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Proteasome regulates transcription-favoring histone methylation, acetylation and ubiquitination in long-term synaptic plasticity

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Abstract

Histone modifications, such as lysine methylation, acetylation and ubiquitination, are epigenetic tags that shape the chromatin landscape and regulate transcription required for synaptic plasticity and memory. Here we show that transcription-promoting histone H3 trimethylated at lysine 4 (H3K4me3), histone H3 acetylated at lysine 9 and 14 (H3K9/14ac), and histone H2B monoubiquitinated at lysine 120 (H2BK120ub) are enhanced after the induction of long-lasting chemically-induced long-term potentiation (cLTP) in the murine hippocampus. While H3K4me3 and H3K9/14ac were transiently upregulated, H2BK120ub levels oscillated after cLTP induction. In addition, we present results showing that blocking the proteasome, a molecular complex specialized for targeted protein degradation, inhibited the upregulation of these epigenetic tags after cLTP. Thus, our study provides the initial steps towards understanding the role of the proteasome in regulating histone modifications critical for synaptic plasticity.

Keywords

Transcription; long-term potentiation; epigenetic modification; proteolysis; ubiquitin

1. Introduction

Histone modification is an important epigenetic mechanism that underlies long-term learning and memory [24]. Posttranslational modifications (PTMs) of histone proteins include lysine acetylation, mono-, di- and tri- methylation, and ubiquitination. Histone lysine acetylation is a transcription-activating tag that is added by histone acetyltransferases (HATs) and is removed by histone deacetylases (HDACs) [4]. Histone lysine mono-, di- and tri- methylation can activate or repress transcription, depending on the location and the number of methyl groups. Methylation is controlled by histone lysine methyltransferases

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(KMTs) and histone lysine demethylases (KDMs) [3]. Attachment of a single ubiquitin (monoubiquitination) to a lysine residue is associated with both transcriptional activation and repression when it occurs on H2B or H2A histones, respectively. Histone H2B monoubiquitination is thought to be a precursor to histone H3 methylation [12, 23].

Histone PTMs in brain regions specialized for learning and memory, such as the amygdala, the hippocampus, the prefrontal cortex, and the entorhinal cortex (EC), provide mechanisms for long-term memory storage as a long-lasting, yet flexible epigenetic code [14, 15]. Decreased transcription-favoring histone acetylation and methylation in the hippocampus of animals is associated with poor performance on hippocampal-dependent memory tasks [21, 31]. Mice deficient in mixed-lineage leukemia 2 (MLL), a KMT responsible for trimethylation of histone 3 at lysine 4 (H3K4me3), have impaired performance in contextual fear conditioning and water maze [16, 21]. Activation of HATs, such as CREB-binding protein (CBP), occurs in the hippocampus during consolidation of spatial memories [5]. To elucidate the mechanisms of chromatin remodeling in synaptic plasticity, we explored the role of the ubiquitin-proteasome pathway (UPP) in regulating histone PTMs.

The UPP specializes in targeted degradation of intracellular proteins. In this pathway, proteins are tagged with a polyubiquitin chain that signals their degradation by the 26S proteasome. The proteasome consists of a 20S catalytic core and two 19S regulatory caps. The 19S cap contains ATPase subunits, Rpt1-6, that unfold the substrate protein and channel it through the 20S core for degradation [17]. Accumulating evidence suggests that the UPP plays an important role in synaptic plasticity and memory [17, 19, 34]. Proteasome inhibition in the hippocampus and the amygdala is associated with impaired consolidation of an inhibitory avoidance memory and fear memory, respectively [20, 26]. Our previous studies show that the maintenance of hippocampal late phase of long-term potentiation (L-LTP), that depends on new transcription and translation and underlies long-term memory, is blocked by a specific proteasome inhibitor *clasto-lactacystin* β -lactone (henceforth, β -lactone) [7, 8]. We previously showed that β -lactone blocks the upregulation of a CREB-mediated gene, *brain-derived neurotrophic factor* (*Bdnf*), in L-LTP, indicating that proteasomal activity is necessary for transcription in synaptic plasticity [8]. The mechanisms by which the proteasome can regulate transcription are still poorly understood.

