REVIEW

The Role of Histone Acetylation in Memory Formation and Cognitive Impairments

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Long-term memory formation requires transcription and protein synthesis. Over the past few decades, a great amount of knowledge has been gained regarding the molecular players that regulate the transcriptional program linked to memory consolidation. Epigenetic mechanisms have been shown to be essential for the regulation of neuronal gene expression, and histone acetylation has been one of the most studied and best characterized. In this review, we summarize the lines of evidence that have shown the relevance of histone acetylation in memory in both physiological and pathological conditions. Great advances have been made in identifying the writers and erasers of histone acetylation marks during learning. However, the identities of the upstream regulators and downstream targets that mediate the effect of changes in histone acetylation during memory consolidation remain restricted to a handful of molecules. We outline a general model by which corepressors and coactivators regulate histone acetylation during memory storage and discuss how the recent advances in high-throughput sequencing have the potential to radically change our understanding of how epigenetic control operates in the brain.

INTRODUCTION

It has long been acknowledged that there can be long-lasting changes in phenotype that are not encoded in the DNA sequence of a cell. The term epigenetics was originally coined by Waddington in 1942 (Waddington, 1942) to explain phenotypic changes that occur from cell to cell during development. It was defined independently by Nanney in 1958 (Nanney, 1958) as 'auxiliary mechanisms involved in determining which specificities are to be expressed in any particular cell.' Allis *et al* (2007b) crystallized the molecular definition of epigenetics as 'the sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (transcription) and silencing from the same genome'. Although once thought as a more or less irreversible process that happens in dividing cells, it is now known that epigenetic mechanisms can be dynamic. In the past decade, considerable evidence has accumulated that the brain utilizes epigenetic marks to encode responses to environmental stimuli and their associated behaviors (Borrelli *et al*, 2008). Both histone acetylation and DNA methylation have been shown to play important roles in memory consolidation. This review focuses on understanding the role of one of these processes, histone acetylation, in the expression of a long-lasting but incredibly malleable behavior, memory. Histone acetylation has been the most characterized epigenetic mechanism involved in memory formation, and its relevance has been shown in both physiological and pathological conditions. However, a full understanding of the mechanisms by which histone acetylation controls the gene expression patterns that encode long-term memory remains elusive.

Histone Acetylation and the Control of Gene Expression

Gene expression in eukaryotes is greatly influenced by the status of chromatin, the complex of proteins and DNA that constitutes chromosomes and allows the linear genome to exist within the nucleus of a cell. Changes in chromatin structure regulate the accessibility and the recruitment of

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the transcriptional machinery to the DNA and hence determine if transcription can occur. The nucleosome, the fundamental unit of chromatin, is composed of an octamer of the four core histones (H3, H4, H2A, and H2B) around which DNA is wrapped (Luger *et al*, 1997). Histone modifications encompass a variety of posttranslational modifications to the tails of histone proteins and are among the most studied forms of epigenetic mechanisms that control gene expression (Kouzarides, 2007). In particular, histone acetylation, which involves the addition of acetyl groups to lysines present in the N-terminal tails on the surface of the nucleosome, is associated with active transcription (Hebbes *et al*, 1988).

Originally, the model by which histone acetylation promotes transcription was based on the assumption that charge neutralization upon acetylation of lysines on histone tails would loosen the electrostatic attraction between DNA and histones and facilitate transcription (Davie and Chadee, 1998). Although it is clear that histone acetylation directly alters chromatin structure and accessibility, experiments in yeast and *in vitro* have suggested that charge neutralization is unlikely the cause (Choi and Howe, 2009). Yeast mutagenesis studies have shown that acetylation site substitutions from lysine to arginine and deletions on histone H3 tails both lead to GAL promoter hyperactivation (Mann and Grunstein, 1992). *In vitro* studies have also shown that the interaction between histone tails and DNA is not weakened by acetylation in physiological conditions (Mutskov *et al*, 1998). This evidence argues that the recognition of the acetylated lysines is likely more important than the change in charge itself, a point that probably applies to mammalian systems as well. More recent evidence has shown that acetylated histones are also able to serve as molecular tags. Proteins with bromodomains, which are frequently found in complexes that posttranslationally modify chromatin including transcriptional coactivators such as CBP, p300, and PCAF (Mujtaba *et al*, 2007; Sanchez and Zhou, 2009; Zeng and Zhou, 2002), are able to bind acetyllysines. Whether histone acetylation promotes transcription through direct chromatin remodeling, such as opening chromatin structure, or recruitment of other factors is still debated. Both mechanisms are not mutually exclusive and likely work in conjunction to affect gene expression.

The fact that histone modifications can recruit other proteins by recognition of the modified histone via protein domains is a central idea of the histone code hypothesis (Allis *et al*, 2007b; Jenuwein and Allis, 2001). The histone code hypothesis refers to the combination of modifications within and between histones that code for information not present in the DNA sequence and predicts that the modification marks on the histone tails should provide binding sites for proteins with regulatory functions that are able to 'read' such marks. How those modifications are established or removed is a key step in epigenetic regulation, and a wealth of work has shown that histone tail modifications are established ('written') or removed
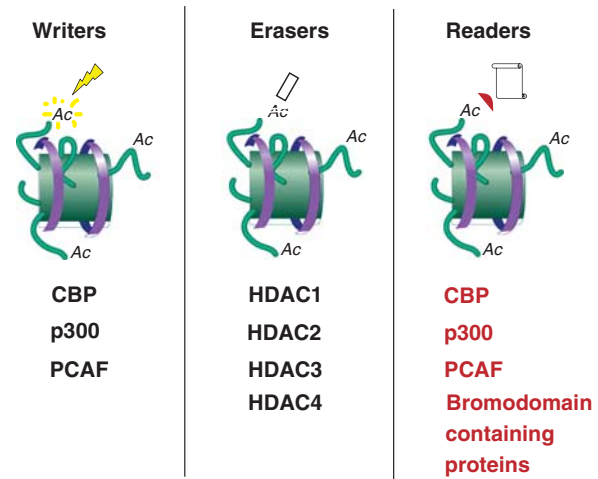

Figure 1.   Writers, Erasers, and Readers of histone marks during long-term memory formation. Molecules that regulate acetylation of histone tails can be conceptually grouped into three categories (Borrelli *et al*, 2008): Writers, the enzymes that are able to add acetyl groups to the lysines in the tails (KATs); Erasers, the enzymes that remove the acetyl groups (KDACs); and Readers, the proteins that possess bromodomains and can recognize acetylated lysines (including KATs). For details on the evidence linking listed writers, erasers, and readers, see Tables 2 and 3.

('erased') by the action of chromatin-associated enzymatic complexes. As 'writers' of histone acetylation marks often possess bromodomains, they are able to 'read' the marks as well, creating the possibility of a positive feedback loop. The context dependence of the function of particular modifications, as well as the overlap between 'readers' and 'writers', has led to the proposal of a histone 'language' rather than a code (Lee *et al*, 2010) (Figure 1). It remains to be proven whether acetyl-lysine/bromodomain interactions are sufficient to mediate targeting of chromatin-modifying complexes to specific regions or require crosstalk with other types of modifications. However, increasing evidence has pointed to recruitment of 'readers' as the key function of histone acetylation (Yun *et al*, 2011). Hence, patterns of histone acetylation can induce transcription by direct recruitment of other proteins and not necessarily by destabilizing the interaction between histones and DNA, although both mechanisms are not mutually exclusive.

