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Review article

Epigenetic codes in cognition and behaviour

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Abstract

The epigenetic marking of chromatin provides a ubiquitous means for cells to shape and maintain their identity, and to react to environmental stimuli via specific remodeling. Such an epigenetic code of the core components of chromatin, DNA and histone proteins, can thus be stable but is also highly dynamic. In the nervous system, epigenetic codes are critical for basic cellular processes such as synaptic plasticity, and for complex behaviours such as learning and memory. At the same time, epigenetic marks can be stably transmitted through mitosis and meiosis, and thereby underlie non-genomic transgenerational inheritance of behavioural traits. In this review, we describe recent findings on the role and mechanisms of epigenetic codes in the brain, and discuss their implication in synaptic plasticity, cognitive functions and psychiatric disorders. We provide examples of transgenerational inheritance of epigenetic marks that affect simple morphological traits or complex processes such as disease susceptibility, and point to the potential implication of epigenetic codes in medicine and evolution.

Keywords: Epigenetic mechanisms; Synaptic plasticity; Memory; DNA methylation; Histone; Cognition; Environment; Transgenerational inheritance

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1. Introduction

The importance of epigenetic modifications has long been recognized in the areas of stem cell research, cancer and developmental biology. But it is only recently that their relevance has also been acknowledged in the field of neuroscience, for both developmental processes and higher-order brain functions such as cognition. Epigenetic modifications of the chromatin are diverse and complex, and their study in neurobiology has been inspired by work in disciplines such as developmental and evolutionary biology. The integration of findings in these diverse fields has stirred new concepts and perspectives for the understanding of the intimate mechanisms of basic and higher brain functions, and for the processes that underlie the heritability of behavioural traits across generations, which is key to evolution.

2. History and terminology

The term epigenetics derives from the Greek prefix "epi" that literally means "above" or "in addition to" genetics. It refers to processes that physically occur with or on genes, and involves the physical support of genetic processes, the chromatin. Originally, long before the notion of chromatin even existed, the developmental biologist Conrad Hal Waddington (1905–1975) defined epigenetics as "... the interactions of genes with their environment which bring the phenotype into being", emphasizing that epigenetic mechanisms vary in response to a given environment [1]. Waddington later referred to an equally important characteristic of epigenetic modifications by stating that "...it is possible that an adaptive response can be fixed without waiting for the occurrence of a mutation..."[2]. This notion of non-genetic transmission of acquired morphological and behavioural traits had already been proposed by Jean-Baptiste Lamarck (1744–1829), but met with fierce criticism, essentially due to Lamarck's inclination to place his observations in the perspective of adaptive evolution. The modern definition of epigenetics now integrates Waddington's early assumptions, but excludes most of Lamarck's views. Epigenetics is nowadays most commonly defined as the ensemble of alterations in gene functions that are heritable through both mitosis and meiosis, but that cannot be explained by changes in the DNA sequence itself [3] (reviewed in [4,5]).

At the molecular level, epigenetic mechanisms are biochemical modifications of the DNA and histone proteins, the major constituents of chromatin (Fig. 1A). Recent findings have revealed that additional mechanisms involving RNA interference and prion proteins also contribute to epigenetic regulation [6], but these mechanisms will not be covered in this review. Chromatin modifications are multiple and complex, and comprise methylation of DNA at cytosine-guanine dinucleotides, and posttranslational modifications of histone proteins. Histones are basic proteins consisting of a core and an N-terminus tail composed of a loosely-structured sequence of amino acids [7]. Posttranslational histone modifications occur primarily on the N-terminus tail, and include acetylation, methylation, phosphorylation, ubiquitination (reviewed in [6]) and sumoylation (Fig. 1B). Because of their chemical properties, these modifications influence the condensation of chromatin, and thereby modulate the accessibility of DNA to the transcriptional machinery (Fig. 2A and B).

DNA methylation occurs throughout the genome but is functionally most relevant when present in sequences rich in CpG dinucleotides, called CpG islands, often found in promoter regions. DNA methylation is commonly associated with transcriptional silencing because it can directly inhibit the binding of transcription factors or regulators, or indirectly recruit methyl-CpG binding proteins (MBPs), which have repressive chromatin-remodeling functions [9,10]. However, DNA methylation can also occur in actively transcribed genes, suggesting a potential positive role in transcription regulation as well [11,12]. Because of the covalent nature of the binding of methyl groups to the C_5 carbon in cytosine, DNA methylation is thought to be the most stable epigenetic mark [9].

Posttranslational modifications of histone tails also play a critical role in the regulation of gene transcription. First, histone acetylation is associated with transcriptional activation. It results in the neutralization of the positive charge of the ε-amino group of lysine (K) residues in the histone tail, which decreases the affinity between the protein tail and the DNA, and relaxes the chromatin structure [13]. In contrast, histone methylation has a dual impact on transcriptional activity and is associated

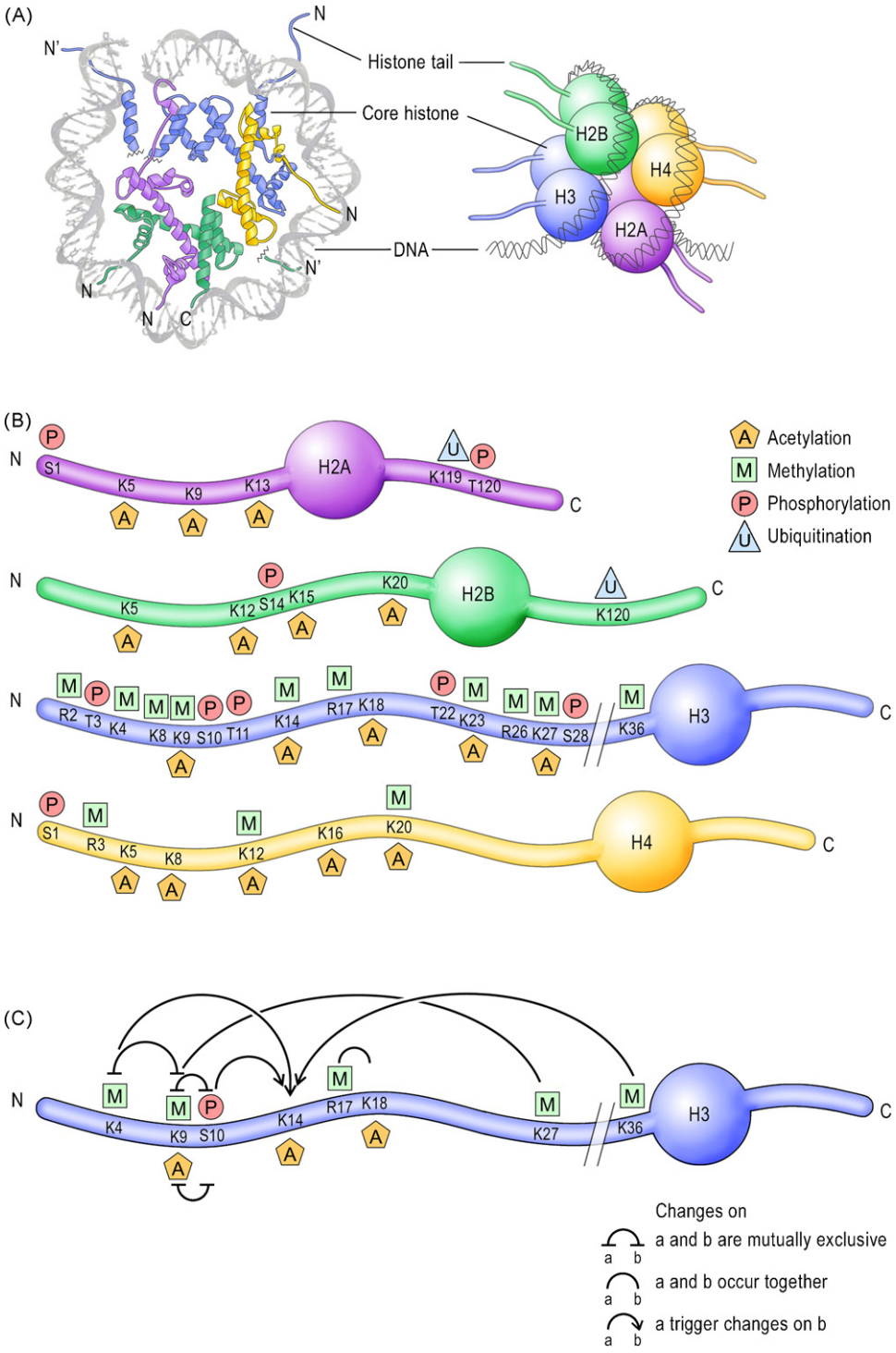

Fig. 1. Epigenetic marks on histone tails and DNA. (A, left) View of the nucleosome down the DNA superhelix axis showing one half of the nucleosome structure; reproduced with permission from [7]. (Right) Schematic representation of the four-nucleosome core histones, H2A, H2B, H3 and H4. (B) Schematic representation of the N- and C-termini of the core histones and their residue-specific epigenetic modifications. (C) Crosstalk between epigenetic modifications on the H3 N-terminus tail. The relationship between the different residues is based on recent literature [20–23]. C, C-terminus; N, N', N-terminal tails.

with both actively transcribed and silenced genes [14,15]. Histone methylation occurs in different forms, from mono-, di- to tri-methylation of lysine residues, the combination of which plays a complex role in the regulation of gene expression [16]. Methyl groups can also be added to arginine (R) residues in both mono- and dimethylated forms, but their impact on the organization of chromatin is poorly understood [16]. Histone phosphorylation, similar to acetylation, is most commonly associated with transcriptional activation [17], presumably because it creates a repulsive force between the negative charges of phospho-histones and DNA. This repulsive force decondenses the chromatin and increases its accessibility to the

