Biol. Chem., Vol. 390, pp. 1145–1153, November 2009 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/BC.2009.131

Review

The epigenetic bottleneck of neurodegenerative and psychiatric diseases

Farahnaz Sananbenesi and Andre Fischer*

Laboratory for Aging and Cognitive Diseases, European Neuroscience Institute, Grisebach Str. 5, D-37077 Göttingen, Germany

*Corresponding author
e-mail: afische2@gwdg.de

Abstract

The orchestrated expression of genes is essential for the development and survival of every organism. In addition to the role of transcription factors, the availability of genes for transcription is controlled by a series of proteins that regulate epigenetic chromatin remodeling. The two most studied epigenetic phenomena are DNA methylation and histone-tail modifications. Although a large body of literature implicates the deregulation of histone acetylation and DNA methylation with the pathogenesis of cancer, recently epigenetic mechanisms have also gained much attention in the neuroscientific community. In fact, a new field of research is rapidly emerging and there is now accumulating evidence that the molecular machinery that regulates histone acetylation and DNA methylation is intimately involved in synaptic plasticity and is essential for learning and memory. Importantly, dysfunction of epigenetic gene expression in the brain might be involved in neurodegenerative and psychiatric diseases. In particular, it was found that inhibition of histone deacetylases attenuates synaptic and neuronal loss in animal models for various neurodegenerative diseases and improves cognitive function. In this article, we will summarize recent data in the novel field of neuroepigenetics and discuss the question why epigenetic strategies are suitable therapeutic approaches for the treatment of brain diseases.

Keywords: Alzheimer's disease; epigenetics; HDAC inhibitors; histone acetylation; learning and memory; neurodegenerative diseases.

Epigenetics: a neuroscientific definition

The human genome encodes approximately 30 000 genes and is identical in all somatic cells of an individual. As such a liver cell harbors the same genome as a neuron, although both cell types are obviously different and serve highly specialized purposes. This is explained by the fact that a liver cell uses the genome differently than a neuron, and at least under physiological conditions each cell type only expresses those genes needed for its proper function. As such, it is the specific gene expression pattern that defines the phenotype of a cell. Consequently, the expression of genes is a tightly controlled process.

The human DNA is organized into pairs of 23 chromosomes containing 3×10^9 base pairs. This adds up to two meters of DNA, which needs to be packed into the nucleus. To accomplish this, DNA is organized into chromatin, a complex of condensed DNA bound to proteins. Interestingly, the three-dimensional structure of chromatin is an important mechanism to regulate gene expression. The very condensed form of chromatin is termed heterochromatin. In this form, the DNA is mainly inaccessible to the transcriptional machinery and gene expression is inhibited. In contrast, euchromatin represents a more open form that is associated with gene expression. As such, the chromatin structure is extremely plastic and represents an important mechanism to translate environmental stimuli into altered gene expression, a process that is often referred to as 'epigenetics'. It was Conrad H. Waddington who first coined the term 'epigenetics'. He added the Greek prefix 'epi' (over; above) to the word 'genetics' to illustrate that it is not simply the DNA sequence that defines a cellular phenotype (Waddington, 1953). Since then the definition of epigenetics has undergone an evolution by itself. The most common way to define epigenetics nowadays is 'the study of heritable changes in gene expression that cannot be explained by changes in DNA Sequence' (Holliday, 1994). However, more recently the players involved with the regulation of epigenetic gene expression have been implicated with various novel functions, such as learning and memory processes in the adult brain (Levenson and Sweatt, 2005). As a result, recent publications and review articles started to use the term 'epigenetics' in a much broader, and sometimes misleading sense. For example, the neuroscientific community is now commonly using the term 'epigenetics' to describe changes in gene expression that are mediated by the molecular machinery involved in epigenetic processes. In postmitotic neurons those changes are, however, seldom heritable.

Chromatin plasticity is mainly mediated via histone proteins that bind DNA to form nucleosomes. The nucleosome is the fundamental building unit of the chromatin and consists of a protein octamer containing two molecules of each core histone (H) H2A, H2B, H3, and H4, around which is wrapped 147 bp of DNA. An additional histone 1 acts as a linker that regulates the packing of nucleosomes to higher order structures. Histones are conserved, small and highly basic proteins composed of a globular domain and a flexible N-terminus that protrudes from the surface of the nucleosome and is often

referred to as 'histone-tail'. The histone tails, particularly those of H3 and H4, are prone to extensive post-translational modifications, such as acetylation, phosphorylation, methylation, ubiquitination, sumoylation, ADP-ribosylation, and biotinylation (Vaquero et al., 2003). Those modifications occur at specific sites and residues and mediate positive and negative changes on gene expression.

The best-studied histone modifications are acetylation and methylation. For example acetylation of H3 or lysine residues (K) 9 and 14, as well as H4K5, 8, 12 or 16 correlate with active gene expression, whereas methylation of H3K27 is a marker for gene repression (Li et al., 2007). Those post-translational changes alter the chromatin structure, for example, by introducing additional negative charges in the case of acetylation which leads to weakened DNA-histone interaction allowing the transcriptional machinery to interact with DNA. Generally, a loose chromatin structure is associated with active gene expression, whereas tightly packed heterochromatin normally does not allow for significant gene expression.

In addition to post-translational modifications, several protein variants of H2A and H3 are known to also modulate gene expression (Henikoff and Ahmad, 2005). Some histone modifications are rather transient, whereas others are stable and can also be inherited to the next generation. They are mediated by the counteracting activities of proteins that either add or remove a certain modification. For example, histone acetylation is regulated by histone acetyltransferases (HAT), which add, and histone deacetylaes (HDAC), which remove acetyl groups from histones. Similarly, histone methyltransferases and histone demethylases regulate histone methylation (Allis et al., 2007).

