2008

Regulation of gene expression in the nervous system

Lezanne Ooi
University of Wollongong, lezanne@uow.edu.au

Ian C. Wood
University of Leeds, i.c.wood@leeds.ac.uk

Regulation of gene expression in the nervous system

Abstract

The nervous system contains a multitude of cell types which are specified during development by cascades of transcription factors acting combinatorially. Some of these transcription factors are only active during development, whereas others continue to function in the mature nervous system to maintain appropriate gene-expression patterns in differentiated cells. Underpinning the function of the nervous system is its plasticity in response to external stimuli, and many transcription factors are involved in regulating gene expression in response to neuronal activity, allowing us to learn, remember and make complex decisions. Here we review some of the recent findings that have uncovered the molecular mechanisms that underpin the control of gene regulatory networks within the nervous system. We highlight some recent insights into the gene-regulatory circuits in the development and differentiation of cells within the nervous system and discuss some of the mechanisms by which synaptic transmission influences transcription-factor activity in the mature nervous system. Mutations in genes that are important in epigenetic regulation (by influencing DNA methylation and post-translational histone modifications) have long been associated with neuronal disorders in humans such as Rett syndrome, Huntington's disease and some forms of mental retardation, and recent work has focused on unravelling their mechanisms of action. Finally, the discovery of microRNAs has produced a paradigm shift in gene expression, and we provide some examples and discuss the contribution of microRNAs to maintaining dynamic gene regulatory networks in the brain.

Keywords

expression, regulation, gene, system, nervous, CMMB

Publication Details

Ooi, L. & Wood, I. C. (2008). Regulation of gene expression in the nervous system. Biochemical Journal, 414 (3), 327-341.

BJ www.biochemj.org

BJ Gene

Biochem. J. (2008) **414**, 327–341 (Printed in Great Britain) doi:10.1042/BJ20080963

multimedia

REVIEW ARTICLE

Regulation of gene expression in the nervous system

Lezanne OOI and Ian C. WOOD[1]

Institute of Membrane and Systems Biology, Faculty of Biological Sciences, Garstang Building, University of Leeds, Leeds LS2 9JT, U.K.

The nervous system contains a multitude of cell types which are specified during development by cascades of transcription factors acting combinatorially. Some of these transcription factors are only active during development, whereas others continue to function in the mature nervous system to maintain appropriate gene-expression patterns in differentiated cells. Underpinning the function of the nervous system is its plasticity in response to external stimuli, and many transcription factors are involved in regulating gene expression in response to neuronal activity, allowing us to learn, remember and make complex decisions. Here we review some of the recent findings that have uncovered the molecular mechanisms that underpin the control of gene regulatory networks within the nervous system. We highlight some recent insights into the gene-regulatory circuits in the development and differentiation of cells within the nervous system and discuss some of the mechanisms by which synaptic transmission influences transcription-factor activity in the mature nervous system. Mutations in genes that are important in epigenetic regulation (by influencing DNA methylation and post-translational histone modifications) have long been associated with neuronal disorders in humans such as Rett syndrome, Huntington's disease and some forms of mental retardation, and recent work has focused on unravelling their mechanisms of action. Finally, the discovery of microRNAs has produced a paradigm shift in gene expression, and we provide some examples and discuss the contribution of microRNAs to maintaining dynamic gene regulatory networks in the brain.

Key words: chromatin, gene expression, microRNA, nervous system, synaptic plasticity, transcription.

INTRODUCTION

The brain is the most complex organ in the human body, containing the largest diversity of cell types of any organ. Collectively, cells that form the nervous system express 80 % of genes in the genome [1]. However, each individual cell type expresses a distinct subset of those genes. Preservation of appropriate expression of these genes is a highly regulated process during development to ensure production of correct numbers of the different cell types and to maintain essential neuronal signalling pathways. Complexity within the brain continues into adulthood, and cells undergo phenotypic changes in response to environmental cues and neuronal signalling. Such plasticity is vital and underlies our higher cognitive functions, such as learning and memory. Development of the nervous system is brought about by waves of transcription factors, which act combinatorially to specify neural gene networks and determine cell fate. Many of these transcription factors are not expressed in the adult brain; rather, they wield their power during development, bringing about lasting gene-expression changes that extend into adulthood. Nevertheless a substantial number of transcription factors are expressed in the brain and are vital for regulating phenotypic plasticity by controlling expression of a multitude of genes. It is now well established that alterations in gene expression are important in learning and memory, and also that inappropriate regulation of gene expression is a cause of a multitude of neuronal diseases. Though many mechanisms that control gene expression in neurons have been uncovered, there is still much work to be done before we fully understand how these individual mechanisms are integrated and feed into neuronal gene networks to create a complex organ that maintains homoeostatic control of our bodies, allows us to interpret our environment and to make complex decisions. Here we review some of the recent advances that have been made in elucidating the mechanisms that regulate neuronal gene expression and highlight the insights that have contributed to our understanding of the progression of neuronal disease.