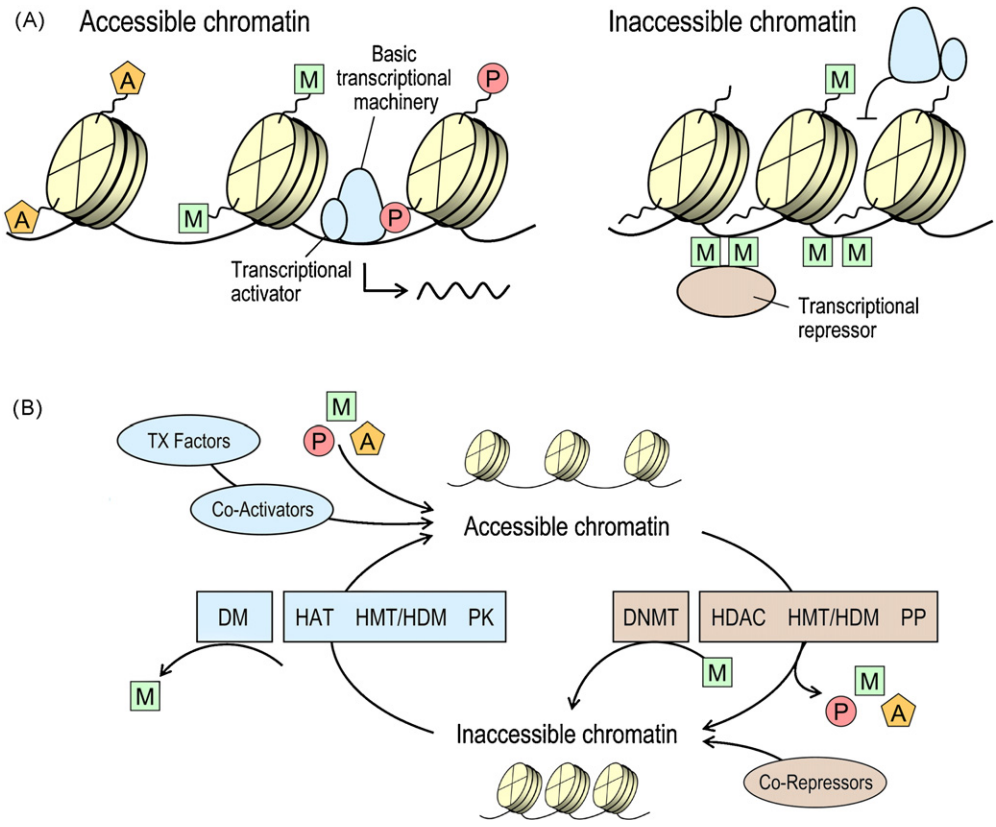

Fig. 2. The transcriptional accessibility of chromatin is governed by specific enzymes. (A) Schematic representation of the differences in chromatin condensation between a transcriptionally active and silent state. When histones are acetylated, phosphorylated, and methylated (depending on the methylated residue), there is an increase in electrostatic repulsion between the histone proteins and the DNA, which results in a less condensed chromatin structure giving access to the transcriptional machinery and co-activators. When DNA and histones are methylated (depending on the methylated residue, and in the simultaneous absence of histone acetylation and phosphorylation), the electrostatic repulsion between the histone proteins and the DNA is decreased, leading to chromatin being less accessible to the transcriptional machinery. In addition, transcriptional co-repressors bind to the methylated DNA. Together, these changes result in transcriptional silencing. (B) Enzymes modulating the epigenetic marking of histones and DNA. Note that the existence of DMs is still hypothetical (but see [149]), and that the implication of PPs in the epigenetic tagging of histones has not yet been demonstrated *in vivo*. A, acetylation; DM, DNA demethylase; DNMT, DNA methyl transferase; HAT, histone acetyl transferase; HDAC, histone deacetylase; HDM, histone demethylase; HMT, histone methyltransferase; M, methylation; P, phosphorylation; PK, protein kinase; PP, protein phosphatase; TX, transcription.

transcriptional machinery. Histone phosphorylation is perhaps the most intriguing epigenetic histone modification in that it provides a functional link between chromatin remodeling and intracellular signaling pathways, both of which involve protein kinases and phosphatases [6]. Protein ubiquitination (also called ubiquitylation) is most commonly associated with the marking of proteins for degradation by the proteasome, but has also been found to occur on histone tails. The ubiquitination of histone tails by attachment of the 76-amino acid ubiquitin peptide correlates with both transcriptional activation and nucleosome loosening, and has also been identified as a prerequisite for subsequent histone methylation [16,18]. However, its precise functions remain unclear. Finally, histone sumoylation is the least understood posttranslational histone modification. In yeast, it occurs on all four core histones and negatively regulates transcription, possibly by interfering with histone acetylation and ubiquitination [8,19]. Its role in mammals has not been established yet.

An important feature of epigenetic marks that is essential for transcriptional regulation is their ability to crosstalk [20,21] (Fig. 1C). Posttranslational histone modifications often act in concert, and multiple feed-forward and feed-back mechanisms involving the same nucleosome or histone, or distinct nucleosomes and histones have been identified [21]. These crosstalks can enhance chromatin condensation when transcriptional silence is required, or chromatin opening when transcriptional activity is needed [22,23]. The repertoire of DNA and histone modifications is controlled by specific enzymes that include DNA methyltransferases (DNMTs), histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone demethylases (HDMs), protein kinases (PKs), protein phosphatases (PPs), ubiquitin- and SUMO-associated enzymes [6,16,19] (Fig. 2B). These enzymes operate both independently and in synergy to establish a "histone code", a highly dynamic and flexible chromatin marking that, in combination with chromatin-associated proteins, determines the pattern of gene expression in response to given external stimuli [24,25]. That way, epigenetic modifications at the level of the chromatin provide a focal point to bridge nuclear events to intracytoplasmic signaling cascades, and a potential molecular means to retain marks of prior transcriptional activity in the nuclear machinery.

3. Epigenetic regulation of synaptic plasticity

Epigenetic mechanisms play a fundamental role in the functions of nerve cells and in the nervous system. They contribute to developmental and differentiation processes, and influence communication and signaling in neuronal networks (for a review see [26]). Recent studies have revealed their central role in the regulation of synaptic plasticity. Synaptic plasticity is the ability of neuronal cells to strengthen or weaken their connections following neuronal activation. Believed to be a cellular correlate of learning and memory in several species, its mechanisms have not only been extensively studied in simple organisms such as the marine mollusk *Aplysia californica,* but also in mammals. Long-term synaptic plasticity involves changes in the expression of genes involved in synaptic functioning (for a review see [27]) and in recent years, evidence has accumulated showing that epigenetic mechanisms are central to such gene regulation in both invertebrates and vertebrates.

3.1. Non-mammalian synaptic plasticity

In *Aplysia*, two major forms of long-term synaptic plasticity have been identified at sensory-motor synapses: long-term facilitation (LTF) and long-term depression (LTD). LTF and LTD represent a persistent increase and a persistent decrease in synaptic transmission, respectively (for a review see [28]). Both forms of plasticity are expressed at the same synapses, which implies that synaptic responses are modulated by a reversible and bidirectional molecular "switch". In an elegant study using cultured sensory-motor neurons in *Aplysia*, Guan et al. [29] demonstrated that acetylated histones constitute such a switch. LTF was found to be accompanied by increased binding of the HAT CREB binding protein (CBP) to the promoter region of C/EBP (CCAAT/enhancer binding protein), a gene downstream of CREB containing several CRE binding sites. Concurrently, acetylation of lysine 14 on histone 3 (H3K14) and lysine 8 on histone 4 (H4K8), and C/EBP transcription were also increased. LTD in contrast, led to an opposite effect mediated in part by the recruitment of HDAC5. Consistently, the administration of the HDAC inhibitor trichostatin A (TSA) resulted in a switch from short- to long-term facilitation after stimulation, suggesting that a global change in acetylation can modify synaptic plasticity in invertebrates.

3.2. Mammalian synaptic plasticity

The most prominent forms of synaptic plasticity in mammals are long-term potentiation (LTP) and LTD. These forms of plasticity reflect respectively, an increase and a decrease in the efficiency of synaptic transmission, and have been extensively studied in the hippocampus, a brain area required for learning and memory (for a review see [30]). The mechanisms of synaptic plasticity are complex and recruit different signaling cascades depending on their localization and temporal phase. They engage pathways such as glutamatergic, dopaminergic and cholinergic signaling as well as nuclear events when long-lasting [31–33]. These different neurotransmitter pathways have therefore been postulated to be involved in the posttranslational regulation of histones. Accordingly, the systemic administration of the dopamine receptor agonist SKF82958, the muscarinic acetylcholine receptor agonist pilocarpine or the kainic glutamate receptor agonist kainate, enhance the phosphorylation of serine 10 on H3 (H3S10) and the acetylation of H3K14 [34]. This enhancement was found to occur in the promoter region of the immediate early gene *c-Fos* in the hippocampus, and correlated with increased gene expression [34]. Interestingly, the pattern of H3 phosphorylation was paralleled by a similar pattern of phosphorylation of the extracellular regulated kinase (ERK), a member of the mitogen-activated protein kinase (MAPK) pathway implicated in memory and synaptic plasticity (reviewed in [35]). Activation of the MAPK cascade was also reported to increase H3K14 acetylation during memory formation [36], pointing to a role for the MAPK cascade in the epigenetic regulation of histones, in addition to its classical functions in neuronal signaling. This dual implication is also observed for epigenetic regulators of developmental processes such as Polycomb (PcG) and trithorax (trxG), in that these proteins influence synaptic plasticity as well [37]. Mice heterozygous for *eed* and for *MII*, members of the epigenetic repressor PcG and activator trxG group respectively, have altered synaptic plasticity. LTP is enhanced in $eed^{+/-}$ mice and decreased in $MII^{+/-}$ mice, which correlates with H4 hypoacetylation of several residues in $MII^{+/-}$ mice [38].

Consistent with findings in *Aplysia*, such epigenetic modifications of histones are not only correlative but also functionally important for synaptic plasticity. For instance, inactivation of the HAT CBP in mice decreases the overall acetylation of H2B and impairs the induction of transcription-dependent late-phase LTP in hippocampal slices without altering transcription-independent early-phase LTP [39]. Treatment of such slices with the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) ameliorates late-phase LTP. Importantly, a similar effect is observed in wild-type rats, in which administration of the HDAC inhibitors trichostatin A (TSA) or sodium butyrate enhances the induction of LTP in the hippocampus [36], suggesting a causal link between histone acetylation and LTP. Further, similar to the hippocampus, systemic administration of TSA also increases LTP in the amygdala, a brain area implicated in emotional memory [40]. Surprisingly, however, such broad administration of an HDAC inhibitor does not alter the entire epigenome but is site-specific [41]. When TSA is applied to hippocampal slices in animals subjected to fear conditioning, only a subset of CRE-containing genes is transcriptionally upregulated [41]. These results point to the existence of specific epigenetic mechanisms of gene regulation that can be modulated by different environmental stimuli, an observation reminiscent of the histone code.

DNA methylation is another epigenetic mechanism that contributes to regulate synaptic plasticity [42]. Blockade of DNA methylation by DNMT inhibitors such as zebularine or 5-aza-2-deoxycytidine (5-aza) impairs the induction of LTP in the hippocampus [42]. Further, a deficiency in MBD1, a methylated DNA-associated transcriptional repressor, reduces hippocampal LTP, presumably through an effect on gene expression [43].

Additionally, activation of protein kinase C (PKC) by administration of phorbol ester also decreases DNA methylation, specifically at the promoter region of *reelin*, a gene implicated in the induction of synaptic plasticity. PKC activation is further accompanied by increased transcriptional activation of *c-Fos*, an immediate early gene involved in synaptic transmission, and of the DNA methyl transferase DNMT3a, suggesting the involvement of DNA methylation in synaptic signalling cascades. Interestingly, PKC activation also increases the acetylation of H3K14, but not H4, an effect that is blocked by zebularine [42]. These findings therefore suggest that DNMT activity is associated with the control of histone acetylation upon PKC stimulation, providing a link between DNA methylation and histone acetylation in synaptic plasticity that is specific for certain histones and certain residues. Although more experiments are needed to clarify this link, these findings are nonetheless important because they show for the first time, that DNA methylation, a process initially thought to be static, is dynamically modulated during synaptic plasticity in the mammalian brain.