In addition to histone modifications, DNA methylation is another important mechanism of epigenetic gene expression. It consists of the addition of a methyl group to a cytosine base in the DNA. In mammals, DNA methylation correlates with non-coding genomic DNA at CpG dinucleotides that are therefore termed CpG islands. Such CpG islands also occur in gene promoters and regulate expression of target genes (Klose and Bird, 2006). DNA methylation is regulated by DNA methyltransferases (DNMT) that catalyze either maintenance DNA methylation, such as DNMT1, or '*de novo*' methylation, such as DNMT3a and DNMT3b. Surprisingly, most recent work suggests that DNA methylation is reversible and that especially in postmitotic neurons it is regulated in a rather dynamic manner (Miller and Sweatt, 2007; Kangaspeska et al., 2008; Lubin et al., 2008; Métivier et al., 2008). DNA methylation is tightly coupled to histone modifications. For example, H3K9 methylation recruits DNMTs that subsequently mediate DNA methylation, which by itself represents a target for protein binding. In fact, methylated CpG islands recruit methylated DNA-binding proteins that interact with HDACs and other proteins to mainly mediate repression of the corresponding target genes (Klose and Bird, 2006). More recently, 22 nucleotide long small RNA molecules (microRNAs, miRNAs) have been implicated with epigenetic gene expression. miRNAs are transcribed as primary miRNAs and are subsequently processed to mature miRNAs. In most cases, they inhibit gene expression by binding to multiple target mRNAs. Interestingly, miRNAs have been particularly implicated with DNA methylation because they regulate the expression of DNMTs. Recent data suggest that miRNAs also directly affect the chromatin structure indicating a novel mechanism of epigenetic gene expression (Chuang and Jones, 2007).

As such, epigenetic changes of the chromatin can be viewed as specific signatures that activate or repress certain parts of the genome. Therefore, each cell has an 'epigenome' which defines the gene expression profile in response to environmental stimuli. The fact that defined changes in chromatin structure translate into distinct patterns of gene expression led to the proposal of an 'epigenetic code'. For example, it is well established that specific histone modifications mark a specific pattern of gene expression and it was Strahl and Allis who first proposed the concept of a 'histone-code' (Strahl and Allis, 2000).

Epigenetic mechanisms in the adult brain

Of the multiple different cell types that contribute to an organism, neurons are most certainly among the most complex. They are essential for the formation and storage of memories and are postmitotic. As such, once generated a neuron remains for the lifetime of the individual. One exemption are neuronal stem cells that are maintained in the neurogenic niche of the subventricular zone and the subgranular zone of the dentate gyrus (Gage et al., 2008). Generally, neurons are extremely plastic and translate environmental stimuli into morphological and functional changes to mediate cognition.

A very interesting example of how epigenetic mechanisms impact on neuronal function and behavior stems from the work of Michael Meaney and coworkers. It is well known that parental care can affect offspring behavior. As such, 'good' parental care has been associated with reduced anxiety and stress responses in offspring. Interestingly, those behavioral adaptations might be mediated via long-lasting changes in gene expression that are maintained via epigenetic mechanisms. For example, in rodents intense maternal care, which is measured as high pup licking and grooming (LG), leads to a better stress response in adult life. Hippocampal glucocorticoid receptors (GRs) are central mediators of the stress response. High LG behavior increases hippocampal serotonin release, which results in higher levels of the Egr-1 transcription factor. Egr-1 enhances the expression hippocampal GRs via binding to the brain specific $GR1_7$ promoter. Whereas Egr-1 levels soon return to baseline, the increased expression of hippocampal GRs is maintained throughout the lifetime of the offspring. This is explained by the finding that high LG eventually leads to increased H3 acetylation and decreased DNA methylation of the $GR1_7$ promoter, which facilitates Egr-1 binding. As a result, the same levels of Egr-1 result in higher expression of GRs in the offspring of high LG mothers compared to low LG mothers (Weaver et al., 2004).

Most recent work indicates that epigenetic mechanisms also play an important role during learning and

memory processes. In rodents, learning and memory can be investigated by a number of established tests, such as the Morris water maze test, Pavlovian fear conditioning or novel object recognition. A unanimous finding derived from such studies is that the consolidation of long-term memories requires differential gene expression and *de novo* protein synthesis (Fischer et al., 2003). On the structural level, the hippocampus, a region that belongs to the limbic system, is of particular importance for the formation of memories in rodents and humans (Kim and Fanselow, 1992). Notably, the hippocampus is also among the first regions to be affected by Alzheimer's disease (AD; Mesulam, 1999). Especially the fear conditioning paradigm helped to elucidate the mechanisms underlying memory formation, because it allows taking a molecular snapshot of the hippocampus during memory consolidation. This is possible because a single training session is sufficient to induce long-term memory consolidation that can be analyzed 24 h later. The formation of associative memories in the fear conditioning paradigm requires differential gene expression within the first 3 h after the training session (Fischer et al., 2003). Using this approach, the laboratory of David Sweatt was able to show that exposing rats to fear conditioning training induces a transient increase in hippocampal levels of DNMT3a and DNMT3b. Interestingly, inhibition of DNA methylation by intrahippocampal injections of 5-Aza or zebularine (drugs that prevent DNA methylation) right before the training severely impaired memory consolidation (Miller and Sweatt, 2007). This correlated with transient changes of DNA methylation and corresponding gene expression of the Reelin and PP1 genes, of which both have been implicated with neuronal plasticity. Those data indicate that DNA methylation might be critically involved in learning and memory processes in the adult brain. In line with this, it was shown that exposing animals to a fear conditioning training protocol induces very selective and reversible changes in the promoter methylation of the BDNF gene (Lubin et al., 2008). Although those results should still be considered preliminary, it is very interesting to think that DNA methylation might be involved in the plastic response of neurons during memory formation.

Similar observations were made for the acetylation of histones. It was observed that rats displayed a transient increase in histone acetylation 1 h after exposure to the fear conditioning paradigm (Levenson et al., 2004). Interestingly, the authors only detected increased H3 but not H4 acetylation. This might, however, be explained by the fact that pan-antibodies detecting multiple acetylation sites were used. Nevertheless, the finding that memory consolidation correlates with increased histone acetylation could be reproduced in mice and is not limited to fear conditioning but was also observed in the novel object recognition paradigm (Fischer et al., 2007; Vecsey et al., 2007; Fontán-Lozano et al., 2008). Interestingly, the transient increase in hippocampal H3 acetylation after fear conditioning is, at least in part, mediated via NMDA receptor-dependent activation of MAPK signaling (Chwang et al., 2006, 2007). Consistent with the finding that learning increased hippocampal histone-acetylation, mouse models for altered HAT activity display memory impairments. For example, loss of CREB (cAMP response element binding protein) binding protein (CBP) impairs hippocampal histone acetylation and leads to impairments in various memory tests, such as novel object recognition and spatial memory (Alarcon et al., 2004; Korzus et al., 2004; Vecsey et al., 2007). Notably, even if the CREB binding site of CBP is mutated severe learning and histone acetylation deficits were observed in those mice (Wood et al., 2005). Interestingly, learning impairment in mutant CBP mice has been linked to the inducible expression of a limited number of genes, namely Nr4a1 and Nr4a2 (Vecsey et al., 2007). In addition to a role of CBP in learning and memory, recent data suggest that HATs, such as P300 and p300/CBP-associated factor, also play an important role in memory consolidation (Oliveira et al., 2007; Maurice et al., 2008). Importantly, administration of HDAC inhibitors, such as suberoylanilide hydroxamic acid (SAHA), were able to restore memory function in mice lacking proper CBP activity (Alarcon et al., 2004). Learning and synaptic plasticity was also facilitated in wild type mice and rats after treatment with HDAC inhibitor, suggesting that increased histone acetylation is an important mechanism in memory formation (Levenson et al., 2004; Fischer et al., 2007; Vecsey et al., 2007).

