EPIGENETIC MECHANISMS IN MEMORY FORMATION

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Abstract | Discoveries concerning the molecular mechanisms of cell differentiation and development have dictated the definition of a new sub-discipline of genetics known as epigenetics. Epigenetics refers to a set of self-perpetuating, post-translational modifications of DNA and nuclear proteins that produce lasting alterations in chromatin structure as a direct consequence, and lasting alterations in patterns of gene expression as an indirect consequence. The area of epigenetics is a burgeoning subfield of genetics in which there is considerable enthusiasm driving new discoveries. Neurobiologists have only recently begun to investigate the possible roles of epigenetic mechanisms in behaviour, physiology and neuropathology. Strikingly, the relevant data from the few extant neurobiology-related studies have already indicated a theme — epigenetic mechanisms probably have an important role in synaptic plasticity and memory formation.

Epigenetic mechanisms typically involve heritable alterations in chromatin structure, which, in turn, regulate gene expression. Fundamental insights about epigenetic heritability have come from studies of cell division and development. However, there is increasing evidence that the regulation of chromatin structure through histone acetylation and DNA methylation might mediate long-lasting behavioural changes in the context of learning and memory. This idea is fascinating because similar mechanisms are used for triggering and storing long-term memories at the cellular level during, for example, cell differentiation. Another intriguing aspect of this hypothesis is that the storage of lifelong behavioural memory might involve lasting changes in the physical, three-dimensional structure of DNA itself.

Epigenetics is unfamiliar to most neurobiologists. Recently, cellular, molecular and behavioural approaches have led to several exciting developments in this area that specifically concern neurobiological systems. In this review, we first introduce the topic of epigenetics and then discuss the idea that the conservation of epigenetic mechanisms for information storage represents a unifying model in biology, with epigenetic mechanisms being used for cellular memory at different levels that range from cellular differentiation to development to behavioural memory.

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What is epigenetics?

Epigenetics and its associated terminology have several connotations, and specific terms need to be defined before we can discuss them in detail. We define the genome as a complete set of haploid DNA and the functional units that it encodes. In the nucleus, DNA exists as a highly compressed structure that consists of DNA and protein, known as chromatin. The epigenome is the sum of both the chromatin structure and the pattern of DNA methylation, which is the result of an interaction between the genome and the environment. Three definitions for the term 'epigenetic' are currently in use in the literature.

The broadest definition includes the transmission and perpetuation of information through meiosis or mitosis that is not based on the sequence of DNA. This process is not restricted to DNA-based transmission and can also be protein-based. This definition is broadly used in the yeast literature, wherein phenotypes that can be inherited by daughter cells are perpetuated past cell division using protein-based mechanisms[1–3].

Developmental biologists and cancer researchers provide a second definition for epigenetic: meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself. The altered patterns of gene expression can occur through

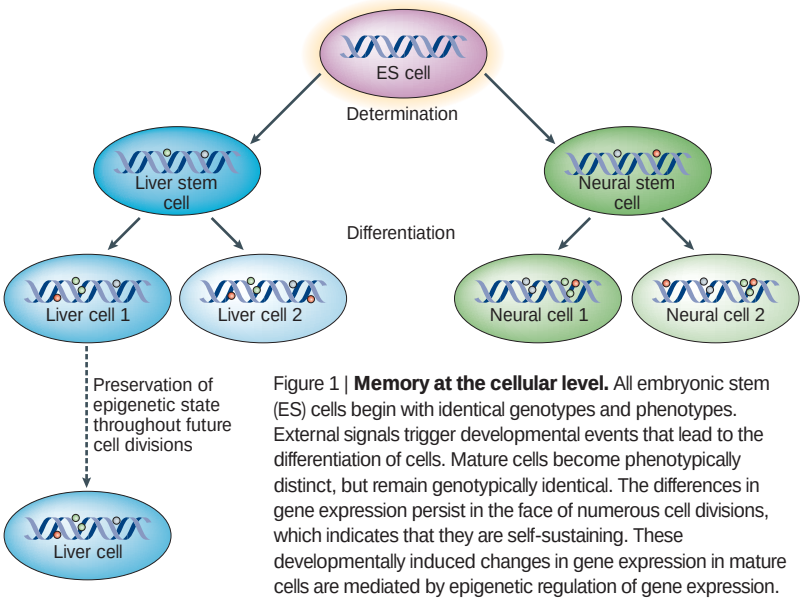

Figure 1 | **Memory at the cellular level.** All embryonic stem (ES) cells begin with identical genotypes and phenotypes. External signals trigger developmental events that lead to the differentiation of cells. Mature cells become phenotypically distinct, but remain genotypically identical. The differences in gene expression persist in the face of numerous cell divisions, which indicates that they are self-sustaining. These developmentally induced changes in gene expression in mature cells are mediated by epigenetic regulation of gene expression.

several mechanisms that are based on DNA, RNA or proteins (see below)[4].

A third definition posits that epigenetics is the mechanism for the stable maintenance of gene expression that involves physically 'marking' DNA or its associated proteins. This allows genotypically identical cells (such as all cells in an individual human) to be phenotypically distinct (for example, a neuron is phenotypically distinct from a liver cell). The molecular and physical basis for this type of change in DNA or chromatin structure[5] is the focus of this review. By this definition, the regulation of chromatin structure is equivalent to epigenetics.

Epigenetics for information storage

Several classic examples illustrate the importance of epigenetic mechanisms in information storage at the cellular level. They indicate that epigenetic mechanisms are widely used for the formation and storage of cellular information in response to transient environmental signals. We present these examples to emphasize that the storage of cellular information is in some ways analogous to memory storage in the adult nervous system. Moreover, the lasting cellular changes are triggered by a transient signal in each case, which is also analogous to the formation of behavioural memory in the CNS.

