatin assembly	CRL4		[56]
HBO1	DNA replication		FbxW15 (L17)		[44]
MORF4	Unknown	Cell senescences	Unknown		[45]
MORF4L1	H2A, H4	Transcription activation	Nedd8	Breast cancer, coronary artery disease	[83]
SRC1	H3K (9,14)	Transcription activation	Unknown	Rhabdomyosarcoma	[87]
HDAC1	H2A, H2B, H3, H4	Transcription repression	Mdm2, Chfr	Colon cancer, COPD	[39,99]
HDAC2	H2A, H2B, H3, H4K16	Transcription repression	RLIM	Cancer, COPD	[35,100]
DNMT1	DNA CpG	Transcription repression	Cdh1, UHRF1	Hereditary sensory neuropathy, deafness	[36,106]
Lpcat1	H4 (ser47)	Transcription repression	FbxW1	Lung cancer, ALI	[37]
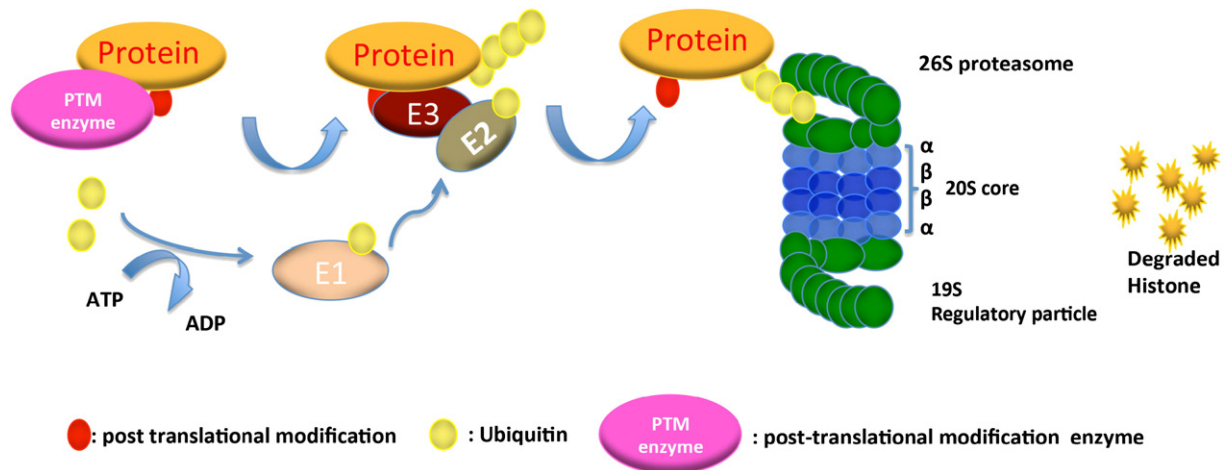
Fig. 1. Schematic representation of protein turnover *via* the ubiquitin proteasome system. The protein destined for ubiquitin proteasomal degradation is polyubiquitinated that involves multi-step enzymatic processes. The targeted protein for degradation is usually post-translationally modified (red circles) by a post-translational modification (PTM) enzyme that facilitates E3 ubiquitin ligase binding. Ubiquitin (yellow circles) is first loaded onto an E1 ubiquitin activation enzyme in an ATP-dependent manner, and then transferred to E2 conjugation enzyme. An E3 ubiquitin ligase interacts with the E2 conjugation enzyme and the targeted protein substrate. The E2 ubiquitin conjugation enzyme then covalently attaches the ubiquitin to the histone modification enzyme *via* an isopeptide linkage. The polyubiquitin chain of the protein is recognized by the lid of 19S regulatory particle and escorted into 20S core for degradation. The β-subunits of 20S core with proteolytic activity hydrolyze the targeted protein into fragments with a size of typically 5–11aa.

to calmodulin kinase II, suggesting the possibility that the proteasome is enriched within specific organelles or regions by anchoring a signaling kinase in the 19S lid [33].