An obvious question that arises from such data is how a global mechanism, such as histone acetylation, can regulate the specific set of genes that are believed to underlie the formation of long-term memories. To this end, it is important to mention that only a limited number of genes are regulated via histone acetylation. For example, treatment of cultured mammalian cells with pan-HDAC inhibitors led to the upregulation of only 2–10% of all genes. Moreover, most recent studies started to apply the novel technique of ChIP-sequencing (the analysis of chromatin immunoprecipitation results by direct sequencing) to the brain and found that the P300 HAT is associated with distinct regions of the genome in the embryonic mouse brain. For example, in the midbrain region isolated from the E11.5 mouse embryo, P300 was associated with only 561 distinct regions within the genome. Interestingly, most of those genomic regions were enhancers located far away from the open reading frame of the genes (Visel et al., 2009).

Taken together, those data suggest that histone acetylation is an important mechanism of memory formation and HDAC inhibitors could be suitable therapeutic strategy to treat cognitive diseases. However, we have to acknowledge that a lot of work is still needed to further elucidate the role of epigenetic mechanisms in memory formation. For example, it is not even entirely clear where the epigenetic machinery is expressed in the adult brain. The human and rodent genome encodes 11 different HDAC proteins. Based on homology those proteins are grouped into three classes. The class I HDACs (HDAC1, 2, 3, and 8) are mainly localized to the nucleus but class II HDACs (HDAC 5, 6, 7, 9, and 10) are known to shuttle between nucleus and cytoplasm. Interestingly, HDAC4 and 5 were shown to translocate from the nucleus to the cytoplasm in response to electric stimulation in cultured neurons (Chawla et al., 2003). HDAC 11 is the sole member of the class IV HDACs and relatively little is known

about its function. Whereas class I, II, and IV HDACs require zinc as a cofactor, class III HDCAs (or Sirtuins; Sirt 1–7) are regulated by nicotinamide adenine dinucleotide. pan-HDAC inhibitors, such as sodium butyrate, trichostatin A (TSA) or SAHA, do not inhibit sirtuins and only affect class I, II, and IV HDACs. To understand the precise role of individual HATs and HDACs in the adult brain is a field of intensive research. A recent study used *in situ* hybridization to describe the expression of the HDAC 1–11 in the adult rat brain (Broide et al., 2007). These data are mostly in line with available gene expression data published as part of the Mouse Allen brain atlas (www.brain-map.org) and allows for some interesting insight. For example, HDAC2 seems to be highly expressed in excitatory neurons of the entire brain. Indeed, a recent study using HDAC2 knockout and HDAC2 overexpressing mice showed that HDAC2 is a negative regulator of memory formation (Guan et al., 2009).

Epigenetic strategies to treat brain diseases

The deregulation of epigenetic mechanisms has been recognized as an important mechanism in the pathogenesis of various cancers (Yoo and Jones, 2006). As such, the basic research in the field of epigenetics dramatically increased throughout the past decade and as a result the first epigenetic drugs have recently been approved for clinical use. For example, the HDAC inhibitor SAHA (Vorinostat) is prescribed to treat cutaneous T-cell lymphoma and is currently tested in phase III trials for other cancers. In addition, several other HDAC inhibitors or modulators of DNA methylation are currently in clinical trials. In retrospect, the time from the discovery of HDAC proteins to the eventual approval of HDAC inhibitors for clinical use is astonishingly short. As such, it is extremely exiting that recent findings indicate a deregulation of epigenetic gene expression during the pathogenesis of neuronal diseases and that HDAC inhibitors facilitate learning and memory in rodents.

For example, Rubinstein-Taybi syndrome (RTS) is a neurological disease inherited in an autosomal dominant manner. The prevalence in the general population is approximately 1 case in 125 000 individuals and the patients usually suffer from mental retardation. Genetically, RTS has been linked to the genomic region encoding the CBP gene and is indeed associated with reduced CBP function leading to a decrease in histone acetylation (Roelfsema and Peters, 2006). Consistently, mice lacking CBP die prenatally but heterozygous $CBP^{+/-}$ mice can survive to adulthood. Interestingly, those mice display several symptoms characteristic for RTS, including severe cognitive deficits. Notably, the administration of the HDAC inhibitor SAHA could reinstate learning abilities and synaptic plasticity in $CBP^{+/-}$ mice, suggesting that HDAC inhibitors might be promising drugs to treat RTS (Alarcon et al., 2004).

Similar to RTS, Rett syndrome is an X chromosomal inherited disorder leading to mental retardation. Rett syndrome affects 1 in 15 000 individuals and is the second-most cause for mental retardation in young women (Chahrour and Zoghbi, 2006). The cause of Rett syndrome is ascribed to mutations in the methyl-CpG binding protein (MeCP2), which is involved in DNA methylation dependent regulation of gene expression. MeCP2 is also a critical regulator of synaptic plasticity and consistently Rett patients also show cognitive deficits (Chahrour and Zoghbi, 2006; Chao et al., 2007). Several mouse models overexpressing mutant MeCP2 have been generated. Although those mutant mice display characteristic symptoms of mental retardation, a recent study demonstrated that HDAC inhibitors could reverse some of the symptoms (Guy et al., 2007). This is in line with data showing that HDAC inhibitors could attenuate learning impairment induced by hypermethylation of DNA (Miller et al., 2008). Taken together, those results suggest the exciting possibility that HDAC inhibitors might also have beneficial effects in diseases that are mainly related to deregulated developmental processes.