Studies in yeast and cancer cells showed that proteasomal subunits bind to promoters of active genes and physically interact with HATs and KMTs to regulate histone PTMs [2, 11, 12, 22, 23]. As a first step towards investigating the role of the proteasome in the regulation of histone PTMs in synaptic plasticity, we studied some of the transcription-favoring epigenetic modifications and monitored their levels after the induction of chemical LTP (cLTP). We show that H3K4me3, H3 acetylated at lysine 9 and 14 (H3K9/14ac), and H2B monoubiquitinated at lysine 120 (H2BK120ub) are dynamically regulated in synaptic plasticity and require proteasomal activity for their upregulation.

2. Materials and Methods

2.1 Induction of chemical long-term potentiation (cLTP)

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences. Male C57/Bl6 mice, 6–12 weeks old, were obtained from Harlan Laboratories (Frederick, MD). Transverse 400 μ m hippocampal slices were prepared using a tissue chopper in an oxygenated and chilled artificial cerebrospinal fluid (ACSF) containing 125 mM NaCl, 3 mM KCl, 2.3 mM CaCl_2 , 1.3 mM MgCl_2 , 25 mM NaHCO_3 , 1.25 mM NaH_2PO_4 , and 10 mM glucose (pH 7.4). After recovery for 2 h, slices were transferred to Mg^{2+} -free ACSF containing NMDA (200 nM) for 10 min followed by Mg^{2+} -free ACSF containing cAMP elevating reagents, for skolin (50 μ M) and rolipram (0.1 μ M), for 15 min and then returned to normal ACSF [8]. Some hippocampal slices were treated with an irreversible proteasome inhibitor, β -lactone (25 μ M) [8], for 30 min (β -lactone alone) while other slices were subjected to cLTP induction after the 30-min β -lactone treatment (β -lactone + cLTP).

2.2 Immunohistochemistry

Immunohistochemistry was carried out as described previously [7] with primary antibodies specific for H3K4me3, H3K9/14ac (EMD Millipore, Temecula, CA) or H2BK120ub (Cell Signaling Technology, Danvers, MA). Images of the CA1 region of the hippocampus were taken using Carl Zeiss LSM510 confocal laser scanning microscope and quantified using ImageJ software (National Institute of Health, Bethesda, MD).

2.3 Statistical analysis

All statistical analyses were carried out using one-way ANOVA followed by a Tukey's Multiple Comparison *post hoc* test. The values are represented as mean $\pm$ standard error. The sample size (n) corresponds to the number of animals used to collect the data, not the number of hippocampal slices.

3. Results

3.1 H3K4me3 and H3K9/14ac levels increase transiently after cLTP induction

The transcription-favoring histone PTMs, such as H3K4me3 and H3K9/14ac, are necessary for the induction of plasticity-related genes and memory formation [14, 31]. To test whether H3K4me3 and H3K9/14ac are upregulated in cLTP, we performed a time-course experiment with hippocampal slices fixed 0, 15, and 30 min after the end of the cLTP protocol. We chose to induce LTP using chemical rather than electrical stimulation to enhance the number of activated synapses and to improve signal-to-noise ratio [8]. Our previous studies, by carrying out electrophysiological recording after cLTP induction, showed that cLTP protocol induces long-lasting LTP similar to electrically-induced L-LTP. Also, plasticity-related genes, such as *Bdnf*, are upregulated in cLTP [8].