Abbreviations used: AA-NAT, acylalkalamine *N*-acetyltransferase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ATRX, α-thalassaemia/mental retardation, X-linked; BMP, bone morphogenetic protein; *Bdnf*, BDNF (brain-derived neurotrophic factor) gene; bHLH, basic helix–loop–helix; BRAF35, BRCA2-associated factor 35; *Calb1*, calbindin gene; CaMKIV, calmodulin kinase IV; Cdk1, cyclin-dependent kinase 1; CK1, casein kinase-1; CREB, cAMP-response-element-binding protein; CBP, CREB-binding protein; CCAT, calcium-channel-associated transcription regulator; CCR, C–C chemokine receptor; ChIP, chromatin immunoprecipitation, CREM, cAMP-responsive-element modulator; DNMT, DNA methyltransferase; DRE, downstream regulatory element; DREAM, downstream- regulatory-element antagonistic modulator; DRG, dorsal root ganglion; DSCR1, Down syndrome critical region gene 1; DYRKIA, dual-specificity tyrosine-phosphorylation-regulated kinase 1A; ERK, extracellular-signal-regulated kinase; ES, embryonic stem; ESET, ERG (ets-related gene)-associated protein with SET (suppressor of variegation, enhancer of zest and trithorax) domain; FGF, fibroblast growth factor; FMRP, fragile X mental retardation protein; FOXO1, forkhead box O1; GABA, γ-aminobutyric acid; Gadd45a, growth arrest and DNA-damage-inducible 45 alpha; GluR2, glutamate receptor subunit 2; *Gria2*, glutamate receptor, ionotropic, AMPA2; GSK3, glycogen synthase kinase 3; H3K4, histone H3 Lys^4 (etc.); HAT, histone acetyltransferase; HDAC, histone deacetylase; HEK-293, human embryonic kidney-293; ICER, inducible cAMP repressor; IP_3R1, Ins(1,4,5)P_3 receptor type 1; JARID1C, jumonji, AT-rich interactive domain 1C; Lmtk1, lemur tyrosine kinase 1; LSD, lysine-specific demethylase; LTP, long-term potentiation; MBD2, methyl-CpG-binding domain protein 2; MeCP2, methyl-CpG binding protein 2; mEPSC, miniature excitatory postsynaptic current; MKP, MAPK (mitogen-activated protein kinase) phosphatase; MSK, mitogen- and stress-activated kinase; MLL, mixed-lineage leukaemia; MOR1, μ-opiod receptor; NFAT, nuclear factor of activated T-cells; NMDA, *N*-methyl-D-aspartate; KChIP, potassium channel-interacting protein; PI3K, phosphoinositide 3-kinase; PP1, protein phosphatase 1; PTBP, polypyrimidine-tract-binding protein; Ptf1a, pancreas-specific transcription factor 1a; REST, repressor element 1-silencing transcription factor; SCA, spinocerebellar ataxia; TDG, thymine-DNA glycosylase; TORC, transducer of regulated CREB activity; VMN, visceral motor neuron.

[1] To whom correspondence should be addressed (email i.c.wood@leeds.ac.uk).

DEVELOPMENT AND DIFFERENTIATION

Cells in the nervous system arise from ES (embryonic stem) cells that develop into neural stem cells that, in turn, differentiate into neurons, astrocytes or oligodendrocytes. Many groups have now identified a core regulatory network of the transcription factors Nanog/Sox2/Oct4 that is important for controlling pluripotency and self-renewal of ES cells (reviewed in [2]). Combinatorially these transcription factors regulate their own expression levels in addition to those of many other genes, and disruption of any component of the network is sufficient to disrupt the whole system. Once initiated, this circuit is self-maintaining, and differentiation of ES cells toward specific cell fates requires external signalling via secreted molecules such as BMP (bone morphogenetic protein). Nanog is critical for maintaining the ES cell state. The expression of Nanog fluctuates in ES cells over time and, when levels of Nanog are low, ES cells are predisposed to cell differentiation [3]. Thus low levels of Nanog provide a window of opportunity for ES cell differentiation that is lost when the levels of Nanog subsequently rise [3]. Sox2 expression is important for maintaining Oct4 levels. In the absence of Sox2, other Sox proteins expressed in ES cells, such as Sox4, 11 and 15, can co-operate with Oct4 to activate expression of target genes, including Oct4 itself [4]. However, loss of Sox2 results in reduced expression of the gene *Nr5a2* (nuclear receptor subfamily 5, group A, member 2), which encodes a steroid hormone receptor that activates Oct4 expression, and increased expression of *Nr2f2* (nuclear receptor subfamily 2, group F, member 2), which encodes a steroid hormone receptor that represses Oct4 expression. Thus despite the ability of Sox4, 11 and 15 to substitute for Sox2 and directly enhance Oct4 expression, loss of Sox2 leads to reduced Oct4 levels and ES cell differentiation. In ES cells that lack Sox2, it is the effect on Oct4 levels that are crucial, as highlighted by the fact that ectopic expression of Oct4 is sufficient to prevent ES-cell differentiation [4]. Oct4 appears to play a more general role than either Sox2 or Nanog. In co-operation with Sox2 and Nanog, Oct4 binds to and regulates the expression of many genes. Unlike Nanog, Oct4 levels do not appear to fluctuate, and redundancy among Oct factors has not been identified. ES cells become committed to neural cell lineages and differentiate toward specific neuronal or glial cell fates in response to a range of signals, including retinoic acid, FGF (fibroblast growth factor), inhibition of BMP signalling, and Notch and Wnt signalling [5]. Cell fate specification towards either neural-stem-cell maintenance or differentiation toward post-mitotic neurons or glia is achieved through a balance of antagonistic transcription factors [6,7].

