Review

Svitlana V. Bach and Ashok N. Hegde*

The proteasome and epigenetics: zooming in on histone modifications

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Abstract: The proteasome is a structural complex of many proteins that degrades substrates marked by covalent linkage to ubiquitin. Many years of research has shown a role for ubiquitin-proteasome-mediated proteolysis in synaptic plasticity and memory mainly in degrading synaptic, cytoplasmic and nuclear proteins. Recent work indicates that the proteasome has wider proteolytic and non-proteolytic roles in processes such as histone modifications that affect synaptic plasticity and memory. In this review, we assess the evidence gathered from neuronal as well as non-neuronal cell types regarding the function of the proteasome in positive or negative regulation of post-translational modifications of histones, such as acetylation, methylation and ubiquitination. We discuss the critical roles of the proteasome in clearing excess histone proteins in various cellular contexts and the possible non-proteolytic functions in regulating transcription of target genes. In addition, we summarize the current literature on diverse chromatin-remodeling machineries, such as histone acetyltransferases, deacetylates, methyltransferases and demethylases, as targets for proteasomal degradation across experimental models. Lastly, we provide a perspective on how proteasomal regulation of histone modifications may modulate synaptic plasticity in the nervous system.

Keywords: epigenetics; histone modifications; proteasome; synaptic plasticity; ubiquitin.

***Corresponding author: Ashok N. Hegde,** Department of Biological and Environmental Sciences, Georgia College and State University, Milledgeville, GA 31061-3375, USA, e-mail: ashok.hegde@gcsu.edu
Svitlana V. Bach: Department of Neurobiology, The University of Alabama at Birmingham, Birmingham, AL 35294, USA

Introduction

The proteasome is a cellular complex that degrades proteins marked by covalent attachment to several molecules of ubiquitin. The linkage of ubiquitin to substrates is precisely regulated by an enzyme that mediates activation of ubiquitin (E1), enzymes that carry the activated ubiquitin (E2s) and enzymes that ligate it to the protein substrate (E3s). The proteolytic portion of the proteasome resides in its 20S (named thus because of its sedimentation coefficient) core to which two 19S regulatory caps (RCs) are attached. The 19S RCs remove the polyubiquitin tag, unfold the substrate protein and thread it through the narrow aperture of the 20S core for degradation into polypeptide fragments (Figure 1) (1).

Protein degradation by the ubiquitin-proteasome pathway (UPP) plays numerous roles in the nervous system. Originally, the role of the UPP was discovered in synaptic plasticity in the invertebrate *Aplysia californica* (2), a marine slug utilized for pioneering discoveries of molecular mechanisms underlying long-term facilitation (3). Subsequently, the role of the UPP was shown in late-phase long-term potentiation in the murine hippocampus, a well-studied model of synaptic plasticity in vertebrates (4, 5). Since then, numerous other investigations have shown that the proteasome is critical for memory formation. For example, proteasome inhibition in the hippocampus hinders consolidation of inhibitory avoidance memory, while blocking proteasome activity in the amygdala interferes with long-term fear memory (6, 7).

What are the mechanisms by which UPP contributes to synaptic plasticity and memory? Although the role of the UPP and the proteasome have been elucidated in regulating protein kinases, transcription factors, neurotransmitter receptors and other molecules critical for changing synaptic strength, much remains to be understood. Recent studies show that the proteasome modulates transcription by regulating epigenetic modifications of the N-terminal tails of histone proteins, such as histone acetylation, methylation and ubiquitination, which are critical for synaptic plasticity and memory.

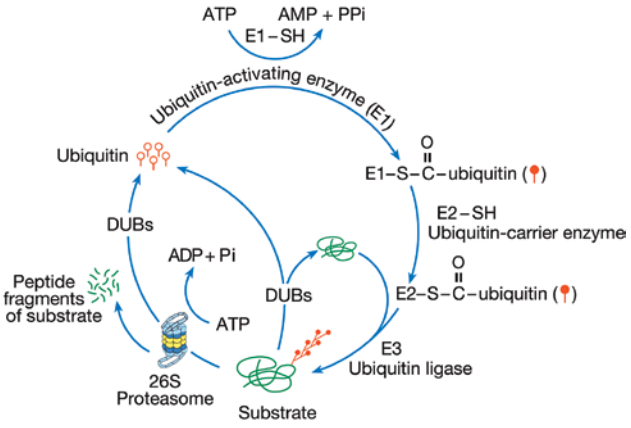

Figure 1: The ubiquitin-proteasome pathway.
In this pathway, ubiquitin (represented by open circles with straight tails) is selectively and covalently liked to the substrate. The enzymatic process of attaching ubiquitin to substrates (called ubiquitination or ubiquitin conjugation) depends on the action of three different classes of enzymes E1, E2 and E3. Initially, ubiquitin is activated by E1 to form a ubiquitin-AMP intermediate. Activated ubiquitin (closed circles with straight tails) is passed on to E2 (ubiquitin carrier enzymes). E2 transfers ubiquitin to an E3 (ubiquitin ligase) which ligates the activated ubiquitin to the substrate. A series of other ubiquitin molecules are attached to the substrate-linked ubiquitin and, thus, a polyubiquitin chain forms. The substrates marked with polyubiquitin tags are degraded by a proteolytic complex called the 26S proteasome in an ATP-dependent reaction. Ubiquitin is not degraded, but the polyubiquitin chain is disassembled and ubiquitin is recycled by deubiquitinating enzymes (DUBs). Prior to being committed for degradation by the proteasome, ubiquitination is reversible. DUBs can disassemble the polyubiquitin chain and prevent the degradation of the substrate.

Roles of the proteasome and ubiquitin

The proteasome is traditionally known to degrade proteins that are marked by attachment of ubiquitin. A single ubiquitin is covalently linked to the side chain of lysine (K) residues in the substrate. Once the first ubiquitin molecule is attached, a second ubiquitin molecule is linked to the K residue in the 48th amino acid sequence position of the first ubiquitin. This process is repeated several times and thus a polyubiquitin chain grows. The polyubiquitin chain is recognized by the proteasome for degradation.

Attachment of ubiquitin molecules to substrate proteins (ubiquitination) is a highly regulated step performed by three specialized enzymes (Figure 1), as explained above. The E1 is the least physiologically regulated enzyme, while the E2 is highly selective for its E3 binding partner and the E3 displays a high degree of substrate specificity. Although the kinetics of ubiquitination have not been extensively investigated, one study showed that transfer of the first ubiquitin by a ligase is the rate-limiting step (8). Genes encoding just one E1, dozens of E2s and hundreds of E3s have been identified in the mammalian genome (9, 10).

Substrates can also be posttranslationally modified with a single ubiquitin (monoubiquitination), or a single ubiquitin attached to several different lysine residues (multi-monoubiquitination). In addition, the type of polyubiquitin linkage determines how a substrate protein is degraded. Ubiquitin molecules covalently liked to each other through their 48th K residues mark a substrate protein for degradation by the proteasome, whereas ubiquitin linkage via K11 leads to endoplasmic reticulum-mediated substrate degradation (11) and linkage via K29 leads to endosomal substrate degradation (12). Monoubiquitination usually causes a conformational change within the protein and is not a signal for substrate degradation, but is associated with regulation of protein activity and protein-protein interactions (13). For example, a monoubiquitin tag attached to histones is an epigenetic modification that changes the landscape of chromatin and alters gene transcription (14, 15). In addition, attachment of a single ubiquitin to different K residues of a substrate protein marks a protein for endocytosis (16). Polyubiquitination via unusual ubiquitin linkage has also been described to regulate signaling pathways and kinase activity; however, its role is not very well understood (17–19). The ubiquitination process can be reversed by deubiquitinating enzymes (DUBs) (Figure 1) (9, 20).

Structure of the proteasome

To appreciate the proteolytic and non-proteolytic roles of the proteasome in histone modifications, it is instructive to describe the structure of the proteasome in detail. The proteasome is a large complex, the parts of which perform different functions not only in proteolysis but also in other functions such as histone modification. Originally, components of the proteasome were characterized by their sedimentation coefficient (21). The full complex is called the 26S proteasome and the catalytic core is termed the 20S proteasome. The catalytic core is a narrow cylinder to the either end of which two 19S RCs are attached. In eukaryotic cells, the 20S core is made up of two outer rings with seven α subunits (α1–α7) in each ring and two inner rings comprising seven β subunits (β1–β7). Three of the seven β subunits (β1, β2 and β5) are responsible for the catalytic activity of the proteasome. The catalytic sites

in these β subunits are located at their N-termini and are positioned inside the catalytic chamber, which has a narrow opening of 13Å in diameter (22). Therefore, only an unfolded substrate can pass through this opening. It is thought that the ATPases that are present in the base of the 19S RC provide the unfolding activity (23).