It was demonstrated early on that histone acetylation is rapid and reversible (DeLange and Smith, 1971). Levels of histone acetylation are controlled by the balance of proteins with histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity. HATs are frequently transcriptional coactivators and possess bromodomains, whereas HDACs are usually part of corepressor complexes. Many HAT and HDAC enzymes have been shown to target nonhistone proteins, and were given a more generic name to reflect this phenomenon: lysine-acetyltransferases (KATs) and lysine deacetylases (KDACs; Allis *et al*, 2007a; Choudhary *et al*, 2009). Although the nomenclature of KATs has been changed to reflect this, the nomenclature of KDACs has remained the same as they already had a coherent nomenclature (Allis *et al*, 2007a). Therefore, individual

KDACs will be named as HDAC1-11, whereas general lysine deacetylase function will be referred as KDAC. The availability of KDAC inhibitors and histone modification-specific antibodies has allowed for a more thorough understanding of histone acetylation dynamics and its relationship with gene expression. Studies have shown that fast turnover of acetylation is tied to transcriptional activation (Waterborg, 2002). Binding of both KATs and KDACs to promoter regions is positively correlated with genome-wide gene expression (Wang *et al*, 2009). It has also been reported that the use of KDAC inhibitors can downregulate gene expression and acetylation levels at some promoters despite global increases in histone acetylation (Rada-Iglesias *et al*, 2007). Hence, the regulation of gene expression by histone acetylation functions likely through rapid turnover of histone marks and the dynamic recruitment of factors to the DNA that can themselves affect the function of the transcriptional machinery. Numerous studies have reported changes in histone acetylation and gene expression following diverse activating signals. However, one of the most notable examples of the role of histone acetylation in the regulation of biological function is its role in learning and memory formation.

HISTONE ACETYLATION AND THE ESTABLISHMENT OF LONG-TERM MEMORY

It has long been known that long-term memory formation requires transcription and protein synthesis *in vivo* (Agranoff *et al*, 1967; Flood *et al*, 1973). That requirement has also been demonstrated in *ex vivo* models of synaptic plasticity such as long-term potentiation (LTP) (Nguyen *et al*, 1994; Stanton and Sarvey, 1984). It has also been shown that this requirement is limited to 'critical periods' after learning (Bourtchouladze *et al*, 1998; Igaz *et al*, 2002), demonstrating that there is a necessary timeline of gene expression events. The regulation of transcription necessary for synaptic plasticity and long-term memory formation has been shown to be dependent on the cAMP response element-binding protein (CREB) as well as the NF-κB family of transcription factors (Albensi and Mattson, 2000; Gutierrez and Davies, 2011; Pittenger *et al*, 2002; Sakamoto *et al*, 2011). Several genes, in particular immediate-early genes (IEGs), have been implicated as targets, including c-fos, BDNF, Egr1, and the Nr4a family of transcription factors (Dragunow, 1996; Hawk and Abel, 2011a; Perez-Cadahia *et al*, 2011). More recently, it has been demonstrated that the regulation of transcription via histone acetylation is essential for memory formation, establishing epigenetic modifications as a potential mechanism for the persistence of long-term memory. However, it is still unclear how epigenetic changes are functionally related to specific transcriptional changes. In this section we summarize the lines of evidence that have linked histone acetylation to memory formation and what we know about the molecular pathways that underlie this phenomenon.

Linking Acetylation to Memory: Global Changes in Histone Acetylation, KDAC Activity, and the Effects of KDAC Inhibitors in Learning

Early studies using radioactive acetate incorporation showed that histone acetylation was increased in the hippocampus after training when compared with untrained controls, whereas histone acetylation was decreased in other brain regions such as the cortex (Schmitt and Matthies, 1979). More than two decades later, histone acetylation was shown to be a critical component of memory formation (Alarcon *et al*, 2004; Korzus *et al*, 2004; Levenson *et al*, 2004). KDAC inhibitors such as Trichostatin A (TSA) and sodium butyrate (NaB) enhance LTP, and systemic injection of NaB enhances memory *in vivo* (Levenson *et al*, 2004). Intrahippocampal injection of TSA immediately after learning produces enhancements in long-term memory without affecting short-term memory (Vecsey *et al*, 2007), suggesting that histone acetylation is necessary for memory consolidation. Studies have also shown that injection of NaB can facilitate the formation of long-term memory for weak stimuli and enhance persistence of long-term memory (Stefanko *et al*, 2009). Hence, histone acetylation has a functional role in long-term memory formation.

Histone acetylation occurs at a variety of lysine positions within the four core histone proteins (Roth *et al*, 2001; Shahbazian and Grunstein, 2007; Suganuma and Workman, 2011). Chromatin immunoprecipitation (ChIP) studies have identified changes in acetylation at particular histones or positions within histones with long-term memory formation and synaptic plasticity. Table 1 summarizes these findings. Initial studies identified an increase in acetylation of histones H3 and H4 after 5-HT facilitation in *Aplysia* (Guan *et al*, 2002). Levenson *et al* (2004) then showed that acetylated levels of histone H3 increased 1 h after contextual fear conditioning in area CA1 of the hippocampus. Latent inhibition, however, increased histone H4 but not H3 acetylation. Forskolin stimulation of hippocampal slices induces acetylation of histone H3K14 but not of H4 (Chwang *et al*, 2007). BDNF treatment also induces H3 acetylation at K9 and K14 (Calfa *et al*, 2011). Studies in rats following Morris water maze training show increase in acetylation histones H2B, H3, and H4, but only increases in H2B and H4 acetylation are exclusive to the hidden platform version of the task (Bousiges *et al*, 2010). Recent studies have shown an increase in acetylation of histones H3K9, K14, H4K5, K8, and K12 (but not K16) at 1 h after contextual fear conditioning in healthy young mice (Peleg *et al*, 2010). A strong correlation has been reported between transcriptional activity and acetylation of the histone H3 and H4 lysine residues (Pokholok *et al*, 2005). The relationship between acetylation of H2B and gene expression is less well known, but it has been reported to mirror that of H3 and H4 only in the most transcribed genes (Myers *et al*, 2003). It is likely that acetylation of H2B, H3, and H4 has a role in memory storage. However, residues of histones H3 and H4 are acetylated and deacetylated by

TABLE 1 Summary of Histone Acetylation Marks Shown to be Altered by Long-Term Memory Formation and Synaptic Plasticity