3. Histone modification enzymes are regulated by ubiquitin proteasomal degradation

Histone modification enzymes catalyze post-translational modifications within histone particularly at the histone NH_2-terminal tails that regulate the chromatin structure and transcriptional activity. In living cells, the chromatin structure and transcriptional activity are dynamically regulated in response to clues that emerge within pathophysiological settings. There is mounting experimental evidence demonstrating that the UPS plays an important role in selective degradation of histone modification enzymes, thus regulating a vast range of life processes [34–40]. Various up-stream recognition signals selectively direct histone modification enzymes for UPS degradation [36,37]. Phosphorylation at certain residues of a given protein is one prerequisite for binding of a substrate to an E3 ubiquitin ligase; this phosphorylation is often an important molecular signature or phosphodegron that is recognized by the ubiquitination apparatus that facilitates proteasomal degradation of targeted proteins. Among various phosphodegrons, E3 ubiquitin ligase subunit FbxW1 recognizes a well-characterized binding motif, S/TXXXS/T, within substrates. Here, the serine or threonine residues are first phosphorylated by distinct kinases that facilitate the ability of FbxW1 to dock with the phosphorylated binding motif leading to substrate ubiquitination [23]. For example, the histone *O*-palmitoylation enzyme Lpcat1 is proteosomally degraded, a process involving its phosphorylation [41]. In other modes of regulation, dephosphorylation in some cases is a signal that leads to UPS degradation. For example, dephosphorylation of the acetyltransferase, p300, at Ser^{1834} triggers its UPS degradation [41]. However, Akt-mediated p300 phosphorylation at Ser^{1384} stabilizes it from degradation [42]. Further, acetylation or methylation also modulates vulnerability of histone modification enzymes for UPS degradation. In addition, acetylation of MORF4L1, an acetyltransferase, stabilizes it from UPS degradation (unpublished observations). Conversely, methylation of the DNA methyltransferase DNMT1 acts as a degron that destabilizes DNMT1 [36]. Thus, histone modification enzymes themselves are marked by a variety of the post-translational alterations at

specific residues that selectively target these enzymes for UPS degradation. These targeting processes are triggered by specific environmental stimuli, thus precisely controlling histone modification enzyme availability within cells that can alter chromatin architecture and transcriptional activity.

In general, histone modification enzymes are distributed both in the cytoplasm and in the nucleus (Fig. 2) and accordingly histone modification enzymes are degraded in the cytoplasm and in the nucleus [34–40,43,44]. Rapid turnover of these enzymes appears essential to reprogram chromatin architecture and transcriptional activity in response to various stimuli. It is conceivable that turnover of histone modification enzymes in the nucleus is a cellular homeostatic control mechanism that efficiently removes enzymes from chromatin, limits their association with transcriptional factors, or restricts their association with co-activators to tightly regulate chromatin architecture and transcriptional activity.

Many histone modification enzymes are also degraded in the cytoplasm [43–45]. UPS degradation of histone post-translational enzymes may reduce the total cellular mass of any individual enzyme that indirectly regulates histone modification by nuclear forms. Second, turnover of the cytoplasmic histone modification enzymes may regulate cytoplasmic functions of these histone modification enzymes. Cytoplasmic histone modification enzymes may represent a storage pool that is also functional. In this regard, histone modification enzymes catalyze post-translational modification of cellular proteins other than histones. For example, p300 acetylates more than seventy cellular proteins other than histone proteins, including control of p53 stability in the cytoplasmic compartment [46]. Turnover of cytoplasmic p300 will terminate the acetylation-dependent regulation of these cellular proteins that may affect many biological processes. Third, turnover of histone modification enzymes in the cytoplasm may impact nucleosome assembly and aggregation. For example, histone proteins are post-translationally modified in the cytoplasmic compartment before the histones enter into the nucleus for their nucleosomal assembly [47]. Thus, it is conceivable that UPS degradation of a histone modification enzyme may regulate nucleosome assembly and aggregation, although this appears speculative at present.