To study histone methylation and acetylation levels after cLTP induction, immunohistochemistry was carried out with antibodies specific for H3K4me3 (Fig. 1A) and H3K9/14ac (Fig. 1C). Quantification of immunoreactivity in the CA1 region showed a

significant upregulation of both methylation and acetylation at 0 min after cLTP induction (Fig. 1B, D; H3K4me3 [all time-points]: $F_{(3,22)}=4.48$, $P=0.01$, $n=6$; H3K4me3 [at 0 min]: 2.59 ± 0.54 fold change, $P<0.05$ compared with control; H3K9/14ac [all time-points]: $F_{(3,23)}=7.21$, $P=0.001$, $n=6$; H3K9/14ac [at 0 min]: 2.42 ± 0.37 fold change, $P<0.01$ compared with control). While histone methylation was no longer significantly upregulated at 15 min after cLTP induction compared to control (Fig. 1B), histone acetylation at 15 min was still significantly elevated (Fig. 1D; H3K9/14ac [at 15 min]: 2.13 ± 0.28 fold change, $P<0.05$ compared with control). By the 30-min time-point, both methylation and acetylation levels returned to baseline. These data show that H3K4me3 and H3K9/14ac are transiently upregulated after cLTP induction.

3.2 Increase in H3K4me3 and H3K9/14ac levels observed during cLTP is regulated by the proteasome

Studies in yeast and cancer cells show that the proteasome regulates transcription by modulating histone PTMs [2, 12, 22, 23]. To test whether proteasomal activity is necessary for histone methylation and acetylation in synaptic plasticity, hippocampal slices were treated with β -lactone and fixed immediately after the end of the cLTP protocol (cLTP 0 min).

We investigated the levels of methylation and acetylation of histones with proteasomal inhibition after cLTP using immunohistochemistry with antibodies specific for H3K4me3 (Fig. 2A) and H3K9/14ac (Fig. 2C). H3K4me3 and H3K9/14ac immunoreactivity was significantly upregulated with cLTP and this upregulation was brought down to control levels by β -lactone (Fig. 2B, D; H3K4me3: $F_{(3,29)}=28.4$, $P<0.0001$, $n=7$; cLTP vs. β -lactone + cLTP $P<0.001$; Control vs. β -lactone + cLTP $P>0.05$; H3K9/14ac: $F_{(3,24)}=9.68$, $P=0.0002$, $n=6$; cLTP vs. β -lactone + cLTP $P<0.05$; Control vs. β -lactone + cLTP $P>0.05$). In addition, H3K4me3 immunoreactivity in the β -lactone + cLTP condition was significantly upregulated compared to the β -lactone alone condition, indicating that proteasome inhibition may only cause a partial hindrance of histone methylation after cLTP (Fig. 2B; β -lactone + cLTP vs. β -lactone $P<0.05$). Moreover, we observed a reduction in H3K9/14ac with β -lactone treatment of control slices; however, the effect of β -lactone was not significant (Fig. 2D; Control vs. β -lactone $P>0.05$; β -lactone + cLTP vs. β -lactone $P>0.05$). Together, these data indicate that activity-dependent histone methylation and acetylation during cLTP require a functional proteasome.

3.3 H2BK120ub levels oscillate after cLTP induction and are regulated by the proteasome

Histone H2B monoubiquitination has been described as a precursor to histone H3 trimethylation [33]. In yeast cells, histone H2B monoubiquitination recruits a KMT, COMPASS (the homolog of the mammalian MLL), which tri-methylates H3K4 and promotes transcription [23]. Several other studies in yeast demonstrated an interaction between specific proteasomal ATPase subunits, Rpt4 and Rpt6, with COMPASS in a ubiquitin-dependent manner [9]. Based on these previous works suggesting that the proteasome facilitates the crosstalk between H2B monoubiquitination and histone H3 methylation [9], we tested the effect of proteasome inhibition on histone ubiquitination.