There are six ATPase subunits in the 19S RC, named regulatory particle ATPase 1–6 (Rpt1–6), which are collectively called the ATPases independent of 20S (APIS) complex. The 19S RC also contains four non-ATPase subunits regulatory particle non-ATPases 1, 2, 10 and 13 (Rpn1, Rpn2, Rpn10 and Rpn13). In addition, the 19S RC has a 'lid' which consists of only non-ATPase subunits (Rpn3, Rpn5, Rpn6–9, Rpn11, Rpn12, and Rpn15) (24, 25). Among the Rpn subunits, Rpn11 (also called Poh1) and Rpn13 (also called Uch37) are DUBs that are an integral part of the 19S RC. Rpn11 and Rpn13 assist in deubiquitination of the substrate as it is unfolded and threaded into the catalytic chamber of the 20S core. One DUB called Usp14 is known to stimulate substrate degradation through deubiquitination by reversibly associating with the Rpn1 subunit in the base (23, 26) and is known to be a critical regulator of long-term memory formation (27).

The 20S proteasome can exist without the 19RC attachment, in which case it cannot degrade ubiquitinated proteins (28). The 20S proteasome by itself possesses chymotrypsin-like, trypsin-like and postglutamyl peptidase activities that cleave after hydrophobic, basic, and acidic residues, respectively (25). The 19S RC recognizes the polyubiquitinated substrate, and its ATPases channel the substrate into the catalytic 20S core of the proteasome. The catalytic core then cleaves the ubiquitinated protein into small peptides. The peptides thus generated are likely to be later hydrolyzed to generate free amino acids by other proteases and amino peptidases (24, 25). The studies carried out so far indicate that the proteolytic activity of the 26S proteasome and the non-proteolytic activity of the 19S RC both have a role in histone modifications, as will be discussed later.

Histone posttranslational modifications (PTMs) and transcription

How do cells containing identical genetic makeup express different sets of genes and differentiate into various cell types with distinct structures and functions? In the past few decades, it has become clear that heritable genetic information is not limited to the DNA sequence and that other processes, which alter the structure of DNA-protein complexes (or chromatin), are crucial in guiding gene expression (29–31). Such dynamic chromatin alterations that determine the spatial and temporal sequence of gene expression in response to environmental factors are referred to as epigenetic modifications. Epigenetic mechanisms include PTMs of N-terminal tails of histone proteins, DNA methylation at cytosine residues, and noncoding RNAs, which collectively remodel chromatin and regulate gene expression. This review will focus on histone PTMs to examine their relationship with the proteasome and the UPP.

Histone proteins can be divided into two groups: replication-dependent (or canonical) and replication-independent (or variant histones) (32). Canonical histone proteins are encoded by a family of replication-dependent genes expressed rapidly during the S phase of the cell cycle. The genes that encode canonical histones are arranged in long clusters containing multiple copies of core histones (H2A, H2B, H3 and H4) and the linker histone H1. Replication-dependent histone mRNAs are the only known mRNAs in eukaryotes that lack a 3′ polyadenylated tail (33). Instead, they end in a 3′ stem-loop sequence that plays an important part in their regulation. On the other hand, replication-independent histone genes are expressed throughout the cell cycle and are polyadenylated. Some of the most commonly studied histone variants include the H3.3 and the H2A.Z, which are known to mark actively remodeled chromatin regions (34, 35).

In eukaryotic cells, DNA wraps around octamers of histones to form DNA-protein structural units called nucleosomes. Each nucleosome is composed of 147 bp of DNA wrapped around two molecules of each core histone (H2A, H2B, H3 and H4) with linker H1 histones occurring in between the nucleosomes (29). The tight packaging allows for space conservation needed to accommodate millions of base pairs of DNA into a small space of the nucleus. This tight packaging, however, restricts the accessibility of DNA by transcriptional machinery and serves as an additional regulatory step in the transcription process (29, 36–38).

The double-stranded DNA can assume two folding states based on how closely it is associated with histone-packaging proteins. In a heterochromatic state, strong DNA-protein interactions lead to tightly coiled chromatin that is transcriptionally inactive (Figure 2). When the DNA assumes its euchromatic state, the chemical interactions between DNA and proteins are weakened, producing loosely coiled chromatin that is transcriptionally active (Figure 2). These different packaging states depend largely

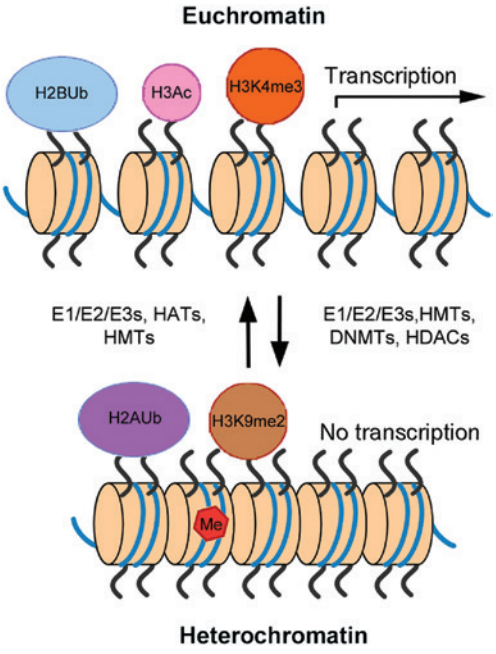

Figure 2: Histone modifications that regulate transcription. Top: In the open 'euchromatin' state, histone H2B is ubiquitinated, histone H3 is acetylated and tri-methylated on lysine 4. These modifications allow the transcription machinery to bind to the chromatin and genes are transcribed. Bottom: In the closed 'heterchromatin' state, histone H2A is ubiquitinated and histone H3 is dimethylated on lysine 9. DNA is methylated at cytosine residues. These modifications prevent the transcription machinery from binding the chromatin and transcription of genes does not occur. E1/E2/E3s: Enzymes that ubiquitinate H2B or H2A; HATs, histone acetyltransferases; HMTs, histone methyltransferases; DNMTs, DNA methyltransferases; HDACs, histone deacetylases.

on PTMs of histone N-terminal tails that protrude from the nucleosomes. Covalent PTMs of histone tails induce conformational changes within the chromatin, allowing it to adopt condensed or relaxed states that inhibit or stimulate transcription, respectively (29, 36, 39, 40) (Figure 2).

Most commonly studied histone PTMs are acetylation and methylation, both of which have been implicated in memory (41–43). During histone acetylation, an acetyl group from an acetyl-CoA molecule is transferred to a K residue within the histone tails. This transfer neutralizes a positively charged histone protein and weakens its affinity for the negatively charged DNA, promoting euchromatin formation (44). Histone acetyltransferase (HAT) enzymes catalyze histone acetylation and stimulate transcription, while histone deacetylase (HDAC) enzymes remove the acetyl groups from histone tails and repress transcription (44). Histone methylation occurs on both K and arginine (R) side-chain groups of H3 and H4 histone tails. Histone methylation is catalyzed by histone methyltransferase (HMT) enzymes and their removal is facilitated by histone demethylase (HDM) enzymes (37, 45, 46). K residues can be mono, di, and trimethylated whereas R residues can be mono and dimethylated. Depending on the methylation site and the number of methyl groups transferred, histone methylation can repress or stimulate transcription. For example, transcriptionally silent genes contain di and trimethylated histone H3 at lysine 9 (H3K9) whereas actively transcribed genes contain di and trimethylated histone H3 at lysine 4 (H3K4) (45, 47–49). On the other hand, R methylation serves for transcriptional activation only (36). A wide variety of HDMs, specific to certain methylation sites and numbers of methyl groups, have been identified (45).