Chorea Huntington is a neurological disease inherited in an autosomal dominant manner. It affects 1 in 100 000 individuals who suffer from movement disorders and impaired cognitive function. Chorea Huntington is caused by mutant Huntingtin protein that harbors an abnormal number of CAG repeats (36–250 times). Although the precise mechanism by which mutant Huntingtin leads to the phenotypes observed in patients is still under intense investigation, it has been shown that it affects neuronal gene expression by inhibiting CBP function. Consistently, expression of mutant Huntingtin is associated with reduced histone acetylation and the administration of HDAC inhibitors was shown to attenuate disease progression in multiple animal models for Huntington's disease. The potential of HDAC inhibitors is currently investigated in clinical trials (Butler and Bates, 2006).

Another neurological disease, which is inherited in an autosomal recessive manner, is Friedreich's ataxia, which affects 1 in 50 000 newborns. It is a severe neurodegenerative disorder that is caused by mutations of the FRDA gene coding for the mitochondrial Frataxin protein. An abnormal number of GAA repeats within the FRDA gene leads to its reduced expression. Additionally, it has been observed that the FRDA gene of patients shows reduced H3/H4 acetylation but increased H3K9 trimethylation, which is associated with heterochromatin. Interestingly, it was shown that the novel HDAC inhibitor 4b could increase FRDA expression, whereas the more common inhibitors SAHA or TSA failed to do so (Herman et al., 2006; Rai et al., 2008). These data further undermine the potential of HDACs as drug targets for neurological disorders but also indicate that in addition to the currently available pan-inhibitors it will be necessary to develop more specific compounds.

In line with this assumption, some but not all tested pan-HDAC inhibitors had beneficial effects in models for spinal muscle atrophy (SMA), a degenerative disease that affects motorneurons. SMA is accompanied by down-regulation of the survival motor neuron 1 (SMN1) and SMN2 genes. Particularly the levels of SMN2 correlate well with disease progression. Interestingly, it was shown that the SMN2 gene promoter is hypermethylated in patients, suggesting a MeCP2 mediated silencing of expression. The fact that HDAC inhibitors can attenuate

DNA, methylation-dependent gene silencing is in line with data showing that SAHA, TSA, and romidepsin were able to increase SMN2 expression. Notably, other pan-HDAC inhibitors, such as phenylbutyrate and valproate, failed to do so (Avila et al., 2007; Hauke et al., 2009).

In addition to the inherited diseases briefly discussed above, it is noteworthy that the majority of neuronal diseases ultimately correlate with altered gene expression. However, the precise cause of those changes is often not well understood. Considering that HDAC inhibitors show beneficial effects in many neurological disorders, one intriguing possibility is that altered histone acetylation might be causally linked to the disease progression. To test this possibility and eventually identify an epigenetic signature of disease progression, a better understanding of HATs, HDACs, and DNMTs in the brain will be necessary. This is of particular importance in sporadic diseases where disease onset and progression is significantly affected by environmental factors.

The most common form of dementia in the elderly is sporadic AD. The major risk factor for sporadic AD is increasing age. Because of increasing life expectancies the number of those afflicted with AD is believed to double by the year 2025. As such, AD is a huge emotional and economical burden to our societies. Despite intensive studies no effective therapy is currently available. The main pathological hallmarks of AD are amyloid plaques, neurofibrillary tangles (NFTs), and neuronal cell death. Amyloid plaques consist of extracellular aggregates of small Aβ-peptides, which are cleaved from the APP precursor protein. In AD patients, a shift towards the production of more toxic Aβ-peptides, such as Aβ42, is commonly observed (Haass and Selkoe, 2007). NFTs are intracellular aggregates that mainly consist of the hyperphosphorylated microtubule binding protein Tau (Schneider and Mandelkow, 2008). The precise mechanism by which amyloid and NFT pathology leads to neuronal cell loss and cognitive impairment is not well understood. Nevertheless, most of the research aiming to find therapeutic approaches against AD focused on the amyloid cascade or Tau pathology. Several studies also indicated that AD correlates with altered gene expression in those brain regions that are first affected. It was observed that particularly genes involved with synaptic plasticity were deregulated during AD. Taken into account that aging is a major risk factor for AD it is interesting to note that several studies showed that aging correlates with a deregulation of 'plasticity genes' in the human brain (Scheff, 2003; Lu et al., 2004; Berchtold et al., 2008). The cause of this deregulation of gene expression is not understood but it can be speculated that epigenetic processes might play a role. In line with this assumption, we could recently show that administration of HDAC inhibitors could reinstate learning behavior in CK-p25 mice, a mouse model for neurodegeneration. We initially found that exposure of CK-p25 mice to environmental enrichment (EE) improved memory formation and retrieval even when up to 25% of forebrain neurons were degenerated (Fischer et al., 2007). EE, a combination of exercise and cognitive training, is a well-known but poorly understood mechanism to facilitate cognitive function in rodents and humans (Green et al., 2006; Nithianantharajah and Hannan, 2006). Interestingly, EE was also found to attenuate disease progression and to improve memory function in mouse models for amyloid pathology. This could be explained by the upregulation of the Neprysilin gene, a protease that degrades amyloid peptides (Jankowsky et al., 2005; Lazarov et al., 2005). Interestingly, exercise and cognitive training also facilitate cognitive function in humans and certain activities, such as dancing, are associated with a decreased risk for developing AD. Nevertheless, care has to be taken when data from animal studies are translated to humans. Therefore, our approach was that a better understanding of the mechanisms that underlie EE could lead to the identification of suitable drug targets to treat AD. Because EE correlates with the upregulation of many genes involved in synaptic function, we hypothesized that a master-regulatory program involving epigenetic mechanisms might be crucial to coordinate the response to EE. In line with this, we could show that mice subjected to EE display a specific upregulation of hippocampal and cortical histone acetylation. Conversely, when HDAC inhibitors were administered to CK-p25 mice histone acetylation was increased and memory formation and retrieval was much improved (Fischer et al., 2007). As such, HDAC inhibitors were able to replicate the effect of EE. A recent study could confirm these results in a mouse model for amyloid pathology (Ricobaraza et al., 2009) (Figure 1).