	Acetylated position	Writer (acetylated)	Eraser (deacetylated)	Reader (recognized)	Change in acetylation after learning/synaptic plasticity
H2A	K5, K7	CBP/p300, HAT1, Esa1 (MYST2)	Rpd3 (HDAC1–2)		Reduced in CBP mutant mice (Valor et al, 2011)
H2B	K5, K12, K15, K20	CBP/p300, GCN5	Rpd3 (HDAC1–2) Hda1 (HDAC4–7)		Reduced in CBP mutant mice (Alarcon et al, 2004; Valor et al, 2011) Induced after MWM (hidden > visible) (Bousiges et al, 2010)
H3	K9, K14, K18, K23, K27, K36	K9: GCN5/PCAF K14: CBP/p300, GCN5/PCAF K18: CBP/p300, GCN5/PCAF K23/K27: GCN5	Rpd3 (HDAC1–2) HDAC8 K9: SIRT1	K9/K14: TAFIID K9: PCAF K14: DPF3b, PCAF K36: PCAF	Induced after LTF in Aplysia (Guan et al, 2002) Not reduced in CBP mutant mice (Alarcon et al, 2004) Induced in hippocampal slices after FSK and PDA (K14, Chwang et al, 2007) Induced after CFC 1 h but not 24 h (Levenson et al, 2004) Induced after cued fear conditioning in amygdala (Monsey et al, 2011) Induced at Nr4a2 and Nr4a1 promoter after CFC+TSA (Vecsey et al, 2007) Induced upon learning in food aversion in mollusks (Danilova et al, 2010) K9, K14: induced 1 h after CFC (Peleg et al, 2010) K9, K14 induced after MWM (both visible and hidden) (Bousiges et al, 2010) K9, K14 induced after BDNF treatment (Calfa et al, 2011) K14 decreased in CBP focal KO (Barrett et al, 2011) Reduced in CBP mutant mice (Valor et al, 2011)
H4	K5, K8, K12, K16, K20	Esa1 (MYST2) K5, 8, 12: CBP/p300, HAT1 K16 – MOF	Rpd3 (HDAC1–2) K16: SIRT1	K5 (deAc): SMRT K8: BRG1 (SWI/SNF) K12: Bdf1 (SWR1) K16: SIRT1, NURF301 (ISWI) K20: CBP	Induced after LTF in Aplysia (Guan et al, 2002) Not reduced in CBP mutant mice (Alarcon et al, 2004) Not induced in hippocampal slices after FSK and PDA (Chwang et al, 2007) Induced after latent inhibition but not CFC (Levenson et al, 2004) Not induced after cued fear conditioning in amygdala (Monsey et al, 2011) Induced at Nr4a2 and Nr4a1 promoters after CFC+TSA (Vecsey et al, 2007) K5, K12: increased in HDAC2 KO mice (Guan et al, 2009) K5, K8, K12: induced 1 h after CFC (but not K16) (Peleg et al, 2010) K12: induced after MWM (hidden > visible) (Bousiges et al, 2010) K8 decreased in CBP focal KO (but not K12; Barrett et al, 2011) Reduced in CBP mutant mice (Valor et al, 2011)

Abbreviations: CFC, contextual fear conditioning; MWM, Morris water maze.
Based on Shahbazian and Grunstein (2007) and Suganuma and Workman (2011).
Human/mouse orthologs of yeast proteins in columns 3, 4, and 5 (in parenthesis) were mapped using the OrthoMCL database (http://www.orthomcl.org). Individual residues in column 6 indicated when tested.

different enzymes and recruit different protein complexes (reviewed in Suganuma and Workman, 2011; summarized in Table 1). Hence, it is possible that different forms of learning will result in different patterns of acetylation at specific promoters that will lead to the activation of an overlapping yet slightly divergent transcriptional program.

Because inhibiting KDACs leads to memory enhancements, it is reasonable to propose that KDACs act as memory-suppressor genes (Abel et al, 1998). There are four classes of KDACs, based on sequence homology and domain structure. The use of selective gene knockout and siRNA gene suppression in conjunction with pharmacological approaches has allowed the study of the role of specific KDACs in memory (see Table 2 for a summary of KDAC involvement in memory formation). TSA and NaB are broad class I and class II KDAC inhibitors (Dokmanovic et al, 2007). Using MS-275, a selective KDAC inhibitor, it has been recently demonstrated that class I KDAC inhibition is sufficient for memory enhancement (Hawk et al, 2011b). Overexpression of HDAC2 (but not HDAC1) decreases synaptic plasticity and memory formation and HDAC2 knockouts display memory enhancements (Guan et al, 2009). Although overexpression of HDAC1 does not disrupt initial memory consolidation, it has been recently shown to affect fear extinction (Bahari-Javan et al, 2012). Down-regulation of HDAC3 using genetic and pharmacological

TABLE 2 Summary of KDAC Involvement in Long-Term Memory Formation

Class	KDAC	Localization	KDAC inhibitor	Brain Expression[a]	Interaction with other HDACs	Involvement in learning and memory
I	HDAC1	Nucleus	TSA, MS275, VPA[b], NaB, SAHA	0.3	HDAC2 (Sin3, NurD, and CoREST complexes)	No effect of overexpression in memory (Guan et al, 2009)
	HDAC2	Nucleus	TSA, MS275, VPA [b], NaB, SAHA	1.6	HDAC1 (Sin3, NurD, and CoREST complexes)	Overexpression suppresses memory and deletion enhances (Guan et al, 2009),
	HDAC3	Nucleus/cytoplasm, plasma membrane	TSA, MS275, VPA[b], NaB, RGFP136, SAHA	0.8	HDACs 4, 5, and 7	Inhibition enhances memory (McQuown et al, 2011)
	HDAC8	Nucleus	TSA, MS275, VPA[b], NaB, SAHA	—		
IIA	HDAC4	Nucleus /cytoplasm	TSA, VAHA, NaB	—	HDAC3/SMRT/N-CoR complex	
	HDAC5	Nucleus/cytoplasm	TSA,VAHA, NaB	7.2	HDAC3/SMRT/N-CoR complex	Recruitment blocks long-term facilitation in Aplysia (Guan et al, 2002)
	HDAC7	Nucleus/cytoplasm	TSA,VAHA, NaB	0.8	HDAC3/SMRT/N-CoR complex	
	HDAC9	Nucleus /cytoplasm	TSA, VAHA, NaB	0.8		
IIB	HDAC6	Cytoplasm	TSA, VAHA, SAHA	4.5		
	HDAC10	Nucleus /cytoplasm	TSA	—		
III	Sirtuins			—		
IV	HDAC11			—		

[a]EST, per/100 000 tags.
[b]VPA does not inhibit class II HDACS at physiological concentrations (Fass et al, 2010).
Based on McQuown and Wood (2011) and Sengupta and Seto (2004).