During histone methylation, a methyl group is transferred from a high-energy donor, S-adenosyl methionine. Unlike histone acetylation, this transfer does not neutralize a positive charge on the histone protein and, therefore, does not necessarily cause direct conformational changes within nucleosomes. It is hypothesized that histone methylation can cause chromatin modifications through binding of effector proteins (36). A particular histone methylation code could serve as a binding surface for effector proteins that recruit other transcription cofactors to regulate transcription (36). For example, studies in yeast have demonstrated that methylation of H3K4 recruits the SAGA protein complex that possesses acetyltransferase enzymes. Therefore, methylation of H3K4 promotes histone acetylation and specific gene transcription (36, 50).

In addition to acetylation and methylation, less well-characterized PTMs of histones include phosphorylation and monoubiquitination. Histone phosphorylation occurs at serine and threonine residues and has been observed in numerous cellular processes such as transcription, mitosis, DNA repair and apoptosis (36, 37, 51). Histone phosphorylation facilitates both condensation and relaxation of chromatin; therefore, it can repress or induce transcription depending on the cellular context. One well-characterized histone phosphorylation event occurs on histone H3 at serine residues 10 and 28 (37, 52). This histone phosphorylation has been correlated to the activation of protein S6 kinase 2 (RSK2), extracellular signaling-regulated kinase (ERK) and mitogen-activated protein kinase 1 (MAPK1) signaling pathways in hippocampal-dependent memory (36, 37, 51, 53).

During histone monoubiquitination, one ubiquitin molecule is attached to the ε-amino group of a K residue. Histone monoubiquitination occurs at histones H2A, H2B and H3 (13, 36, 37). The addition of a large and bulky moiety, such as ubiquitin, to histone proteins leads to radical structural changes within the chromatin and, depending on the monoubiquitination site, recruits

different co-factors to facilitate up or downregulation of transcription. For example, monoubiquitination of histone H2A is associated with silenced gene expression (54), while monoubiquitination of histone H2B may recruit transcriptional co-activators and promote histone acetylation, methylation and gene transcription (55, 56).

Interestingly, transcription of replication-dependent histones themselves is also regulated by epigenetic histone modification. For instance, in yeast, H2B phosphorylation at tyrosine 37 occurs in a region of chromatin upstream of the histone gene cluster called *Hist1* (or *HIST1* in mammalian cells) and serves to inhibit transcription of multiple histone genes during the late S phase (57). In *Drosophila*, a molecular switch, called histone gene-specific epigenetic repressor in late S phase (HERS), binds to histone regulatory elements and blocks transcription of histone genes by inducing H3K9 methylation (58). In human mammary epithelial cells, a histone modifying enzyme, Pygopus 2, binds to promoters of histone genes and upregulates the acetylation of H3K56, previously associated with transcriptional activation (59). Moreover, the enzyme Set8, known to monomethylate H4K20 and repress histone gene expression during the late S phase of the cell cycle, is ubiquitinated and degraded by the proteasome, indicating the importance of the UPP in the regulation of histone gene expression and cell cycle regulation (60).

Many transcription factors and co-factors such as HATs, HDACs and HMTs are ubiquitinated and degraded by the proteasome (61). Moreover, histone proteins as well as their variants have been identified as targets of the UPP in different cellular contexts, ranging from DNA damage repair (62) to synaptic plasticity (34). Detailed investigations of the mechanisms behind how key regulatory proteins are targeted for proteasomal degradation in the nucleus to regulate transcription are still lacking. In addition to protein degradation, some studies suggest an alternative, non-proteolytic role of the UPP in the regulation of chromatin folding and transcription (56). In this review, we discuss key studies across different fields and model systems that describe a strong relationship between the UPP and histone modifications, specifically focusing on the nervous system.

Histone degradation by the proteasome

Several studies demonstrate that histone proteins themselves can be degraded by the proteasome, although the underlying mechanisms of this process are still unclear (63). *In vitro* analysis of histone H3 degradation by the UPP indicates that it may be independent of polyubiquitin chain formation or even E3 activity (64). Other *in vitro* studies report ATP- and ubiquitin-independent proteasomal degradation of histones, damaged by oxidative stress that occurs during antitumor chemotherapy (65). In K562 human hematopoietic cells, a nuclear proteasome-activating pathway that specifically targets oxidatively damaged histones has been identified (65, 66).

Later studies confirmed the ATP- and polyubiquitination-independent degradation of histones by the proteasome and identified that histone degradation is dependent upon histone acetylation (67). This mechanism was found to be important for DNA repair mechanisms in developing sperm (67). Qian and colleagues showed that the special type of proteasomes, containing the PA200 activating complex bound to the 20S particle that are predominant in sperm and, therefore, termed ‘spermatoproteasomes’, are inefficient at degrading polyubiquitinated proteins. PA200 binds acetylated core histone proteins and targets them for degradation by the proteasome. This process is promoted by DNA double-stranded breaks induced by γ-irradiation in yeast (67).

In addition to the PA200-dependent proteasomal degradation of histone proteins, some studies also describe polyubiquitination-dependent histone degradation by the UPP. Some argue that in order for new histone incorporation to be possible, old evicted histones must be degraded (63); however, the mechanistic details of that process are still poorly understood. Studies in the budding yeast, *Saccharomyces cerevisiae*, show the importance of tight regulation of excess histones by the UPP. In their yeast model, Singh and colleagues demonstrated that non-chromatin bound histones undergo phosphorylation, polyubiquitination and proteasome-dependent degradation (68). In addition, they identified specific E2s (Ubc4 and Ubc5) as well as E3s (Tom1, Pep5, Snt2, Hel1 and Hel2) associated with the ubiquitination of excess histones (68, 69). These studies highlight the importance of clearing excess histone proteins, as non-chromatin-bound histones are known to interfere with cell viability and promote cytotoxicity (70). The corresponding mechanism of histone clearing in the mammalian cells is not yet apparent.

One recent study found that histone variant H3.3 is polyubiquitinated and degraded by the proteasome in mouse embryonic neurons (34). In neurons and glial cells, the H3.3 variant has been found to rapidly accumulate with age, replacing most of the canonical H3 histone (34). H3.3 incorporation produced highly dynamic and

transcriptionally active chromatin in both humans and rodents (34). This histone turnover was identified to be essential for controlling cell type-specific gene expression as well as synaptic connectivity (71). In addition, efficient H3.3 incorporation and eviction from chromatin were dependent upon clearing of histones by the proteasome, as H3.3 turnover was significantly reduced by inhibition of the proteasome. Therefore, this suggests that active histone degradation regulates activity-dependent gene expression and may provide life-long synaptic and behavioral plasticity (34).

Another histone variant, H2A.Z, has been identified as a target for UPP-dependent degradation and has been examined in clinically relevant work. Studies in rat cardiac myocytes and prostate cancer cell lines identified an upregulated level of H2A.Z variant in both diseases (62, 72). Both studies suggest that H2A.Z is a target of the UPP and that a class III HDAC, sirtuin 1 (Sirt1), promotes deacetylation and subsequent degradation of H2A.Z in both disease models (62, 72). Such studies provide evidence for the therapeutic potential of compounds, which enhance HDAC activity (such as resveratrol), in combination with other chromatin-remodeling compounds that increase H2A.Z degradation by the proteasome. Alternatively, compounds aimed to upregulate proteasomal activity directly could be useful for the same purposes.

Recently, Zovkic and colleagues described an important role of H2A.Z in relation to cognitive function (35). H2A.Z was identified as a negative regulator of memory consolidation in the hippocampus and cortex, by showing that it is evicted from transcriptionally active chromatin in response to fear conditioning, a widely used behavioral paradigm of learning and memory. Even though the proteolytic degradation of the H2A.Z variant was not investigated in this learning context, the authors did suggest that the reduction of the H2A.Z protein after fear conditioning might be mediated by the UPP (73), therefore implicating the role of the UPP in chromatin remodeling necessary for memory consolidation.

Proteasome-dependent degradation of epigenetic remodeling machinery

Histone acetylation is intricately linked to proteasomal degradation of proteins in regulating gene expression. The proteasome has been found to degrade key regulatory proteins required for histone acetylation, such as HATs (74, 75) and HDACs (76). For example, the transcriptional co-activator with HAT activity, cyclic adenosine monophosphate (cAMP) response element-binding (CREB) binding protein (CBP), that is essential for cell proliferation and embryonic development is a known target for UPP-mediated degradation in specialized nuclear compartments, called the promyelocytic leukemia (PML) bodies (74, 75, 77). Moreover, CBP's homolog p300 displays nucleo-cytoplasmic shuttling and has been identified as a target of the UPP both in the cytoplasm and the nucleus, allowing for even more stringent regulation of its activity by the proteasome (78).