3.3. Epileptiform activity

Epileptic seizures represent a form of excessive synaptic plasticity that often occurs following uncontrolled electrical activity in the central nervous system. Seizures have been associated with alterations in the epigenetic regulation of several genes, including the glutamate receptor 2 (*GluR2*) and brain-derived neurotrophic factor (*BDNF*) (reviewed in [26]). Pilocarpine-induced seizures in rat hippocampal slices lead to *GluR2* downregulation and to an overall hypoacetylation of H4 in its promoter region, which can be reversed by TSA [44]. In contrast, *BDNF* expression is significantly upregulated and H4 hyperacetylated at *BDNF* promoter P2 by pilocarpine treatment. Electroconvulsive seizures (ECS) also modulate H4 acetylation in the promoter region of *c-Fos*, *BDNF* and *CREB* genes, and correlate with altered mRNA expression in that H4 hyperacetylation is associated with increased gene expression and vice versa [45]. However, there is no clear correlation for H3 acetylation on K9 or K14, or for the combined phosphorylation/acetylation of Ser10 and K14, suggesting a general role for H4 acetylation but a more

Table 1
Epigenetic mechanisms in synaptic plasticity and memory

Epigenetic modification	Context	Effect	Brain area	Organism	References
DNA methylation	Synaptic plasticity	MeCP1 deficiency reduces LTP	Hippocampus	Mice	[43]
		Inhibition of DNMT1 activity blocks LTP	Hippocampus	Mice	[42]
	Spatial memory	MeCP1 deficiency impairs spatial memory	Hippocampus	Mice	[43]
		DNA methylation of *PP1*, a memory suppressor gene, is increased while methylation of *reelin*, a memory promoting gene, is decreased by context learning	Hippocampus	Rats	[48]
Histone acetylation	Synaptic plasticity	Increased H3K14 and H4K8 acetylation on *C/EBP* induced by the HAT CBP accompanies LTF	Sensory-motor neurons	*Aplysia*	[29]
		NMDA receptor activation is accompanied by H3K14 hyperacetylation	Hippocampus	Rats	[36]
		MII (a member of the TX repressor group of trxG proteins) deficiency is accompanied by H4 hypoacetylation	Hippocampus	Mice	[38]
		CBP deficiency results in H2B hypoacetylation and late-phase LTP impairment	Hippocampus	Mice	[39]
	Seizures	H4 hyperacetylation on *Bdnf*, H4 hypoacetylation on *GluR2* observed in acute and chronic, H3 hyperacetylation only in chronic seizures	Hippocampus	Rats	[44,45]
	Taste memory	Indirect evidence that MAPK is regulating H2A and H4 acetylation in novel taste learning	Insular cortex	Mice	[49]
	Spatial memory	CBP deficiency and haploinsufficiency inhibit spatial memory	Hippocampus and forebrain	Mice	[39,50]
		p300 deficiency impairs spatial memory	Hippocampus	Mice	[51]
		H3K14 acetylation is increased by spatial memory formation	Hippocampus	Rats	[36]
	Emotional memory	H4 hyperacetylation on *Bdnf* promoters is increased by conditioned fear	Prefrontal cortex	Mice	[55]
Histone phosphorylation	Emotional memory	H3S10 phosphorylation is regulated by the ERK/ MAPK pathway and increased upon contextual fear conditioning	Hippocampus	Rats	[53]

Bdnf, brain-derived neurotrophic factor; CBP, cyclic-AMP response-element binding protein; C/EBP, CCAAT/enhancer binding protein; DNMT1, DNA methyl transferase 1; ERK, extracellular regulated kinase; GluR2, glutamate receptor 2; HAT, histone acetyl transferase; LTF, long-term facilitation; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; MeCP1, methyl-CpG binding protein 1; NMDA, *N*-methyl D-aspartate; PP1, protein phosphatase 1.

specific, albeit unknown role for H3 acetylation and phosphorylation. Interestingly, ECS appear to induce different epigenetic mechanisms when administered acutely or chronically. While both acute and chronic ECS alter H4 acetylation at the promoter of *BDNF* (P2), *c-Fos*, and *CREB*, chronic ECS selectively alters H3 acetylation, at least at the *BDNF* P2 promoter [45].

These results overall support the hypothesis that synaptic activity recruits complex mechanisms of epigenetic regulation of gene expression, and that different types of synaptic activity may activate distinct epigenetic processes involving both histone proteins and DNA (Table 1). This in turn, extends the notion of a "histone code" to an "epigenetic code" in the brain, where distinct epigenetic programs seem to be recruited by different forms of synaptic activity.

4. Epigenetic mechanisms in cognition

The idea that epigenetic mechanisms play a role in memory and cognition was first proposed by Francis Crick (1916–2004) in 1984, when he stated that "memory might be coded in alterations to particular stretches of chromosomal DNA" [46]. In 1999, Holliday [47] followed this concept, and refined the idea of epigenetics to the process of DNA methylation. It took, however, another 8 years until DNA methylation was found to be a dynamic process in the brain that is critical for memory formation [48]. Meanwhile, several studies fostered the notion that posttranslational histone modifications are associated with learning and memory by showing that histone acetylation and phosphorylation regulate multiple aspects of memory and cognition (Table 1).

4.1. Learning and memory in rodents

4.1.1. Novel taste learning

One of the first indications that epigenetic mechanisms are involved in memory stems from a study examining histone acetylation in the insular cortex, a brain region involved in novel taste learning. In this study, TSA treatment of insular cortex samples from animals after novel taste learning was shown to selectively increase the acetylation of histones H2A and H4, but had no effect on H2B or H3 [49]. These results indicate that novel taste learning can induce a specific set of posttranslational histone modifications. However, the possibility that TSA is residue-specific and does not act similarly on all histone residues cannot be excluded (see [41]). Therefore, more refined analyses are required to determine the real effect of the drug. Novel taste learning has also been shown to be associated with increased phosphorylation of MAPK [49], indicating that the intracytoplasmic signaling MAPK pathway may as well be implicated in the regulation of histone acetylation during memory formation.

4.1.2. Object recognition, spatial and contextual memory

Several studies have addressed the importance of histone acetylation in object recognition, spatial and contextual memory. Korzus et al. [50] expressed an inducible, dominant-negative form of the HAT CBP in the mouse forebrain and demonstrated that the resulting HAT deficiency impairs long-term memory formation in a novel object recognition (NOR) task. The behavioural deficit could be rescued by TSA, confirming its association with acetylation. In a second study, Alarcon et al. [39] showed that mice haploinsufficient for *CBP* (i.e., mice lacking one CBP allele), have deficits in long-term memory in both NOR and contextual fear conditioning and that the deficit in contextual fear conditioning could be reversed by the HDAC inhibitor SAHA. Importantly, in both studies, transcription-independent short-term memory was not affected, suggesting that epigenetic mechanisms are primarily recruited for transcriptional-dependent memory formation. Recently, mice expressing a truncated form of p300, another transcriptional co-activator with HAT activity further supported these findings by showing that p300 deficiency induces similar behavioural deficits in long-term but not short-term spatial memory [51]. The requirement of histone acetylation for memory was further highlighted in two studies showing that increasing histone acetylation itself is sufficient to improve memory in wild-type animals. Systemic administration of sodium butyrate, a broad HDAC inhibitor before contextual fear conditioning enhances long-term but not short-term memory. This finding is accompanied by *in vitro* evidence of increased acetylation of H3K14 and H4 (several residues) [36]. Likewise, local administration of TSA into the hippocampus improves spatial long-term memory [41]. However, a drawback of these studies remains that the drugs have limited specificity and target all HDACs in every cellular compartment. More refined approaches need to be developed to determine which residues are selectively involved, and which forms and temporal phases of memory are associated with histone acetylation.

Histone phosphorylation constitutes another important epigenetic process implicated in spatial memory. In particular, H3 phosphorylation is thought to serve as a molecular integrator of intracytoplasmic signaling pathways and nuclear events (reviewed in [52]), and has been shown to be critical for gene transcription associated with memory [53]. Major signaling cascades activated in neuronal cells involve multiple protein kinases and protein phosphatases, which balance each other to control synaptic plasticity and memory [28,35,54]. Recent studies have shown that protein kinases of the MAPK family contribute to the regulation of histone phosphorylation in memory formation. For instance, the protein kinase ERK is activated during contextual fear conditioning and is associated with a transient increase in H3S10 phosphorylation and H3K14 acetylation in the rat hippocampus [53]. This increase is blocked by an inhibitor of MEK (MAP kinase/ERK), a kinase upstream of ERK, suggesting that the observed epigenetic changes are regulated by ERK/MAPK signaling. Intriguingly, H3 acetylation remains unchanged after latent inhibition, a behavioural test based on the inhibition of conditioning, whereas H4 acetylation (on several residues) is increased [36]. These findings point to the fact that, similar to synaptic plasticity, different memory tasks activate distinct signaling cascades that lead to differential epigenetic regulation of chromatin [6,26].

Finally, DNA methylation is also implicated in the formation of spatial and contextual memory. Initial indirect evidence

showed that mice lacking MBD1, a transcriptional co-repressor that binds methylated DNA, have impaired spatial memory on a water maze [43]. A more direct link was recently established by the demonstration that contextual fear conditioning is associated with increased methylation and decreased transcription of the memory suppressor gene *PP1,* and at the same time, with decreased methylation and increased transcription of *reelin*, a memory promoting gene [48]. These findings were further supported by the observation that DNMT inhibitors reverse the hypermethylation of the *PP1* promoter and restore normal transcription. Together, these results delineated for the first time a crucial role for DNA methylation in both spatial and contextual memory.

4.1.3. Tone fear conditioning

The role of histone acetylation in emotional memory has been examined in the hippocampus using a cued fear conditioning task. In the hippocampus, cued fear conditioning seems to be histone acetylation-independent because the administration of HDAC inhibitors did not ameliorate this form of memory. For instance, in transgenic mice with an inducible dominant-negative form of CBP [50], in mice haploinsufficient for *CBP* [39], or in wild-type animals [41], these inhibitors have no effect. In contrast, in the insular cortex, histone acetylation is associated with emotional memory. In this brain structure, the extinction of conditioned fear is accompanied by a significant increase in the overall H4 acetylation at promoter 4 of *BDNF*, and in the transcription of *BDNF* exon I and IV [55]. This effect is specific for H4 since acetylation on H3K9 and H3K14 was not affected. Consistently, the systemic administration of the HDAC inhibitor valproic acid increases H4 but not H3 acetylation at *BDNF* promoters 1 and 4, which correlates with an increased transcription of exon IV, and enhanced long-term memory [55]. Valproic acid and sodium butyrate were also shown to improve emotional memory in wild-type mice whereas TSA does not [41]. These studies therefore suggest that HDAC inhibitors may differ in their mode of action or ability to induce changes in histone acetylation to ultimately influence behaviour. As a result, studies using these drugs need to be combined with more precise analyses of the effect of the drug in different brain regions and different memory tasks. Nevertheless, these findings all support the hypothesis that a specific histone code is associated with different types of memory in distinct brain structures.

4.2. Cognitive dysfunctions in human

Cognition is a highly complex process that engages multiple brain functions, most of which remain not fully understood. Initial insight into these mechanisms has been brought forward by clinical studies in patients with cognitive disorders. Their study has established a central role for a complex interplay between several epigenetic mechanisms, a disruption of which can lead to severe cognitive impairments. Yet, on the basis of their reversibility, epigenetic codes might also represent promising targets for future therapeutic approaches (Table 2).

4.2.1. Rubinstein–Taybi Syndrome (RTS)

RTS is a rare congenital disorder (1:100,000–125,000 prevalence in the US) characterized by short stature, skeletal abnormalities, and mild to severe mental retardation. In most patients, the disease is caused by several types of autosomal mutations in the gene coding for CBP [56]. Many of the mutations found in RTS patients lead to the formation of a protein that lacks part of the HAT domain and activity [57–59]. In several RTS cases, this results in abnormal transcription of genes that require CBP as co-activator, such as CREB-dependent genes [58]. Several mouse models with *CBP* mutations were generated and confirmed the involvement of CBP in the molecular mechanisms of the disease [39,50,60,61]. Importantly, these models had deficits in object, contextual and spatial memory, reminiscent of the cognitive impairments observed in RTS patients, which confirs that histone acetylation is an important posttranslational modification in the etiology of this disease.