In addition to neurodegenerative diseases epigenetic mechanisms of gene expression have been associated with the pathogenesis of various neuropsychiatric disorders, such as schizophrenia. It is estimated that 1% of the world population suffers from schizophrenia which affects mainly young individuals and leads to life-long impairments of life quality in 80% of patients. The etiology of schizophrenia is still under intense investigation but most recent work suggests that deregulation of epigenetic mechanisms might play an important role. For example, by using postmortem tissue samples of patients that suffered from schizophrenia it was reproducibly shown that the levels of GAD67 and reelin are decreased in GABAergic neurons of the prefrontal cortex. This finding correlated with increased DNA methylation in the promoter regions of GAD67 and reelin genes (Costa et al., 2006). Consistently, the levels of DNMT1 and DNMT3a were increased in schizophrenia (Costa et al., 2007; Zhubi et al., 2009). Interestingly, when mice were injected with the HDAC inhibitor valproate, gene expression and H3 acetylation of GAD67 and reelin was increased (Sharma et al., 2006). These data indicate that deregulation of DNA methylation but also histone acetylation might play a role in the pathogenesis of schizophrenia. Interestingly, a recent report indicates that levels of HDAC1 might be increased in schizophrenia (Sharma et al., 2008). Consistent with this finding, administration of the HDAC1 specific inhibitor MS-275 to mice was found to increase the expression of reelin and GAD67 more efficiently than valproate (Simonini et al., 2006; Dong et al., 2007). In addition to specific epigenetic changes associated with reelin and GAD67, bulk changes in histone acetylation were observed among schizophrenia patients and control individuals (Akbarian et al., 2005; Sharma et al., 2006; Gavin et al., 2008). Although

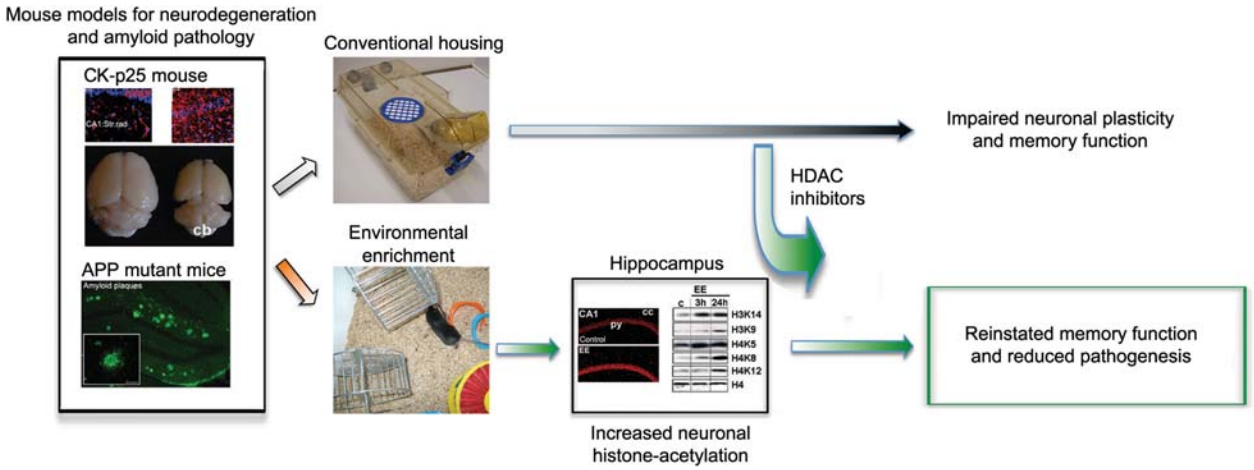

Figure 1 Environmental enrichment and HDAC inhibitors have neuroprotective and neuroregenerative properties in animal models for Alzheimer's disease.
In animal models for neurodegeneration and Alzheimer's disease, such as the CK-p25 or APP mutant mice, housing in an enriched environment attenuates disease progression and cognitive decline. Interestingly, it was shown that enrichment leads to increased neuronal histone acetylation ultimately resulting in increased expression of plasticity genes. In line with these data, administration of HDAC inhibitors recapitulated the effect of an enriched environment strongly suggesting that deregulated chromatin plasticity is involved in the pathogenesis of Alzheimer's disease.

obviously more work is needed to appreciate the impact of DNA methylation and histone acetylation during the pathogenesis of schizophrenia, the present data indicate that HDAC inhibitors might also have beneficial effects in those patients.

Therapeutic strategies to increase learning ability seem very suitable to treat cognitive impairments. However, it has to be noted that also unwanted behaviors, such as drug addiction, often involve learning processes. To this end, it was shown that altered chromatin plasticity is linked to the development of cocaine addiction (Kumar et al., 2005). In addition, the formation of strong aversive memories is believed to underlie the pathogenesis of anxiety disorders, which affect more than 12 million Europeans. Treatment of anxiety diseases, such as post-traumatic stress disorder, often involves cognitive behavior therapy, where the patient is repeatedly confronted with its traumatic memory. As a result the aversive behavior declines, a process termed fear extinction. In the laboratory, the extinction of learned fear is mainly investigated using the fear conditioning paradigm where specific cues are associated with an electric foot shock. Based on associative learning, rodents show high levels of aversive freezing behavior when re-exposed to the conditioning context 24 h after training. However, when repeatedly presented with the conditioned context on subsequent days the freezing behavior gradually declines (Myers and Davis, 2007; Fischer and Tsai, 2008). The present data suggest that fear extinction involves new learning but also adaptive processes, such as habituation. A better understanding of the mechanisms underlying fear extinction is believed to help identifying therapeutic approaches for treating anxiety diseases (Sananbenesi et al., 2007). Interestingly, two recent publications showed that HDAC inhibitors, such as TSA and valproate, facilitate learning of fear extinction in mice (Lattal et al., 2007; Bredy and Barad, 2008).

Conclusion

Neuroepigenetics is a young but fast growing field of research. This is mainly explained by the fact that epi-