approaches in the hippocampus is sufficient to enhance contextual fear memory (McQuown et al, 2011). HDAC5 recruitment blocks long-term facilitation in Aplysia (Guan et al, 2002). Class II KDACs (HDACs 4, 5, 7, and 10) are known to interact with class I HDACs, in particular with HDAC3-containing complexes (McQuown and Wood, 2011). It is likely that class I KDACs: HDAC1, HDAC2, and HDAC3, are involved in the epigenetic regulation of long-term memory; however, their individual contribution remains unclear. KDACs do not bind DNA directly but are a part of transcriptional multiprotein corepressor complexes (Sengupta and Seto, 2004). HDAC1 and HDAC2 are found together in corepressor complexes containing Sin3a, NurD, and CoREST. HDAC3 requires binding with the nuclear receptor corepressors N-CoR or SMRT for activity. In vitro studies have demonstrated that recruitment of Sin3 corepressor complex results in deacetylation of histones H3 and H4, whereas recruitment of the N-CoR/SMRT complex results in deacetylation of histone H3 only (Vermeulen et al, 2004). These findings provide evidence that distinct KDAC-containing corepressor complexes may play different roles in the regulation of transcription. It is also known that N-CoR interacts with Sin3a (Jones et al, 2001), and hence dissecting which of the KDACs found on those complexes is responsible for the transcriptional repression present in neurons may not be possible. It is likely that recruitment of both HDAC2 and HDAC3 corepressor complexes is relevant for memory consolidation but may vary with different learning or synaptic plasticity paradigms in a way that mirrors reported changes in H3 or H4 acetylation. An interesting observation is that the induction of H3 acetylation by BDNF treatment requires

KDAC activity and chronic TSA treatment actually inhibits further induction of histone acetylation and dendritic spine formation by BDNF (Calfa et al, 2011). Although this could be because of a ceiling effect, it is important to note that although KDAC inhibition produces memory enhancements in the short term, it could have the opposite effect with long-term exposure. Last, but not the least, the effects of KDAC inhibitors in learning may not be mediated exclusively through their effects on histone proteins, as acetylation of NF-κB has been shown to improve long-term memory retention (Yeh et al, 2004). More studies are needed to understand the specific roles of HDAC2 and HDAC3, as well as other components of the corepressor complexes, in learning and memory formation.

Levels of histone acetylation have been shown to be changed in other brain regions besides the hippocampus, such as the lateral amygdala (Adachi et al, 2009; Monsey et al, 2011). Normal aging-related memory impairment is associated with the lack of histone H4 acetylation, which can be rescued by treatment with KDAC inhibitors to restore memory function (Peleg et al, 2010). Histone acetylation has also been shown to be induced after food aversion training in mollusks (Danilova et al, 2010), and overexpression as well as RNAi-mediated knockdown of histone deacetylase Rpd3 has been shown to impair long-term memory in Drosophila (Fitzsimons and Scott, 2011). Thus, the evidence argues that histone acetylation is a general mechanism of memory storage. It has been shown that the proportion of genes whose expression is responsive to KDAC inhibition in cell culture is $\sim$2% of expressed transcripts (Van Lint et al, 1996), and although histone acetylation is usually correlated with transcriptional activ-

ity, it was demonstrated early on that TSA can differentially regulate CREB target genes by contributing to either activation or cessation of transcription (Fass et al, 2003). As the memory enhancement effects of KDAC inhibitors are dependent on transcription and translation, it is likely that the effects of histone acetylation in memory are restricted to a small subset of the genes that are altered. In the next section we discuss what is known regarding the molecular pathways that mediate the effects of histone acetylation in memory and their targets.

Defining the Molecular Pathways that Lead to Changes in Histone Acetylation

The regulation of transcription necessary for synaptic plasticity and long-term memory formation has been shown to be dependent on transcription factors CREB and NF-κB (Albensi and Mattson, 2000; Gutierrez and Davies, 2011; Pittenger et al, 2002; Sakamoto et al, 2011). CREB binding-protein (CBP), a transcriptional coactivator with KAT activity, has also been shown to be essential for long-term memory formation. Several lines of CBP mutant mice have confirmed its essential role in learning; and memory and mutations in CBP cause Rubinstein–Taybi syndrome (RSTS), a neurodevelopmental disorder characterized by cognitive impairments (Alarcon et al, 2004; Bourtchouladze et al, 2003; Oike et al, 1999; Tanaka et al, 1997; Wood et al, 2005). Early on, it was demonstrated that CBP KAT activity was a critical component of memory consolidation (Korzus et al, 2004). It was also shown that the CREB-binding domain (KIX) is required for long-term memory (Wood et al, 2006). The effect of KDAC inhibitors in long-term memory has been shown to be CBP dependent and involve its interaction with CREB (Alarcon et al, 2004; Haettig et al, 2011; Korzus et al, 2004; Vecsey et al, 2007). Although all studies of CBP mutant mice have found memory impairments, the results regarding specific tasks have been contradictory. Varying performance in spatial navigation has been observed in different CBP mutant mice (Alarcon et al, 2004; Korzus et al, 2004). Despite multiple studies demonstrating a role for CBP in fear conditioning (Alarcon et al, 2004; Barrett et al, 2011; Chen et al, 2010; Korzus et al, 2004; Vecsey et al, 2007; Wood et al, 2005), some studies have not seen deficits in long-term fear conditioning (Valor et al, 2011), making object recognition the only task that is consistently affected. Not all deletions are complete and each of the mutants studied were not on identical inbred genetic backgrounds, which has been shown to be essential in studies of CREB (Graves et al, 2002). Therefore, a small population of CBP-positive neurons may be sufficient for learning paradigms that either involve several trials or generate persistent memories. It is also hard to determine whether the defects observed in the above models are because of developmental compensation, as most studies have relied on transgenic mice.

Recent studies have tried to address the effect of deleting CBP from the postnatal brain in a space-restricted manner,

to isolate the role of CBP in learning away from its developmental effects. Chen et al (2010) showed that CBP seems to be required in excitatory neurons of the postnatal forebrain for both short-term and long-term memory formation. This is the first study to report short-term memory deficits in CBP mutants; however, the B6/129 hybrid background used in the study has been shown to exhibit short-term memory deficits when combined with CREB mutations (Graves et al, 2002), and hence it is unclear whether the deletion in CBP is indeed the cause of the deficit in short-term memory. Valor et al (2011) observed defects in novel object recognition but not in other memory tasks, such as contextual fear conditioning and Morris water maze. Focal deletion of CBP in hippocampal area CA1 using viral vectors impairs L-LTP (5 θ bursts) and long-term memory for contextual fear and object recognition (Barrett et al, 2011). Although it has been reported that CBP$^{+/-}$ transgenics only show deficits in acetylation of H2B, but not of H3 or H4 (Alarcon et al, 2004), focal deletion of CBP in the hippocampus produces deficits in H2BK12, H3K14, and H4K8 acetylation (Barrett et al, 2011) and forebrain deletion shows deficits in H2A, H2B, H3 and H4 acetylation (Valor et al, 2011). It is possible that global transgenic models show compensation during development or that newer lysine residue-specific antibodies are more sensitive. The deficit reported by Barrett et al (2011) is indeed greater in H2B acetylation, but H4K8 acetylation is affected whereas H4K12 is not, and hence an antibody that recognizes various forms of acetylated H4 will not be able to detect the difference. As mentioned before, different learning tasks seem to show differences in which specific histone marks are altered by training. Because H2B acetylation seems to be preferentially affected in CBP mutants, it is possible that learning paradigms whose transcriptional program is more dependent on H2B acetylation are affected to a greater extent. Overall, although it is clear that CBP plays a role in memory, and that the role is dependent on its KAT activity, the exact mechanism remains elusive. Determining the genes regulated by CBP is a crucial next step to understanding its role in memory.