4.2.2. Rett Syndrome (RS)

RS is a relatively common X-linked developmental disease (1:10,00–15,000 prevalence in the US) characterized by arrested or retarded neurological development, microcephaly and cognitive decline. Most cases of RS are caused by mutations in the gene coding for methyl-CpG binding protein 2 (MeCP2) [62], a member of the MBP family involved in long-term gene silencing (for a review see [9]). Brain-specific deletion of MeCP2 in mice mimics RS; it leads to reduced brain size and impairs locomotor activity [63,64]. MeCP2 is also important for cognitive functions. MeCP2 deficiency increases anxiety, and a two-fold overexpression of human MeCP2 in mice carrying a truncated endogenous form of MeCP2 was shown to enhance synaptic plasticity in the hippocampus and to improve spatial memory [65]. These functions of MeCP2 were recently shown to require MeCP2 phosphorylation [66], implicating neuronal signaling cascades involving protein kinases and phosphatases in MeCP2-dependent epigenetic regulation. This has been further corroborated by two studies showing that neuronal activity in particular, membrane depolarization, induces calcium-dependent MeCP2 phosphorylation, which in turn leads to its dissociation from the *BDNF* promoter region, and ultimately increases *BDNF* transcription [67,68]. Additionally, HDAC1 was shown to dissociate from *BDNF* promoter regions, suggesting a simultaneous action of DNA methylation and histone acetylation in RS [67]. A deficiency in MeCP2 also correlated with increased overall acetylation of H3 in the cerebral cortex and cerebellum [69], further strengthening the idea that MeCP2 exerts its repressive function, in part, by recruiting histone-modifying enzymes that reinforce the compaction of chromatin [70].

4.2.3. Fragile X Syndrome (FXS)

FXS is a common heritable disease (prevalence in the US 1:8000 in women, 1:4000 in men) characterized by mental retardation and learning disabilities. FXS is caused by abnormal expansion of the trinucleotide repeats CGG and CCG at the 5′-end of the genes encoding fragile X mental retardation protein 1 and 2 (FMRs), respectively [71–73]. These expan-

Table 2
Epigenetic mechanisms in cognitive and psychiatric diseases

Pathology	Implicated gene	Epigenetic modification	Potential treatment	References
Cognitive				
Rubinstein–Taybi	*CBP*	Mutations in the HAT *CBP* cause aberrant histone acetylation	HDAC inhibitors TSA and SAHA reverse cognitive deficits	[39,50,58]
Rett Syndrome	*MeCP2*	Mutations in the methylated DNA-binding protein *MeCP2* cause aberrant DNA methylation and histone acetylation	None suggested	[62–65,69]
Fragile X Syndrome	*FMR1* and *FRM2*	Trinucleotidic repeats within these genes cause their repression via increased methylation	DNA demethylating drug 5-aza rescues secondary acetylation changes	[71–73,75]
Alzheimer's disease	*APP*	AICD, the cleavage product of APP recruits the HAT TIP60, suggesting a potential hyperacetylation	None suggested	[77]
		APP-induced death of cortical neurons provokes H3 and H4 hypoacetylation	None suggested	[79]
	PS1	*PS1* mutations prevent CBP degradation, resulting in abnormal gene expression, potentially through hyperacetylation	Substitution of PS1-mediated enzymatic activity	[78]
		PS1 conditional knock-out mice have decreased CBP level and CBP-mediated gene expression, e.g., *c-Fos*, *Bdnf*, potentially through hypoacetylation	None suggested	[80]
		Hypomethylation of *PS1* promoter increases APP formation	Methyl-donor SAM administration reverses hypomethylation	[81]
Huntington's disease	*Htt*	*Htt* polyglutamine extension binds the HAT CBP and inhibits its function, leading to reduced acetylation	HDAC inhibitors SAHA, sodium butyrate and phenylbutyrate rescue aberrant acetylation	[84–87]
Psychiatric				
Schizophrenia	*Reln*	*Reln* hypermethylation and transcriptional downregulation involved in schizophrenia	5-aza and TSA increase *reln* expression	[90–92]
Cocaine addiction	*c-Fos*, *Cdk5*, *FosB*, *Bdnf*	Acute cocaine administration in rat leads to hyperacetylated *c-Fos and FosB*; chronic administration to hyperacetylated *FosB*, *Bdnf* and *Cdk5*	Viral-mediated *HDAC4* transfer abolishes cocaine place conditioning	[94]
Alcohol addiction	*α-synuclein*	Alcohol consumption in chronic patients leads to hypermethylation of *α-synuclein*	None suggested	[101]
Depression	*Bdnf*	In a rat model of depression, *Bdnf* splice variants are repressed and histone methylation is increased	Antidepressant imipramine reverses *Bdnf* repression and increases histone acetylation	[102]
Predisposition to stress	*GR*	Poor maternal care decreases *GR* expression through increased DNA methylation	SAM precursor l-methionine reverses the maternal care effect on DNA methylation	[108,111]

AICD, APP intracellular domain; APP, amyloid precursor protein; Bdnf, brain-derived neurotrophic factor; CBP, cyclic-AMP response-element binding protein; Cdk5, cyclin-dependent kinase 5; c-Fos, FBJ osteosarcoma oncogene; FosB, FBJ osteosarcoma oncogene B; FMR, fragile X mental retardation; GR, glucocorticoid receptor; HAT, histone acetyl transferase; HDAC, histone deacetylase; Htt, huntingtin; MeCP2, methyl-CpG binding protein 2; PS1, presenilin 1; Reln, reelin; SAHA, suberoylanilide hydroxamic acid; SAM, S-adenosylmethione; TSA, trichostatin A; 5-aza, 5-aza-2-deoxycytidine.

sions increase the methylation and repression of the genes [71–73], and result in protein deficiency and ultimately malfunctions of the nervous system. In mice, an FMR2 deficiency induced by gene knock-out impairs spatial memory, however, it enhances LTP [74]. Although such dissociation between impaired memory and enhanced LTP was previously reported ([74] and references therein), the mechanisms underlying these alterations and their biological implication remain unknown. Interestingly, DNA methylation-dependent silencing of *FMR1* appears to be reinforced by epigenetic modifications of histones. Treatment of FMR1-deficient cell lines with the DNMT inhibitor 5-aza decreases H3K9 dimethylation but increases H3K4 dimethylation, H3K9 and H3K14 acetylation, and overall H4 acetylation [75]. These findings highlight the combined and complex contribution of multiple epigenetic mechanisms in FXS pathology.

4.2.4. Alzheimer's disease (AD)

AD is a late onset neurodegenerative disease with high prevalence (approx. 1:100 in the US), mainly characterized by severe dementia. One of the major landmarks of AD is the deposition of neurotoxic β-amyloid peptides forming Aβ plaques in the brain (for a review see [76]). These peptides are produced by β- and γ-secretase-mediated enzymatic cleavage of the amyloid

precursor protein (APP), which also results in the production of the APP intracellular domain (AICD). AICD recruits the nuclear adaptor protein Fe65 and the HAT TIP60, and in combination with these proteins, acts as a transcriptional activator [77]. In addition, AD-related mutations in *presenilin 1* (*PS1*), the gene coding for the catalytic subunit of the γ-secretase complex, lead to sustained HAT activity of CBP, suggesting that a general increase in acetylation is associated with AD [78]. However, controversial evidence argues that AD is rather associated with a reduction in histone acetylation. For instance, cell death induced by activation of the APP signaling pathway results in decreased acetylated H3 and CBP levels [79]. Conditional knock-out mice deficient for both *PS1* and *PS2* in the forebrain not only have severe memory defects and signs of neurodegeneration, but also reduced expression of CBP and CREB/CBP-dependent target genes such as *c-Fos* and *BDNF* [80]. Altogether, these studies suggest a role for histone acetylation in AD, but further research is required to identify the precise mechanisms involved.

Intriguingly, DNA methylation may also be implicated in the etiology of AD. Hypomethylation of the promoter region of *PS1* increases gene expression and PS1 enzymatic activity, and rises β-amyloid production [81]. In cell culture, this hypomethylation can be partially reversed by S-adenosylmethionine (SAM) administration, a common metabolic methyl donor, indicating the possibility for future therapeutic intervention using such a compound (reviewed in [82]).

4.2.5. Huntington's disease (HD)

HD is a progressive brain disorder (1:100,000 prevalence in Europe) characterized by uncontrollable movements, emotional dysfunctions and cognitive deficits. HD is caused by a mutation in the gene *huntingtin* (*htt*) that leads to an abnormally high number of CAG repeats producing a polyglutamine extension of the protein huntingtin (for a review see [83]). This extension directly binds and inhibits the HAT domain of CBP and p300/CBP-associated factor (P/CAF) [84], which ultimately leads to transcriptional dysregulation. In cultured cells of *Drosophila*, overexpression of the polyglutamine-containing domain reduces the overall level of H3 and H4 acetylation, an effect that can be reversed by the application of SAHA or sodium butyrate [84]. In mouse models of HD, the administration of several types of HDAC inhibitors significantly attenuates motor deficits [85,86] and neuronal atrophy [87], which are accompanied by increased histone acetylation and decreased histone methylation. This suggests the involvement of histone acetylation, and possibly of histone methylation in HD and by extension, points to the potential usefulness of HDAC inhibitors in therapeutic treatments.

5. Epigenetic mechanisms in psychiatric disorders and predisposition to stress

In addition to cognitive disorders, epigenetic mechanisms have been implicated in the etiology of psychiatric diseases such as schizophrenia, addiction, depression, and stress-related disorders (reviewed in [88]) (Table 2).

5.1. Psychiatric disorders

5.1.1. Schizophrenia

Schizophrenia is a multifactorial disease with 1% prevalence worldwide characterized by a disruption of cognitive and emotional processes, motor disturbances and severe deterioration of daily life. Recent evidence has indicated that the epigenetic downregulation of the extracellular matrix protein reelin is causally linked to the disease (reviewed in [89]). *Reln*, the gene coding for reelin, contains a high number of CpG dinucleotide repeats in its promoter region, and is strongly susceptible to regulation by DNA methylation [90]. Both *in vitro* and *in vivo,* DNA hypermethylation decreases *reln* expression, an effect that is reversed by 5-aza *in vitro* [90,91]. Furthermore, TSA and valproic acid *in vitro* (but also *in vivo* for valproic acid) can activate *reln* expression, an effect that *in vivo* correlates with an overall increase in H3 acetylation [91]. TSA also prevents the hypermethylation of the *reln* promoter [92], possibly because hyperacetylated chromatin is inaccessible to DNA binding proteins such as DNMT1 [93]. Overall, both DNA methylation and histone acetylation are involved in the control of *reelin* expression and in turn, may be involved in schizophrenic symptoms. Given the multigenic character of the disease, however, it is likely that genes other than *reln* are subject to regulation by DNA methylation and histone acetylation (see also [93]). Initial evidence has indeed been provided for *glutamic acid decarboxylase* [91,92], but more work is needed to extend these findings.