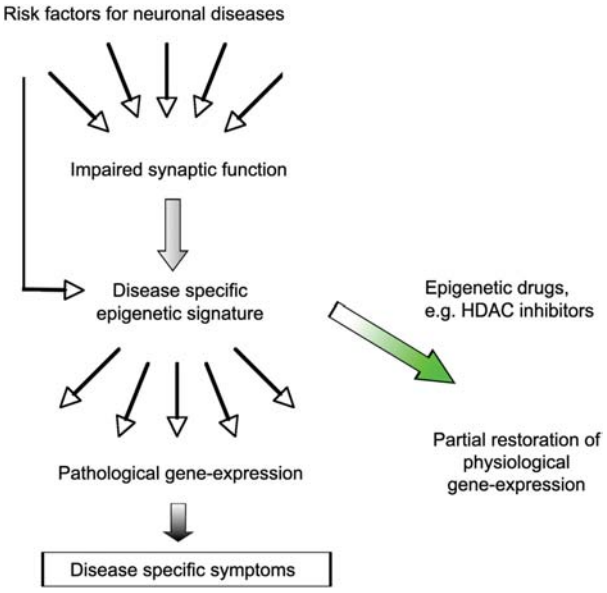

Figure 2 Therapeutic strategies targeting chromatin plasticity might be suitable to treat brain diseases.
Risk factors for a number of neuronal diseases are known to directly impact on chromatin plasticity. However, especially for sporadic diseases a combination of genetic predisposition and chronic exposure to environmental risk factors eventually leads to altered synaptic function. Owing to intimate signaling between the synapse and the nucleus this will further cause deregulation of chromatin plasticity ultimately leading to a disease-specific epigenetic signature. The resulting pathological gene expression profile will contribute to the disease-specific symptoms. Drugs, such as HDAC inhibitors, help to reinstate a physiological gene expression profile thereby affecting disease progression and reinstate cognitive function.

genetic strategies, especially HDAC inhibitors, have great potential for the treatment of various neurodegenerative and neuropsychiatric diseases. Future research will show if the current enthusiasm is justified. However, it appears that HDAC inhibitors are more than just another class of drugs that improve learning and memory in rodents. For a number of inherited but also sporadic brain diseases deregulation of epigenetic gene expression seems to be causally involved in disease progression. This might explain why HDAC inhibitors display neuroprotective but also neuroregenerative properties. It is therefore of utmost importance to further study the role of HATs, HDACs, DNMTs, and other key regulators of epigenetic gene expression in the adult brain.

However, a puzzling question is why inhibition of HDACs has beneficial effects in very different neuronal disorders, such as AD or schizophrenia. Considering that epigenomic stability is an important factor for cellular homoeostasis, it is important to note that the neuron is characterized by extreme plasticity at all levels. As such, the consolidation of memories involves plastic changes at the synapse, which mediates short-term memory. Long-term memory consolidation, however, requires intimate signaling among the synapse and the nucleus leading to altered gene expression. As such, risk factors that affect synaptic plasticity would, if persistent for a prolonged time period, ultimately lead to changes in chromatin plasticity and gene expression. Therefore, as a working hypothesis, we propose that risk factors for sporadic neuronal disorders, such as AD, will ultimately cause a distinct epigenetic signature that leads to pathological gene expression and manifestation of disease-specific symptoms. If true, epigenetic changes in gene expression could be viewed as the bottleneck of neurodegenerative and neuropsychiatric diseases (Figure 2).

Acknowledgments

This work was made possible owing to the generous support of A.F. by the European Science Foundation, Hans and Ilse Breuer Stiftung, Schram-Stiftung and ERA-Net Neuron. F.S. is supported by the German Research Foundation (DFG). The European Neuroscience Institute is jointly funded by the University Medicine Göttingen and the Max Planck Society.

References

Akbarian, S., Ruehl, M.G., Bliven, E., Luiz, L.A., Peranelli, A.C., Baker, S.P., Roberts, R.C., Bunney, W.E., Conley, R.C., Jones, E.G., et al. (2005). Chromatin alterations associated with down-regulated metabolic gene expression in the prefrontal cortex of subjects with schizophrenia. Arch. Gen. Psychiatry *62*, 829–840.

Alarcon, J.M., Malleret, G., Touzani, K., Vronskaya, S., Ishii, S., Kandel, E.R., and Barco, A. (2004). Chromatin acetylation, memory, and LTP are impaired in $CBP^{+/-}$ mice: a model for the cognitive deficit in Rubinstein-Taybi syndrome and its amelioration. Neuron *42*, 947–959.

Allis, C.D., Berger, S.L., Cote, J., Dent, S., Jenuwein, T., Kouzarides, T., Pillus, L., Reinberg, D., Shi, Y., Shiekhattar, R., et al. (2007). New nomenclature for chromatin-modifying enzymes. Cell *131*, 633–636.

Avila, A.M., Burnett, B.G., Taye, A.A., Gabanella, F., Knight, M.A., Hartenstein, P., Cizman, Z., Di Prospero, N.A., Pellizzoni, L., Fischbeck, K.H., et al. (2007). Trichostatin. J. Clin. Invest. *117*, 659–671.

Berchtold, N.C., Cribbs, D.H., Coleman, P.D., Rogers, J., Head, E., Kim, R., Beach, T., Miller, C., Troncoso, J., Trojanowski, J.Q., et al. (2008). Gene expression changes in the course of normal brain aging are sexually dimorphic. Proc. Natl. Acad. Sci. USA *105*, 15605–15610.

Bredy, T.W. and Barad, M. (2008). The histone deacetylase inhibitor valproic acid enhances acquisition, extinction, and reconsolidation of conditioned fear. Learn. Mem. *15*, 39–45.

Broide, R.S., Redwine, J.M., Aftahi, N., Young, W., Bloom, F.E., and Winrow, C.J. (2007). Distribution of histone deacetylases 1–11 in the rat brain. J. Mol. Neurosci. *31*, 47–58.

Butler, R. and Bates, G.P. (2006). Histone deacetylase inhibitors as therapeutics for polyglutamine disorders. Nat. Rev. Neurosci. *7*, 784–796.

Chahrour, M. and Zoghbi, H.Y. (2006). The story of Rett syndrome: from clinic to neurobiology. Neuron *56*, 422–437.

Chao, H.T., Zoghbi, H.Y., and Rosenmund, C. (2007). MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. Neuron *56*, 58–65.

Chawla, S., Vanhoutte, P., Arnold, F.J., Huang, C.L., and Bading, H. (2003). Neuronal activity-dependent nucleocytoplasmic shuttling of HDAC4 and HDAC5. J. Neurochem. *85*, 151–159.

Chuang, J.C. and Jones, P.A. (2007). Epigenetics and microRNAs. Pediatr. Res. *61*, 25R–29R.

Chwang, W.B., O'Riordan, K.J., Levenson, J.M., and Sweatt, J.D. (2006). ERK/MAPK regulates hippocampal histone phosphorylation following contextual fear conditioning. Learn. Mem. *13*, 322–328.

Chwang, W.B., Arthur, J.S., Schumacher, A., and Sweatt, J.D. (2007). The nuclear kinase mitogen- and stress-activated protein kinase 1 regulates hippocampal chromatin remodeling in memory formation. J. Neurosci. *27*, 12732–12742.