Emerging studies, describing many transcriptional co-factors that are targets of the UPP, are rapidly accumulating. Among such chromatin remodeling proteins that associate with HATs are mortality factor on human chromosome 4 (MORF4), a cellular senescence factor that regulates cell division (79, 80) and p300/CBP-associated factor (PCAF), a protein important for the transcriptional regulation of p53 and many other genes (77). Further investigation of the UPP-targeted transcriptional activating complexes will help decipher the mechanisms by which the proteasome regulates transcriptional activation in many cellular processes.

The UPP plays an equally important role in regulating transcriptional-silencing enzymes, such as HDACs and HMTs. One of the most fascinating examples of how the proteasome regulates histone deacetylation and gene expression is found in the immune system, as some viruses manipulate gene expression of the host through proteasomal degradation of HDACs. A recent study found that an HIV-1 accessory protein, Vpr, physically interacts with the class I HDACs, HDAC1, 2, 3 and 8, to direct them for proteasome-dependent degradation (76). This removal of HDACs facilitates histone hyperacetylation at the HIV-1 promoter and drives infection in primary macrophages (76).

Additional indirect evidence for the function of UPP-mediated proteolysis of transcriptional-silencing complexes has been obtained by the use of an HDAC inhibitor, valproic acid (VPA). Over the past three decades, VPA has been used for treatments of seizures (74), some cancers (81) and, more recently, has been described as a potent memory enhancer (82). VPA inhibits HDAC activity by either direct binding or by the stimulation of HDAC degradation by the UPP. Interestingly, the facilitation of UPP to degrade HDACs has been described as a selective process, specifically affecting HDAC2, but not other class I HDACs, suggesting a possible utility for targeted application of this drug (83). Moreover, HDAC2 has been shown to be subject to NEDD8 conjugation, or NEDDylation, which is a prerequisite for its degradation by the UPP (84). NEDDylation-activating enzymes have

been proposed as novel therapeutic targets for upregulating proteasomal activity (84).

Transcriptional-silencing histone mono and dimethyl transferases, such as G9a and GLPs, are targeted for proteasome-mediated degradation in response to DNA damage in human fibroblasts (85). A role for the proteasome has also been found in degrading HDMs associated with heterochromatin, such as JARD1C (86). Degradation of polyubiquitinated JARD1C/SMCX by the proteasome promotes H3K4 trimethylation and gene expression. The earlier studies were carried out in yeast and the observations on the UPP-mediated degradation of Jhd2 (the yeast counterpart of JARD1C) such as the requirement for the ligase Not4 hold true for human cells as well (86). Mutations in the human *SMCX* gene are linked to mental retardation (87–89) and therefore it is likely that the regulation of HDMs by the UPP plays a significant physiological role in the nervous system.

It seems counterintuitive that the UPP is responsible for the degradation of transcription-silencing histone modifiers, such as HDACs, HMTs and HDMs, as well as proteolysis of transcription-promoting histone modifiers such as HATs. It is highly likely, therefore, that the cellular context and signaling affects what molecules are to be degraded by the proteasome and, thus, determines the transcriptional outcome.

The 19S RC and histone modifications

The best described role of the 19S ATPases, outside of their function as a part of the proteasome, is in the transcriptional regulation of genes. Studies in yeast and cancer cells showed that ATPase subunits bind to promoters of active genes and physically interact with chromatin-remodeling transcriptional machinery (87, 90–95). The 19S ATPases, Rpt4 and Rpt6, are known to regulate epigenetic histone PTMs and control gene expression (96). These studies led to the hypothesis that ATPase subunits facilitate transcription independently of the 20S catalytic core. Other studies, however, found that both the 19S cap and the 20S core are recruited to active chromatin (97). The APIS complex and the 20S proteasomal subunits bind at promoters and gene bodies, independently of one another (98). Also, the entire 26S proteasome has been shown to co-immunoprecipitate with RNA polymerase II, supporting both the proteolytic and the non-proteolytic roles of the proteasome in transcriptional regulation (99).

The proteasome, histone modifications and synaptic plasticity

Numerous studies across various model systems have yielded a wealth of information supporting the role of the UPP in the regulation of synaptic plasticity, learning and memory (25, 100–103). Our previous studies showed that the maintenance of murine hippocampal late phase of long-term potentiation (L-LTP), that underlies long-term memory, is blocked by a specific proteasome inhibitor β-lactone (4, 104). We showed that proteasome inhibition with β-lactone stabilizes translational activators early, followed by translational repressors later in L-LTP, illustrating the proteasome's dual role in mediating signaling pathways in synaptic plasticity at the level of dendrites (104) (Figure 3). A recent study in cultured hippocampal neurons showed that only 20% of the proteasomes are engaged in substrate processing at baseline, leaving ample room for activity-dependent increase in protein degradation, upon sufficient environmental stimulation (105). Taken together, these studies suggest an activity-triggered system in which the proteasome regulates plasticity-related proteins to produce an appropriate synaptic response within the hippocampal neurons after synaptic stimulation.

The proteasome also plays a critical role in mediating gene expression in synaptic plasticity. In the nucleus, proteasome inhibition blocks gene expression induced by CREB, a transcription factor that is crucial for long-term synaptic plasticity and memory (106). Treatment of hippocampal slices with β-lactone prior to chemically induced LTP (cLTP) or electrically induced L-LTP with theta-burst protocol (TBP) blocks the upregulation of a CREB-inducible gene, *brain-derived neurotrophic factor* (*Bdnf*), necessary for the maintenance of L-LTP (4, 107). This observation was supported by the finding that a CREB repressor, activating transcription factor 4 (ATF4), is degraded by the proteasome during cLTP (4). Thus, proteasome inhibition causes a buildup of transcriptional repressors, such as ATF4, which blocks the upregulation of *Bdnf* and other plasticity-related genes, and blocks the maintenance of L-LTP (4). The connection between proteasome-dependent protein regulation in the dendrites and the nucleus during synaptic plasticity remains uncharacterized. Future studies addressing proteasome-mediated retrograde signaling in synaptic plasticity will perhaps provide some mechanistic details.

Furthermore, we identified a novel role of the proteasome in modulating transcription-favoring epigenetic

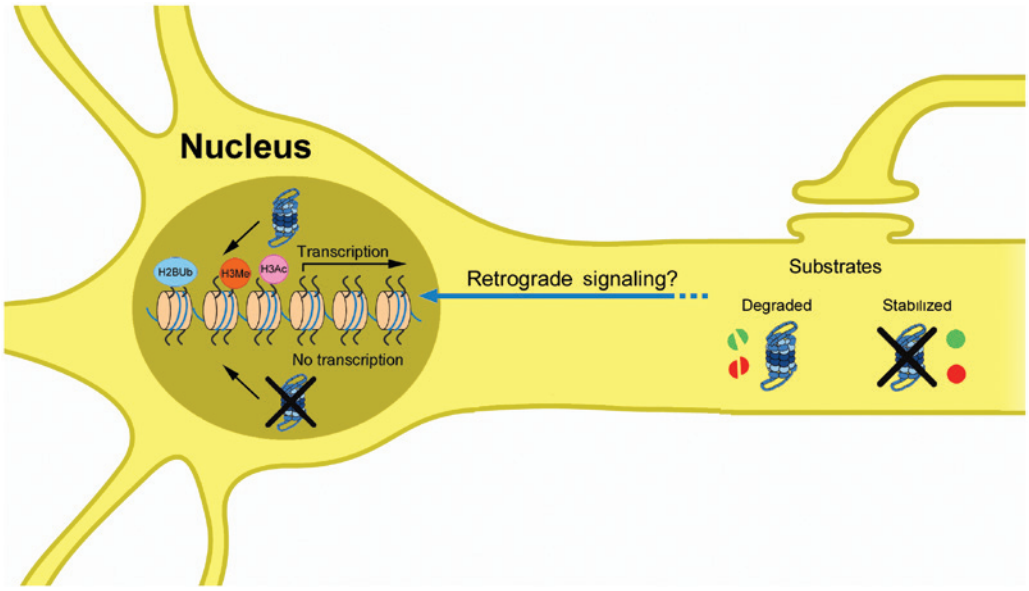

Figure 3: Histone-modifying roles of the proteasome in synaptic plasticity.
In the nucleus, when the proteasome is active (depicted at the top), proteasome facilitates histone acetylation, methylation and ubiquitination. When the proteasome is inactive (depicted with an X mark at the bottom), histone modifications are blocked. The proteasome might also have an indirect effect on histone modification through the regulation of protein degradation in or near dendrites (broken circles), which in turn is expected to affect retrograde signaling to the nucleus. When the proteasome is inactive and the substrates are stabilized (solid circles), retrograde signaling to the nucleus may not occur.

histone modifications, which are known to control gene transcription in synaptic plasticity, learning and memory. Our study demonstrated that the trimethylation of histone 3 at lysine 4 (H3K4me3), acetylation of histone H3 at lysines 9 and 14 (H3K9/14ac), and monoubiquitination of histone H2B at lysine 120 (H2BK120ub) are enhanced immediately after cLTP induction and their enhancement is blocked by β-lactone pretreatment (108) (Figure 3).