Transcription factors play a key role in specifying neuronal identity upon neuronal differentiation, and much work has been undertaken in an attempt to understand the transcription-factor network that defines specific neuronal lineages. Combinations of transcription factors result in different, but specific, cell fates. The co-ordinated function of homoeodomain and bHLH (basic helix–loop–helix) transcription factors, including Mash1, neurogenin and Math1, are involved in differentiation of neural progenitors into neurons and specification of neuronal subtype [6]. Most neurons differentiate toward either a glutamatergic (excitatory neuron) or GABAergic (inhibitory neuron; GABA is γ-aminobutyric acid) phenotype, and the actions of a range of transcription factors have been implicated in implementing this decision in different regions of the nervous system. Expression of the bHLH gene Mash1 promotes generation of GABAergic neurons from neural stem cells of the subependymal zone [8], whereas the bHLH transcription factor Ptf1a (pancreas-specific transcription factor 1a) [as a heterodimer with RBJ (rab- and DnaJ-domain containing) protein] defines GABAergic neurons in the cerebellum [9]. In the absence of Ptf1a, only glutamatergic neurons are formed in the cerebellum, whereas ectopic expression of Ptf1a in glutamatergic precursors is sufficient to switch neurons to the GABAergic phenotype [10]. The homoeobox containing transcription factors Tlx1 and Tlx3 promote specification of glutamatergic neurons, inhibit GABAergic differentiation in spinal-cord neurons [11] and antagonize the functions of Lbx1, which promotes GABAergic differentiation [12]. Lbx1 is expressed in glutamatergic neurons, but its actions to promote GABAergic differentiation are inhibited by the expression of Tlx1 and Tlx3. Mice lacking Tlx3 show increased GABAergic differentiation, which is due to the presence of Lbx1, but interestingly, normal glutamatergic differentiation is restored in mice that lack both Tlx3 and Lbx1 [12].

Transcription factors operate combinatorially and can promote different cell fates as a result of interactions with other transcription factors in specific cells. Neurogenin2 expression in the forebrain promotes the generation of glutamatergic neurons [13] and in the spinal cord in association with Olig2, promotes motor-neuron differentiation [14]. Combinations of Pax6, Olig2 and Nkx2.2 and their inhibitors, Id and Hes, define both neuronal, and then glial, differentiation [7], while Dlx1 and Dlx2 promote neurogenesis by inhibiting Olig2 in mouse forebrain progenitors [15]. Transcription factors such as Pax6, Olig2 and Nkx2.2 can also act combinatorially in specifying cell fate towards motor neurons, oligodendrocytes and dopaminergic neurons [16–18]. Nkx2.2 promotes differentiation toward serotonergic neurons [19], whereas Olig2 promotes a motor-neuron fate [20]. In fact Nkx2.2 and Olig2 function antagonistically, and both transcription factors repress each other's expression during the differentiation process [16]. Though many such mutually antagonistic relationships between individual transcription factors are known, the complete identification of all target genes for a particular factor during development has not been carried out. Such information, though technically challenging to obtain, would provide the first steps towards really understanding the transcription-factor networks that specify the multitude of neuronal cell types.

The timing of transcription-factor activity is important during the generation of many cell types; neuronal subtypes are often produced sequentially from the same pool of multipotent progenitors. One such example is found in the hindbrain, where VMNs (visceral motor neurons) and serotonergic neurons are generated sequentially from the same set of progenitor cells [21]. This mechanism requires the actions of two transcription factors, Phox2b and Mash1, that are required for VMN and serotonergic differentiation respectively. Foxa2 is also required for serotonergic specification and, as with Nkx2.2 and Olig2, Foxa2 and Phox2b mutually repress each other's expression [21]. The activity of Phox2b dominates that of Foxa2, meaning that initially VMNs are produced and a switch to serotonergic differentiation is initiated only by increased expression of Foxa2 [21] through an unidentified mechanism. The increased levels of Foxa2 repress expression of Phox2b and activate serotonergic differentiation. Foxa2 therefore acts as a key molecular switch and in its absence, serotonergic neurons are not produced.

In addition to roles in guiding neuronal progenitors toward specific neuronal fates, other transcription factors are important for regulating more general aspects of neuronal phenotype. One such transcription factor is the REST [repressor element 1-silencing transcription factor, also known as NRSF (neural restrictive silencer factor)]. Reduced expression of the transcriptional repressor REST is an important step in neuronal differentiation. REST is expressed in ES cells [22–24], and down-regulation of REST is required prior to neuronal differentiation

[22,24,25]. Removal of mitogens and addition of retinoic acid to cultures of ES cells results in loss of REST expression both by reduced mRNA levels and enhanced targeting of REST protein for degradation and a concomitant differentiation of cells into neurons [22,24,26]. REST can recruit multiple chromatin-modifying enzymes via interactions with at least two independent co-repressor complexes containing mSin3 and CoREST [27–29] (for a recent review on chromatin and REST, see [30]), which are utilized to repress its predicted 1800 target genes [31,32]. Many of these genes are normally expressed in differentiated neurons and are important for neuronal functions such as neurotransmitter release [24,33–35] and axon guidance [36].