Finally, histone modification enzymes reversibly shuttle between the nucleus and cytoplasm and this is tightly coordinated with histone
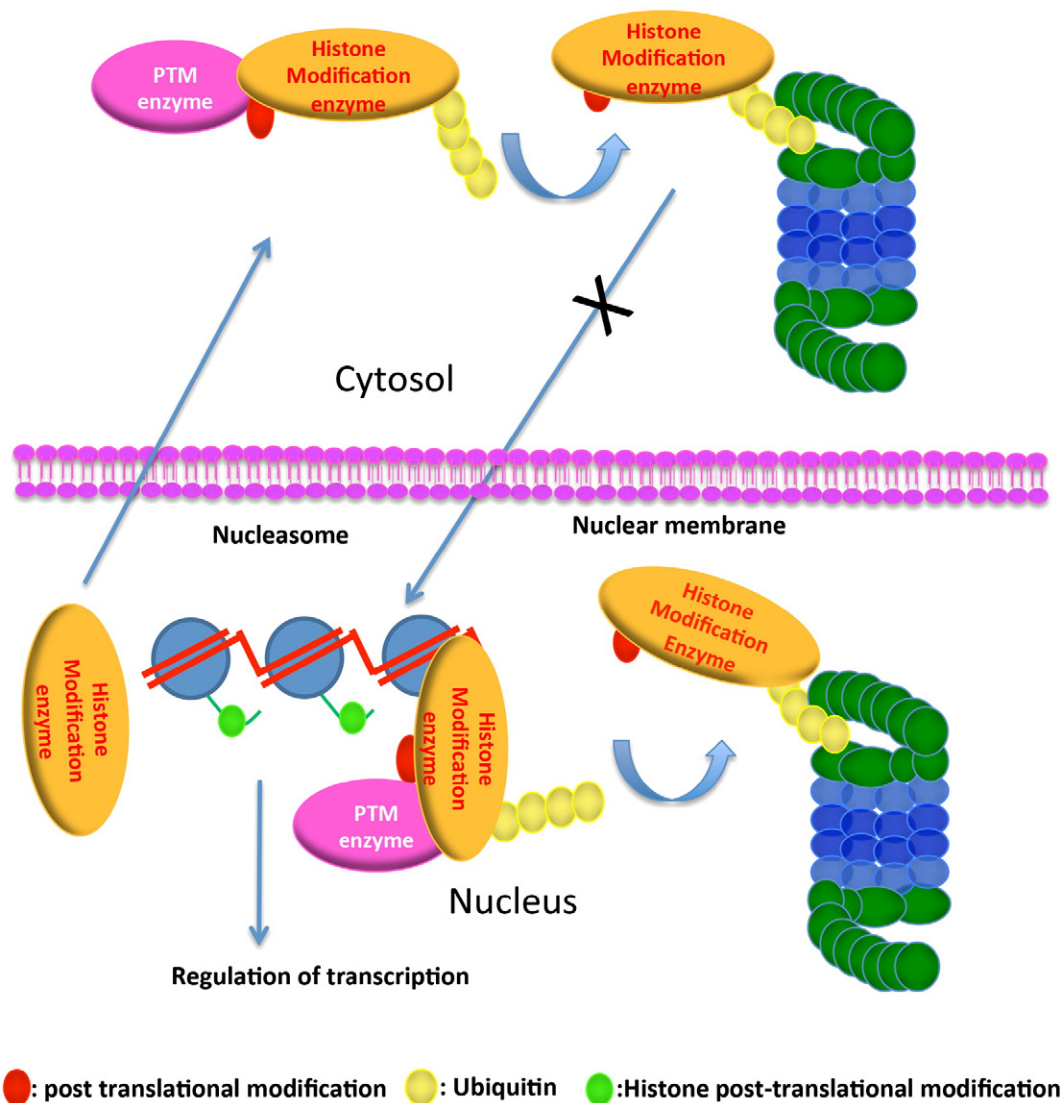
Fig. 2. The ubiquitin proteasome system spatially degrades histone modification enzymes to regulate gene transcriptional activity. Histone modification enzymes are ubiquitinated and proteosomally degraded in the cytoplasm or in the nucleus. Degradation of histone modification enzymes in the cytoplasm limits the mass of the enzyme that may enter into the nucleus but also modulates the cytoplasmic function of the enzyme. Rapid elimination of a histone modification enzyme in association with chromatin in the nucleus limits the extent of a histone modification and may result in a shift in transcriptional expression of genes in response to an environmental stimulus. In some cases, the histone modification enzymes shift into the cytoplasmic compartment for ubiquitination. Ubiquitin–proteasomal turnover of histone modification enzymes terminates their function impacting transcriptional activity.

modification and gene transcriptional activity. Nuclear histone modification enzymes can translocate from the nucleus into the cytoplasm for ubiquitination thereby preventing the enzyme from exerting important epigenetic roles [48].