H3K4me3 and H3K9/14ac are transcription-favoring epigenetic tags that have been identified as critical regulators of learning and memory in behavioral rodent models (46, 109–112); however, the mechanisms behind their addition and removal are still poorly understood. Our study illustrated the dynamic nature of these modifications, since both H3K4me3 and H3K9/14ac were upregulated immediately after cLTP induction and returned back to baseline after 30 min of recovery. This histone remodeling time-course was surprisingly fast. Transient histone modifications have been studied in the timescale of hours or days after synaptic stimulation or behavioral training (113, 114). It is also known, however, that histone modifications can occur much more rapidly, in the timescale of minutes (115–117). It has been previously hypothesized that lasting cellular changes in synaptic plasticity can be triggered by a transient histone modification signal (37). Previous results in *Aplysia* show that transient acetylation of histone H3 is critical during long-term synaptic plasticity (118). Therefore, transient spikes of proteasome-dependent histone acetylation and methylation may be sufficient to trigger long-lasting upregulation of plasticity-related genes.

Furthermore, we investigated the role of the transcription-favoring H2BK120ub in synaptic plasticity. We demonstrated that H2BK120ub levels oscillate after the induction of cLTP, as an increase of monoubiquitination was observed immediately after cLTP induction and at 30 min after cLTP induction, but not at 15 min. This finding is consistent with previous studies of histone H2B monoubiquitination in yeast transcriptional regulation, where multiple rounds of histone ubiquitination and deubiquitination are required for transcription initiation and elongation, respectively (119–121). Histone H2B monoubiquitination has also been described as a precursor for other histone modifications (55, 95). If H2BK120ub is the first link in a chain of events that precede the initiation of transcription in synaptic plasticity, manipulating histone monoubiquitination at promoters of active genes may serve as a therapeutic target for memory impairments.

Collectively, these studies are in agreement with the idea that a combination of all epigenetic tags at promoters of genes, brought upon by environmental stimulation, control gene expression and modify behavior (109, 122). In the hippocampus, the regulation of chromatin structure through PTMs of histones may represent a ‘molecular

code' for long-term memory (37). This flexible 'molecular code' may mediate long-term physiological and behavioral changes by controlling the transcription of genes (37). In our work, we observed the dynamic nature of global histone modifications in cLTP, which suggests that the role of histone modifications in synaptic plasticity may be more complex than previously thought. It appears that the proteasome (and the UPP) has a role in regulating the process of histone modifications in synaptic plasticity underlying memory. Many key questions regarding the exact nature of the proteasome's role remain to be answered.

Numerous lines of evidence support the importance of both protein degradation by the proteasome and histone modifications in behavioral learning and memory models. Proteasome inhibition in the hippocampus and the amygdala is associated with an impaired consolidation of an inhibitory avoidance memory and long-term fear memory, respectively (6, 7). In addition, retrieval of either an auditory or a contextual fear memory results in an increase of the degradation-specific protein polyubiquitination in the amygdala (7). Epigenetic histone modifications, such as acetylation and methylation, are likely to be part of the molecular mechanisms necessary for persistent gene expression to support long-term memory formation and promote memory storage (109, 123, 124). Studies delineating how the two critical systems – the UPP and histone modification – may interact to drive memory formation *in vivo* are still lacking.

Future directions

In the past several years, parallel lines of research have found evidence for the roles of the UPP and histone modifications in synaptic plasticity and memory. It is only recently that the investigations on the UPP and epigenetics have begun to intersect. The main challenge for the future is to elucidate the mechanistic details on how different components of the UPP, the ubiquitin conjugating enzymes and the proteasome regulate histone modification. Given that the UPP can control both transcription-favoring and transcription-repressing types of histone modifications, it would be important to investigate the signaling pathways in neurons that control these opposing effects. There is also a necessity to understand the dynamic nature of some of the histone modifications (such as H2BK120ub) and how they relate to gene expression underlying synaptic plasticity and memory. Temporal regulation of histone PTMs is beginning to be understood in non-neuronal systems (125) and the same kind of in-depth analysis would be beneficial for understanding the physiological functions of the nervous system, including synaptic plasticity and memory.

Conclusion

In this review, we provided a brief summary on the current knowledge connecting two seemingly unrelated cellular processes, the UPP and epigenetic histone modifications, in multiple cell types and model systems. We discussed studies describing proteolytic degradation of canonical histones as well as their variants. We also described studies on proteolytic degradation of histone-remodeling machinery, such as HATs, HDACs, HMTs and HDMs, as well as transcriptional co-factors that form complexes with epigenetic remodeling enzymes. Although not very well described in the literature, we attempted to summarize the current knowledge of non-proteolytic roles of the proteasomal APIS complex in modulating transcription from yeast studies. Finally, we provided a perspective on proteasomal regulation of histone modifications in the nervous system by discussing studies on synaptic plasticity, learning and memory. As the relatively new and still developing field of neuroepigenetics continues to grow, we look forward to exciting future studies that incorporate the roles of the proteasome in modulating synaptic plasticity by regulating epigenetic modifications.

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List of abbreviations

ATF4	Activating transcription factor 4
ATP	adenosine triphosphate
APIS	ATPases independent of 20S
Bndf	Brain-derived neurotrophic factor
cAMP	cyclic adenosine monophosphate
CREB	cAMP response element-binding
cLTP	chemically induced long-term potentiation
CBP	CREB binding protein
DUB	deubiquitinating enzyme
E1	ubiquitine activating enzyme

E2	ubiqutine carrier enzyme
E3	ubiquitine ligase enzyme
HAT	histone acetyltransferase
HDAC	histone deacetylase
HDM	histone demethylase
H2BK120ub	histone H2B monoubiquitinated at lysine 120
H3K9/14ac	histone H3 acetylated at lysine 9 and 14
HMT	histone methyltransferase
K	lysine
L-LTP	late phase of long-term potentiation
PTM	posttranslational modification
R	arginine
RC	regulatory cap
Rpt	regulatory particle ATPase
Rpn	regulatory particle non-ATPase
TBP	theta-burst protocol
H3K4me3	trimethylated histone H3 at lysine 4
UPP	ubiquitin-proteasome pathway
VPA	valproic acid