A prototype example is mammalian cellular differentiation. Once an embryonic precursor cell is triggered to differentiate into a particular cell type (for example, a liver cell), that cell and its subsequent daughter cells might be required to undergo thousands of cell divisions over the lifetime of the animal. How does a liver cell remember that it is a liver cell when, over the course of cell division, it must replicate *de novo* its entire genome? The information clearly cannot be contained in the DNA sequence itself. As mentioned above, the answer to this question involves heritable epigenetic mechanisms that allow the cell's identity to be manifest as the subset of genomic DNA that it expresses (FIG. 1). The genome is marked by, for example, DNA methylation or histone acetylation (or lack thereof) at specific sites that are acquired as part of the differentiation process but are self-perpetuating during DNA replication and cell division. Several hepatic nuclear factors have been shown to be involved in mediating liver-specific gene expression during development through the regulation of histone acetylation[6–8]. Moreover, developmentally-induced changes in histone acetylation are stably propagated from mother to daughter cells in mammals[9]. So, a liver cell perpetuates its specific acquired pattern of gene expression across cellular generations and over time through these heritable epigenetic marks — an example of lasting memory at the cellular level.

The formation of epigenetic memory is not limited to mammalian cells. Plants are induced to flower by a process known as vernalization that also involves epigenetic mechanisms (for a review, see REF. 10). For example, a biennial plant must experience a period of cold weather between its first and second years of existence for its flowering to be triggered. Exposure to cold in biennial plants results in the activation of epigenetic mechanisms that involve methylation of DNA-binding proteins and acetylation of histones, and these processes trigger mitotically stable changes in the pattern of gene expression. In this way, plant cells 'remember' their exposure to the winter cold and are prepared for the plant to flower during the next spring.

Another example involves T cells of the mammalian immune system. The commitment of T-lymphocyte precursors to a wide variety of differentiated states with different patterns of gene expression is triggered by numerous epigenetic mechanisms that involve DNA methylation and histone modifications (for a review, see REF. 11). These processes are important in the formation of long-lasting immunological memory in response to a transient signal from the environment.

Are these epigenetic mechanisms also extant and operable in non-dividing, terminally differentiated neurons in the adult CNS? Adult neurons no longer have the problem of heritability. However, are the basic epigenetic mechanisms that are important for information storage during development also important for storing memories that manifest themselves behaviourally in the adult? We predict that these mechanisms are conserved in the adult nervous system, where they have been co-opted to serve the formation of behavioural memories. Epigenetic mechanisms subserve changes in neuronal function in the adult that are components of memory at the behavioural level. We propose that epigenetic processes constitute a unified set of molecular mechanisms that allow information storage in systems as diverse as yeast, plants, cellular differentiation and memory storage in the mammalian CNS. To this end, we view chromatin as a dynamic structure that can integrate potentially hundreds of signals from the cell surface and effect a coordinated and appropriate transcriptional response. In the following sections, we explore this hypothesis in more mechanistic detail, focusing on epigenetic mechanisms at the cellular level.

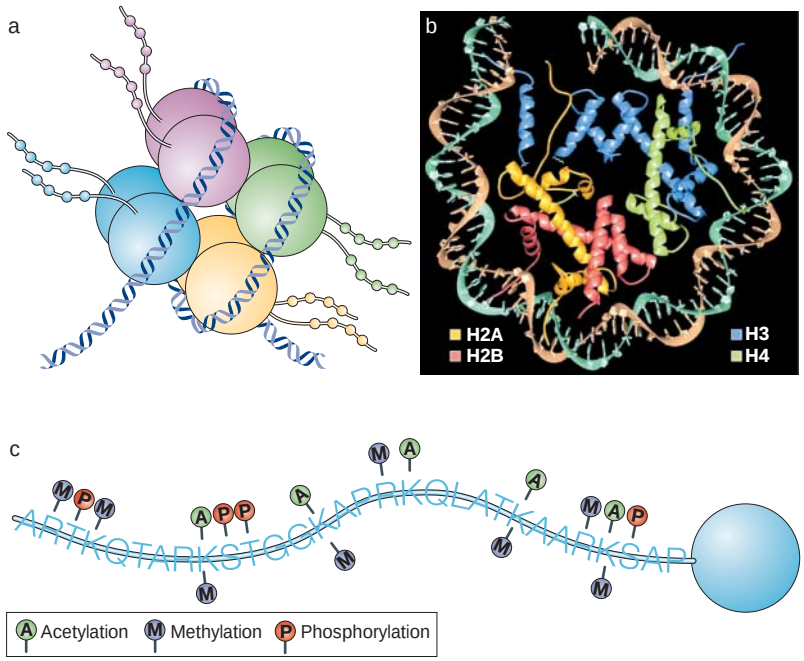

Figure 2 | **The nucleosome and the histone code. a** | Each nucleosome comprises an octamer of histone molecules, which consists of an $H3_2$–$H4_2$ tetramer and two H2A–H2B dimers. The amino (N) termini of histones project out of the nucleosome core and interact with DNA. These histone tails can be epigenetically modified, and function as signal integration platforms. **b** | Crystal structure of the nucleosome depicting the interaction of DNA with histones. Reproduced, with permission, from REF. 12 © (1997) Macmillan Magazines Ltd. **c** | The first 30 amino acids in the N terminus of the human histone H3 are illustrated. Many sites in the N terminus can be targets for epigenetic tagging by, for example, acetylation, phosphorylation and methylation. Regulation of each site is independent, and the integration of epigenetic tags elicits a finely tuned transcriptional response. The integration of signalling at the level of epigenetics is commonly referred to as the histone code[13].

Molecular mechanisms

Epigenetic tagging of histones. Histones are highly basic proteins that function to compress DNA within the nucleus and provide a platform for regulating gene transcription. Modification of histones is a mechanism for epigenetic tagging of the genome. Histone modification can occur as a secondary consequence of DNA methylation, or can be mediated by mechanisms that are independent of DNA methylation and controlled by intracellular signalling.