4. Ubiquitin proteasomal degradation of histone modification enzymes

4.1. GCN5 (general control non-derepressible 5)

Histone acetyltransferases (HATs) are a group of histone modification enzymes that attach an acetyl group to the ε-amino group of lysine residues of histones or other proteins. Histone NH_2-terminal tail acetylation relaxes the chromatin structure near promoter regions to activate gene transcription. HATs include more than 100 members categorized into at least several subfamilies, the GCN5-related *N*-acetyltransferases (GNATs); p300/CREB-binding protein (CBP) and p300/CBP-associated factor (PCAF); the MYST proteins that include

HBO1 (histone acetyltransferase binding to origin recognition complex), an acetyltransferase that modulates DNA replication origin licensing; others include the general transcription factor HATs including the TFIID subunit TBP-associated factor-1 (TAF1), and the nuclear hormone-related HATs, SRC1 and ACTR (SRC3).

Histone acetyltransferase general control non-derepressible 5 (GCN5) globally acetylates the histone NH_2-terminal free tail at several molecular sites including H3K14, H4K8, and H4K16, thus linking the acetyltransferase to transcriptional activation. In *Saccharomyces cerevisiae*, GCN5 is active as a component in the histone acetylation protein complex containing the Spt-Ada-GCN5 acetyltransferase complex (SAGA) [49], Saga altered Spt8 absent acetyltransferase complex (SALSA) [50], and GCN5/Ada acetyltransferase complex (Ada) [51] that exhibit distinct functions. The GCN5/SAGA complex exerts acetyltransferase activity involved in RNA polymerase II-dependent transcriptional regulation that is required for recruitment of the basal transcription machinery to the promoter. GCN5/SAGA activity regulates acetylation of nucleosomes at H2B, and histone H3 (K9, 14, 18, and 23) [52,35]. In the SAGA complex,

GCN5 also associates with another histone acetyltransferase, PCAF. GCN5/SALSA is a distinct complex that stimulates gene transcription [52]. GCN5/Ada complex selectively catalyzes histone H3 (K14, 18) and H2B acetylation leading to transcriptional activation [52]. GCN5 is involved in diverse cellular processes including proliferation and tumorigenesis [53–55]. Interestingly, GCN5 itself is unstable and ubiquitinated in human cancer cells [56]. Inhibition of the proteasome by addition of a proteasomal inhibitor, MG132, stabilizes GCN5 protein levels. Cullin4-RING E3 ubiquitin ligase (CRL4) cellular depletion by siRNA results in stabilization of GCN5 levels whereas overexpression of CRL4 reduces GCN5 protein levels [56]. CRL4 is sufficient to ubiquitinate GCN5 both in vivo and in vitro. Interestingly, acidic nucleoplasmic DNA binding protein 1 (And-1) that is elevated in tumorogenic cells impacts GCN5 protein stability. And-1 and CRL4 competitively bind to the carboxyl-terminus of GCN5 [56]. And-1 stabilizes GCN5 by impairing the interaction between GCN5 and CRL4 thereby preventing GCN5 ubiquitination and degradation. The ubiquitination acceptor site within the GCN5 molecule has not yet been identified, nor does the upstream signal that triggers CRL and GCN5 binding to modulate acetylation in vivo.

4.2. p300/CBP (CREB-binding protein)

The p300/CBP proteins are a sub-family of histone acetyltransferases that catalyze global acetylation of lysine residues in histone proteins H2AK5, H2B (K5, K12, K15, K20), H3 (K14, K18, K23), and H4 (K5, K8, K12). p300/CBP also acetylates histones inside the core structures, specifically histone H3 at K56. Aside from histone proteins, p300/CBP selectively acetylates more than 70 transcriptional regulators [57]. p300/CBP mediated histone NH_2-terminal tail acetylation relaxes the chromatin structure in the promoter region to activate gene transcription. The p300/CBP complex also recruits the basal transcriptional machinery to the promoter region and acts as an adaptor molecule to bind multiple proteins to regulate transcriptional activity [58]. p300/CBP factors participate in many pathophysiological processes including cell proliferation [59], development [60], tumorogenesis [61], and apoptosis [62].