References

1. Sun L, Johnston SA, Kodadek T. Physical association of the APIS complex and general transcription factors. Biochem Biophys Res Commun 2002; 296: 991–9.
2. Hegde AN, Goldberg AL, Schwartz JH. Regulatory subunits of cAMP-dependent protein kinases are degraded after conjugation to ubiquitin: a molecular mechanism underlying long-term synaptic plasticity. Proc Natl Acad Sci USA 1993; 90: 7436–40.
3. Greenberg SM, Castellucci VF, Bayley H, Schwartz JH. A molecular mechanism for long-term sensitization in *Aplysia*. Nature 1987; 329: 62–5.
4. Dong C, Upadhya SC, Ding L, Smith TK, Hegde AN. Proteasome inhibition enhances the induction and impairs the maintenance of late-phase long-term potentiation. Learn Mem 2008; 15: 335–47.
5. Lee SH, Choi JH, Lee N, Lee HR, Kim JI, Yu NK, Choi SL, Lee SH, Kim H, Kaang BK. Synaptic protein degradation underlies destabilization of retrieved fear memory. Science 2008; 319: 1253–6.
6. Lopez-Salon M, Alonso M, Vianna MR, Viola H, Mello e Souza T, Izquierdo I, Pasquini JM, Medina JH. The ubiquitin-proteasome cascade is required for mammalian long-term memory formation. Eur J Neurosci 2001; 14: 1820–6.
7. Jarome TJ, Werner CT, Kwapis JL, Helmstetter FJ. Activity dependent protein degradation is critical for the formation and stability of fear memory in the amygdala. PLoS One 2011; 6: e24349.
8. Pierce NW, Kleiger G, Shan SO, Deshaies RJ. Detection of sequential polyubiquitylation on a millisecond timescale. Nature 2009; 462: 615–9.
9. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 2002; 82: 373–428.
10. Ciechanover A, Brundin P. The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. Neuron 2003; 40: 427–46.
11. Zattas D, Hochstrasser M. Ubiquitin-dependent protein degradation at the yeast endoplasmic reticulum and nuclear envelope. Crit Rev Biochem Mol Biol 2015; 50: 1–17.
12. Chastagner P, Israel A, Brou C. Itch/AIP4 mediates Deltex degradation through the formation of K29-linked polyubiquitin chains. EMBO Rep 2006; 7: 1147–53.
13. Nakagawa T, Nakayama K. Protein monoubiquitylation: targets and diverse functions. Genes Cells 2015; 20: 543–62.
14. Fuchs G, Oren M. Writing and reading H2B monoubiquitylation. Biochim Biophys Acta 2014; 1839: 694–701.
15. Groothuis TA, Dantuma NP, Neefjes J, Salomons FA. Ubiquitin crosstalk connecting cellular processes. Cell Div 2006; 1: 21.
16. Huang T, Lehmann MJ, Said A, Ma G, Osterrieder N. Major histocompatibility complex class I downregulation induced by equine herpesvirus type 1 pUL56 is through dynamin-dependent endocytosis. J Virol 2014; 88: 12802–15.
17. Shi CS, Kehrl JH. Tumor necrosis factor (TNF)-induced germinal center kinase-related (GCKR) and stress-activated protein kinase (SAPK) activation depends upon the E2/E3 complex Ubc13-Uev1A/TNF receptor-associated factor 2 (TRAF2). J Biol Chem 2003; 278: 15429–34.
18. Hofmann RM, Pickart CM. Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. Cell 1999; 96: 645–53.
19. Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature 2001; 412: 346–51.
20. Hegde AN, DiAntonio A. Ubiquitin and the synapse. Nat Rev Neurosci 2002; 3: 854–61.
21. Tanahashi N, Tsurumi C, Tamura T, Tanaka K. Molecular structure of 20S and 26S proteasomes. Enzyme Protein 1993; 47: 241–51.
22. Cheng Y. Toward an atomic model of the 26S proteasome. Curr Opin Struct Biol 2009; 19: 203–8.
23. Peth A, Besche HC, Goldberg AL. Ubiquitinated proteins activate the proteasome by binding to Usp14/Ubp6, which causes 20S gate opening. Mol Cell 2009; 36: 794–804.
24. Marques AJ, Palanimurugan R, Matias AC, Ramos PC, Dohmen RJ. Catalytic mechanism and assembly of the proteasome. Chem Rev 2009; 109: 1509–36.
25. Hegde AN. The ubiquitin-proteasome pathway and synaptic plasticity. Learn Mem 2010; 17: 314–27.
26. Leggett DS, Hanna J, Borodovsky A, Crosas B, Schmidt M, Baker RT, Walz T, Ploegh H, Finley D. Multiple associated proteins regulate proteasome structure and function. Mol Cell 2002; 10: 495–507.
27. Jarome TJ, Kwapis JL, Hallengren JJ, Wilson SM, Helmstetter FJ. The ubiquitin-specific protease 14 (USP14) is a critical regulator of long-term memory formation. Learn Mem 2014; 21: 9–13.
28. Rechsteiner M, Hoffman L, Dubiel W. The multicatalytic and 26 S proteases. J Biol Chem 1993; 268: 6065–8.
29. Borrelli E, Nestler EJ, Allis CD, Sassone-Corsi P. Decoding the epigenetic language of neuronal plasticity. Neuron 2008; 60: 961–74.
30. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003; 33 (Suppl): 245–54.
31. Martin C, Zhang Y. Mechanisms of epigenetic inheritance. Curr Opin Cell Biol 2007; 19: 266–72.
32. Marzluff WF, Wagner EJ, Duronio RJ. Metabolism and regulation of canonical histone mRNAs: life without a poly(A) tail. Nat Rev Genet 2008; 9: 843–54.

33. Marzluff WF. Metazoan replication-dependent histone mRNAs: a distinct set of RNA polymerase II transcripts. Curr Opin Cell Biol 2005; 17: 274–80.
34. Maze I, Wenderski W, Noh KM, Bagot RC, Tzavaras N, Purushothaman I, Elsässer SJ, Guo Y, Ionete C, Hurd YL, Tamminga CA, Halene T, Farrelly L, Soshnev AA, Wen D, Rafii S, Birtwistle MR, Akbarian S, Buchholz BA, Blitzer RD, Nestler EJ, Yuan ZF, Garcia BA, Shen L, Molina H, Allis CD. Critical role of histone turnover in neuronal transcription and plasticity. Neuron 2015; 87: 77–94.
35. Zovkic IB, Paulukaitis BS, Day JJ, Etikala DM, Sweatt JD. Histone H2A.Z subunit exchange controls consolidation of recent and remote memory. Nature 2014; 515: 582–6.
36. Berger SL. The complex language of chromatin regulation during transcription. Nature 2007; 447: 407–12.
37. Levenson JM, Sweatt JD. Epigenetic mechanisms in memory formation. Nat Rev Neurosci 2005; 6: 108–18.
38. Lubin FD. Epigenetic gene regulation in the adult mammalian brain: multiple roles in memory formation. Neurobiol Learn Mem 2011; 96: 68–78.
39. Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. Cell 2000; 103: 263–71.
40. Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000; 403: 41–5.
41. Kramer JM. Epigenetic regulation of memory: implications in human cognitive disorders. Biomol Concepts 2013; 4: 1–12.
42. Park CS, Rehrauer H, Mansuy IM. Genome-wide analysis of H4K5 acetylation associated with fear memory in mice. BMC Genomics 2013; 14: 539.
43. Graff J, Woldemichael BT, Berchtold D, Dewarrat G, Mansuy IM. Dynamic histone marks in the hippocampus and cortex facilitate memory consolidation. Nat Commun 2012; 3: 991.
44. Kimura A, Matsubara K, Horikoshi M. A decade of histone acetylation: marking eukaryotic chromosomes with specific codes. J Biochem 2005; 138: 647–62.
45. Martin C, Zhang Y. The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol 2005; 6: 838–49.
46. Jarome TJ, Lubin FD. Histone lysine methylation: critical regulator of memory and behavior. Rev Neurosci 2013; 24: 375–87.
47. Margueron R, Trojer P, Reinberg D. The key to development: interpreting the histone code? Curr Opin Genet Dev 2005; 15: 163–76.
48. Sims RJ, 3rd, Nishioka K, Reinberg D. Histone lysine methylation: a signature for chromatin function. Trends Genet 2003; 19: 629–39.
49. Vermeulen M, Mulder KW, Denissov S, Pijnappel WW, van Schaik FM, Varier RA, Baltissen MP, Stunnenberg HG, Mann M, Timmers HT. Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4. Cell 2007; 131: 58–69.
50. Pray-Grant MG, Daniel JA, Schieltz D, Yates JR, 3rd, Grant PA. Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. Nature 2005; 433: 434–8.
51. Perez-Cadahia B, Drobic B, Khan P, Shivashankar CC, Davie JR. Current understanding and importance of histone phosphorylation in regulating chromatin biology. Curr Opin Drug Discov Devel 2010; 13: 613–22.
52. Crosio C, Cermakian N, Allis CD, Sassone-Corsi P. Light induces chromatin modification in cells of the mammalian circadian clock. Nat Neurosci 2000; 3: 1241–7.
53. Carter SD, Mifsud KR, Reul JM. Distinct epigenetic and gene expression changes in rat hippocampal neurons after Morris water maze training. Front Behav Neurosci 2015; 9: 156.
54. Endoh M, Endo TA, Endoh T, Isono K, Sharif J, Ohara O, Toyoda T, Ito T, Eskeland R, Bickmore WA, Vidal M, Bernstein BE, Koseki H. Histone H2A mono-ubiquitination is a crucial step to mediate PRC1-dependent repression of developmental genes to maintain ES cell identity. PLoS Genet 2012; 8: e1002774.
55. Lee JS, Shukla A, Schneider J, Swanson SK, Washburn MP, Florens L, Bhaumik SR, Shilatifard A. Histone crosstalk between H2B monoubiquitination and H3 methylation mediated by COMPASS. Cell 2007; 131: 1084–96.
56. Bhat KP, Greer SF. Proteolytic and non-proteolytic roles of ubiquitin and the ubiquitin proteasome system in transcriptional regulation. Biochim Biophys Acta 2011; 1809: 150–5.
57. Mahajan K, Fang B, Koomen JM, Mahajan NP. H2B Tyr37 phosphorylation suppresses expression of replication-dependent core histone genes. Nat Struct Mol Biol 2012; 19: 930–7.
58. Ito S, Fujiyama-Nakamura S, Kimura S, Lim J, Kamoshida Y, Shiozaki-Sato Y, Sawatsubashi S, Suzuki E, Tanabe M, Ueda T, Murata T, Kato H, Ohtake F, Fujiki R, Miki T, Kouzmenko A, Takeyama K, Kato S. Epigenetic silencing of core histone genes by HERS in *Drosophila*. Mol Cell 2012; 45: 494–504.
59. Gu B, Watanabe K, Dai X. Pygo2 regulates histone gene expression and H3 K56 acetylation in human mammary epithelial cells. Cell Cycle 2012; 11: 79–87.
60. Abbas T, Shibata E, Park J, Jha S, Karnani N, Dutta A. CRL4(Cdt2) regulates cell proliferation and histone gene expression by targeting PR-Set7/Set8 for degradation. Mol Cell 2010; 40: 9–21.
61. Zou C, Mallampalli RK. Regulation of histone modifying enzymes by the ubiquitin-proteasome system. Biochim Biophys Acta 2014; 1843: 694–702.
62. Baptista T, Graca I, Sousa EJ, Oliveira AI, Costa NR, Costa-Pinheiro P, Amado F, Henrique R, Jerónimo C. Regulation of histone H2A.Z expression is mediated by sirtuin 1 in prostate cancer. Oncotarget 2013; 4: 1673–85.
63. Chen YS, Qiu XB. Transcription-coupled replacement of histones: degradation or recycling? J Genet Genomics 2012; 39: 575–80.
64. Haas A, Reback PM, Pratt G, Rechsteiner M. Ubiquitin-mediated degradation of histone H3 does not require the substrate-binding ubiquitin protein ligase, E3, or attachment of polyubiquitin chains. J Biol Chem 1990; 265: 21664–9.
65. 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 USA 1999; 96: 6223–8.
66. Catalgol B, Wendt B, Grimm S, Breusing N, Ozer NK, Grune T. Chromatin repair after oxidative stress: role of PARP-mediated proteasome activation. Free Radic Biol Med 2010; 48: 673–80.
67. Qian MX, Pang Y, Liu CH, Haratake K, Du BY, Ji DY, Wang GF, Zhu QQ, Song W, Yu Y, Zhang XX, Huang HT, Miao S, Chen LB, Zhang ZH, Liang YN, Liu S, Cha H, Yang D, Zhai Y, Komatsu T, Tsuruta F, Li H, Cao C, Li W, Li GH, Cheng Y, Chiba T, Wang L, Goldberg AL, Shen Y, Qiu XB. Acetylation-mediated proteasomal degradation of core histones during DNA repair and spermatogenesis. Cell 2013; 153: 1012–24.
68. Singh RK, Kabbaj MH, Paik J, Gunjan A. Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis. Nat Cell Biol 2009; 11: 925–33.