Compensation of CBP function by expression of CBP paralogs may also be a confounding factor in the analysis of these different mutant mice. This is particularly a problem for constitutive CBP mutants, but compensatory induction of CBP paralogs could also occur on a short-time scale. p300, the closest homolog of CBP, is often able to acetylate the same residues. In addition to CBP, mutations in p300 have also been linked to RSTS (van Belzen et al, 2011). Studies of p300$^{+/-}$ mice have suggested that the role of p300 in memory is less critical than that of CBP, as their cognitive impairments were mild (Viosca et al, 2010). However, expression of an inhibitory form of p300 as well as forebrain-specific postnatal knockdown has shown that p300 is required for long-term recognition and contextual fear memory (Oliveira et al, 2007, 2011). The role of p300 in memory is likely different from the role of CBP, as CREB/CBP interaction is needed for motor learning whereas

TABLE 3 Summary of KAT Involvement in Long-Term Memory Formation

KAT group	KAT (and complexes associated)	Interactions with other KATs	Involvement in learning and synaptic plasticity
GNAT	KAT1/HAT1 KAT2A/GCN5 (SAGA, ADA, A2) KAT2B/PCAF KAT9/ELP3 (elongator) LAT10/Hap2	p300; CBP	PCAF is required for short-term memory in young mice and long-term in old mice (Maurice et al, 2008)
MYST	KAT5/TIP60/PLIP KAT6/Sas3 (NuA3) KAT6A/MOZ/MYST3 KAT6B/MORF/MYST4 KAT7/HBO1/MYST2 (ORC) KAT8/HMOF/MYST1 (MSL) KAT13D/CLOCK		
p300/CBP	KAT3A/CBP KAT3B/p300	PCAF; GCN5	p300 is required for long-term recognition and contextual fear memory (Oliveira et al, 2007, 2011, Viosca et al. 2010) Memory impairments observed in several types of CBP mutant mice: Hetereozygous knockout (Tanaka et al, 1997; Alarcon et al, 2004) Dominant-negative (Oike et al, 1999; Bourtchouladze et al, 2003) Spatially restricted transgenic dominant-negative (Wood et al, 2005) Spatially and temporally restricted conditional transgenic dominant-negative (Korzus et al, 2004) Homozygous knockin (Wood et al, 2006) Forebrain restricted postnatal knockout (Chen et al, 2010; Valor et al, 2011) Local deletion using viral injections (Barrett et al, 2011)
Basal transcription factors	KAT4/TAF1 (TFIID) KAT12/TFIIIC90		
Nuclear receptor cofactors	KAT13A/SRC1 KAT13B/ACTR KAT13C/P160		

Based on Allis et al (2007a) and Roth et al (2001).

CREB/p300 is not (Oliveira et al, 2006). It has also been reported that loss of p300/CBP-associated factor (PCAF) leads to short-term memory deficits in young mice and long-term memory deficits in older mice (Maurice et al, 2008). Table 3 summarizes our knowledge of the involvement of KATs in memory consolidation. However, unlike KDACs, the role of other KATs besides CBP/p300 in learning and synaptic plasticity remains largely unexplored. Figure 1 summarizes the known 'writers', 'readers', and 'erasers' of histone acetylation marks. Although the involvement of class I KDACs in learning and memory is clear, in particular HDAC2 and HDAC3, the role of KATs other than CBP/p300 deserves further investigation. CBP, p300, and PCAF all possess bromodomains and are likely readers of the histone marks as well as writers. The role of other bromodomain-containing proteins in memory has not been explored and warrants further investigation. Nonetheless, the largest unexplored areas regarding the role of histone acetylation in memory are the upstream signals that lead to changes in histone acetylation and the identity of the promoters of the genes that are regulated by such events.

Upstream Pathways that Regulate Histone Acetylation in Memory Formation

Several pathways have been shown to be required for long-term memory formation, including cyclic AMP/protein kinase A (cAMP/PKA), Ca2 + /calmodulin-dependent kinase (CAMK), and mitogen-activated protein kinase (MAPK) signaling (Morgado-Bernal, 2011). NMDA receptor activity and MAPK ERK signaling have been shown to be essential for histone H3 acetylation after contextual fear conditioning (Levenson et al, 2004). Other upstream components shown to be involved in learning-dependent chromatin remodeling are mitogen- and stress-activated protein kinase 1 (MSK1, RPS6KA5) and protein phosphatase 1 (PP1). Mice lacking MSK1, a nuclear kinase downstream of ERK, display impaired contextual fear learning and show decrease histone acetylation and phosphorylation after learning (Chwang et al, 2007). Interestingly, MSK1 has been implicated in the induction of several IEGs that have also been reported to be induced after learning. MSKs are required for the transcription of the nuclear orphan receptors Nur77, Nurr1, and Nor1 (Nr4a1, Nr4a2, and Nr4a3; Darragh et al, 2005). BDNF signaling has also been shown to induce histone acetylation changes (Zeng et al, 2011), and BDNF-induced expression of CREB-regulated genes c-fos and Nurr1 (Nr4a2) is impaired in MSK-deficient cortical neurons (Arthur et al, 2004). MSK1 is required for the phosphorylation of CREB, NF-κB, and histone H3 (Arthur, 2008). However, the relationship between MSK1 and histone acetylation is less clear. As MSK1 activates CREB, it is possible that the increase in acetylation is

mediated by CBP/p300 recruitment. PP1 is usually involved in dephosphorylating histone H3, among other substrates, and it has been shown to interact with KDACs (Brush *et al*, 2004). Selective inhibition of nuclear PP1 in forebrain neurons decreases KDAC activity in the hippocampus, increases acetylation of H4K5 and H2B, and enhances memory for object recognition (Koshibu *et al*, 2009). Overexpression of PP1 decreases acetylation of H3K14 and H4K5, and could be mediated through KDAC recruitment. It has been shown that histone H3 phosphorylation and acetylation are synergistic modifications. Hence, the observed effect of both MSK1 and PP1 in histone acetylation and learning could be a result of crosstalk between histone acetylation and phosphorylation. Indeed, histone phosphorylation has also been shown to be associated with the consolidation of memory (Chwang *et al*, 2006, 2007).

What Are the Genes Affected by Changes in Histone Acetylation During Learning?