In the nucleus, DNA is tightly packaged into chromatin. The interaction between histone, in the form of the nucleosome (an octamer of histones), and DNA is mediated in part by the amino (N)-terminal tail of histone proteins (FIG. 2a). Structural studies indicate that the N-terminal tails of histones protrude beyond the chromosomes[12] (FIG. 2b). The current hypothesis is that these histone tails serve as signal integration 'platforms', whereby post-translational modifications are combined in a 'histone code' that ultimately directs the activity of numerous transcription factors, co-factors and the transcriptional machinery in general[13] (FIG. 2c). The histone code is the specific pattern of post-translational modifications of a given histone octamer in chromatin. This code, or pattern, is read out as an influence on the specific level of expression of the associated gene(s).

There are several specific sites of post-translational modification within the N-terminal tails of histone proteins, and modifications of these sites modulate the overall structure of chromatin. Currently, four post-translational modifications of histone tails have been characterized: acetylation, methylation, ubiquitylation and phosphorylation, all of which can serve as epigenetic tags.

Acetylation is the best characterized of the post-translational modifications on histones. Acetylation of lysine residues occurs on the amino group in their side chain, which effectively neutralizes their positive charge. The reaction is catalysed by histone acetyltransferases (HATs),which transfer an acetyl group from acetyl-coenzyme A to the ε-NH^+ group of a Lys residue within a histone[14–17]. The process is reversible, and the enzymes that catalyse the reversal of histone acetylation are known as histone deacetylases (HDACs).

Histone methylation — first discovered 40 years ago[18] — is another histone-directed epigenetic tag. Similar to acetylation, methylation of histones occurs on ε-NH^+ groups of Lys residues, and is mediated by histone methyltransferases (HMTs). Unlike acetylation, methylation of Lys preserves their positive charge. In addition, Lys can accept up to three methyl groups. Arginine residues within histones can also be mono- or dimethylated on their guanidine nitrogen. This reaction is catalysed by protein Arg methyltransferases (PRMTs).

Ubiquitylation of histones was identified 29 years ago[19] but has only recently begun to be characterized in detail. Ubiquitin, a protein of 76 amino acids that is named for its ubiquitous distribution in all cell types and high degree of conservation across species, is usually, but not always, attached to proteins as a signal for degradation by the proteasome[20]. Similar to other proteins, histones are ubiquitylated through the attachment of a ubiquitin to the ε-NH^+ group of a Lys residue[21]. Ubiquitylation of histones H1, H2A, H2B and H3 has been observed[19,22–24]. Most histones seem to be mono-ubiquitylated, although there is evidence for poly-ubiquitylation[21].

Phosphorylation of histones H1 and H3 was first observed more than 30 years ago in the context of chromosome condensation during mitosis[25,26]. H3 was the first histone whose phosphorylation was characterized in response to the activation of mitogenic signalling pathways[27]. Phosphorylation of serine 10 on H3 is mediated by ribosomal protein S6 kinase 2 (RSK2), which is downstream of extracellular signal-regulated kinase (ERK), mitogen- and stress-activated protein kinase 1 (MSK1), which is downstream of both ERK and mitogen-activated protein kinase 1 (MAPK1 or p38), and the aurora kinase family member increase in ploidy 1 (IPL1) (REFS 28–31). Recent evidence also implicates aurora kinases in the phosphorylation of Ser28 in histone H3 (REF. 32). In order to reverse these phosphorylation events, phosphatases remove phosphate groups from histones[27,33]. So far, the phosphatases PP1 and PP2A have been shown to regulate levels of phorphorylation on H3 (REFS 30,34).

Although a great deal of attention has been given to the N termini of the histones that comprise the nucleosome, increasing evidence indicates that other sites exist for the epigenetic modulation of the genome. Some histones can be modified at domains other than their N termini. For example, disruptor of telomeric silencing 1 protein (DOT1P) has been shown to methylate histone H3 on Lys79, a residue that lies within the globular domain[35]. In addition, higher-order chromatin folding is also undoubtedly involved in the regulation of gene expression. In this regard, there is increasing evidence that the linker histone H1 has a role in the modulation of chromatin structure[36].

Epigenetic tagging of DNA. As mentioned before, the genome can be epigenetically marked by DNA methylation and modifications of histone. Methylation of DNA is catalysed by a class of enzymes known as DNA methyltransferases (DNMTs)[37]. DNMTs transfer methyl groups to cytosine (C) residues within a continuous stretch of DNA, specifically at the 5-position of the pyrimidine ring[38]. Not all cytosines can be methylated; usually they must be immediately followed by a guanine (G) residue to be methylated[39,40]. These 'CpG' dinucleotide sequences are highly underrepresented in the genome relative to that which would be predicted by chance; however, about 70% of the existing CpG dinucleotides are methylated[41]. The rest of the normally unmethylated CpG dinucleotides occur in small clusters, known as 'CpG islands'[42]. For purposes of this review, we focus on epigenetic mechanisms that, we speculate, involve reversible DNA methylation, and not the semi-permanent DNA methylation that occurs during cellular differentiation.

DNA methylation leads to marked changes in the structure of chromatin that ultimately result in significant downregulation of transcription. It can directly interfere with the ability of transcription factors to bind to regulatory elements. The transcription factor erythroblastosis 1 (ETS1) and the boundary element CCCTC-binding factor (CTCF) can efficiently bind to non-methylated, but not methylated DNA[43,44]. Moreover, several proteins recognize and bind to methylated CpG residues independent of DNA sequence. The five proteins that are known to bind to methylated CpGs are methyl CpG-binding protein 2 (MECP2), methyl CpG-binding domain protein 1 (MBD1), MBD2, MBD4 and Kaiso[45,46]. These proteins might mediate transcriptional repression by recruiting chromatin-remodelling enzymes. For example, MECP2 directly associates with the transcriptional co-repressor SWI-independent 3A (SIN3A) and histone deacetylase[47,48].