69. Singh RK, Gonzalez M, Kabbaj MH, Gunjan A. Novel E3 ubiquitin ligases that regulate histone protein levels in the budding yeast Saccharomyces cerevisiae. PLoS One 2012; 7: e36295.
70. Singh RK, Liang D, Gajjalaiahvari UR, Kabbaj MH, Paik J, Gunjan A. Excess histone levels mediate cytotoxicity via multiple mechanisms. Cell Cycle 2010; 9: 4236–44.
71. Wenderski W, Maze I. Histone turnover and chromatin accessibility: critical mediators of neurological development, plasticity, and disease. Bioessays 2016; 38: 410–9.
72. Chen IY, Lypowy J, Pain J, Sayed D, Grinberg S, Alcendor RR, Sadoshima J, Abdellatif M. Histone H2A.z is essential for cardiac myocyte hypertrophy but opposed by silent information regulator 2alpha. J Biol Chem 2006; 281: 19369–77.
73. Walters BJ, Zovkic IB. Building up and knocking down: an emerging role for epigenetics and proteasomal degradation in systems consolidation. Neuroscience 2015; 300: 39–52.
74. St-Germain JR, Chen J, Li Q. Involvement of PML nuclear bodies in CBP degradation through the ubiquitin-proteasome pathway. Epigenetics 2008; 3: 342–9.
75. Chen J, Li Q. Life and death of transcriptional co-activator p300. Epigenetics 2011; 6: 957–61.
76. Romani B, Shaykh Baygloo N, Hamidi-Fard M, Aghasadeghi MR, Allahbakhshi E. HIV-1 Vpr protein induces proteasomal degradation of chromatin-associated class I HDACs to overcome latent infection of macrophages. J Biol Chem 2016; 291: 2696–711.
77. Jin Y, Zeng SX, Lee H, Lu H. MDM2 mediates p300/CREB-binding protein-associated factor ubiquitination and degradation. J Biol Chem 2004; 279: 20035–43.
78. Chen J, Halappanavar S, Th' ng JP, Li Q. Ubiquitin-dependent distribution of the transcriptional coactivator p300 in cytoplasmic inclusion bodies. Epigenetics 2007; 2: 92–9.
79. Tominaga K, Tominaga E, Ausserlechner MJ, Pereira-Smith OM. The cell senescence inducing gene product MORF4 is regulated by degradation via the ubiquitin/proteasome pathway. Exp Cell Res 2010; 316: 92–102.
80. Pena AN, Pereira-Smith OM. The role of the MORF/MRG family of genes in cell growth, differentiation, DNA repair, and thereby aging. Ann NY Acad Sci 2007; 1100: 299–305.
81. Blaheta RA, Michaelis M, Driever PH, Cinatl J, Jr. Evolving anticancer drug valproic acid: insights into the mechanism and clinical studies. Med Res Rev 2005; 25: 383–97.
82. Bredy TW, Barad M. The histone deacetylase inhibitor valproic acid enhances acquisition, extinction, and reconsolidation of conditioned fear. Learn Mem 2008; 15: 39–45.
83. Kramer OH, Zhu P, Ostendorff HP, Golebiewski M, Tiefenbach J, Peters MA, Brill B, Groner B, Bach I, Heinzel T, Göttlicher M. The histone deacetylase inhibitor valproic acid selectively induces proteasomal degradation of HDAC2. EMBO J 2003; 22: 3411–20.
84. Pandey D, Hori D, Kim JH, Bergman Y, Berkowitz DE, Romer LH. NEDDylation promotes endothelial dysfunction: a role for HDAC2. J Mol Cell Cardiol 2015; 81: 18–22.
85. Takahashi A, Imai Y, Yamakoshi K, Kuninaka S, Ohtani N, Yoshimoto S, Hori S, Tachibana M, Anderton E, Takeuchi T, Shinkai Y, Peters G, Saya H, Hara E. DNA damage signaling triggers degradation of histone methyltransferases through APC/C(Cdh1) in senescent cells. Mol Cell 2012; 45: 123–31.
86. Mersman DP, Du HN, Fingerman IM, South PF, Briggs SD. Polyubiquitination of the demethylase Jhd2 controls histone methylation and gene expression. Genes Dev 2009; 23: 951–62.
87. Jensen LR, Amende M, Gurok U, Moser B, Gimmel V, Tzschach A, Janecke AR, Tariverdian G, Chelly J, Fryns JP, Van Esch H, Kleefstra T, Hamel B, Moraine C, Gecz J, Turner G, Reinhardt R, Kalscheuer VM, Ropers HH, Lenzner S. Mutations in the JARID1C gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. Am J Hum Genet 2005; 76: 227–36.
88. Santos C, Rodriguez-Revenga L, Madrigal I, Badenas C, Pineda M, Mila M. A novel mutation in JARID1C gene associated with mental retardation. Eur J Hum Genet 2006; 14: 583–6.
89. Tzschach A, Lenzner S, Moser B, Reinhardt R, Chelly J, Fryns JP, Kleefstra T, Raynaud M, Turner G, Ropers HH, Kuss A, Jensen LR. Novel JARID1C/SMCX mutations in patients with X-linked mental retardation. Hum Mutat 2006; 27: 389.
90. Bhat KP, Turner JD, Myers SE, Cape AD, Ting JP, Greer SF. The 19S proteasome ATPase Sug1 plays a critical role in regulating MHC class II transcription. Mol Immunol 2008; 45: 2214–24.
91. Koues OI, Mehta NT, Truax AD, Dudley RK, Brooks JK, Greer SF. Roles for common MLL/COMPASS subunits and the 19S proteasome in regulating CIITA pIV and MHC class II gene expression and promoter methylation. Epigenet Chromatin 2010; 3: 5.
92. Koues OI, Dudley RK, Truax AD, Gerhardt D, Bhat KP, McNeal S, Greer SF. Regulation of acetylation at the major histocompatibility complex class II proximal promoter by the 19S proteasomal ATPase Sug1. Mol Cell Biol 2008; 28: 5837–50.
93. Uprety B, Lahudkar S, Malik S, Bhaumik SR. The 19S proteasome subcomplex promotes the targeting of NuA4 HAT to the promoters of ribosomal protein genes to facilitate the recruitment of TFIID for transcriptional initiation *in vivo*. Nucleic Acids Res 2012; 40: 1969–83.
94. Ferdous A, Gonzalez F, Sun L, Kodadek T, Johnston SA. The 19S regulatory particle of the proteasome is required for efficient transcription elongation by RNA polymerase II. Mol Cell 2001; 7: 981–91.
95. Gonzalez F, Delahodde A, Kodadek T, Johnston SA. Recruitment of a 19S proteasome subcomplex to an activated promoter. Science 2002; 296: 548–50.
96. Ezhkova E, Tansey WP. Proteasomal ATPases link ubiquitylation of histone H2B to methylation of histone H3. Mol Cell 2004; 13: 435–42.
97. Geng F, Tansey WP. Similar temporal and spatial recruitment of native 19S and 20S proteasome subunits to transcriptionally active chromatin. Proc Natl Acad Sci USA 2012; 109: 6060–5.
98. Rasti M, Grand RJ, Yousef AF, Shuen M, Mymryk JS, Gallimore PH, Turnell AS. Roles for APIS and the 20S proteasome in adenovirus E1A-dependent transcription. EMBO J 2006; 25: 2710–22.
99. Gillette TG, Gonzalez F, Delahodde A, Johnston SA, Kodadek T. Physical and functional association of RNA polymerase II and the proteasome. Proc Natl Acad Sci USA 2004; 101: 5904–9.
100. Fioravante D, Byrne JH. Protein degradation and memory formation. Brain Res Bull 2011; 85: 14–20.
101. Jarome TJ, Helmstetter FJ. The ubiquitin-proteasome system as a critical regulator of synaptic plasticity and long-term memory formation. Neurobiol Learn Mem 2013; 105: 107–16.
102. Hegde AN, Haynes KA, Bach SV, Beckelman BC. Local ubiquitin-proteasome-mediated proteolysis and long-term synaptic plasticity. Front Mol Neurosci 2014; 7: 96.
103. Fischer DF, van DR, van TP, Hobo B, Verhage MC, van der Schors RC, Li KW, van Minnen J, Hol EM, van Leeuwen FW. Long-term