The link between histone acetylation and specific down-stream targets has been limited to a small subset of genes, and there is not always a correlation with gene expression changes. The *Bdnf* promoter has been shown to be responsive to changes in histone acetylation after learning and synaptic plasticity. Transcription from *Bdnf* promoters 1 and 4 is activated when histone deacetylation is inhibited *in vivo* (Tsankova *et al*, 2006). Fear conditioning leads to an increase in histone H3 acetylation at *Bdnf* promoters 1 and 4 in rat prefrontal cortex, whereas extinction of conditioned fear is accompanied by an increase in histone H4 acetylation around promoter 4 (Bredy *et al*, 2007). However, increases in exon I and IV mRNA expression was only observed after extinction training. In rat hippocampal cultures, TSA increases *Bdnf* exon I transcription and H3K9/14 histone acetylation (Tian *et al*, 2010) and rescues age-dependent decline in hippocampal LTP and BDNF gene expression. It has also been shown that CBP is recruited to the *c-fos* promoter in an activity-dependent manner (Impey *et al*, 2002), and CBP KAT activity is required for *c-fos* gene expression (Korzus *et al*, 2004). However, *c-fos* induction after contextual fear conditioning did not change after TSA treatment (Vecsey *et al*, 2007), arguing that although histone acetylation may be necessary for *c-fos* induction after learning, it is not likely to mediate the TSA memory enhancements. On the other hand, CREB target genes *Nr4a1* and *Nr4a2* expression as well as promoter acetylation are selectively increased after TSA inhibition (Vecsey *et al*, 2007). Interestingly, *Nr4a2* binds to *Bdnf* promoter 4, and silencing *Nr4a2* by shRNA leads to a decrease in BDNF protein levels and a reduction of the effect of NMDA in neuronal survival (Barneda-Zahonero *et al*, 2012). CREB-dependent neuroprotection has also been shown to be mediated by NR4A orphan receptors (Volakakis *et al*, 2010).The *Creb* promoter itself changes acetylation status after learning. Object recognition increases H3K14 acetylation at the *Creb* promoter whereas it decreases H3K9 and

H4K5 acetylation 24 h after training, a change that mirrors the increase in *Creb* gene expression (Koshibu *et al*, 2009). Overall, despite the great amount of evidence regarding the involvement of histone acetylation in long-term memory formation, candidate genes and promoters are lacking. Novel high-throughput sequencing technologies such as ChIP-seq are promising avenues to discover such candidates and shed further light on the molecular basis of long-term memory storage. It has been shown that sodium butyrate and TSA can often decrease acetylation levels at specific promoters despite their global increases in acetylation levels (Rada-Iglesias *et al*, 2007), and hence it is hard to predict what direction of change will be observed genome-wide, and it is possible that genes that are functionally implicated in the establishment of long-term memory will show either increase or decrease of histone acetylation at their promoters.

Overall, several players have been uncovered in the regulation of genes expression by histone acetylation after learning. It is clear that KDACs and corepressor complexes play an important role in the repression of promoters in basal conditions, and their effect is regulated in an activity dependent-manner via recruitment of coactivators such as CBP and p300. Figure 2 presents a model for the regulation of histone acetylation during memory consolidation based on the data discussed. The regulation of the NR4A family of nuclear receptors has been proven to be one of the main targets of histone acetylation during memory formation. Hence, known components of their regulation (Hsu *et al*, 2004) have been incorporated into the model, adding elements of the corepressor complexes that may warrant exploration. For example, Cabin1, a calcineurin-dependent repressor that can recruit KDACs, can compete with p300 for binding of transcription factors such as MEF that can induce expression of *Nr4a* genes (Youn and Liu, 2000), establishing a direct link between calcium-dependent gene activation and histone acetylation.

When Acetylation Goes Wrong: Dysregulation of Histone Acetylation and Cognitive Impairment

In previous sections we have summarized the data that establish histone acetylation as an essential player in the processes that lead to long-term memory in physiological conditions. Dysregulation of histone acetylation has been reported in a variety of pathological conditions that impair cognitive function. Reports include mutations in known molecular players, changes in their expression, and/or changes in acetylation levels of specific histones.

Neurodevelopmental Disorders

As mentioned before, mutations of CBP and p300 have been linked to RSTS (van Belzen *et al*, 2011). RSTS is a well-defined syndrome with facial abnormalities, broad thumbs, broad big toes, and intellectual disability as the main clinical features (Rubinstein and Taybi, 1963). RSTS1 is
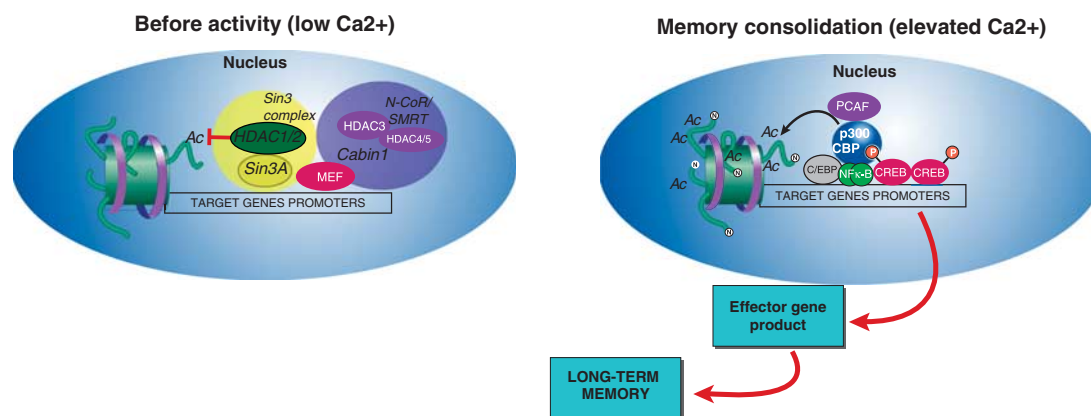
Figure 2. Regulation of gene expression by histone acetylation in the brain. On the left, a model of the proposed status of chromatin and corepressor complexes in basal state (low calcium) is presented, based on the data discussed in the review and the corepressor complexes known to regulate *Nr4a1* expression. On the right, a model of the effect of neuronal activity and calcium influx on chromatin is presented, including coactivators. For simplicity, corepressor complexes are not depicted on the right; however, it is unclear whether they actually dissociate from chromatin.

defined by mutations in CBP and is found in 50–70% of the cases. RSTS2 is defined by mutations in p300 and is found in 3% of the cases (Bartsch *et al*, 2010b). A small percentage of cases remain in which no genetic mutation has been identified, although other CBP paralogs have never been tested. It has also been shown that the degree of severity of the disease can be variable in relatives carrying the same mutation because of variable expression and somatic mosaicism (Bartsch *et al*, 2010a). This finding suggests that normal CBP expression is likely variable among cell types; hence, the role of CBP could be different in different regions of the brain. Floating-harbor syndrome (FLHS; Lacombe *et al*, 1995), which shows phenotypic overlap with RSTS, is caused by mutation in the *Srcap* gene, a coactivator of CBP. Interestingly, the only reported neurodevelopmental deficit in kids with FLHS is an expressive language delay. Therefore, it is possible that only a subset of the genes regulated by CBP, defined by specific CBP coactivators, have a role in memory formation.