The exact role of REST repression in defining neuronal gene expression is still not entirely clear, though it is able to contribute to the deposition of epigenetic marks in neuronal genes, and these effects persist even after REST expression is lost. Indeed, loss of REST from promoters of some genes during neuronal differentiation does not lead to their immediate de-repression and the CoREST–MeCP2 (methyl-CpG binding protein 2) complex recruited by REST may remain bound to the promoter [22]. In this way repression of *Calb1* (calbindin) and *Bdnf* [BDNF (brain-derived neurotrophic factor)] genes is maintained until released by other events, such as membrane depolarization, which results in MeCP2 phosphorylation and/or DNA demethylation, loss of MeCP2 binding and gene activation [22].

Thus, during development and differentiation, combinatorial actions of transcription factors regulate specification of neural-cell type as well as the acquisition of general features associated with the neuronal phenotype. Appropriate control of gene regulation remains important in the mature nervous system, the difference being that the outcomes of gene regulation switch from specification and differentiation to regulating gene expression in response to neuronal activity.

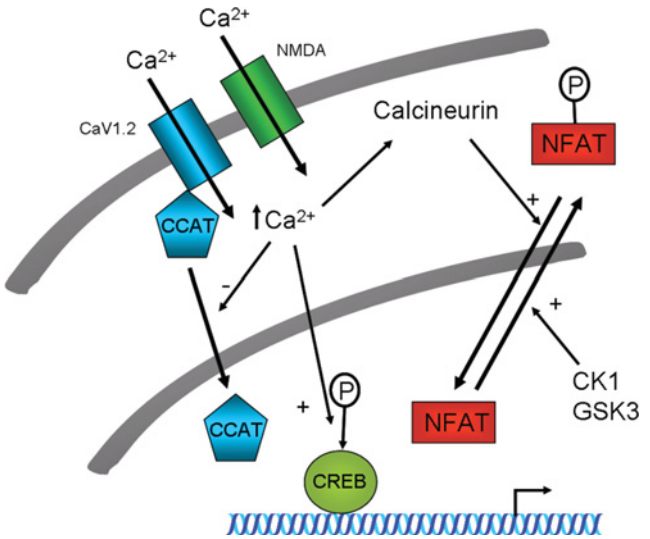

Figure 1 Neuronal activity and calcium influx regulate the function of several transcription factors in neurons

Depolarization of neurons results in the entry of Ca^{2+} through voltage-gated calcium channels such as Cav1.2, whereas glutamate stimulates NMDA receptor activation and Ca^{2+} influx through NMDA receptors of depolarized neurons. Increased intracellular calcium has many effects and underlies many neuronal responses to synaptic activity. Of particular relevance to the present review are: (i) the phosphorylation of CREB, which results in recruitment of CBP and activation of CREB responsive genes; (ii) the activation of the protein phosphatase calcineurin, which dephosphorylates NFAT, allowing NFAT to enter the nucleus, bind to DNA and regulate transcription; (iii) the inhibition of the cleavage of Cav1.2, which prevents the cleaved C-terminal region CCAT from moving to the nucleus to regulate transcription. An animated version of this Figure can be found at http://www.BiochemJ.org/bj/414/0327/bj4140327add.htm.

GENE EXPRESSION CHANGES IN RESPONSE TO NEURONAL ACTIVITY

Neuronal activity results in the influx of calcium and a rise in intracellular calcium levels in neurons. Influx of calcium and changes in the intracellular calcium levels influence the function of several transcription factors (Figure 1).

CREB (cAMP response element binding protein)

CREB is a key modulator in regulating gene expression programs in response to neuronal activity and is pivotal in mediating long-term memory and synaptic plasticity [37]. Neuronal activity and calcium entry through synaptic NMDA (*N*-methyl-D-aspartate) receptors results in phosphorylation of CREB at Ser^{133} and recruitment of the transcriptional co-activator CBP (CREB-binding protein) [38]. Transcriptional activation is also regulated by phosphorylation of CBP by CaMKIV (calmodulin kinase IV), and CBP activates transcription via its intrinsic HAT (histone acetyltransferase) activity [39]. Another co-activator of CREB is TORC (transducer of regulated CREB activity). There are three members of the TORC family encoded by individual genes, one of which (TORC1) is expressed in neurons [40]. TORC1 can interact with CREB independently of the phosphorylation status of Ser^{133} and potentiates CREB-mediated transcriptional activation and is required for LTP (long-term potentiation) in hippocampal neurons [40] (LTP is the process whereby communication between two neurons is strengthened as a result of both neurons being active at the same time. The effects are long-lasting and are mediated by increased neurotransmitter signalling between the two neurons. LTP is thought to be a good candidate for the molecular mechanism that underlies memory formation). The requirement for TORC1 in LTP provides another layer of CREB regulation, because TORC1 is normally located in the cytoplasm and translocates to the nucleus in response to phosphorylation by an unidentified kinase. Both calcium entry (through NMDA receptors or voltage-gated calcium channels) and stimulation of cAMP is required for phosphorylation of TORC1 and transcriptional activation by CREB [41]. Thus TORC1 acts as a coincidence detector for activity, neither calcium entry nor increased cAMP levels alone being sufficient to stimulate activity.