RNA interference. RNA interference (RNAi) is a mechanism by which the expression of cognate genes is disrupted through the action of double-stranded RNA molecules[49]. Recent studies have indicated that the RNAi machinery is used in the nucleus and is involved in the formation of heterochromatin and epigenetic tagging of histones in yeast. Genetic disruption of the RNAi pathway leads to relaxation of heterochromatin around centromeres, which causes erroneous expression of normally silent transcripts and a decrease in the methylation of histone H3 (REFS 50,51). Small RNAs that are produced by a specialized ribonuclease might associate with the DNA and direct the formation of a protein complex that promotes the formation of heterochromatin[52]. It is not known whether activity-dependent regulation of neuronal gene expression can be mediated by RNAi. There are a few examples that implicate RNAi in the regulation of gene expression in eukaryotes. The best known example is the expression of the non-coding RNAs XIST (sense) and TSIX (antisense) in X-chromosome inactivation[53,54]. Another example in which RNAi might be involved in regulation of gene expression is in the generation of circadian rhythmicity. In the silkmoth and *Neurospora*, the expression of core *clock* genes seems to be regulated by endogenous antisense RNA[55,56]. A final example in which RNAi might have a role in the regulation of gene expression involves microRNAs (miRNA). miRNAs are small, non-coding RNAs that have been identified in several metazoans and seem to be involved in development, differentiation and apoptosis[57].

Other mechanisms of epigenetic tagging. We have so far focused on epigenetic mechanisms that are DNA-centric, which result in the modification of either the DNA itself or chromatin structure. According to the broadest definition of epigenetics, which includes any non-DNA-sequence-based system for the perpetuation of information, any protein-based system for the storage of cellular memory is also epigenetic. Prions represent such a viable, protein-based system for epigenetic memory. Once a protein has been converted into its prion form, that protein promotes the transition of other cognate proteins into the prion form. A provocative series of studies has shown that, in *Aplysia californica*, the cytoplasmic polyadenylation element-binding protein (CPEB) assumes a prion-like conformation after synapses are strengthened[3]. By assuming a prion-like conformation, CPEB can maintain a stable synaptic state in the face of protein turnover (see REFS 2,3 for reviews on protein-based mechanisms of epigenetic tagging).

Epigenetics in neural function

In the following sections, we explore the functional significance of epigenetics in various aspects of neural function, with an emphasis on chromatin-associated mechanisms. The theme of our discussion will be that synaptic input or other environmental stimuli lead to changes in epigenetic state and ultimately neural function (FIG. 3).

Neural development and differentiation. Neurons express a complement of proteins that are important for their function, but would adversely affect physiological function in other cell types. These include proteins that are involved in excitability, transmitter release and the maintenance of transmembrane potential. Genes that are to be expressed in neurons, but not in other cell

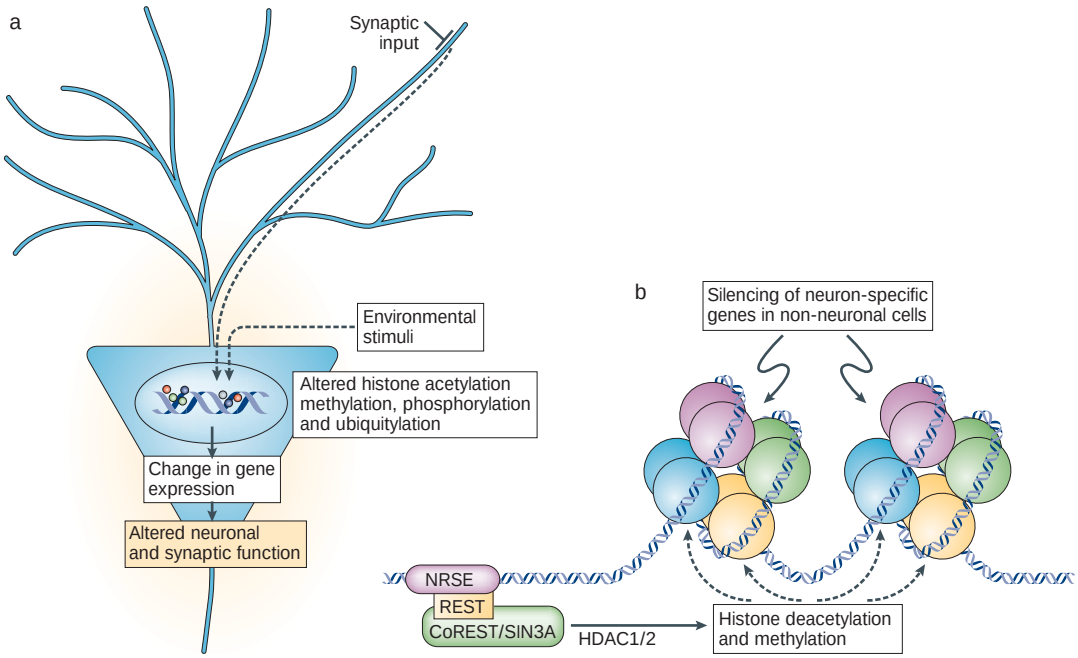

Figure 3 | **Epigenetics in the adult nervous system. a** | Regulation of the epigenetic state of the genome in adult neurons occurs in response to synaptic inputs and/or other environmental stimuli. These external stimuli result in changes in the transcriptional profile of the neuron and, ultimately, neural function. **b** | The RE1-silencing transcription factor (REST)/REST co-repressor (CoREST) system. The neuron-restrictive silencer element (NRSE) is upstream of genes to be silenced in non-neuronal cells and recruits REST as a mediator of transcriptional repression. SIN3A, CoREST and REST function with additional factors such as histone deacetylase 1 (HDAC1) and HDAC2 to lead to chromatin condensation and gene silencing.

types, have a neuron-restrictive silencer element (NRSE) in their promoter[58–60]. This regulatory element, which is approximately 21–24 base pairs long, can completely silence a gene in non-neuronal cells[60].