Several signaling pathways have been identified that regulate p300 turnover. UPS degradation of p300 was first reported in human cardiac myocytes that were exposed to doxorubicin [63]. Mitogen-activated protein kinases (MAPK) including p38, and other kinases such as Ras and Akt induce phosphorylation of p300 to regulate proteasome-mediated p300 degradation [43,64–66]. In contrast to the mechanism of phosphorylation mediated protein degradation, dephosphorylation of p300 at Ser^{1834} triggers p300 UPS degradation. Specifically, the B56γ3 (PPP2R5C) regulatory subunit of protein phosphatase 2A (PP2A) targets p300 for degradation through the 26S proteasomal pathway in the nucleus [41]. Reciprocally, Akt catalyzed phosphorylation at Ser^{1834} stabilizes p300 [66]. Interestingly, cytosolic p300 exhibits ubiquitin E3 ligase activity itself particularly with regard to p53 protein stability and signal transduction [67]. Several E3 ubiquitin ligases are involved in p300 turnover in response to distinct upstream signals. The E3 ubiquitin ligase Fbxo3 mediates p300 degradation which forms a complex with PML, Skp1, Cullin 1 and p300 [68]. One study demonstrated a previously unrecognized role of E3 ligase function of the breast cancer metastasis suppresser 1 (BRMS1) protein on histone acetyltransferase p300 stability [69]. BRMS1 induces polyubiquitination and proteasome-mediated degradation of p300. BRMS1 appears to belong to a bacterial IpaH E3 ligase family with an evolutionarily conserved CXD motif that may be responsible for its E3 ligase function. Mutation of this E3 ligase motif abolishes BRMS1-induced p300 polyubiquitination and degradation [69]. Levels of p300 are tightly and spatially regulated to maintain normal cellular homeostasis through UPS degradation. p300/CBP is degraded both in the cytoplasm and in the nucleus but via distinct mechanisms. For example Fbxo3 mediates p300 turnover in the nucleus [68]. p300 also shuttles from the nucleus into the cytoplasm for

ubiquitination [48]. The degradation of CBP is less studied. The upstream signals that trigger CBP degradation are not known, nor is the E3 ubiquitin ligase(s) that might modulate CBP lifespan in cells. Interestingly, one study reported that promyelocytic leukemia (PML) nuclear bodies appear to be linked to UPS degradation of CBP [70].

4.3. PCAF (p300/CREB-binding protein-associated factor)

PCAF was first reported as a protein that interacts with CBP. PCAF contains intrinsic histone acetyltransferase activity and functions as a coactivator for a number of transcriptional activators [71]. PCAF exhibits histone acetyltransferase activity that potentially acetylates free histone H3, histone H4, and nucleosomal H3 and other transcription factors to regulate transcriptional activity. Yeast PCAF forms multi-subunit protein complexes with several transcription factors including TATA box-binding protein-associate factors and GCN5 to exert its histone modification functions. PCAF has been proposed to facilitate long-distance transcriptional enhancement by direct association with enhancer sequences and interacts with actin and RNA polymerase II to maintain efficient transcriptional elongation. PCAF plays a role in transcriptional activation, cell cycle progression, and cellular differentiation. PCAF is regulated by Mdm2 mediated UPS degradation [34]. E3 ubiquitin ligase Mdm2 ubiquitinates the NH_2-terminus of PCAF in vitro and in cells. PCAF ubiquitination occurs in the nucleus, as MDM2 devoid of its nuclear localization signal is unable to degrade nuclear PCAF. PCAF levels are higher in Mdm2-deficient mouse embryonic fibroblast (MEF) cells compared to Mdm2-containing MEF cells, indicating that Mdm2 regulates the stability of PCAF by ubiquitin proteasomal processing. In addition, PCAF has intrinsic E3 ubiquitin ligase activity to regulate protein stability.

4.4. HBO1 (histone acetyltransferase binding to origin recognition complex)

HBO1 is a member of the MYST histone acetyltransferase family that modulates cell cycle progression and proliferation through epigenetic mechanisms [72–75]. In mammals, HBO1 associates with Mcm2-7 helicase, cdc6, and Cdt1 to constitute the origin recognition complex [74]. HBO1 may also interact with ING5 and JADE1 to form a complex to regulate cell proliferation and cell survival [75]. HBO1 acetylates histone H3 at H3K14 and histone H4 to load the origin recognition complex onto chromatin to initiate DNA replication licensing and trigger DNA replication during the late G1 phase. Knockdown of HBO1 by specific shRNA results in reduced S phase entry and cell death, confirming the pivotal function of HBO1 in initiation of DNA synthesis and cell proliferation [44]. Despite the importance of HBO1's function in DNA replication, the regulation of HBO1 protein level in cells is less studied. One recent study identified that HBO1 is a short lived protein with a half-life of approximately 3 h [44]. HBO1 is UPS degraded evidenced by its stabilization after exposure to the proteasome inhibitor, MG132. A relatively uncharacterized ubiquitin E3 ligase component, Fbxw15, directly interacts with HBO1 to mediate its ubiquitination at K^{338}. As opposed to its predicted role in regulating epigenetic functions in the nucleus, HBO1 degrades predominantly in the cytoplasmic compartment raising the possibility of nuclear–cytoplasmic cross-talk in regulation of HBO1 lifespan. Fbxw15 mediated HBO1 degradation requires mitogen-activated protein kinase 1 (Mek1), which triggers HBO1 phosphorylation and degradation in epithelial cells. Disruption of Fbxw15 by silencing blocks Mek1 ability to trigger HBO1 degradation [44]. Modulation of Fbxw15 levels was able to differentially regulate histone H3K14 acetylation and cellular proliferation by altering HBO1 levels. Hence, Fbxw15 is an ubiquitin E3 ligase subunit that mediates HBO1 depletion in cells to control cell replicative capacity. The detailed information of the molecular signals within HBO1 that regulate its stability remains unknown.