5.1.2. Addiction

Drugs of abuse such as cocaine and alcohol profoundly influence human behaviour by altering the brain's reward pathways. Their effect is often persistent and associated with lasting alterations in gene expression, pointing to the potential involvement of epigenetic regulatory mechanisms. Recent studies have indeed demonstrated that both acute and chronic cocaine administration involve epigenetic regulation of gene expression. Acute administration of cocaine increases H4 acetylation in the promoter region of *c-Fos* and *FosB*, transcription factors implicated in addiction, in the rat striatum [94]. In addition, acute cocaine exposure is associated with H3 phosphoacetylation in the promoter region of *c-Fos* but not *FosB*. This is also true for H4K5 acetylation and H3S10 phosphorylation as demonstrated in an independent study [95]. The effect on acetylation is presumably mediated by the HAT CBP, which is bound to the promoter of *FosB* upon acute cocaine administration [96], while phosphorylation may be caused by mitogen- and stress-activated protein kinase 1 (MSK1), since MSK1 knock-out mice do not have any H3 phosphorylation when acutely administered cocaine [95]. Chronic cocaine treatment by self-administration also leads to H3 hyperacetylation in the promoter region of *FosB*, as well as of *Bdnf* and *Cdk5* [94], which are activated by chronic cocaine exposure in the striatum [97,98]. Further, it increases the expression of *MeCP2* and *MBD1*, and induces H3 hypoacetylation in sub-areas of the striatum and cortex, suggesting that both DNA methylation and histone acetylation contribute to gene regulation upon chronic cocaine exposure [99]. Notably, acute and chronic cocaine exposures differ in their respective epigenetic

programming [88], with acetylated H3 being a preferential mark for chronic administration. This specificity is reminiscent of that elicited by acute and chronic ECS, in which chronic but not acute treatment alters H3 acetylation [45] (see above). Hence, H3 acetylation might represent an epigenetic mark that preferentially targets continuously activated genes. Finally, this acetylation and cocaine-induced conditioning can be reversed by virus-mediated overexpression of HDAC4 [94], suggesting a means for potential therapeutic intervention in cocaine addiction.

Similar to drug addiction, both acute and chronic exposure to alcohol were shown to activate epigenetic mechanisms. The acute administration of ethanol in rats increases H3K9 acetylation in liver, lung and spleen, but not in brain [100]. Further, alcohol consumption in chronic alcoholic patients was found to be associated with hypermethylation in the promoter region of *α-synuclein*, a gene implicated in craving, in peripheral blood, suggesting that DNA methylation may control the degree of craving [101]. However, more studies are required to define more precisely the epigenetic mechanisms associated with alcoholism.

5.1.3. Depression

Depression is a common and persistent mood disorder characterized by despair, helplessness and social withdrawal. These symptoms can be attenuated by antidepressants, however only when administered repeatedly over a long period of time, suggesting that depression is mediated by stable molecular changes [88]. Modeling of depression-like phenotypes in the mouse can be achieved by different manipulations, in particular by exposing the animals to chronic social defeat stress [102]. Chronic exposure to an aggressor results in pronounced social avoidance, prolonged downregulation of two splice variants of *Bdnf*, *BdnfIII* and *BdnfIV* in the hippocampus and increased promoter dimethylation of H3K27 [102], a mark of transcriptional repression [20]. H3K27 dimethylation persists over several weeks after removal of the aggressor, indicating that this repression mark induced by chronic stress on *Bdnf* transcription is stable. Moreover, the effect of chronic stress on histones is residue-specific, since it does not alter the dimethylation of H3K9. Strikingly, treatment with the antidepressant imipramine reverses gene repression and increases promoter H3K9/K14 acetylation and H3K4 dimethylation [102], markers of transcriptional activation [20], however, dimethylation of H3K27 is not affected, which points to the need for finding additional antidepressants. Interestingly, HDAC inhibitors such as sodium butyrate might represent potent candidates [103], since virus-mediated *HDAC5* overexpression reverses the imipramine-induced H3 hyperacetylation and *BdnfIII* and *BdnfIV* transcription [88]. Finally, modification of H3K4 dimethylation might constitute another potential target for antidepressants [104], providing further support for the hypothesis that more than one epigenetic modification is involved in depression-like phenotypes, and that each of them might be a relevant target for antidepressant treatments.

5.2. Predisposition to stress

Early life environment during both pre- and postnatal development can have profound and life-long effects on adult behaviour. In mammals, maternal care and nutrition are important factors that determine the quality of the environment in early life. In rodents, maternal care is characterized by complex behaviours such as arched-back nursing (ABN) and licking and grooming (LG) that vary greatly between individuals and strains [105]. These maternal behaviours strongly influence the development of proper behavioural responses such as the level of anxiety and stress responsiveness in the offspring [106]. Remarkably, female rat pups raised by mothers with high nurturing abilities (high LG–ABN) become high LG–ABN mothers themselves, but this phenomenon can be reversed by cross-fostering, indicating that transmission of this behavioural trait is independent of the genetic make-up of the individual [107]. Behaviourally, high maternal care decreases fear and stress reactivity in the offspring, an effect that at a molecular level is associated with alteration of stress pathways involving the glucocorticoid receptor (GR). Accordingly, the offspring of high LG–ABN mothers have increased *GR* expression, specifically of splice variant *exon* I_7 [108], and reduced reactivity to stress. In contrast, the offspring of low LG–ABN mothers have decreased *GR* expression and increased stress reactivity [109].

The differential expression of *GR* in these animals appears to be mediated by epigenetic mechanisms. The offspring of high LG–ABN mothers have reduced DNA methylation and increased H3K9 acetylation at the binding site for the transcription factor NGFI-A (also known as Egr-1 or Zif268) in the promoter region of *GR*, an effect that persists into adulthood [108]. Conversely, the offspring of low LG–ABN have increased promoter methylation but no change in acetylation. Recent evidence further suggests that NGFI-A itself may mediate these epigenetic changes since its binding to the *GR* promoter region is necessary for these changes [110]. Although stable, the epigenetic modifications can be reversed by cross-fostering or treatment with TSA resulting in histone hyperacetylation and DNA hypomethylation at the *GR* promoter in low LG–ABN offspring [108]. Likewise, maternal programming of stress responses via glucocorticoid receptors can be reversed by methyl supplementation via the administration of L-methionine, a SAM precursor [111]. Both pharmacological treatments result in differential changes in transcriptional activity in the hippocampus in high and low LG–ABN offspring, suggesting that histone acetylation and DNA methylation influence the expression of multiple genes affected by maternal care [112].

Notably, stressful events in adulthood also have a strong impact on the epigenetic marking of chromatin. Psychological stressful stimuli such as inescapable swim stress significantly increase H3S10 phosphorylation in the dentate gyrus of rats and mice [113]. Interestingly, such change appears to be specific to certain types of stress since exposure to ether or cold temperature, two conditions known to induce stress, does not alter histone modifications.

6. Transgenerational epigenetics

With their relatively stable binding to chromatin, epigenetic modifications are prone to be inherited not only through mitosis, but also through meiosis, despite the substantial chromosomal

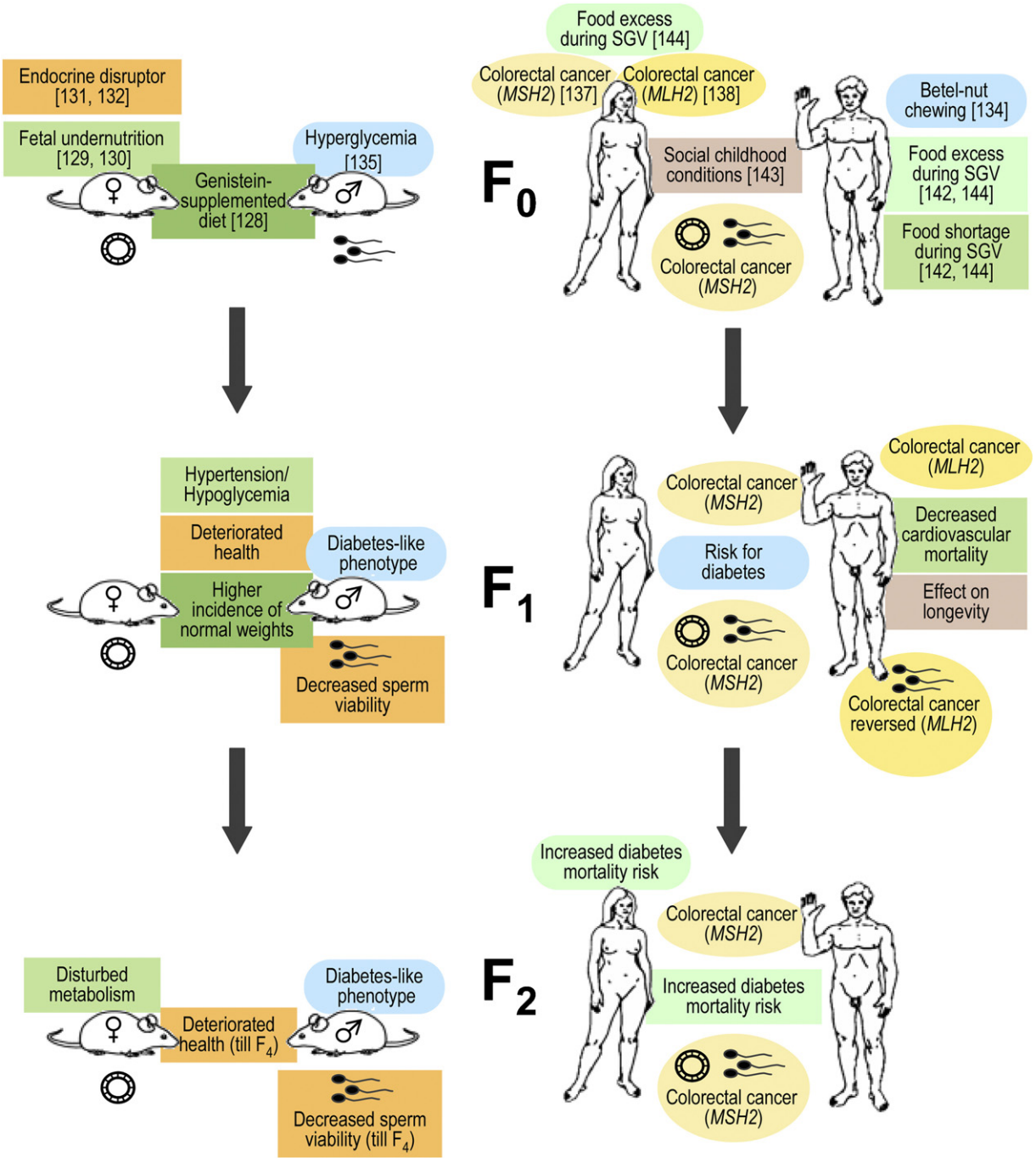

Fig. 3. Disease-related transgenerational epigenetic inheritance. Depicted are examples of transgenerational diseases over three successive generations in both mice and humans and the environmental stimulus in the F_0, which had caused them. Environmental stimuli and their impact in following generations are colour-matched; green for nutrition-related observations; yellow and orange for observations where epigenetic modifications were also detected in the germline; blue for glycemia-related phenotypes; brown for social childhood conditions. Note that for some of the examples, such as nutritional circumstances in the F_0, parallels exist between mice and humans, but the effect can be species-dependent. SGV, slow growth period. See text for gene names.

reorganization occurring during meiosis. Transgenerational epigenetic inheritance is known to occur in plants and invertebrates [114,115], but evidence in vertebrates is still scarce [116]. In vertebrates, transgenerational epigenetic inheritance is defined as "soft inheritance" and can be divided in two types; heritable epigenetic modifications that influence morphological appearance, and modifications that impact on disease susceptibility (see [117] and references therein), both of which can be altered by environmental factors (Fig. 3).