The first step towards understanding how NRSEs confer tissue-specific regulation of gene expression was the identification of the transcription factors that bind to this regulatory element (FIG. 3). The RE1-silencing transcription factor (REST or NRSF) was the first transcription factor shown to bind to NRSEs and repress gene expression[61]. The REST protein is ubiquitously expressed in cells outside the nervous system, where it acts to repress the expression of neuronal genes[61]. Deletion of the REST gene or functional inhibition of the protein in non-neuronal tissues leads to erroneous expression of neuronal genes and embryonic lethality, whereas ectopic expression of REST in the nervous system inhibits expression of neuronal genes, and results in developmental dysfunction[61–63]. Therefore, REST is important in determining whether a cell has a neuronal phenotype.

REST-dependent gene silencing requires the action of transcriptional co-repressors, two of which have been identified as the REST-binding proteins SIN3A and the REST co-repressor (CoREST)[64–66]. The cellular expression pattern of SIN3A is almost identical to that of REST, which indicates that most REST-dependent gene repression might be co-mediated by SIN3A[67]. The expression of CoREST is more restricted, which indicates that it might be important in mediating specific gene expression patterns in subtypes of cell.

REST-mediated gene silencing requires the modulation of chromatin structure. REST/SIN3A repressor complexes are associated with HDAC1, whereas REST/CoREST complexes are associated with HDAC2 (REFS 65,66,68,69). So, REST-dependent gene silencing with either co-repressor seems to involve decreases in histone acetylation. CoREST has also been shown to associate with members of the switch–sucrose non-fermenting (SWI–SNF) complex, which is an ATP-dependent chromatin remodelling complex[70]. Interestingly, REST/CoREST-dependent chromatin remodelling, including decreases in histone acetylation and increases in DNA methylation, does not seem to be restricted to the immediate region around an NRSE silencer sequence; rather, the formation of heterochromatin extends across several genes that flank an NRSE[71]. These observations indicate that REST-dependent gene silencing, and therefore cellular differentiation, involves the action of several proteins, which, through decreases in histone acetylation and/or increases in DNA methylation, ultimately mark DNA epigenetically for repression.

Circadian rhythmicity. The physiology of most organisms is modulated throughout the day. These daily rhythms persist in the absence of external environmental cues, have a period of approximately 24 hours and are commonly referred to as circadian rhythms. Circadian rhythms are generated endogenously by a biological timekeeping mechanism known as the circadian clock, which comprises intricate feedback loops of transcription and translation[72]. In addition, the

mechanisms that are responsible for entrainment of the circadian clock to the environment rely on signalling mechanisms that induce changes in transcription. In mammals, the master circadian clock resides in the suprachiasmatic nucleus (SCN), which is situated in the anterior hypothalamus[73,74]. Many peripheral tissues have also been shown to have endogenous circadian clocks[74,75].

The heart of any circadian clock lies in the transcription–translation feedback loop, which could potentially be modulated by epigenetic mechanisms. So, it is possible that the genome undergoes daily changes in its epigenetic state. The acetylation of histones H3 and H4 associated with the promoters of genes that form part of the core molecular clock mechanism are differentially regulated during a circadian cycle[76]. Moreover, infusion of the HDAC inhibitor trichostatin A into the SCN increases the expression of the mouse clock genes period 1 (*Per1*) and *Per2*, which indicates that epigenetic states directly modulate the expression of the molecular components of the circadian clock[76].

Adjusting the phase of the circadian clock also requires transcription. The most salient phase-resetting environmental stimulus is light, and pulses of light induce changes in the transcription of several genes that comprise the molecular clock[72]. Epigenetic mechanisms seem to be associated with this regulation, as discrete pulses of light induce increases in acetylation of histones H3 and H4 associated with the promoters of *Per1* and *Per2* (REF. 76). Moreover, discrete light pulses induce significant increases in the phosphorylation of histone H3 in the SCN *in vivo*[77]. These observations indicate that regulation of the epigenetic state of the nucleus is a core molecular mechanism of the circadian clock that is used to generate rhythmic gene expression and to establish a stable phase relationship between gene expression, an animal's behaviour and physiology, and the environment.

Seizures. Different patterns of action potential firing can lead to differential regulation of gene expression. A particularly noteworthy example is seizures, which are widespread bursts of abnormal excitatory synaptic activity in the CNS. Seizures induce many changes in gene expression in the nervous system, which can lead to the development of chronic epilepsy and/or neurodegeneration. Early studies revealed that transcription of the immediate early gene *c-fos* was upregulated in many regions of the brain after seizures[78]. Electron microscopy studies showed that c-Fos protein is preferentially localized to the euchromatic regions of chromosomes, which indicates that part of the transcriptional response to seizures involves changes in chromatin structure[79].

Other studies have shown that expression of the glutamate receptor 2 (GluR2), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor subunit and brain-derived neurotrophic factor (BDNF) are also regulated by seizures[80–87]. Expression of GluR2 mRNA is downregulated by seizures, whereas expression of BDNF mRNA is upregulated[82,85–87]. If seizure-induced changes in gene expression are due to changes in chromatin structure, then changes in chromatin structure should occur around the genes that are known to be regulated. Two important studies have used the chromatin immunoprecipitation (ChIP) approach to monitor post-translational modifications of histones that are located in the promoters of the *BDNF* and *GluR2* genes. In the first study[88], pilocarpine-induced seizures significantly decreased acetylation of histone H4 in the GluR2 promoter, whereas acetylation of H4 in the P2 promoter of BDNF was significantly increased. In a separate study[89] that modelled a form of human antidepressant therapy, electroconvulsive seizures (ECS) also increased the acetylation of H4 at the P2 promoter of BDNF, and acetylation and phospho-acetylation of H3 were regulated within the P2 and P3 promoters of BDNF. These results indicate that ECS induces complex regulation of the epigenetic state of the BDNF promoters. ECS also had significant effects on the acetylation of H4 and phospho-acetylation of H3 in the c-Fos promoter, and on the acetylation of H3 and H4 in the promoter of cyclic-AMP response-element-binding protein (CREB)[89]. These data indicate that the synaptic activity or action potential firing that occurs during seizures results in the complex regulation of the epigenetic state of chromatin.