4.5. MORF4 (mortality factor on human chromosome 4)

Most histone acetyltransferases activate transcriptional activity and promote cell proliferation. In contrast, MORF4 was first cloned as a senescence gene from human chromosome 4 [76,77]. Introduction of MORF4 into cells causes significant cell death, although the underlying mechanism is unknown. There are three members of the MORF4 protein family, MORF4, MORF4L1 (MRG15), and MORF4L2 (MRX) [78–80]. Similar to most histone acetyltransferases, MORF4L1 is a component of the NuA4 histone acetyltransferase complex. The MORF4L1/NuA4 complex acetylates histone proteins H2B and H4 thereby probably impacting activation of oncogene and proto-oncogene directed transcription. Sequence analysis indicates that MORF4 lacks 128 aa within its NH_2-terminus that typically contains a chromodomain; chromodomains specifically bind to methylated lysine residues and are well conserved in histone acetyltransferases. MORF4L1 is a multi-functional histone acetyltransferase that regulates senescence, apoptosis, cell cycle progression, and DNA repair. Genome wide studies reveal that MORF4L1 is also involved in neoplasia and atherosclerosis [81,82]. These studies suggest that MORF4 may exert its epigenetic function by regulating a distinct gene population from MORF4L1 to regulate cell viability. MORF4 protein is a short life protein with a half-life of ~30 min [45]. Addition of MG132 accumulates MORF4, suggesting that MORF4 is degraded *via* the UPS. Experimental evidence suggests that MORF4 is degraded in the cytoplasm, because nuclear retention of this acetyltransferase extends its lifespan in cells. Results from mass spectrometry suggest that Nedd8 may participate in MORF4L1 ubiquitination [83].

4.6. SRC-1 (steroid receptor coactivator 1)

SRC-1 is a transcriptional co-regulatory protein with intrinsic histone acetyltransferase activity and several nuclear receptor interaction domains that acetylates histone H3 and H4 [84–86]. SRC1 directly binds to nuclear receptors and stimulates transcriptional activities in a hormone-dependent fashion. SRC1 is recruited to promoter regions of genes by hormones or ligand activated nuclear receptors to acetylate histones at lysine residues to positively modulate transcriptional activity. SRC1 also recruits CBP as an adaptor to co-activate transcription [86]. SRC1 plays a central role in forming multi-subunit complexes that remodel chromatin by recruiting a variety of transcription factors to control the energy balancing gene population. Degradation of SRC-1 is a UPS mediated process, as proteasomal inhibitors stabilize the protein [87]. The majority of SCR-1 is degraded in the cytoplasmic compartment where SRC-1 colocalizes with proteasomal antigens [87]. SRC-1 degradation is ligand-dependent and involves two degradation motifs, as residues 2–16 corresponding to a PEST motif and residues 41–136 located in the bHLH domain of the coactivator impact protein stability [87]. The upstream signals that trigger the protein for ubiquitination are not yet known, nor is the E3 ubiquitin ligase involved in SRC-1 proteasomal degradation.