proteasome dysfunction in the mouse brain by expression of aberrant ubiquitin. Neurobiol Aging 2009; 30: 847–63.
104. Dong C, Bach SV, Haynes KA, Hegde AN. Proteasome modulates positive and negative translational regulators in long-term synaptic plasticity. J Neurosci 2014; 34: 3171–82.
105. Asano S, Fukuda Y, Beck F, Aufderheide A, Forster F, Danev R, Baumeister W. Proteasomes. A molecular census of 26S proteasomes in intact neurons. Science 2015; 347: 439–42.
106. Benito E, Barco A. The Neuronal Activity-Driven Transcriptome. Mol Neurobiol 2015; 51: 1071–88.
107. Barco A, Patterson SL, Alarcon JM, Gromova P, Mata-Roig M, Morozov A, Kandel ER. Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. Neuron 2005; 48: 123–37.
108. Bach SV, Tacon PR, Morgan JW, Hegde AN. Proteasome regulates transcription-favoring histone methylation, acetylation and ubiquitination in long-term synaptic plasticity. Neurosci Lett 2015; 591: 59–64.
109. Day JJ, Sweatt JD. Epigenetic mechanisms in cognition. Neuron 2011; 70: 813–29.
110. Zovkic IB, Guzman-Karlsson MC, Sweatt JD. Epigenetic regulation of memory formation and maintenance. Learn Mem 2013; 20: 61–74.
111. Gupta S, Kim SY, Artis S, Molfese DL, Schumacher A, Sweatt JD, Paylor RE, Lubin FD. Histone methylation regulates memory formation. J Neurosci 2010; 30: 3589–99.
112. Morse SJ, Butler AA, Davis RL, Soller IJ, Lubin FD. Environmental enrichment reverses histone methylation changes in the aged hippocampus and restores age-related memory deficits. Biology (Basel) 2015; 4: 298–313.
113. Gupta-Agarwal S, Franklin AV, Deramus T, Wheelock M, Davis RL, McMahon LL, Lubin FD. G9a/GLP histone lysine dimethyltransferase complex activity in the hippocampus and the entorhinal cortex is required for gene activation and silencing during memory consolidation. J Neurosci 2012; 32: 5440–53.
114. Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD. Regulation of histone acetylation during memory formation in the hippocampus. J Biol Chem 2004; 279: 40545–59.
115. Lopez-Atalaya JP, Ito S, Valor LM, Benito E, Barco A. Genomic targets, and histone acetylation and gene expression profiling of neural HDAC inhibition. Nucleic Acids Res 2013; 41: 8072–84.
116. Riffo-Campos AL, Castillo J, Tur G, Gonzalez-Figueroa P, Georgieva EI, Rodriguez JL, López-Rodas G, Rodrigo MI, Franco L. Nucleosome-specific, time-dependent changes in histone modifications during activation of the early growth response 1 (Egr1) gene. J Biol Chem 2015; 290: 197–208.
117. Buro LJ, Chipumuro E, Henriksen MA. Menin and RNF20 recruitment is associated with dynamic histone modifications that regulate signal transducer and activator of transcription 1 (STAT1)-activated transcription of the interferon regulatory factor 1 gene (IRF1). Epigenet Chromatin 2010; 3: 16.
118. Guan Z, Giustetto M, Lomvardas S, Kim JH, Miniaci MC, Schwartz JH, Thanos D, Kandel ER. Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. Cell 2002; 111: 483–93.
119. Minsky N, Shema E, Field Y, Schuster M, Segal E, Oren M. Monoubiquitinated H2B is associated with the transcribed region of highly expressed genes in human cells. Nat Cell Biol 2008; 10: 483–8.
120. Weake VM, Workman JL. Histone ubiquitination: triggering gene activity. Mol Cell 2008; 29: 653–63.
121. Wyce A, Xiao T, Whelan KA, Kosman C, Walter W, Eick D, Hughes TR, Krogan NJ, Strahl BD, Berger SL. H2B ubiquitylation acts as a barrier to Ctk1 nucleosomal recruitment prior to removal by Ubp8 within a SAGA-related complex. Mol Cell 2007; 27: 275–88.
122. Woldemichael BT, Bohacek J, Gapp K, Mansuy IM. Epigenetics of memory and plasticity. Prog Mol Biol Transl Sci 2014; 122: 305–40.
123. Sweatt JD. Experience-dependent epigenetic modifications in the central nervous system. Biol Psychiatry 2009; 65: 191–7.
124. Jarome TJ, Lubin FD. Epigenetic mechanisms of memory formation and reconsolidation. Neurobiol Learn Mem 2014; 115: 116–27.
125. Dantuma NP, van Attikum H. Spatiotemporal regulation of posttranslational modifications in the DNA damage response. EMBO J 2016; 35: 6–23.