One of the most well-studied targets of CREB transcriptional activation is BDNF, whose identification provided the first insights into the mechanisms by which CREB activation could modulate synaptic activity and neuronal survival [42]. The subsequent availability of complete genome sequences has allowed a more holistic approach in trying to predict the response to CREB activity. Although a genome-wide analysis to identify CREB target genes in neurons has not yet been performed, data from several other cell types can shed light on the gene targets and mechanisms of action of CREB. Using a genome-wide ChIP–SACO (chromatin immunoprecipitation–serial analysis of chromatin occupancy) technique, Impey et al. [43] identified 6302 sites that were bound by CREB in the rat pheochromocytoma cell line PC12. In a separate study, Zhang et al. [44] used a ChIP microarray analysis in HEK-293 (human embryonic kidney-293) cells and predicted that CREB is bound at approx. 4000 sequences, on the basis of their observations of 2811 bound promoters from a selection of 16000 genes. Binding of CREB to genes does not appear to be regulated by its phosphorylation status. It would appear that at most promoters CREB is phosphorylated

in response to increased cAMP; however, CBP is recruited, and transcription is activated, at only a subset of those genes [44]. Given the wide-ranging roles of CREB outside as well as within the nervous system, it is not surprising that it has the potential to regulate such a large number of genes. The evidence would suggest that it is the recruitment of CBP that dictates which CREB-regulated genes are responsive in any particular cell type. Although CBP recruitment is known to require phosphorylation of Ser^{133} in CREB there must be additional mechanisms (perhaps further cofactor interactions) that are also required for CBP recruitment *in vivo*, as increased cAMP can increase Ser^{133} phosphorylation but not CBP recruitment to all CREB-bound genes [44].

CCAT (calcium-channel-associated transcription regulator)

As previously highlighted, influx of calcium is pivotal for activity-dependent changes in neuronal gene expression. The L-type voltage-gated calcium channel Cav1.2 contributes to this mechanism by its ability to allow calcium into the cell but, in addition, a region of its C-terminus can also be cleaved to create a transcription factor (Figure 1). CCAT is a 75 kDa protein which is the product of cleavage of the C-terminus of the Cav1.2 channel [45]. The cleavage has been characterized in a subset of inhibitory (GABAergic, GAD65-positive) neurons in the rat cortex and the peptide produced translocates to the nucleus. Within the nucleus, CCAT binds to the nuclear protein p54(nrb)/NonO, associates with gene promoters and activates the expression of some target genes such as *Gjb5* (connexin 31.1) and *Ntn4* (netrin4), while it repress the expression of other genes such as *Trpv4* (transient receptor potential vanilloid-4) and *Kcnn3* (potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3). In fact many of the genes regulated by CCAT encode proteins that play a role in neuronal excitability, thereby providing another mechanism by which neuronal depolarization results in a remodelling event that impacts upon future activity [45]. Calcium entry through Cav1.2 or NMDA, but not AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, was shown to promote relocalization of CCAT from the nucleus to the cytoplasm. Such relocalization of CCAT is inhibitory, as it prevents its ability to influence transcription. The *in vivo* function of CCAT is not clear, though there is some evidence to support a role for regulation of the neuronal cytoskeleton, suggesting it may play a role in modulating neuronal connectivity. When expressed in HEK-293 cells [46] and in Purkinje neurons [47], the P/Q voltage-gated calcium channel Cav2.1 is also cleaved and produces a 74 kDa proteolytic fragment constitutively, which accumulates in the nucleus. The truncated fragment contains the polymorphic CAG repeat region, expansion of which is responsible for SCA6 (spinocerebellar ataxia type 6), and fragments containing expanded CAG repeats are toxic and result in cell death. Though there is no evidence that toxicity is mediated via changes in gene expression, such a mechanism would be consistent with proposed mechanisms of toxicity of CAG expansions in other types of SCAs such as SCA1 [48,49] and neurodegenerative diseases such as Huntington's disease [50]. Cleavage of another L-type calcium channel, Cav1.3, is stimulated by calcium entry through NMDA receptors and appears to produce a channel with increased flow of calcium [51]. Whether the proteolytic fragment may also function as a transcription factor, or whether its loss is only required for altering Cav1.3 properties, has not been studied. Other examples of membrane proteins that are cleaved to produce transcription factors include PKD1 (polycystic kidney disease 1) [52] and the APP (β-amyloid precursor protein) [53].