4.7. HDAC1 (histone deacetyltransferase 1)

Histone deacetyltransferases (HDACs) are a group of enzymes that remove acetyl groups from modified lysine residues on histones that results in gene transcriptional repression [88–90]. Based on their sequence similarity, HDACs can be classified into three classes: Class I, Class II, and Class III. Class I contains HDAC1, HDAC 2, HDAC3 and HDAC8 which are primarily located in the nucleus. Class II HDAC enzymes include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10, which can be shuttled into the nucleus. Class III consists of Sirt 1–7. HDAC is involved in diverse cellular processes such as control of the cell cycle [91], cell proliferation, tumorigenesis [92], differentiation, and development [93]. HDAC functions *via* the formation of large multiprotein complexes. For example, the HDAC-containing multiprotein complex, Sin3, consists of six core subunits, HDAC1/2, RbAp46, RbAp48,

Sin3A/Sin3B, SAP18, and SAP30 in mammalian cells [94–96]. Another complex, NURD, consists of HDAC1/2, RbAp46, RbAp48, CHD3/CHD4 (Mi-2), MBD2/MBD3, MTA1/MTA2/MTA3, and p66α/p66 in human [97,98].

HDACI is a Class I histone deacetyltransferase that is labile ($t_{1/2}$ ~ 3 h) and degraded by the UPS. In the presence of the androgen receptor, the E3 ubiquitin ligase Mdm2 associates with and ubiquitinates HDAC1 at the prostate specific antigen promoter region [39]. HDAC1 ubiquitination requires androgen treatment and depends upon the E3 ligase activity of Mdm2. Mdm2 mediated HDAC1 UPS degradation may represent a refined level of regulation to terminate androgen receptor activated transcriptional activity. HDAC1 also directly interacts with the carboxyl terminal region of Chfr, an E3 ubiquitin ligase which functions in the mitotic checkpoint. Chfr ubiquitinates HDAC1 *in vitro* and *in vivo*. Overexpression of Chfr accelerates HDAC1 degradation and this is attenuated by addition of MG132. Chfr downregulates HDAC1 levels to stimulate p21 transcription in cancer cells that is normally suppressed by HDAC1 [99]. Valproic acid, an inhibitor of Class I and II HDAC enzymes, destabilizes HDAC2 by activating Ubc8 E2 conjugating enzyme gene expression [100]. Valproic acid-induced HDAC2 turnover critically depends on the E3 ubiquitin ligase RLIM. RLIM specifically ubiquitinates HDAC2 for proteasomal degradation by coordinate actions of Ubc8 and the RLIM E3 ligase [100]. Thus, polyubiquitination and proteasomal degradation through an E2–E3 ligase interaction provide an isoenzyme-selective mechanism for downregulation of HDAC2 protein levels. Interestingly, cigarette smoke induces HDAC2 phosphorylation and proteasomal degradation [35]. Cigarette smoke extract exposure induces HDAC2 CKII-dependent phosphorylation, ubiquitination, and proteasomal degradation. CKII and proteasome inhibitors stabilize HDAC2 from its degradation. The E3 ubiquitin ligase involved in this process has not been identified.

4.8. DNMT1 (DNA methyltransferase 1)

DNMT1 is a DNA methyltransferase that plays a critical role in chromatin modification and gene expression [101]. DNMT1 mediated DNA methylation results in transcriptional repression and can antagonize actions of histone acetyltransferases [102]. DNMT1 methylates CpG residues with a preference for hemimethylated DNA [103]. Human DNMT1 plays a critical role in maintenance of gene activity by maintaining repressed chromatin through recruitment and interaction with histone deacetyltransferase complexes; this process modulates cell cycle progression that may impact neoplasia [104,105]. Studies show that DNMT1 is unstable and regulated by the UPS [105]. The HDAC inhibitor LBH589 down-regulates DNMT1 by mediating DNMT1 UPS degradation. The NH_2-terminal 120 aa within DNMT1 are essential for its proteasomal processing. Deletion of this fragment of DNMT1 abrogates LBH589-induced ubiquitination [105]. SET7, a histone methyltransferase, monomethylates DNMT1 at K^{142} and this modified DNMT1 is unstable and prone to proteasomal degradation [36]. In contrast, Akt-mediated phosphorylation of DNMT1 at serine residues stabilizes DNMT1 [36]. Cross-talk between monomethylation and phosphorylation alter DNMT1 protein levels that influence DNA replication and S phase-G2 transition within the cell cycle [36].