To determine whether H2BK120ub is upregulated after cLTP induction, we performed a time-course immunohistochemistry experiment with an antibody specific to H2BK120ub (Fig. 3A, C). An increase in H2BK120ub was observed at all cLTP time-points as compared to control slices; however, only cLTP 0 and 30 min time-points showed statistical significance (Fig. 3B; H2BK120ub [all time-points]: $F_{(3,24)}=17.61$, $P<0.0001$, $n=6$; H2BK120ub [at 0 min]: 3.24 ± 0.81 fold change, $P<0.01$ compared with control; H2BK120ub [at 30 min]: 5.09 ± 0.48 fold change, $P<0.001$ compared with control). Furthermore, the 30 min time-point of cLTP showed a significant upregulation of H2BK120ub compared to both cLTP 0 and 15 min (Fig. 3B; cLTP 0 min vs. cLTP 30 min $P<0.05$; cLTP 15 min vs. cLTP 30 min $P<0.01$). These data indicate that amounts of H2BK120ub might oscillate because of successive ubiquitination and deubiquitination events. Consistent with this idea, previous studies in yeast suggested that rapid changes in H2B ubiquitination status are required for consecutive transcription initiation and elongation [29, 35, 36].

Next, to determine whether histone ubiquitination is mediated by the proteasome, we compared immunostaining in the control, cLTP, β -lactone and β -lactone + cLTP hippocampal slices collected 30 min after the end of the cLTP protocol (Fig. 3C). The 30-min time-point was chosen because it was the peak of H2BK120ub expression, compared to the other time-points examined. Upregulation of H2BK120ub immunoreactivity after cLTP was significantly inhibited by β -lactone (Fig. 3D; H2BK120ub: $F_{(3,23)}=19.22$, $P<0.0001$, $n=6$; cLTP vs. β -lactone + cLTP $P<0.001$). H2BK120ub levels in the β -lactone alone group were not different from control or β -lactone-treated cLTP groups (Fig. 3D; Control vs. β -lactone $P>0.05$; β -lactone + cLTP vs. β -lactone $P>0.05$). These data indicate that the increase in transcription-promoting H2BK120ub during cLTP is dependent on proteasomal activity, but basal monoubiquitination is not affected by the proteasome.

4. Discussion

In the present study, we show that H3K4me3, H3K9/14ac and H2BK120ub are rapidly upregulated in the CA1 region of the hippocampus after induction of cLTP. The increase in methylation, acetylation and ubiquitination levels was dependent on proteasomal activity, since an irreversible proteasome inhibitor, β -lactone, blocked the upregulation of these epigenetic tags in cLTP. While H3K9/14ac and H2BK120ub appeared to be completely blocked by β -lactone, H3K4me3 showed only a partial inhibition with β -lactone pretreatment, indicating that other factors may contribute to the increase in H3K4me3 in synaptic plasticity. These results implicate the proteasome as a key regulator of transcription-favoring histone acetylation, methylation and ubiquitination.

Furthermore, we showed that while H3K4me3 and H3K9/14ac increase immediately after cLTP induction and decrease to baseline by 30 min, the levels of H2BK120ub appear to oscillate after cLTP induction peaking at 30 min. These findings are in agreement with the previous results in *Aplysia* showing that transient acetylation of histone H3 was critical during long-term synaptic plasticity [13, 24]. Therefore, it appears that lasting cellular changes in synaptic plasticity can be triggered by a transient histone modification signal.

Rapid, stimulation-induced changes in histone modifications at promoters of genes have been previously described in other model systems [6, 25, 32].

Although it is still unclear how proteasomal activity regulates histone modifications, there are several possible explanations. The most likely scenario is that proteasomal inhibition with β -lactone inhibits proteolytic degradation of chromatin remodeling enzymes. For example, one KDM responsible for the removal of H3K4 di- and tri- methylation, KDM5C (also known as JARID1C), has been identified as a target for polyubiquitination and degradation by the proteasome [27]. Mutations in the KDM family of enzymes have been associated with neurological disorders such as X-linked mental retardation and autism [1, 18]. Thus, removal of transcription-repressing KDM5C by the proteasome may be necessary for normal synaptic plasticity and memory.