NFAT (nuclear factor of activated T-cells)

NFAT proteins were originally identified as transcription factors that promote expression of target genes in response to activation of immune cells [54]. However, in actuality they are expressed in many cell types, including neurons. NFATc proteins shuttle to and from the nucleus as a consequence of their phosphorylation status, which is altered in response to changes in intracellular calcium levels. Phosphorylated NFATc is retained in the cytoplasm and is inactive. Increases in intracellular calcium activate the calcium binding phosphatase calcineurin, which dephosphorylates and activates NFATc, causing it to translocate to the nucleus, whereupon it up-regulates target gene expression (Figure 1). However, activation of NFATc can also repress expression of target genes in other systems. For example, in mouse cardiomyocytes, activated NFAT results in reduced expression of the potassium channel gene *Kcnd2* that encodes Kv4.2 [55]. However, whether this is a direct effect of NFATc is not clear. Nuclear NFAT is inactivated by phosphorylation by the kinases CK1 (casein kinase 1) [56] and GSK3 (glycogen synthase kinase 3) [57]. There are four members of the family, NFATc1–NFATc4, which have functions in many regions of the brain. NFATc is activated in hippocampal neurons in response to neuronal signalling [57], in superior cervical ganglion neurons by repetitive action potentials [58], in spinal neurons by substance P or neurotrophins [59,60] and in developing cochlear neurons by de-afferentation [61] or cocaine [62]. Just as the stimuli for NFAT activation are varied, so are the functional outcomes, which include neuronal survival [60], neuronal death [61] and pain-sensitivity [63].

In mouse striatal neurons, stimulation of dopamine D1 receptors activates NFATc4 via calcium entry through L-type calcium channels [62]. Two potential targets for activated NFATc4 are IP_3R1 [Ins(1,4,5)P_3 receptor type 1] and GluR2 (glutamate receptor subunit 2), both of which can be activated by nuclear NFATc4 and show increased mRNA levels after dopamine-receptor activation [62]. The dopamine receptor signalling pathway is important in the development of addiction, particularly the addiction to some drugs of abuse, such as cocaine [64]. Exposure of mice to repeated, but not a single, injection of cocaine over a 5-day period resulted in enhanced nuclear localization of NFATc4 in striatal neurons and an approx. 2-fold increased expression of IP_3R1 and GluR2 mRNA [62]. Increased expression of these genes should result in enhanced synaptic transmission, and thus NFATc4 activation may underlie some of the gene-expression changes that play a role in the remodelling of neuronal transmission mediated by repeated exposure to addictive drugs. Interestingly, although increased IP_3R1 expression is mediated via NFAT activation in striatal neurons [62] and hippocampal neurons [60], in spinal neurons increased IP_3IR expression occurs independently of NFAT activation [63].

NFATc1–NFATc4 genes are all expressed in rat DRG (dorsal root ganglion) neurons, and NFAT activation induced the expression of the CCR2 and CCR5 (C–C chemokine receptors type 2 and 5) in DRG neurons in response to depolarising stimuli [65]. Increased expression of CCR2 in neurons has been linked to the development of allodynia and neuropathic pain [66] (allodynia is a painful response to a normally non-painful stimulus. It is the result of the increased sensitivity and excitability of sensory neurons. Neuropathic pain is a chronic pain resulting from some problem within the neuron. Unlike nociceptive pain, in which neurons send pain signals to the brain in response to tissue damage, in neuropathic pain the signal is generated within the neurons themselves). The stimulation of CCR2 expression by NFAT activation is likely to be direct, as the CCR2 promoter region contains an evolutionary conserved NFAT-binding site

which is required for activation by NFAT [65]. Increased Cox2 (cyclo-oxygenase 2) expression is also associated with pain and in spinal neurons increased *Cox2* mRNA levels are dependent on NFATc activation [63].

Down's syndrome is the most common cause of mental retardation and results in major developmental defects that affect the face, heart and gastrointestinal tract, among others. It results from a trisomy of chromosome 21, and the perturbation of NFAT signalling makes a significant contribution to the phenotype. Increased expression of the genes encoded within a critical region of chromosome 21, covering about 3 Mb, can replicate all of the facial features of Down's syndrome and two of the genes within this region negatively regulate NFATc activity. Expression of *DSCR1* (Down syndrome critical region gene 1) is higher in foetuses of Down-syndrome patients and interacts directly with calcineurin, inhibiting its function and reducing activation of NFATc [67]. A second gene, *DYRK1A*, encodes dual-specificity tyrosine phosphorylation-regulated kinase 1A, a serine/threonine kinase that phosphorylates NFATc in a regulatory region, priming NFATc for phosphorylation by CK1 and GSK3, leading to nuclear export and inactivation [68]. Thus increased expression of both *DSCR1* and *DYRK1A* inhibits NFATc activation and leads to decreased levels of nuclear NFATc [69]. Furthermore, mice lacking NFATc2 or NFATc4 show skull and jawbone abnormalities similar to those seen in the Down syndrome phenotype, suggesting that inhibition of NFAT signalling by increased DSCR1 and DYKIA makes a functional contribution to the Down's-syndrome phenotype [69]. Clearly these data provide compelling evidence that NFAT signalling plays a central role in the development of the nervous system and regulates many of its pathways. The challenge now is to identify those genes that are regulated by NFATc signalling in the different neuronal subtypes and understand how NFATc can have such disparate and apparently opposing functions that are dependent on cell type as well as developmental stage [66]. Whether the individual NFATc isoforms have specific functions is not known. However, the fact that loss of individual NFATc genes does not result in any major abnormalities and that different NFATc members can recognize the same DNA sequence suggests that members of the NFATc family have overlapping functions [70].