Memory formation. In psychological terms, memory describes the processes that are used by the brain for the long-term storage of information. Early studies implicated both transcription and translation as important for the formation of long-term memories[90–94]. Subsequent studies have shown that the formation of long-term memories is a complex process that involves many signalling pathways and the regulation of numerous genes[95–97].

A recent study has shown that the same processes that lead to the formation of long-term behavioural memories also lead to epigenetic marking of the genome[98]. Contextual fear conditioning is a hippocampus-dependent learning model by which an animal learns to associate a novel context with an aversive stimulus[99,100]. Acetylation of histone H3, but not H4, is significantly increased after an animal undergoes contextual fear conditioning[98]. The formation of long-term contextual fear memories requires NMDA (*N*-methyl-D-aspartate)-receptor-dependent synaptic transmission and the MEK–ERK/MAPK signalling cascade (where MEK referes to MAPK/ERK kinase) in the hippocampus[101–103], and inhibition of either of these processes blocks the increase in acetylation of H3 (REF. 98). These observations were the first to indicate that epigenetic tagging of the genome occurs during consolidation of long-term memories. Interestingly, a different form of long-term memory — latent inhibition — has been associated with altered acetylation of H4 but not H3 (REF. 98). This finding indicates that there might be a histone code for memory formation, whereby specific types of memory are associated with specific patterns of histone modification (FIG. 4; BOX 1).

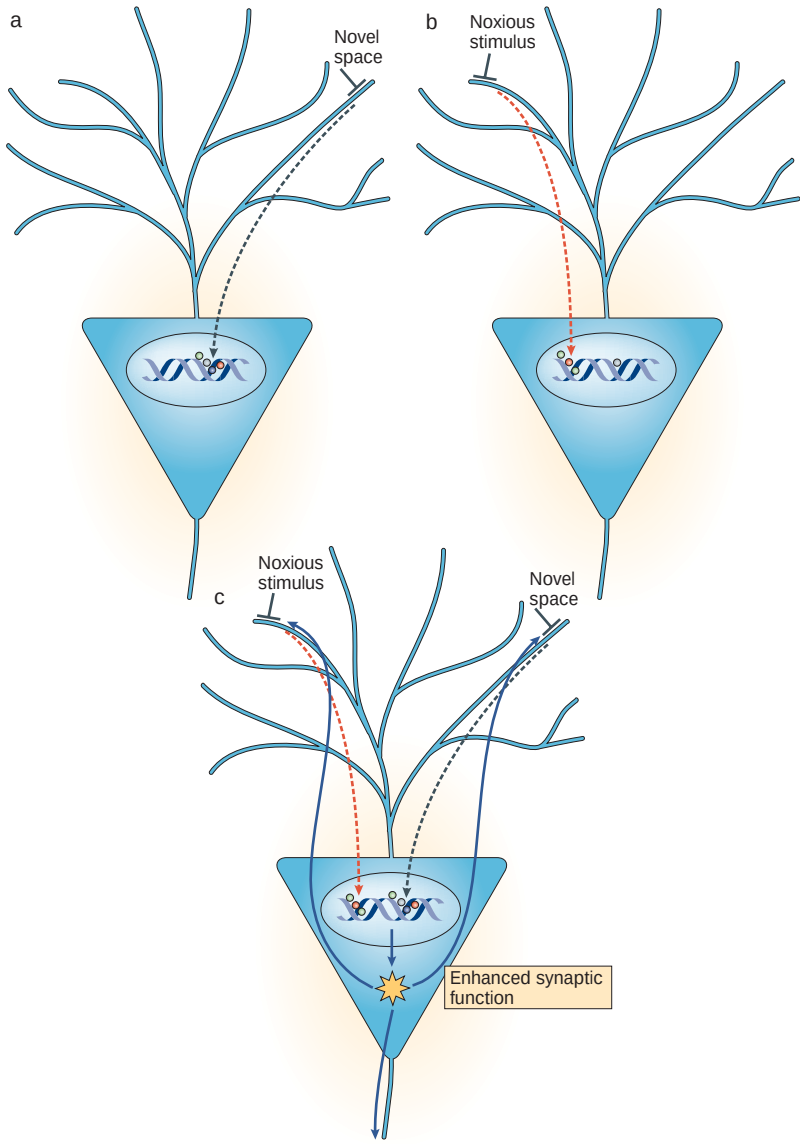

Figure 4 | **Model for epigenetics in contextual fear memory — a histone code for memory formation?** Exposure of a test subject to various environmental conditions leads to changes in the epigenetic profile of the genome in neurons that reside in relevant brain regions. In this example, we focus on pyramidal neurons in area CA1 of the hippocampus. **a** | Exposure of a subject to a novel environment leads to epigenetic changes and formation of novel spatial memories. **b** | Exposure to a noxious stimulus leads to epigenetic changes and formation of novel fear memories. **c** | Coupling the presentation of the novel environment with the noxious stimulus results in integration of the epigenetic responses, and formation of specific contextual fear memories.

As mentioned above, the addition of acetyl groups to Lys residues in histone proteins is catalysed by HATs. If acetylation of histones were important for the consolidation of long-term memories, then disruption of the activity of HATs would interfere with long-term memory formation. CREB-binding protein (CBP) is a transcriptional co-activator that has endogenous HAT activity[104]. Several studies have investigated long-term memory formation in transgenic mice with impaired CBP function. Mice that are heterozygous for a dominant-negative form of truncated CBP ($CBP_{DN}^{+/-}$)[105] have significant deficits in various forms of long-term memory, including step-through passive avoidance, novel object recognition and cued fear conditioning[105,106]. Although these studies provided the first evidence that CBP might be important in long-term memory formation, the wide-ranging developmental abnormalities that are seen in $CBP_{DN}^{+/-}$ mice make it difficult to interpret the performance of these animals in various memory tasks[105].