At least two E3 ubiquitin ligases are involved in DNMT1 degradation. One is Cdh1, an APC (anaphase-promoting complex)/cyclosome ubiquitin ligase. Cdh1 co-localizes with hyperphosphorylated DNMT1 in the nucleus and ubiquitinates DNMT1, suggesting that Cdh1 degrades DNMT1 *via* an unidentified phosphodegron. Depletion of Cdh1 using siRNA increases DNMT1 levels that block 5-aza-CdR-induced degradation [40,106]. Another relevant E3 ligase is UHRF1. The protein Tip60 acetylates DNMT1 triggering ubiquitination by UHRF1, thereby targeting DNMT1 for proteasomal degradation. In contrast, DNMT1 is stabilized by HDAC1 and the deubiquitinase herpes virus-associated ubiquitin-specific protease.

4.9. Lpcat1 (acyl-CoA:lysophosphatidylcholine acyltransferase I)

Lpcat1 was initially characterized as a lung surfactant phospholipid synthetic enzyme from alveolar type II epithelial cells [107]. Lpcat1 is an acyltransferase that adds a palmitate group to the glycerol backbone of phospholipids to generate the major surfactant lipid, dipalmitoylphosphatidylcholine. Targeted disruption of the gene encoding Lpcat1 in mice increases newborn mortality [108]. Interestingly, Lpcat1, a cytosolic protein, migrates into the nucleus to associate and modify histone proteins for their palmitoylation to regulate transcriptional activity [14,15]. Thus, Lpcat1 mediates histone lipidation as a novel epigenetic mark. Lpcat1 is unstable with a half-life of approximately 3.5 h and it is degraded *via* the UPS machinery. Bacterial infection or endotoxin treatment triggers glycogen synthase kinase3-β activation in murine lung epithelia that specifically phosphorylates Lpcat1 at $Ser^{178-182}$. This phosphorylation event stimulates recruitment of an E3 subunit, Fbxw1. This E3 protein directly docks onto the phosphodegron within Lpcat1 that results in Lpcat1 ubiquitination at K^{221} and proteasomal degradation [37]. Lpcat1 mainly localizes in the cytoplasmic compartment and migrates into the nucleus upon stimulation. Though FbxW1 isoforms distribute in both the cytoplasm and nucleus, it is not clear where Lpcat1 is degraded.

5. Summary

Studies of UPS regulation of the histone modification enzymes are still in their infancy. Among hundreds of histone modification enzymes, only a few have been fully characterized with regard to their UPS processing. Mass-spectrometric analysis shows that many proteins are multi-site ubiquitinated. Furthermore, because lysine residues serve as acceptor sites for ubiquitination, acetylation, and methylation, interplay among these modifications increases the complexity of mechanisms that may impact histone modification enzyme availability. Each residue within these histone regulating enzymes that is covalently modified by ubiquitination may impact a distinct function altering gene activity indirectly after a specific pathophysiological stimulus. In the future, it will be important to clarify how UPS targeting of these enzymes impacts cellular behavior. The localization of the UPS degradation of the histone modification enzymes is also largely yet to be elucidated. Among the vast array of the histone modification enzymes, some of them are degraded in the cytoplasmic compartments, whereas others are degraded in the nucleus. This may reflect a targeting mechanism to optimize efficiency to tightly control concentrations of enzymes based on the individual needs of a cell to maintain homeostasis. The specific ubiquitin E3 ligase or ligases that mediate ubiquitination of a specific histone modification enzyme also needs to be defined. This latter issue is critical as there are now emerging therapeutic entities either in preclinical or early phase trials testing modulators of the UPS [109]. Such interventions could conceivably be used as novel therapeutic strategies for inflammation, cell survival, or neoplasia through altering abundance of histone modifying enzymes.

Regulation of gene transcriptional activity requires the orchestrated actions of a variety of histone modification enzymes targeting the histone NH_2-terminal tails, and specific transcriptional machinery with coregulators at targeted DNA loci. Rapid removal of these histone modification enzymes from the specific histone tails or DNA loci is essential to activate or repress any given gene set. Degradation of histone modification enzymes at the protein level enables transcriptional reprogramming in response to various stimuli depending on a specific circumstance. There is mounting evidence supporting the UPS as a mechanistic pathway that controls the availability of several histone modifying enzymes that are critical in regulation of gene expression. Hence, the UPS indirectly may alter the epigenetic code in mammalian systems by controlling expression of genes linked to inflammation, cell growth and proliferation, and survival. Deciphering the molecular basis for UPS degradation of histone post-translational modification enzymes in different pathophysiological settings will help us understand how histone modification enzymes respond to signaling cascades and exert their diverse functions. Future advances in this area might lead to devising pharmacotherapeutic interventions directed at the UPS to control fundamental biological processes through histone modification.

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