KChIP/DREAM (potassium channel interacting protein/downstream regulatory element antagonistic modulator)

Calcium influx has extensive effects on gene expression programmes in neurons, and the modulation is mediated by several independent mechanisms. Perhaps the most direct effect of calcium on regulation occurs via the transcriptional repressor DREAM (also known as KCHIP2). DREAM is a calcium-binding protein containing four EF-hands which bind calcium and interact with DNA via an undetermined region that binds to a specific sequence known as a DRE (downstream regulatory element) [71]. DREAM is abundantly expressed throughout the nervous system in humans and mice [71,72] as well as in non-neuronal tissues [71,73]. It binds to DNA as a tetramer and represses transcription in a calcium- and cAMP-dependent manner. In the absence of calcium, DREAM binds to the DRE and inhibits gene expression, while increases in calcium permit calcium binding by DREAM, resulting in the dissociation of the repressor from the DNA and a consequent derepression of target genes [71]. DREAM is regulated by alternative splicing, phosphorylation and degradation via caspase signalling [72]. Phosphorylation via a component of the PI3K (phosphoinositide 3-kinase) pathway is required for DNA binding of DREAM in haematopoietic progenitor cells,

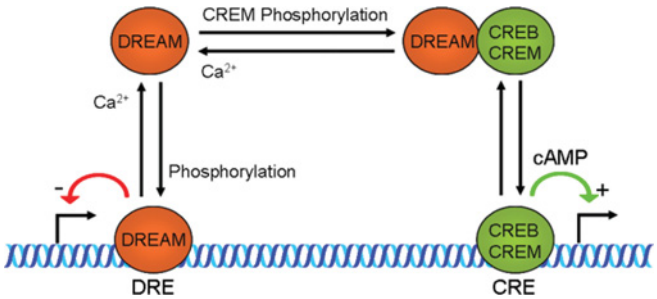

Figure 2 Interactions between DREAM and CREM/CREB integrate Ca^{2+} and cAMP signalling

In the absence of calcium, DREAM binds to a DRE sequence found downstream of target genes and represses expression. In haematopoetic cells, binding of DREAM is enhanced by PI3K-dependent phosphorylation, although whether such a mechanism is important in the nervous system is not known. DREAM contains four EF-hands, which bind Ca^{2+} and in response to increased calcium, DREAM dissociates from DNA, resulting in de-repression of gene expression. DREAM also interacts with the cAMP-responsive transcription factors CREB and CREM. These interactions occur in the absence of Ca^{2+} and inhibit CREB and CREM interactions with DNA, preventing cAMP-mediated transcriptional activation. Increases in Ca^{2+} promote dissociation of the DREAM–CREM/CREB complex, whereas phosphorylation of CREM promotes its interaction with DREAM and inhibits its binding to DNA.

although the candidate kinase responsible has yet to be identified [73]. In glia, cellular localization of DREAM is modulated via serum-response pathways. In the absence of serum, it is found exclusively in the nucleus, where it represses expression of the c-*fos* gene [74]. Upon glutamate or serum stimulation, DREAM is relocalized to the cytoplasm and c-*fos* expression is de-repressed, in turn leading to activation of a panel of c-*fos* target genes [74]. Unlike in neurons, where c-*fos* expression is stimulated in response to glutamate via CREB, ERK (extracellular-signal-related kinase) and/or CaMKIV pathways, in glia it appears that this inhibition of DREAM is the important pathway for c-*fos* induction. Whether increases in calcium concentration alone or a post-translational modification, such as phosphorylation, are important in the inhibition and relocalization of DREAM in glia has not been determined. Expression of DREAM also impacts upon cAMP signalling. In the absence of calcium, DREAM interacts with CREB and CREM (cAMP-responsive-element modulator), preventing them from binding cAMP response elements [75] (Figure 2). The interaction between DREAM and CREB occurs within the kinase-inducible domain of CREB and prevents the interaction between CREB and the co-activator CBP, thus preventing cAMP-mediated transcriptional activation [75]. The ability of DREAM to bind DNA is also inhibited by its interaction with CREM, and phosphorylation of CREM in response to cAMP signalling increases this interaction with DREAM and potentiates de-repression of DREAM target genes [76].