To further determine the role of CBP in long-term memory formation, two recent studies have generated CBP-deficient mice that lack the severe developmental problems of the $CBP_{DN}^{+/-}$ animals. The first study linked the dominant-negative allele of CBP to an inducible promoter ($CBP_{I\text{-}DN}^{+/-}$)[107]. Activation of the dominant-negative allele after animals had developed normally led to impaired learning of the spatial water maze task and novel object recognition[107]. In the second study, mice that lacked one allele of CBP ($CBP^{+/-}$) had impairments in contextual and cued fear memory, and novel object recognition[108]. In both studies, administration of an HDAC inhibitor restored normal long-term memory formation, which indicates that the balance of HAT/HDAC activity was altered in these mice and that this caused the memory deficits[107,108].

These studies indicate that histone acetylation is regulated by HAT activity and that functional disruption of this process can impair the formation of long-term memories. This might mean that any perturbation in the processes that regulate chromatin structure can affect the formation of long-term memories *in vivo*. However, can increases in histone acetylation enhance memory formation? To test this directly, two studies investigated the effect of HDAC inhibitors on the formation of long-term memories. Direct infusion of the HDAC inhibitor trichostatin A into the amygdala significantly enhanced the formation of fear-potentiated startle memory[109]. In addition, systemic administration of the HDAC inhibitor sodium butyrate enhanced the formation of contextual fear memories[98]. In both studies, HDAC inhibitors did not affect short-term memory[98,109]. In these studies, it was possible that the drugs that were used affected other cellular processes. However, taking all the data into consideration, these studies indicate that long-term behavioural memory regulates, and is regulated by, the epigenome (FIG. 4).

Synaptic plasticity. Synaptic plasticity — activity-dependent changes in synaptic strength — is widely believed to underlie the formation of long-term memories. Many studies have characterized the mechanisms that are responsible for the induction, expression and maintenance of synaptic plasticity in several organisms[110–112], and one striking observation is that these mechanisms are similar to those that are involved in the formation of long-term memories. So, induction of synaptic plasticity might involve epigenetic mechanisms like those that are involved in long-term memory.

The sensorimotor synapse of the marine mollusc *Aplysia* shows two forms of plasticity. Long-term facilitation (LTF) refers to the lasting enhancement of synaptic transmission, whereas long-term depression (LTD) is a lasting decrease in synaptic transmission.

Box 1 | Mother's day — every day of your life

Historically, mothers have not been prone to underestimate their lasting impact on their children's behaviour. A recent finding should strengthen their conviction even further[136].

Mouse mothers that show strong nurturing behaviour towards their pups, for example, by frequently licking and grooming their offspring, produce lasting alterations in the patterns of DNA methylation in the CNS of their pups, which apparently persist throughout adulthood[136]. There is evidence that these changes in DNA structure result in decreased anxiety and a strong maternal nurturing instinct in the adult offspring.

Although a detailed review of this landmark study is beyond the scope of this article, this paper is pertinent to the present discussion for several reasons. First, the study indicates that alterations in DNA methylation affect behaviour in the adult. Second, the persistence of neonatally acquired patterns of DNA methylation in the mature CNS is consistent with the hypothesis that epigenetic mechanisms contribute to lasting cellular effects — that is, cellular memory in the CNS. Finally, and perhaps most importantly, the study indicates a specific epigenetic mechanism in the CNS for perpetuating an acquired behavioural characteristic across generations — a particularly robust example of behavioural memory that is potentially subserved by epigenetics.

Acetylation of histone H4 around the promoter of the *Aplysia* CCAAT/enhancer-binding protein (C/EBP) was transiently increased during LTF, but transiently decreased during LTD[113]. Therefore, two opposing forms of plasticity induced opposing changes in histone acetylation in *Aplysia*.

Increases in histone acetylation during LTF in *Aplysia* seem to have functional consequences. Artificial elevation of basal levels of histone acetylation by the HDAC inhibitor trichostatin A transforms short-term facilitation, which does not require the transcription of new genes for its induction, into LTF[113]. So, changes in the overall state of the epigenome can modulate the induction of synaptic plasticity in invertebrates.

Plasticity-induced epigenetic changes are also observed in mammalian models of synaptic plasticity. Long-term potentiation (LTP) is a form of synaptic plasticity whereby synaptic strength is enhanced in response to high-frequency synaptic activity. Several forms of LTP require the activation of NMDA receptors and engagement of the MEK–ERK/MAPK signalling cascade[114–116]. Direct activation of NMDA receptors in the hippocampus leads to an increase in acetylation of histone H3 (REF. 98), which can be blocked by inhibition of the MEK–ERK/MAPK cascade[98]. In addition, activation of dopaminergic, cholinergic and glutamatergic signalling pathways in the hippocampus induces ERK-dependent increases in the phosphorylation of histone H3 (REF. 117). These results suggest that the induction of mammalian synaptic plasticity leads to ERK-dependent increases in histone acetylation and phosphorylation in the hippocampus — an interesting parallel to the observations in *Aplysia*.

Recent studies have directly examined whether histone-modifying enzymes and histone acetylation are necessary for mammalian synaptic plasticity. The induction of early-phase LTP and LTD — forms of plasticity that do not require transcription — was not affected in $CBP^{+/-}$ animals[108]. However, the induction of late-phase LTP, which requires transcription, was significantly impaired in $CBP^{+/-}$ animals[108]. Treatment of hippocampal slices from $CBP^{+/-}$ animals with the HDAC inhibitor suberoylanilide hydroxamic acid significantly improved late-phase LTP induction, which indicates that inhibition of HDACs had compensated for HAT haploinsufficiency[108]. In other studies using hippocampal slices, induction of LTP using high-frequency stimulation was significantly enhanced by two HDAC inhibitors, trichostatin A and sodium butyrate[98]. In addition, LTP in the amygdala that was induced by forskolin was also enhanced by the HDAC inhibitor trichostatin A[109]. These studies indicate that the epigenetic state of the genome affects the induction of long-term forms of mammalian synaptic plasticity.