Costa, E., Dong, E., Grayson, D.R., Ruzicka, W.B., Simonini, M.V., Veldic, M., and Guidotti, A. (2006). Epigenetic targets in GABAergic neurons to treat schizophrenia. Adv. Pharamcol. *54*, 95–117.

Costa, E., Dong, E., Grayson, D.R., Guidotti, A., Ruzicka, W., and Veldic, M. (2007). Reviewing the role of DNA (cytosine-5) methyltransferase overexpression in the cortical GABAergic dysfunction associated with psychosis vulnerability. Epigenetics *2*, 29–36.

Dong, E., Guidotti, A., Grayson, D.R., and Costa, E. (2007). Histone hyperacetylation induces demethylation of reelin and 67-kDa glutamic acid decarboxylase promoters. Proc. Natl. Acad. Sci. USA *104*, 4676–4681.

Fischer, A. and Tsai, L.H. (2008). Counteracting Molecular Pathways Regulating the Reduction of Fear: Implications for the Treatment of Anxiety Diseases. Post Traumatic Stress Disorders. Basic Science and Clinical Disorder, P.J. Shiromani, T.M. Keane, J.E. LeDouxand, eds. (New Jersey, USA: Humana Press, Springer), pp. 79–104.

Fischer, A., Sananbenesi, F., Spiess, J., and Radulovic, J. (2003). Cdk5 in the adult non-demented brain. Curr. Drug Targets CNS Neurol. Disord. *6*, 375–381.

Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M., and Tsai, L.H. (2007). Recovery of learning and memory after neuronal loss is associated with chromatin remodeling. Nature *447*, 178–182.

Fontán-Lozano, A., Romero-Granados, R., Troncoso, J., Múnera, A., Delgado-García, J.M., and Carrión, A.M. (2008). Histone deacetylase inhibitors improve learning consolidation in young and in KA-induced-neurodegeneration and SAMP-8-mutant mice. Mol. Cell Neurosci. *39*, 193–201.

Gage, F.H., Kempermann, G., and Song, H.J. (2008). Adult Neurogenesis. Cold Spring Harbor Monograph (New York, USA: Cold Spring Harbor Laboratory Press), pp. 1–6.

Gavin, D.P., Kartan, S., Chase, K., Grayson, D.R., and Sharma, R.P. (2008). Reduced baseline acetylated histone 3 levels, and a blunted response to HDAC inhibition in lymphocyte cultures from schizophrenia subjects. Schizophr. Res. *103*, 330–332.

Green, R.E., Melo, B., Christensen, B., Ngo, L., and Skene, C. (2006). Evidence of transient enhancement to cognitive functioning in healthy young adults through environmental enrichment: implications for rehabilitation after brain injury. Brain Cogn. *60*, 201–203.

Guan, J.S., Haggarty, S.J., Giacometti, E., Dannenberg, J.H., Joseph, N., Gao, J., Nieland, T.J., Zhou, Y., Wang, X., Mazitschek, R., et al. (2009). HDAC2 negatively regulates memory formation and synaptic plasticity. Nature *459*, 55–60.

Guy, J., Gan, J., Selfridge, J., Cobb, S., and Bird, A. (2007). Reversal of neurological defects in a mouse model of Rett syndrome. Science *315*, 1143–1147.

Haass, C. and Selkoe, D.J. (2007). Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat. Rev. Mol. Cell Biol. *8*, 112–116.

Hauke, J., Riessland, M., Lunke, S., Eyüpoglu, I.Y., Blümcke, I., El-Osta, A., Wirth, B., and Hahnen, E. (2009). Survival motor neuron gene 2 silencing by DNA methylation correlates with spinal muscular atrophy disease severity and can be bypassed by histone deacetylase inhibition. Hum. Mol. Genet. *18*, 304–317.

Henikoff, S. and Ahmad, K. (2005). Assembly of variant histones into chromatin. Annu. Rev. Cell Dev. Biol. *21*, 133–153.

Herman, D., Jenssen, K., Burnett, R., Soragni, E., Perlman, S.L., and Gottesfeld, J.M. (2006). Histone deacetylase inhibitors reverse gene silencing in Friedreich's ataxia. Nat. Chem. Biol. *2*, 551–558.

Holliday, R. (1994). Epigenetics: an overview. Dev. Genet. *15*, 453–457.

Jankowsky, J.L., Melnikova, T., Fadale, D.J., Xu, G.M., Slunt, H.H., Gonzales, V., Younkin, L.H., Younkin, S.G., Borchelt, D.R., and Savonenko, A.V. (2005). Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. J. Neurosci. *25*, 5217–5224.

Kangaspeska, S., Stride, B., Métivier, R., Polycarpou-Schwarz, M., Ibberson, D., Carmouche, R.P., Benes, V., Gannon, F., and Reid, G. (2008). Transient cyclical methylation of promoter DNA. Nature *452*, 112–115.

Kim, J.J. and Fanselow, M.S. (1992). Modality-specific retrograde amnesia of fear. Science *256*, 675–677.

Klose, R.J. and Bird, A.P. (2006). Genomic DNA methylation: the mark and its mediators. Trends Biochem. Sci. *31*, 89–97.

Korzus, E., Rosenfeld, M.G., and Mayford, M. (2004). CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron *42*, 961–972.

Kumar, A., Choi, K.H., Renthal, W., Tsankova, N.M., Theobald, D.E., Truong, H.T., Russo, S.J., Laplant, Q., Sasaki, T.S., Whistler, K.N., et al. (2005). Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron *48*, 303–314.

Lattal, K.M., Barrett, R.M., and Wood, M.A. (2007). Systemic or intrahippocampal delivery of histone deacetylase inhibitors facilitates fear extinction. Behav. Brain Res. *121*, 1125–1131.

Lazarov, O., Robinson, J., Tang, Y.P., Hairston, I.S., Korade-Mirnics, Z., Lee, V.M., Hersh, L.B., Sapolsky, R.M., Mirnics, K., and Sisodia, S. (2005). Environmental enrichment reduces Aβ levels and amyloid deposition in transgenic mice. Cell *120*, 572–574.

Levenson, J.M. and Sweatt, J.D. (2005). Epigenetic mechanisms in memory formation. Nat. Rev. *6*, 108–119.

Levenson, J.M., O'Riordan, K.J., Brown, K.D., Trinh, M.A., Molfese, D.L., and Sweatt, J.D. (2004). Regulation of histone acetylation during memory formation in the hippocampus. J. Biol. Chem. *279*, 40545–40559.