6.1. Variable morphological appearance

Differences in the methylation pattern of transposable elements within a gene can modify the impact of that gene on an

individual's morphology. The best known examples of such a mechanism are the mouse alleles A^{vy} (viable yellow agouti) and *Axin*^{Fu} (axin-fused) implicated in coat pigmentation and kinked tail formation, respectively (reviewed in [118]). *Agouti* encodes a signaling molecule that causes hair follicle melanocytes to produce phaeomelanin (yellow) instead of eumelanin (black). The gene contains a retrotransposable intracisternal A-particle (IAP) in its 5′ region that interferes with the functions of the endogenous promoter, and places its expression under the control of the retrotransposon promoter in the IAP long-terminal repeat (LTR) [118]. Differential methylation of the LTR results in different coat colour ranging from yellow when the LTR is demethylated, to black or pseudoagouti when the LTR is hypermethylated [119]. Coat colour can readily be manipulated by feeding gestating females with a methyl-supplemented diet [120], through a mechanism involving differential LTR methylation [121,122]. Strikingly, such induced epigenetic modification can be passed to subsequent generations through both maternal [119] and paternal [123] germline, suggesting a DNA methylation-dependent transgenerational epigenetic inheritance induced by nutrition. A similar phenomenon occurs at the Axin^{Fu} locus, a regulator of early embryonic axis formation. *Axin*^{Fu} expression is altered by differential methylation of a retrotransposable DNA element [118]. The dominant *Axin*^{Fu} allele contains an IAP in intron 6, which, when expressed leads to the production of several aberrant RNA molecules and a kinked-tail phenotype [124]. Analogous to A^{vy}, hypermethylation of the IAP's LTR prevents erroneous gene expression and results in normal tail morphology [125]. Remarkably, the epigenetic state of *Axin*^{Fu} can also be inherited through both maternal and paternal germline [125], providing a second example of transgenerational epigenetic inheritance.

Of particular interest in this process is the finding that the methylation state of the *Axin*^{Fu} allele in mature sperm reflects the methylation pattern in somatic tissue. This suggests similar regulatory mechanisms in the soma and the germline, or the existence of a yet unknown mechanism of communication of epigenetic marks between these cellular systems [125]. Such a communication could be mediated by molecules that, in response to an epigenetic modification in somatic tissue, are secreted and travel across the germline barrier to implement the same epigenetic change in germ cells. Intriguingly, Charles Darwin (1809–1882) had proposed the existence of such particles, which he gladly called "gemmules" ([126] and references therein). Although still theoretical and not yet identified, these particles could be circulating DNA, RNAs, or prion proteins. Overall, the results of these studies provide strong evidence for the existence of dynamic mechanisms of gene regulation in response to environmental factors that can perpetuate their effect across generations.

6.2. Disease susceptibility

Experimental evidence suggests that epigenetic mechanisms are implicated in the transgenerational transmission of different types of diseases in both rodents and human (for a review see [127]) (Fig. 3).

6.2.1. Obesity

Correlative evidence has shown that supplementation of maternal diet with genistein, an estrogenic compound, not only shifts the methylation profile at the IAP LTR of A^{vy} and coat colour, but also affects the offspring's body weight and health [128]. Genistein increases DNA methylation, and increases the ratio of pseudoagouti offspring and the number of animals with normal body weight. A similar effect has also been observed across two generations following fetal undernutrition [129,130]. These results therefore link methyl-supplemented nutrition during gestation to weight-related disease susceptibility in the offspring. However, more systematic analyses of survival rate and cancer development, and of DNA methylation and gene expression in the offspring are required to provide causal evidence for these observations.

6.2.2. Male infertility

Exposure of gestating rats during gonadal sex determination to the endocrine disruptors vinclozolin, an antiandrogenic compound, and methoxychlor, an estrogenic compound, has been shown to substantially decrease the number and viability of sperm cells, and to increase the incidence of male infertility across four generations [131]. Moreover, vinclozolin treatment impairs the health of all four generations, since these animals have increased level of cholesterol, enhanced tumor development and abnormalities in several tissues [132]. Notably, these effects correlate with altered DNA methylation in the male germline [131], occurring in both non-coding and coding DNA sequences [133]. This is intriguing, but it remains to be determined whether the epigenetic differences are truly causative or simply correlative of the observed phenotype. For instance, it would be critical to investigate whether the methylation pattern in germ cells reflects that in somatic tissue. Although this has been demonstrated for the retroviral element within *Axin*^{Fu} [125], no direct evidence for an environmentally-induced epigenetic mark in both the soma and the germline exists so far.

6.2.3. Glucose intolerance

Glucose intolerance or the risk to develop diabetes is one of the rare examples of transgenerational epigenetic inheritance found to occur in both rodents and humans. The consumption of betel nut has long been known to lead to diabetes, but a recent study newly suggests a paternal effect for the risk of diabetes-like syndrome in F1 offspring in a Taiwanese community [134]. This risk is dose-dependent and correlates with the quantity and the duration of paternal betel nut chewing. Importantly, it occurs even if the offspring does not consume betel nut itself. In mice, similar results were obtained in F1 and F2 males born to hyperglycemic fathers but not in females [135], indicating paternal germline inheritance. Since sperm mainly consists of DNA and has low protein content, it is conceivable that this transgenerational inheritance is caused by altered DNA methylation.

6.2.4. Cancer

Epigenetic mechanisms, specifically DNA methylation, are widely recognized to be a major factor in the etiology of various types of cancer (for a review see [136]). Two recent

studies suggested that the heritability of at least one type of cancer, i.e., nonpolyposis colorectal cancer, might also be of epigenetic origin [137,138], although this remains controversial [139-141]. The first study describes that hypermethylation of the DNA mismatch repair gene *mutS homologue 2* (*MSH2*) across three generations in somatic tissue can be inherited through the germline [137]. In contrast, the second study shows that this epigenetic mark is reversible, since the sperm of sons that had epigenetically inherited a hypermethylated state of another DNA mismatch repair gene, *mutL homologue 1* (*MLH1*), no longer display the same pattern of methylation [138]. These findings might provide novel insight into the etiology and potential cure of some types of cancer that develop even in the absence of a genetic predisposition.

6.2.5. Mortality risk ratio and longevity

Mortality risk and lifespan might be conditioned by the ancestors' food availability during the slow growth period (SGV), defined as age 8–10 in girls and 9–12 in boys [142–144]. Several studies have documented this phenomenon based on thorough records of food supply, disease and cause of death over three generations in a genetically homogeneous north-Swedish community. Food shortage of the father during SGV was shown to decrease mortality by cardiovascular diseases in sons. In contrast, a surplus of food for the paternal grandfather during SGV increased mortality risk due to diabetes in both daughters and sons [142,144]. A similar phenomenon was observed for the paternal grandmother's food supply during SGV, but only in granddaughters [144]. In addition, conditions of social childhood such as number of siblings were found to increase the offspring's longevity, but only in sons, and only when the ancestors' food supply was abundant [143]. This last finding is somewhat unexpected, since sibling size is anticipated to be a determining factor for longevity only when food availability is low. Nevertheless, these studies indicate that nutrition during SGV, i.e., before the onset of puberty influences the risk for disease and mortality in the offspring. This in turn delineates an important time window when environmentally-induced epigenetic changes can become fixed and subsequently transgenerationally inherited. At SGV, germline formation is still active and not complete, and is therefore susceptible to alterations. Unfortunately, no precise information about the type of food available to the ancestors was collected. In this respect, it would be interesting to determine whether the effects observed were due to methyl-supplemented diets, in which case transgenerational disease risks could be tested on laboratory animals. The mechanisms by which environment-induced epigenetic changes in the germline occur and how they are transmitted remain elusive, but will hopefully be the subject of future research.

7. Conclusions

Epigenetic mechanisms play a crucial role in many brain functions and, in light of the their complexity, the initial notion of a "histone code" may now be extended to that of an "epigenetic code". This epigenetic code engages multiple and distinct processes depending on the brain function that is activated, the brain area involved and environmental contingencies. The characteristics of epigenetic mechanisms in the nervous system are reminiscent of developmental processes such as the differentiation of embryonic stem cells, in that they allow cells to respond and adapt to their environment, and keep a cellular memory of previous activity. It has indeed been suggested that the nervous system has "co-opted" comparable mechanisms and that this "co-option" happened in different phylogenetic branches ranging from invertebrates such as *Aplysia,* to rodents and humans [6,26].

Epigenetic codes may help answer one of the most prominent questions in the field of cognition, which is the question of the storage of long-term memory. Epigenetic mechanisms have been suggested to promote the storage of memory because they can stably control gene expression over long periods of time. However, this may be difficult to be reconciled with the concept of retrieval/reconsolidation, during which a memory trace is temporarily destabilized when retrieved, then reconsolidated for further maintenance (for a review see [145]). Rather, it is conceivable that each step involved in the formation and the maintenance of a memory trace is governed by a specific set of epigenetic codes. At a molecular level, this could involve epigenetic codes for transcriptional initiation, elongation, termination or re-initiation of genes that promote memory, and/or for transcriptional repression of genes that prevent memory. A recent study in human embryonic stem cells indeed suggested that epigenetic codes differ between transcriptional initiation and elongation, and that not all transcribed genes have the same epigenetic marks [146]. In addition, epigenetic mechanisms involving DNA methylation were demonstrated to be dynamic processes in the adult brain [48], suggesting that long-term memory traces may be stabilized by a complex and flexible interplay of multiple molecular marks. Future studies examining the time course of epigenetic codes during successive phases of memory should elucidate this interplay.

In contrast, even though epigenetic codes are often initially established by the influence of environmental cues, they can be stably transmitted through mitosis [148] and meiosis [131], and therefore mediate epigenetic inheritance across generations (Fig. 3) [147]. This has been referred to as "soft inheritance" ([117] and references therein) and can reflect an adaptive or a pathological process. Adaptive examples of soft inheritance include the decreased incidence of cardiovascular disease in humans following paternal food shortage during SGV [142], or decreased stress responses of rat offspring to high maternal care [111]. Non-adaptive examples include male infertility following vinclozolin exposure during gonadal sex determination [131] or nonpolyposis colorectal cancer [137,138]. In both cases, the phenotypes acquired through epigenetic mechanisms strongly influence the progenitor's fitness, and thus have a perpetuating incidence.

A final interesting aspect of epigenetic codes is their potential influence on the mutational rate of genes. The density of CpG dinucleotides in the promoter region of a gene has been suggested to be associated with the stability of the gene's transcriptional activity, which in turn, can determine the mutation rate of that gene ([147] and references therein). Although there

is no experimental evidence for a relationship between the influence of environmental factors on epigenetic marks and the subsequent mutation rate of a given gene, it is tempting to speculate that epigenetic mechanisms may have an impact on the genetic code itself. Environmentally-mediated epigenetic codes therefore not only underlie an individual's developmental and cognitive abilities, but may also be a critical mediator of evolutionary processes.