Another way in which the proteasome can regulate PTMs in synaptic plasticity is through its non-proteolytic roles [2]. From work on yeast and cancer cells, proteasomal 19S ATPases are known to bind to promoters of actively transcribed genes [12]. The 19S ATPase subunits are found to regulate histone methylation and acetylation in yeast by interacting with HATs and KMTs [22]. They may also facilitate the crosstalk between histone H2B monoubiquitination and histone H3 methylation [9]. To inhibit the proteasome in our study we used β -lactone, which covalently modifies a specific subunit of the 20S catalytic core and irreversibly inhibits its chymotrypsin-like and trypsin-like activities, but is not known to affect the 19S. It has been hypothesized, however, that β -lactone could cause a conformational change of the proteasome that is transmitted to other active sites [10]. Therefore, our data would suggest that the 19S regulates histone PTMs in synaptic plasticity if β -lactone were to inhibit the 19S allosterically.

Other components of the UPP, such as free ubiquitin, could also influence histone PTMs in synaptic plasticity. Inhibition of the proteasome causes the accumulation of polyubiquitinated substrates and depletion of free ubiquitin pools [28]. Prevention of H2B monoubiquitination because of a lack of free ubiquitin could prevent recruitment of transcription activating complexes, such as MLL, to active chromatin and alter transcription [23, 30, 36].

5. Conclusion

Taken together, our study indicates that transcription-promoting histone methylation, acetylation and ubiquitination are dynamically regulated in synaptic plasticity and are modulated by the proteasome. Our study provides the first evidence that the proteasome can regulate epigenetic tags in synaptic plasticity.

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Figure 1.

H3K4me3 and H3K9/14ac increase transiently after cLTP induction. Confocal images showing H3K4me3 (A) and H3K9/14ac (C) immunoreactivity in the nuclei of the CA1 region of untreated (control) hippocampal slices and hippocampal slices subjected to cLTP and fixed 0, 15, and 30 min after the end of cLTP induction protocol. Scale bars: 20 μm and 5 μm in low and high-power magnification images, respectively. Quantification of H3K4me3 (B) and H3K9/14ac (D) immunoreactivities for the four experimental conditions is shown at right. The values are expressed as mean ± SE. * $P < 0.05$, ** $P < 0.01$ comparison with control (depicted by a dashed line); $n = 6$; one-way ANOVA; Tukey's *post hoc*.

Figure 2.

Increase in H3K4me3 and H3K9/14ac seen during cLTP is inhibited by β-lactone. Confocal images showing H3K4me3 (A) and H3K9/14ac (C) immunoreactivity in the nuclei of the CA1 region of hippocampal slices in control, cLTP, cLTP after β-lactone pretreatment (β-lactone + cLTP), and β-lactone alone conditions. All slices were collected and fixed immediately after the end of cLTP induction protocol (0 min). Scale bars: 20 μm and 5 μm in low and high-power magnification images, respectively. Quantification of H3K4me3 (B) and H3K9/14ac (D) immunoreactivities for the four experimental conditions is shown at right. The values are expressed as mean ± SE. ** $P < 0.01$, *** $P < 0.001$ comparison with control (depicted by a dashed line); # $P < 0.05$, ### $P < 0.001$ (comparison between two groups indicated by a horizontal line); ns: not significant; H3K4me3 $n = 7$; H3K9/14ac $n = 6$; one-way ANOVA; Tukey's *post hoc*.

Figure 3.

H2BK120ub levels oscillate after cLTP induction and are reduced by β -lactone. Confocal images showing H2BK120ub (A, C) immunoreactivity in the nuclei of the CA1 region of control hippocampal slices and hippocampal slices subjected to cLTP 0, 15, and 30 min after the end of cLTP induction protocol. Some hippocampal slices were pretreated with β -lactone prior to cLTP induction (β -lactone + cLTP) or with β -lactone alone (C). Scale bars: 20 μ m and 5 μ m in low and high-power magnification images, respectively. Quantification of H2BK120ub (B, D) immunoreactivities for the experimental conditions is shown at right. The values are expressed as mean $\pm$ SE. ** P < 0.01 and *** P < 0.001 comparison with control (depicted by a dashed line); # P < 0.05, ## P < 0.01 and ### P < 0.001 (comparison between two groups indicated by a horizontal line); ns: not significant; n = 6; one-way ANOVA; Tukey's *post hoc*.