Li, B., Carey, M., and Workman, J.L. (2007). The role of chromatin during transcription. Cell *128*, 707–719.

Lu, T., Pan, Y., Kao, S.Y., Li, C., Kohane, I., Chan, J., and Yankner, B.A. (2004). Gene regulation and DNA damage in the ageing human brain. Nature *429*, 883–891.

Lubin, F.D., Roth, T.L., and Sweatt, J.D. (2008). Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. J. Neurosci. *28*, 10576–10586.

Maurice, T., Duclot, F., Meunier, J., Naert, G., Givalois, L., Meffre, J., Célérier, A., Jacquet, C., Copois, V., Mechti, N., et al. (2008). Altered memory capacities and response to stress in p300/CBP-associated factor (PCAF) histone acetylase knockout mice. Neuropsychopharmacology *33*, 1584–1602.

Mesulam, M.M. (1999). Neuroplasticity failure in Alzheimer's disease: bridging the gap between plaques and tangles. Neuron *24*, 521–529.

Métivier, R., Gallais, R., Tiffoche, C., Le Péron, C., Jurkowska, R.Z., Carmouche, R.P., Ibberson, D., Barath, P., Demay, F., Reid, G., et al. (2008). Cyclical DNA methylation of a transcriptionally active promoter. Nature *452*, 45–50.

Miller, C.A. and Sweatt, J.D. (2007). Covalent modification of DNA regulates memory formation. Neuron *53*, 857–869.

Miller, C.A., Campbell, S.L., and Sweatt, J.D. (2008). DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. Neurobiol. Learn. Mem. *89*, 599–603.

Myers, K.M. and Davis, M. (2007). Mechanisms of fear extinction. Mol. Psychiatry *12*, 120–150.

Nithianantharajah, J. and Hannan, A.J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat. Rev. Neurosci. *7*, 697–709.

Oliveira, A.M., Wood, M.A., McDonough, C.B., and Abel, T. (2007). Transgenic mice expressing an inhibitory truncated form of p300 exhibit long-term memory deficits. Learn. Mem. *14*, 564–572.

Rai, M., Soragni, E., Jenssen, K., Burnett, R., Herman, D., Coppola, G., Geschwind, D.H., Gottesfeld, J.M., and Pandolfo, M. (2008). HDAC inhibitors correct frataxin deficiency in a Friedreich ataxia mouse model. PLoS ONE *3*, e1958.

Ricobaraza, A., Cuadrado-Tejedor, M., Pérez-Mediavilla, A., Frechilla, D., Del Río, J., and García-Osta, A. (2009). Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. Neuropsychopharmacology *34*, 1721–1732.

Roelfsema, J.H. and Peters, D.J. (2006). Rubinstein-Taybi syndrome: clinical and molecular overview. Expert Rev. Mol. Med. *9*, 1–16.

Sananbenesi, F., Fischer, A., Wang, X., Schrick, C., Neve, R., Radulovic, J., and Tsai, L.H. (2007). A hippocampal Cdk5 pathway regulates extinction of contextual fear. Nat. Neurosci. *10*, 1012–1019.

Scheff, S. (2003). Reactive synaptogenesis in aging and Alzheimer's disease: lessons learned in the Cotman laboratory. Neurochem. Res. *11*, 1625–1630.

Schneider, A. and Mandelkow, E. (2008). Tau-based treatment strategies in neurodegenerative diseases. Neurotherapeutics *5*, 443–457.

Sharma, R.P., Rosen, C., Kartan, S., Guidotti, A., Costa, E., Grayson, D.R., and Chase, K. (2006). Valproic acid and chromatin remodeling in schizophrenia and bipolar disorder: preliminary results from a clinical population. Schizophr. Res. *88*, 227–231.

Sharma, R.P., Grayson, D.R., and Gavin, D.P. (2008). Histone deactylase 1 expression is increased in the prefrontal cortex of schizophrenia subjects: analysis of the National Brain Databank microarray collection. Schizophr. Bull. *98*, 111–117.

Simonini, M.V., Camargo, L.M., Dong, E., Maloku, E., Veldic, M., Costa, E., and Guidotti, A. (2006). The benzamide MS-275 is a potent, long-lasting brain region-selective inhibitor of his-

tone deacetylases. Proc. Natl. Acad. Sci. USA *103*, 1587–1592.

Strahl, B.D. and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41–45.

Vaquero, A., Loyola, A., and Reinberg, D. (2003). The constantly changing face of chromatin. Sci. Aging Knowledge Environ. *14*, RE4.

Vecsey, C.G., Hawk, J.D., Lattal, K.M., Stein, J.M., Fabian, S.A., Attner, M.A., Cabrera, S.M., McDonough, C.B., Brindle, P.K., Abel, T., et al. (2007). Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. J. Neurosci. *27*, 6128–6140.

Visel, A., Blow, M.J., Li, Z., Zhang, T., Akiyama, J.A., Holt, A., Plajzer-Frick, I., Shoukry, M.W., Wright, C., Chen, F., et al. (2009). ChIP-seq accurately predicts tissue-specific activity of enhancers. Nature *457*, 854–858.

Waddington, C.H. (1953). Epigenetics and evolution. Symp. Soc. Exp. Biol. *7*, 186–199.

Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., and Meaney, M.J. (2004). Epigenetic programming by maternal behavior. Nat. Neurosci. *7*, 847–854.

Wood, M.A., Kaplan, M.P., Park, A., Blanchard, E.J., Oliveira, A.M., Lombardi, T.L., and Abel, T. (2005). Transgenic mice expressing a truncated form of CREB-binding protein (CBP) exhibit deficits in hippocampal synaptic plasticity and memory storage. Learn. Mem. *12*, 111–119.

Yoo, C.B. and Jones, P.A. (2006). Epigenetic therapy of cancer: past, present and future. Nat. Rev. Drug Discov. *5*, 37–50.

Zhubi, A., Veldic, M., Puri, N.V., Kadriu, B., Caruncho, H., Loza, I., Sershen, H., Lajtha, A., Smith, R.C., Guidotti, A., et al. (2009). An upregulation of DNA-methyltransferase 1 and 3a expressed in telencephalic GABAergic neurons of schizophrenia patients is also detected in peripheral blood lymphocytes. Schizophr. Res. *111*, 115–122.

Received May 15, 2009; accepted August 7, 2009