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References

[1] Waddington CH. Organisers and genes. Cambridge: Cambridge University Press; 1940.
[2] Waddington CH. Canalization of development and the inheritance of acquired characters. Nature 1942;150:563–5.
[3] Russo VEA, Martienssen RA, Riggs AD, editors. Epigenetic mechanisms of gene regulation. Woodbury: Cold Spring Harbour Laboratory Press; 1996.
[4] Bird A. Perceptions of epigenetics. Nature 2007;447(7143):396–8.
[5] Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:245–54.
[6] Levenson JM, Sweatt JD. Epigenetic mechanisms in memory formation. Nat Rev Neurosci 2005;6(2):108–18.
[7] Davey CA, Sargent DF, Luger K, Maeder AW, Richmond TJ. Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution. J Mol Biol 2002;319(5):1097–113.
[8] Nathan D, Ingvarsdottir K, Sterner DE, Bylebyl GR, Dokmanovic M, Dorsey LA, et al. Histone sumoylation is a negative regulator in *Saccharomyces cerevisiae* and shows dynamic interplay with positive-acting histone modifications. Gene Dev 2006;20(8):966–76.
[9] Bird A. DNA methylation patterns and epigenetic memory. Gene Dev 2002;16(1):6–21.
[10] Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci 2006;31(2):89–97.
[11] Fan M, Yan PS, Hartman-Frey C, Chen L, Paik H, Oyer SL, et al. Diverse gene expression and DNA methylation profiles correlate with differential adaptation of breast cancer cells to the antiestrogens tamoxifen and fulvestrant. Cancer Res 2006;66(24):11954–66.
[12] Weber M, Hellmann I, Stadler MB, Ramos L, Paabo S, Rebhan M, et al. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet 2007;39(4):457–66.
[13] Brownell JE, Allis CD. Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. Curr Opin Genet Dev 1996;6(2):176–84.
[14] Klose RJ, Zhang Y. Regulation of histone methylation by demethylimination and demethylation. Nat Rev Mol Cell Biol 2007;8(4):307–18.
[15] Peters AH, Schubeler D. Methylation of histones: playing memory with DNA. Curr Opin Cell Biol 2005;17(2):230–8.
[16] Shilatifard A. Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. Annu Rev Biochem 2006;75:243–69.
[17] Loury R, Sassone-Corsi P. Histone phosphorylation: how to proceed. Methods 2003;31(1):40–8.
[18] He H, Lehming N. Global effects of histone modifications. Brief Funct Genomic Proteomic 2003;2(3):234–43.
[19] Shiio Y, Eisenman RN. Histone sumoylation is associated with transcriptional repression. Proc Natl Acad Sci USA 2003;100(23):13225–30.
[20] Kouzarides T. Chromatin modifications and their function. Cell 2007;128(4):693–705.
[21] Latham JA, Dent SY. Cross-regulation of histone modifications. Nat Struct Mol Biol 2007;14(11):1017–24.
[22] Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007;128(4):707–19.
[23] Ruthenburg AJ, Allis CD, Wysocka J. Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. Mol Cell 2007;25(1):15–30.
[24] Jenuwein T, Allis CD. Translating the histone code. Science 2001;293(5532):1074–80.
[25] Turner BM. Cellular memory and the histone code. Cell 2002;111(3):285–91.
[26] Levenson JM, Sweatt JD. Epigenetic mechanisms: a common theme in vertebrate and invertebrate memory formation. Cell Mol Life Sci 2006;63(9):1009–16.
[27] Pittenger C, Kandel ER. In search of general mechanisms for long-lasting plasticity: *Aplysia* and the hippocampus. Philos Trans R Soc B 2003;358(1432):757–63.
[28] Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. Science 2001;294(5544):1030–8.
[29] Guan ZH, Giustetto M, Lomvardas S, Kim JH, Miniaci MC, Schwartz JH, et al. Integration of long-term-memory-related synaptic plasticity involves bidirectional regulation of gene expression and chromatin structure. Cell 2002;111(4):483–93.
[30] Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993;361(6407):31–9.
[31] Bortolotto ZA, Collingridge GL. Characterisation of LTP induced by the activation of glutamate metabotropic receptors in area CA1 of the hippocampus. Neuropharmacology 1993;32(1):1–9.
[32] Frey U, Matthies H, Reymann KG, Matthies H. The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region *in vitro*. Neurosci Lett 1991;129(1):111–4.
[33] Huerta PT, Lisman JE. Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. Nature 1993;364(6439):723–5.
[34] Crosio C, Heitz E, Allis CD, Borrelli E, Sassone-Corsi P. Chromatin remodeling and neuronal response: multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. J Cell Sci 2003;116(Pt 24):4905–14.
[35] Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem 2001;76(1):1–10.
[36] Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD. Regulation of histone acetylation during memory formation in the hippocampus. J Biol Chem 2004;279(39):40545–59.
[37] Orlando V. Polycomb, epigenomes, and control of cell identity. Cell 2003;112(5):599–606.
[38] Kim SY, Levenson JM, Korsmeyer S, Sweatt JD, Schumacher A. Developmental regulation of Eed complex composition governs a switch in global histone modification in brain. J Biol Chem 2007;282(13):9962–72.
[39] Alarcon JM, Malleret G, Touzani K, Vronskaya S, Ishii S, Kandel ER, et al. Chromatin acetylation, memory, and LTP are impaired in CBP+/−mice: a model for the cognitive deficit in Rubinstein–Taybi syndrome and its amelioration. Neuron 2004;42(6):947–59.
[40] Yeh SH, Lin CH, Gean PW. Acetylation of nuclear factor-kappa B in rat amygdala improves long-term but not short-term retention of fear memory. Mol Pharmacol 2004;65(5):1286–92.
[41] Vecsey CG, Hawk JD, Lattal KM, Stein JM, Fabian SA, Attner MA, et al. Histone deacetylase inhibitors enhance memory and synaptic plas-

ticity via CREB:CBP-dependent transcriptional activation. J Neurosci 2007;27(23):6128–40.
[42] Levenson JM, Roth TL, Lubin FD, Miller CA, Huang IC, Desai P, et al. Evidence that DNA (cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. J Biol Chem 2006;281(23):15763–73.
[43] Zhao X, Ueba T, Christie BR, Barkho B, McConnell MJ, Nakashima K, et al. Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. Proc Natl Acad Sci USA 2003;100(11):6777–82.
[44] Huang Y, Doherty JJ, Dingledine R. Altered histone acetylation at glutamate receptor 2 and brain-derived neurotrophic factor genes is an early event triggered by status epilepticus. J Neurosci 2002;22(19):8422–8.
[45] Tsankova NM, Kumar A, Nestler EJ. Histone modifications at gene promoter regions in rat hippocampus after acute and chronic electroconvulsive seizures. J Neurosci 2004;24(24):5603–10.
[46] Crick F. Memory and molecular turnover. Nature 1984;312(5990):101.
[47] Holliday R. Is there an epigenetic component in long-term memory? J Theor Biol 1999;200(3):339–41.
[48] Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. Neuron 2007;53(6):857–69.
[49] Swank MW, Sweatt JD. Increased histone acetyltransferase and lysine acetyltransferase activity and biphasic activation of the ERK/RSK cascade in insular cortex during novel taste learning. J Neurosci 2001;21(10):3383–91.
[50] Korzus E, Rosenfeld MG, Mayford M. CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron 2004;42(6):961–72.
[51] Oliveira AMM, Wood MA, McDonough CB, Abel T. Transgenic mice expressing an inhibitory truncated form of p300 exhibit long-term memory deficits. Learn Mem 2007;14:564–72.
[52] Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. Cell 2000;103(2):263–71.
[53] Chwang WB, O'Riordan KJ, Levenson JM, Sweatt JD. ERK/MAPK regulates hippocampal histone phosphorylation following contextual fear conditioning. Learn Mem 2006;13(3):322–8.
[54] Hedou G, Mansuy IM. Inducible molecular switches for the study of long-term potentiation. Philos Trans R Soc B 2003;358(1432):797–804.
[55] Bredy TW, Wu H, Crego C, Zellhoefer J, Sun YE, Barad M. Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learn Mem 2007;14(4):268–76.
[56] Petrij F, Giles RH, Dauwerse HG, Saris JJ, Hennekam RC, Masuno M, et al. Rubinstein–Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature 1995;376(6538):348–51.
[57] Roelfsema JH, White SJ, Ariyurek Y, Bartholdi D, Niedrist D, Papadia F, et al. Genetic heterogeneity in Rubinstein–Taybi syndrome: mutations in both the CBP and EP300 genes cause disease. Am J Hum Genet 2005;76(4):572–80.
[58] Kalkhoven E, Roelfsema JH, Teunissen H, den Boer A, Ariyurek Y, Zantema A, et al. Loss of CBP acetyltransferase activity by PHD finger mutations in Rubinstein–Taybi syndrome. Hum Mol Genet 2003;12(4):441–50.
[59] Murata T, Kurokawa R, Krones A, Tatsumi K, Ishii M, Taki T, et al. Defect of histone acetyltransferase activity of the nuclear transcriptional coactivator CBP in Rubinstein–Taybi syndrome. Hum Mol Genet 2001;10(10):1071–6.
[60] Bourtchouladze R, Lidge R, Catapano R, Stanley J, Gossweiler S, Romashko D, et al. A mouse model of Rubinstein–Taybi syndrome: defective long-term memory is ameliorated by inhibitors of phosphodiesterase 4. Proc Natl Acad Sci USA 2003;100(18):10518–22.
[61] Oike Y, Hata A, Mamiya T, Kaname T, Noda Y, Suzuki M, et al. Truncated CBP protein leads to classical Rubinstein–Taybi syndrome phenotypes in mice: implications for a dominant-negative mechanism. Hum Mol Genet 1999;8(3):387–96.
[62] Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 1999;23(2):185–8.
[63] Chen RZ, Akbarian S, Tudor M, Jaenisch R. Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. Nat Genet 2001;27(3):327–31.
[64] Guy J, Hendrich B, Holmes M, Martin JE, Bird A. A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. Nat Genet 2001;27(3):322–6.
[65] Collins AL, Levenson JM, Vilaythong AP, Richman R, Armstrong DL, Noebels JL, et al. Mild overexpression of MeCP2 causes a progressive neurological disorder in mice. Hum Mol Genet 2004;13(21):2679–89.
[66] Zhou Z, Hong EJ, Cohen S, Zhao WN, Ho HY, Schmidt L, et al. Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neuron 2006;52(2):255–69.
[67] Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, et al. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science 2003;302(5646):890–3.
[68] Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, et al. Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science 2003;302(5646):885–9.
[69] Shahbazian M, Young J, Yuva-Paylor L, Spencer C, Antalffy B, Noebels J, et al. Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. Neuron 2002;35(2):243–54.
[70] Hong EJ, West AE, Greenberg ME. Transcriptional control of cognitive development. Curr Opin Neurobiol 2005;15(1):21–8.
[71] Ashley CT, Sutcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, et al. Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. Nat Genet 1993;4(3):244–51.
[72] Gecz J, Gedeon AK, Sutherland GR, Mulley JC. Identification of the gene FMR2, associated with FRAXE mental retardation. Nat Genet 1996;13(1):105–8.
[73] Gu Y, Shen Y, Gibbs RA, Nelson DL. Identification of FMR2, a novel gene associated with the FRAXE CCG repeat and CpG island. Nat Genet 1996;13(1):109–13.
[74] Gu Y, McIlwain KL, Weeber EJ, Yamagata T, Xu B, Antalffy BA, et al. Impaired conditioned fear and enhanced long-term potentiation in Fmr2 knock-out mice. J Neurosci 2002;22(7):2753–63.
[75] Tabolacci E, Pietrobono R, Moscato U, Oostra BA, Chiurazzi P, Neri G. Differential epigenetic modifications in the FMR1 gene of the fragile X syndrome after reactivating pharmacological treatments. Eur J Hum Genet 2005;13(5):641–8.
[76] Cummings JL. Alzheimer's disease. N Engl J Med 2004;351(1):56–67.
[77] Cao X, Sudhof TC. A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. Science 2001;293(5527):115–20.
[78] Marambaud P, Wen PH, Dutt A, Shioi J, Takashima A, Siman R, et al. A CBP binding transcriptional repressor produced by the PS1/epsilon-cleavage of N-cadherin is inhibited by PS1 FAD mutations. Cell 2003;114(5):635–45.
[79] Rouaux C, Jokic N, Mbebi C, Boutillier S, Loeffler JP, Boutillier AL. Critical loss of CBP/p300 histone acetylase activity by caspase-6 during neurodegeneration. EMBO J 2003;22(24):6537–49.
[80] Saura CA, Choi SY, Beglopoulos V, Malkani S, Zhang D, Shankaranarayana Rao BS, et al. Loss of presenilin function causes impairments of memory and synaptic plasticity followed by age-dependent neurodegeneration. Neuron 2004;42(1):23–36.
[81] Scarpa S, Fuso A, D'Anselmi F, Cavallaro RA. Presenilin 1 gene silencing by S-adenosylmethionine: a treatment for Alzheimer disease? FEBS Lett 2003;541(1–3):145–8.
[82] Scarpa S, Cavallaro RA, D'Anselmi F, Fuso A. Gene silencing through methylation: an epigenetic intervention on Alzheimer disease. J Alzheimer's Dis 2006;9(4):407–14.
[83] Bates GP. History of genetic disease: the molecular genetics of Huntington disease – a history. Nat Rev Genet 2005;6(10):766–73.
[84] Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL, et al. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. Nature 2001;413(6857):739–43.