A link between histone acetylation and the expression of the *Fmr1* gene has been reported in Fragile X syndrome (FXS). FXS is caused by a trinucleotide expansion that produces the silencing of *Fmr1* through DNA methylation (Pieretti *et al*, 1991). Treatment with class III HDAC inhibitors have been shown to induce *Fmr1* expression in cells derived from FXS patients (Biacsi *et al*, 2008). It has been shown that DNA methylation is coupled to histone acetylation and methylation in FXS patients (Tabolacci *et al*, 2008). These findings warrant further exploration of the role of histone acetylation in disorders in which DNA methylation is known to be affected, such as Rett Syndrome.

Neurodegenerative Disorders

Deficits in the expression of CBP, p300, and associated proteins have also been reported to be involved in the cognitive aspects of neurodegenerative disorders such as Alzheimer's disease (AD) and Huntington's disease (HD).

HD is caused by a CAG expansion in the coding region of the *huntingtin* gene that leads to a protein with an expanded polyglutamine track. The expansion has been shown to bind and inhibit the activity of the HAT domain of CBP and PCAF (Steffan *et al*, 2001). The long-term memory deficits in HD are correlated with reduced CBP levels and can be rescued by TSA administration (Giralt *et al*, 2012). Abnormal repression of *Bdnf* expression has been shown to be mediated by Sin3A/KDAC complexes in cortical neurons expressing the mutant huntingtin protein (Landles and Bates, 2004). SAHA, a class I KDAC inhibitor, decreases HDAC2 and HDAC4 levels *in vivo* and improves molecular phenotypes in the R6/2 mouse model of HD (Mielcarek *et al*, 2011). Hence, it is clear that defects in CBP function are linked to the pathophysiology of HD.

Presenilin 1 and 2, the two primary genes known to cause familial AD, are able to stimulate transcriptional activation by CBP and p300. This ability is disrupted in the AD mutant variants (Francis *et al*, 2007; Francis *et al*, 2006) implicating abnormal CBP/p300-mediated gene expression in the molecular basis of AD pathology. Nuclear localization of p300 interacting inhibitor of differentiation 1 (EID1), a CBP/p300 inhibitory protein, has also been shown to play a role in AD pathogenesis (Liu *et al*, 2012). Systemic injection of HDAC inhibitors is able to rescue age-dependent memory deficits in a mouse model of AD (Kilgore *et al*, 2010). Increasing histone acetylation has been shown to reverse consolidation deficits in mouse models of induced neurodegeneration (Fischer *et al*, 2007; Fontan-Lozano *et al*, 2008), suggesting that manipulating the brain acetyl epigenome may be an effective way to treat the cognitive aspects of several brain disorders.

Pharmacological Approaches for Manipulating the Brain Acetyl Epigenome and the Treatment of Psychiatric Disorders

KDAC inhibitors have been shown to be neuroprotective in several mouse models of disease of the nervous

system, including HD (Ferrante *et al*, 2003; Gardian *et al*, 2005), spinal muscular atrophy (Minamiyama *et al*, 2004), amyotrophic lateral sclerosis (Ryu *et al*, 2005), ischemia (Qi *et al*, 2004), Parkinson's disease (Gardian *et al*, 2004), and AD (Kilgore *et al*, 2010; Ricobaraza *et al*, 2009). The fact that several KDAC inhibitors are currently available and FDA approved to treat other disorders makes this class of drugs especially attractive. The possibility of using KDAC inhibitors to treat cognitive disorders has been extensively discussed and reviewed elsewhere (Fischer *et al*, 2010; Kazantsev and Thompson, 2008), and it is seen as a rational therapeutic approach based on the evidence discussed. Although the development of novel, potent, and specific KDAC inhibitor compounds is being actively pursued (Fass *et al*, 2010), their main drawback remains. As it has been made clear in this review, deacetylases are responsible for regulating diverse and important biological functions, and adverse effects are expected. Chronic KDAC treatment has been shown to lead to cognitive impairments (Adachi *et al*, 2009) and the failure of BDNF to induce dendrite outgrowth (Calfa *et al*, 2011). Most currently available KDAC inhibitors are pan-inhibitors, but it is not clear whether class-specific or isoform-specific inhibitors will be more efficacious.

The fact that *Nr4a1* and *Nr4a2* gene expression as well as promoter acetylation are selectively increased after TSA inhibition (Vecsey *et al*, 2007) suggests that the regulation of orphan nuclear receptors and their targets is an important downstream event in the regulation of long-term memory formation by histone acetylation. *Nr4a1* has been extensively studied as a drug target in cancer, as compounds that induce *Nr4a1* expression, including KDAC inhibitors, can induce apoptosis in cancerous cells (Safe *et al*, 2011). Several Nr4a family agonists have been described. These include: Cytosporone B (Csn-B), an agonist that activates the ligand-binding domain of NR4A1 and induces nuclear NR4A1-dependent gene expression (Zhan *et al*, 2008); 6-Mercaptopurine (6-MP) and anticancer agent that induces *Nr4a* receptor expression and NR4A2 and NR4A3 transactivation (Ordentlich *et al*, 2003; Wansa *et al*, 2003; Yoo *et al*, 2007); and substituted benzimidazoles (Dubois *et al*, 2006) and isoxazolopyridinones (Hintermann *et al*, 2007). The consequences of activating NR4A receptor signaling pharmacologically in cognitive function remains unexplored, but it constitutes a promising new avenue for the rational design of compounds to treat cognitive disorders. Although the focus of therapeutic development has been mostly on more specific and potent KDAC inhibitors, the fact that a single family of effector proteins such as NR4A receptors can provide several potential new drug targets exemplifies why knowing more about the downstream genes affected by histone acetylation is a promising avenue for the development of novel rationally designed drugs that treat cognitive impairments.

FUTURE RESEARCH DIRECTIONS: THE IMPACT OF NEXT-GENERATION SEQUENCING ON THE STUDY OF THE MEMORY EPIGENOME

Despite the considerable advances in the understanding of the histone acetylation changes caused by long-term memory formation, the identity of the genes subject to epigenetic regulation remains a largely unexplored question. The use of genome-wide high-throughput technologies for discovery-based studies in now commonplace in biomedical research. Hence, there is tremendous potential for the discovery of novel candidates whose histone acetylation state is a functional consequence of memory consolidation using these technologies. A great amount of the study of the regulation of genome-wide gene expression involves the identification of protein/DNA interactions, which include the binding patterns of transcription factors and modified histones to the genome. ChIP is usually the basis of such experiments, which are then followed by a genome-wide identification of the DNA elements that are enriched by the precipitation step. Although originally done using DNA arrays (ChIP-chip), advancement in high-throughput sequencing technologies have replaced the use of arrays for sequencing. ChIP-seq is the sequencing of the genomic DNA fragments that coprecipitate with a DNA-binding protein that is under study. As the technique does not rely on prior knowledge of precise DNA-binding sites, theoretically, ChIP-seq can identify in an unbiased manner all DNA segments in the genome physically associated with a specific DNA-binding protein. The details of ChIP-seq technology have been extensively reviewed elsewhere (Kharchenko *et al*, 2008; Park, 2009; Pepke *et al*, 2009; Zhou *et al*, 2011a) and are beyond the scope of this review, but in order to evaluate their potential in helping define the memory epigenome, it is useful to understand its strengths and caveats. Genomic approaches are rarely unbiased, which, combined with the number of simultaneous experiments performed, limits their ability to actually answer the hypotheses we wish to test. A genome-wide perspective relies heavily on computational processing and the results more often than not defy conventional wisdom. The development of new technologies for genome-wide studies is fast paced, but its use and the development of the bioinformatics infrastructure for the processing of the data that these technologies generate often lags behind. In this section we will briefly discuss what has been learned from the study of genome-wide chromatin regulation outside the brain and the advantages and pitfalls of the application of ChIP-seq to the study of long-term memory *in vivo*.