Epigenetics in human cognition

There is a considerable body of evidence, albeit indirect, implicating the disruption of epigenetic mechanisms as a causal basis for human cognitive dysfunction. In this section, we briefly describe several instances in which derangements in molecular components of the epigenetic apparatus have been implicated in human cognitive disorders. In interpreting these findings in the present context, an important caveat applies. When considering these cases, it is important to distinguish between a developmental need for epigenetic mechanisms, to allow formation of a normal nervous system, versus an ongoing need for these mechanisms as part of cognitive processing *per se* in the adult. Most of the attention so far has justifiably focused on developmental roles for epigenetics in establishing the capacity for cognitive function in the adult. However, the experimental results outlined above implicate an ongoing and active role for epigenetic mechanisms in memory formation in the adult. So, we believe that it is timely and worthwhile to consider a possible component of cognitive disruption in those disorders outlined below to be due to a loss of the active use of epigenetic mechanisms that are necessary for normal cognition in the mature CNS.

Several disorders of human cognition can be attributed, at least partly, to dysfunction in the mechanisms that underlie epigenetic marking of the genome. Rubinstein–Taybi syndrome (RTS), an inherited autosomal dominant disease, is due to mutations of the transcriptional co-activator, HAT and CBP[118,119]. Several studies using animal models to investigate the molecular basis of RTS indicate that deficiency in CBP has severe consequences for long-term memory formation[105–108].

Rett syndrome (RS) is an inherited, X-linked disease that seems to be due, at least in part, to a mutation of MECP2 (REFS 120–122). Using animal models, it was discovered that overexpression of MECP2 enhanced long-term memory formation and the induction of hippocampal LTP, indicating that MECP2 modulates memory formation and the induction of synaptic plasticity[123].

Fragile X syndrome, the most commonly inherited form of mental retardation, is brought about by an abnormal expansion of repeated trinucleotide sequences within one of two Fragile X genes: *FMR1* and *FMR2* (REFS 124,125). *FMR1* and *FMR2* each contain a polymorphic trinucleotide repeat (CGG and CCG,

Table 1 | Epigenetics in human cognitive disorders

Disease	Gene	Function	Epigenetic effect	References
Rubinstein–Taybi Syndrome	*CBP*	Histone acetyltransferase	↑ histone acetylation	118,119
Rett Syndrome	*MECP2*	Binds to CpG dinucleotides and recruits HDACs	↓ histone acetylation	120–123, 137,138
Fragile X mental retardation	*FMR1* and *FMR2**	Expansion of CGG or CCG repeats results in aberrant DNA methylation around *FMR1* and *FMR2* genes	↑ DNA methylation ↑ histone acetylation	124–127
Alzheimer's disease	*APP*	*APP* intracellular domain acts as a Notch-like transcription factor; associated with the HAT TIP60	↓ histone acetylation	128–133
Schizophrenia	reelin	An extracellular matrix protein, involved in synapse development	↑ DNA methylation around the reelin gene	134,135

*Trinucleotide expansions in *FMR1* and *FMR2*. *APP*, amyloid precursor protein; *CBP*, cyclic-AMP response-element-binding protein; *FMR*, fragile X mental retardation; HAT, histone acetyltransferases; HDAC, histone deacetylase; *MECP2*, methyl CpG-binding protein 2; TIP60, HIV-1 Tat interactive protein, 60kDa.

respectively) in their 5′-untranslated regions that are responsible for the loss of gene expression[126,127]. Expansion of these repeats results in hypermethylation of these regions and flanking CpG islands, leading to transcriptional silencing of the *FMR* and surrounding genes.

The most widespread of senile dementias, Alzheimer's disease, seems to be due, in part, to an increase in soluble β-amyloid peptides in the brain[128]. These peptides are created by endo-proteolytic cleavage of the transmembrane amyloid precursor protein (APP) by β- and γ-secretases[129]. Interestingly, cleavage of APP results in the production of not only an extracellular β-amyloid fragment, but also an intracellular fragment, the APP intracellular domain (AICD). AICD regulates transcription through recruitment of the adaptor protein FE65 and the HAT TIP60 (HIV-1 Tat interactive protein, 60kDa), which indicates that some of the pathology of Alzheimer's disease is due to misregulation of histone acetylation[130–133].

Finally, schizophrenia is a serious disorder of cognition, rendering sufferers unable to function normally in social situations and in performing everyday cognitive tasks. An emerging body of evidence indicates that deficiencies in the extracellular matrix protein reelin are responsible for the aetiology of schizophrenia[134]. The promoter of reelin contains several sites for DNA methylation, and inhibitors of HDAC and DNMT activity increase expression of reelin, indicating that epigenetic mechanisms govern the expression of this protein[135]. All these observations indicate that dysfunction of the normal epigenetic status of the genome can have marked consequences on normal cognitive function (TABLE 1). These studies also indicate that drugs that target the epigenome might represent viable therapies for treating various diseases that affect cognition.

Conclusions

Chromatin is a dynamic structure that integrates potentially hundreds of signals from the cell surface and effects a coordinated and appropriate transcriptional response. It is increasingly clear that epigenetic marking of chromatin and DNA itself is an important component of the signal integration that is performed by the genome as a whole. Moreover, changes in the epigenetic state of chromatin can have lasting effects on behaviour. We propose that the CNS has co-opted mechanisms of epigenetic tagging of the genome for use in the formation of long-term memories. Moreover, many disorders of human cognition might involve dysfunctions of epigenetic tagging. In our estimation, understanding the epigenetic regulation of neural function will be vital for fully understanding the molecular processes that govern memory formation and human cognition.

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