[85] Ferrante RJ, Kubilus JK, Lee J, Ryu H, Beesen A, Zucker B, et al. Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. J Neurosci 2003;23(28):9418–27.
[86] Hockly E, Richon VM, Woodman B, Smith DL, Zhou X, Rosa E, et al. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. Proc Natl Acad Sci USA 2003;100(4):2041–6.
[87] Gardian G, Browne SE, Choi DK, Klivenyi P, Gregorio J, Kubilus JK, et al. Neuroprotective effects of phenylbutyrate in the N171-82Q transgenic mouse model of Huntington's disease. J Biol Chem 2005;280(1):556–63.
[88] Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. Nat Rev Neurosci 2007;8(5):355–67.
[89] Costa E, Chen Y, Davis J, Dong E, Noh JS, Tremolizzo L, et al. REELIN and Schizophrenia: a disease at the interface of the genome and the epigenome. Mol Interv 2002;2(1):47–57.
[90] Chen Y, Sharma RP, Costa RH, Costa E, Grayson DR. On the epigenetic regulation of the human reelin promoter. Nucleic Acids Res 2002;30(13):2930–9.
[91] Tremolizzo L, Carboni G, Ruzicka WB, Mitchell CP, Sugaya I, Tueting P, et al. An epigenetic mouse model for molecular and behavioral neuropathologies related to schizophrenia vulnerability. Proc Natl Acad Sci USA 2002;99(26):17095–100.
[92] Dong E, Agis-Balboa RC, Simonini MV, Grayson DR, Costa E, Guidotti A. Reelin and glutamic acid decarboxylase67 promoter remodeling in an epigenetic methionine-induced mouse model of schizophrenia. Proc Natl Acad Sci USA 2005;102(35):12578–83.
[93] Grayson DR, Chen Y, Costa E, Dong E, Guidotti A, Kundakovic M, et al. The human reelin gene: transcription factors (+), repressors (−) and the methylation switch (±) in schizophrenia. Pharmacol Ther 2006;111(1):272–86.
[94] Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DEH, Truong HT, et al. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. Neuron 2005;48(2):303–14.
[95] Brami-Cherrier K, Valjent E, Herve D, Darragh J, Corvol JC, Pages C, et al. Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. J Neurosci 2005;25(49):11444–54.
[96] Levine AA, Guan Z, Barco A, Xu S, Kandel ER, Schwartz JH. CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. Proc Natl Acad Sci USA 2005;102(52):19186–91.
[97] Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y. Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci 2003;23(3):742–7.
[98] Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, et al. Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature 2001;410(6826):376–80.
[99] Cassel S, Carouge D, Gensburger C, Anglard P, Burgun C, Dietrich JB, et al. Fluoxetine and cocaine induce the epigenetic factors MeCP2 and MBD1 in adult rat brain. Mol Pharmacol 2006;70(2):487–92.
[100] Kim JS, Shukla SD. Acute *in vivo* effect of ethanol (binge drinking) on histone H3 modifications in rat tissues. Alcohol Alcohol 2006;41(2):126–32.
[101] Bonsch D, Lenz B, Kornhuber J, Bleich S. DNA hypermethylation of the alpha synuclein promoter in patients with alcoholism. Neuroreport 2005;16(2):167–70.
[102] Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 2006;9(4):519–25.
[103] Schroeder FA, Lin CL, Crusio WE, Akbarian S. Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. Biol Psychiatry 2007;62(1):55–64.
[104] Lee MG, Wynder C, Schmidt DM, McCafferty DG, Shiekhattar R. Histone H3 lysine 4 demethylation is a target of nonselective antidepressive medications. Chem Biol 2006;13(6):563–7.
[105] Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav 2003;79(3):359–71.
[106] Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 2001;24:1161–92.
[107] Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science 1999;286(5442):1155–8.
[108] Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. Nat Neurosci 2004;7(8):847–54.
[109] Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. Science 1997; 277(5332):1659–62.
[110] Weaver IC, D'Alessio AC, Brown SE, Hellstrom IC, Dymov S, Sharma S, et al. The transcription factor nerve growth factor-inducible protein a mediates epigenetic programming: altering epigenetic marks by immediate-early genes. J Neurosci 2007;27(7):1756–68.
[111] Weaver ICG, Champagne FA, Brown SE, Dymov S, Sharma S, Meaney MJ, et al. Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. J Neurosci 2005;25(47):11045–54.
[112] Weaver IC, Meaney MJ, Szyf M. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. Proc Natl Acad Sci USA 2006;103(9): 3480–5.
[113] Bilang-Bleuel A, Ulbricht S, Chandramohan Y, De Carli S, Droste SK, Reul JMHM. Psychological stress increases histone H3 phosphorylation in adult dentate gyrus granule neurons: involvement in a glucocorticoid receptor-dependent behavioural response. Eur J Neurosci 2005;22(7):1691–700.
[114] Molinier J, Ries G, Zipfel C, Hohn B. Transgeneration memory of stress in plants. Nature 2006;442(7106):1046–9.
[115] Xing Y, Shi S, Le L, Lee CA, Silver-Morse L, Li WX. Evidence for transgenerational transmission of epigenetic tumor susceptibility in *Drosophila*. PLoS Genet 2007;3(9):e151.
[116] Chong SY, Whitelaw E. Epigenetic germline inheritance. Curr Opin Genet Dev 2004;14(6):692–6.
[117] Richards EJ. Opinion – inherited epigenetic variation – revisiting soft inheritance. Nat Rev Genet 2006;7(5):395–402.
[118] Rakyan VK, Preis J, Morgan HD, Whitelaw E. The marks, mechanisms and memory of epigenetic states in mammals. Biochem J 2001;356:1–10.
[119] Morgan HD, Sutherland HG, Martin DI, Whitelaw E. Epigenetic inheritance at the agouti locus in the mouse. Nat Genet 1999;23(3):314–8.
[120] Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in A(vy)/a mice. FASEB J 1998;12(11):949–57.
[121] Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 2003; 23(15):5293–300.
[122] Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr 2002;132(Suppl. 8), 2393S-400S.
[123] Cropley JE, Suter CM, Beckman KB, Martin DI. Germ-line epigenetic modification of the murine A^{vy} allele by nutritional supplementation. Proc Natl Acad Sci USA 2006;103(46):17308–12.
[124] Vasicek TJ, Zhen L, Guan X-J, Zhang T, Costantini F, Tilghman SM. Two dominant mutations in the mouse *fused* gene are the result of transposon insertions. Genetics 1997;147(2):777–86.
[125] Rakyan VK, Chong S, Champ ME, Cuthbert PC, Morgan HD, Luu KV, et al. Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. Proc Natl Acad Sci USA 2003;100(5):2538–43.
[126] Liu Y. Like father like son. EMBO Rep 2007;8(9):798–803.
[127] Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet 2007;8(4):253–62.

[128] Dolinoy DC, Weidman JR, Waterland RA, Jirtle RL. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ Health Perspect 2006;114(4):567–72.
[129] Anderson CM, Lopez F, Zimmer A, Benoit JN. Placental insufficiency leads to developmental hypertension and mesenteric artery dysfunction in two generations of Sprague-Dawley rat offspring. Biol Reprod 2006;74(3):538–44.
[130] Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, Rodriguez-Gonzalez GL, et al. Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. J Physiol 2005;566(Pt 1):225–36.
[131] Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 2005;308(5727):1466–9.
[132] Anway MD, Leathers C, Skinner MK. Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. Endocrinology 2006;147(12):5515–23.
[133] Chang HS, Anway MD, Rekow SS, Skinner MK. Transgenerational epigenetic imprinting of the male germline by endocrine disruptor exposure during gonadal sex determination. Endocrinology 2006;147(12): 5524–41.
[134] Chen THH, Chiu YH, Boucher BJ. Transgenerational effects of betelquid chewing on the development of the metabolic syndrome in the Keelung Community-based Integrated Screening Program. Am J Clin Nutr 2006;83(3):688–92.
[135] Boucher BJ, Ewen SWB, Stowers JM. Betel Nut (*Areca catechu*) consumption and the induction of glucose-intolerance in adult Cd1 mice and in their F1 and F2 offspring. Diabetologia 1994;37(1):49–55.
[136] Esteller M. Epigenetic gene silencing in cancer: the DNA hypermethylome. Hum Mol Genet 2007;16:R50–9. Spec. No. 1.
[137] Chan TL, Yuen ST, Kong CK, Chan YW, Chan ASY, Ng WF, et al. Heritable germline epimutation of MSH2 in a family with hereditary nonpolyposis colorectal cancer. Nat Genet 2006;38(10):1178–83.
[138] Hitchins MP, Wong JJ, Suthers G, Suter CM, Martin DI, Hawkins NJ, et al. Inheritance of a cancer-associated MLH1 germ-line epimutation. N Engl J Med 2007;356(7):697–705.
[139] Hitchins MP, Ward RL. Erasure of MLH1 methylation in spermatozoa-implications for epigenetic inheritance. Nat Genet 2007;39(11): 1289.
[140] Suter CM, Martin DI, Ward RL. Germline epimutation of MLH1 in individuals with multiple cancers. Nat Genet 2004;36(5):497–501.
[141] Suter CM, Martin DI, Ward RL. Addendum: Germline epimutation of MLH1 in individuals with multiple cancers. Nat Genet 2007;39(11): 1414.
[142] Kaati G, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. Eur J Hum Genet 2002;10(11):682–8.
[143] Kaati G, Bygren LO, Pembrey M, Sjostrom M. Transgenerational response to nutrition, early life circumstances and longevity. Eur J Hum Genet 2007;15(7):784–90.
[144] Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, et al. Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 2006;14(2):159–66.
[145] Alberini CM, Milekic MH, Tronel S. Mechanisms of memory stabilization and de-stabilization. Cell Mol Life Sci 2006;63(9):999–1008.
[146] Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA. A chromatin landmark and transcription initiation at most promoters in human cells. Cell 2007;130(1):77–88.
[147] Jablonka E, Lamb MJ. Epigenetic inheritance in evolution. J Evolution Biol 1998;11(2):159–83.
[148] Cavalli G, Paro R. Epigenetic inheritance of active chromatin after removal of the main transactivator. Science 1999;286(5441):955–8.
[149] Gerken T, Girard CA, Loraine Tung Y-C, Webby CJ, Saudek V, Hewitson KS, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 2007;318(5855):1469–72.