Studying Genome-Wide Gene Regulation: What Have We Learned?

Systematic identification of DNA elements involved in the regulation of gene expression through high-throughput approaches has been a relatively recent concern in higher

eukaryotes. Early ChIP-chip studies established that high-resolution maps of the state of histone tail modifications in human cells and *Drosophila* can provide insights on the regulatory state of chromatin (Bernstein *et al*, 2005; Schubeler *et al*, 2004). The first published results of The ENCyclopedia of DNA Elements (ENCODE) project provided further insight into the relationship between histone modifications and genome-wide transcriptional activation (Birney *et al*, 2007). Acetylation of histone H3, among other marks, was found to be highly predictive of gene activity (Koch *et al*, 2007), whereas at the same time administration of KDAC inhibitor butyrate was found to decrease histone acetylation at transcription start sites and downregulate associated genes (Rada-Iglesias *et al*, 2007). The findings seem contradictory at first, as blocking KDACs increases global acetylation that theoretically should upregulate many genes. However, although KDAC inhibitor treatment regulates ~5–20% of the transcriptome, it was shown previously that it often downregulates equal or greater number of genes than it upregulates (Daly and Shirazi-Beechey, 2006) (de Ruijter *et al*, 2005; Peart *et al*, 2005). Recent data have shown that butyrate-induced acetylation in H3K9 and H3K27 changes the sequence-based binding preference of histone H3 and may explain the observed pattern of gene expression regulation induced by KDAC inhibitors (Shin *et al*, 2012). The ENCODE findings highlight the fact that the meaning of areas that are enriched in histone acetylation in a given sample ('Peaks') when compared with nonprecipitated DNA ('Input') is not the same as the meaning of the changes in histone acetylation enrichment between samples with different treatments. In other words, although peaks in histone acetylation are usually found at the transcription start site of most genes that will be expressed by a particular cell type, a particular stimulus can induce subtle changes in the size and shape of those peaks that will affect expression levels at a given time. In essence, this reflects the difference between the role of histone acetylation in the maintenance of a particular cell type-specific expression profile and its dynamic role in transcriptional regulation. Most of the available ChIP-seq analysis software focus on the detection of peaks relative to input DNA control; they are in essence peak-finding tools. However, no available algorithm exists that will detect statistically significant differences in peak shape and size between treatments, such as learning and controls. The development of bioinformatics tools for next-generation sequencing analysis, however, is extremely fast paced, and hence this is likely to change in the near future.

The Challenges of Defining the Learning Epigenome

There are several reasons that make the analysis of ChIP-seq data a complicated task, and some issues are particularly difficult for the study of genome-wide changes in histone acetylation induced by learning. Several steps of the Chip-seq procedure introduce biases (Park, 2009); hence, the use

of controls is essential and has been shown to have a huge impact in peak-calling (Ho *et al*, 2011). Another important source of variation in the analysis of ChIP-seq profiles originates from the use of different analysis algorithms, and it has been shown that the variability introduced by using different algorithms can be as high as the variability between using ChIP-chip vs. ChIP-seq (Ho *et al*, 2011). The ability to detect a peak, biases aside, depends directly on the signal-to-noise ratio. The fact that genome-wide approaches test tens of thousands of events simultaneously, and hence require multiple testing correction, makes this requirement even stricter.

One of the biggest challenges when studying a physiological stimulus like learning *in vivo* is that the signal is subtle and the noise is high. At any given time it is likely that some other stimulus besides the learning experience is causing changes in gene expression or histone acetylation (eg, the presence of females in the holding room if the subjects are male) as it is virtually impossible to isolate the animal from its environment. The only way to control this is to randomize all possible sources of variation so that they can be removed at a later point in the analysis. An effective way to improve the detection of signal over noise is to increase the number of biological replicates in the study; however, the high cost of sequencing makes this difficult to achieve. Also, not all the cells that are collected in a sample are responding to the stimulus of interest, which dilutes the signal. Because we assume that activity-dependent changes occur in activated neurons, an alternative approach would be to tag histones in the cell populations of interest. For example, H2B-GFP mice generated by Jiang *et al* (2008) can be used to isolate excitatory neurons in the hippocampus. In those mice, histone 2B-GFP fusion protein is expressed under the control of forebrain-specific *CamkII* promoter. Sequential ChIP for GFP and then the histone modification of interest can be used to isolate DNA for further sequencing.

In summary, proper randomization of the experimental design combined with a high number of biological replicates and enrichment in the cell population of interest will greatly increase the chance of a successful ChIP-seq experiment in the context of learning. A handful of recent studies have use ChIP-seq technology to study changes in epigenetic marks (including transcription factor binding) in the context of brain and behavior (Kramer *et al*, 2011; Tang *et al*, 2011; Zhou *et al*, 2011b), and only one has studied changes in histone acetylation after learning (Peleg *et al*, 2010). The reproducibility of the results given the lack of biological replicates remains in question, as there are not reported *P*-values for the detected differences. For example, although H4K12 was shown to be increased in the promoters of genes upregulated by fear conditioning in 3-month-old mice by ChIP-seq (Peleg *et al*, 2010), the study fails to reproduce the result using ChIP-PCR at the promoters of the candidate genes tested. An interesting point to note is that the integration of other types of genome-wide data sets (in this case, gene expression), can

often help in narrowing down functionally meaningful results. The biggest hurdle in the use of ChIP-seq technology for the study of learning and memory remains the fact that the interesting question is not finding the peaks, but what the differences in peak size and shape induced by learning are. Unfortunately, the algorithms to ask that question taking account statistical significance are not yet available.

Whole genome expression studies should, when possible, accompany ChIP-seq studies, and although RNA-seq has its own inherent biases, the challenges in terms of experimental design are the same as outlined above for ChIP-seq studies. The immense amount of data produced by next-generation sequencing means a heavy reliance on computational methods, which in turn requires controls to evaluate their performance. Hence, candidate-gene approaches should be pursued in parallel, not only for the validation of genome-scale studies but preferably before the execution of genome-wide sequencing studies to serve as positive controls. The integration of 'top-down' and 'bottom-up' approaches will likely constitute the future avenue of research for many years to come as we strive to define the epigenetic language of long-term memory. Overall, there is great promise in the use of next-generation sequencing technologies to define the downstream candidates of histone acetylation that are relevant for memory formation. Identifying the downstream effectors of the changes in histone acetylation produced by memory consolidation will in turn lead to a better chance toward the rational design of drugs that target the epigenome to treat brain disorders that alter cognitive function.

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