DREAM is a member of a family of four calcium binding proteins (KCHIP1– KCHIP4) that were initially characterized by their interactions with voltage-gated potassium channels and their ability to enhance cell-surface expression of potassium channels and increase potassium-channel activity in a calcium-dependent manner. Each of the KCHIPs can bind to a DRE sequence *in vitro* [77], and oscillation of DRE-binding activity in the pineal gland (whose function is important in regulating a circadian rhythm and sleep/wake cycle) is thought to be responsible for oscillatory expression of three genes: *fra2* (Fos-related antigen), *ICER* (inducible cAMP repressor) and *AA-NAT* (acylalkalamine *N*-acetyltransferase) [77]. What regulates the rhythm of DREAM is unknown, although the fact that mRNA and protein levels remain constant while the levels of DRE binding to DNA fluctuate simultaneously, suggests that a post-translational mechanism may

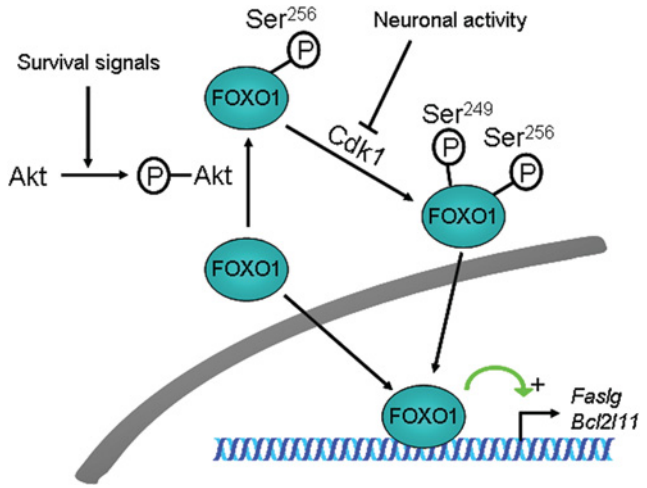

Figure 3 Regulation of FOXO1 activity

FOXO1 is phosphorylated at Ser^{256} by phospho-Akt in response to activation of the PI3K pathway. Phosphorylation of Ser^{256} creates a binding site for 14-3-3 proteins, which sequester FOXO1 in the cytoplasm, preventing it from functioning as a transcription factor. FOXO1 is also constitutively phosphorylated by Cdk1 on Ser^{249}. Phosphorylation of Ser^{249} inhibits 14-3-3 binding, thus allowing FOXO1 to enter the nucleus and activate expression of genes such as those coding for Fas ligand (*Faslg*) and Bim (*Bcl2l11*), proteins which promote apoptosis. Neuronal activity inhibits Cdk1 activity and promotes cytoplasmic sequestration of FOXO1 and neuronal survival. An animated version of this Figure can be found at http://www.BiochemJ.org/bj/414/0327/bj4140327add.htm.

be responsible. The diverse functions of DREAM as both a potassium-channel modulator and a transcription factor do not appear to have any co-regulatory connections, and so far the data are consistent with separate nuclear and cytoplasmic roles for DREAM. Whereas the binding of calcium promotes the loss of DREAM from DNA, it is not known whether it leaves the nucleus. Furthermore, interactions between DREAM and potassium channels seem to be constitutive, suggesting that the cytoplasmic pool of DREAM would not have the opportunity to translocate to the nucleus. Whether DREAM really does have two distinct functions and the mechanisms by which DREAM is partitioned between the nucleus and cytoplasm within a given cell remain undetermined. However, increases in the expression of the A-type (Kv1 family) potassium channels could potentially increase the cytoplasmic pools of DREAM and result in decreased nuclear DREAM with the consequence of a de-repression of DREAM target gene expression. Conversely, diminished A-type potassium channel expression (either in response to normal signals or in knockout mouse models) would have the opposite effect and could potentially decrease the expression of DREAM target genes.

GENE EXPRESSION CHANGES IN RESPONSE TO NEURONAL INACTIVITY

Excessive neuronal activity results in glutamate excitotoxicity and cell death. However, some neuronal activity and NMDA receptor activation is important for neuronal survival (reviewed in [78]). Recently the transcription factor FOXO1 (forkhead box O1) has been implicated in promoting cell death in the absence of neuronal activity [79]. Deprivation of neuronal activity activates the protein kinase Cdk1 (cyclin-dependent kinase 1) which phosphorylates FOXO1 at Ser^{249} (Figure 3) [79]. Phosphorylation at this residue prevents the interaction of FOXO1 with 14-3-3 proteins (which otherwise sequester it in the cytoplasm), leading to the nuclear accumulation of FOXO1. In the nucleus, FOXO1 acts as a transcriptional activator and increases expression of pro-apoptotic target genes such as *Fslg* (Fas ligand) and *Bcl2l11* (Bim), which promote neuronal cell death [79]. FOXO1 is also regulated through the PI3K pathway and is phosphorylated by activated Akt (protein kinase B) at Thr^{24}, Ser^{256} and Ser^{319} [80]. Phosphorylation at these sites promotes interaction of FOXO1 with 14-3-3 proteins and is required for sequestration of FOXO1 in the cytoplasm. Thus PI3K signalling inhibits FOXO1 activity, and this is one of many mechanisms by which PI3K activity promotes neuronal cell survival, some others being the phosphorylation and inactivation of BAD (Bcl-2 antagonist of cell death) [81] and the activation of nuclear factor κB [82].

Epigenetic regulation

Regulation of gene expression via epigenetic mechanisms is important during normal development, providing a potential mechanism for cellular memory and the inheritance of gene-expression-pattern information during mitosis. DNA methylation is the prototypical epigenetic marker and is required for repressing gene transcription, X-inactivation, genomic imprinting and maintaining chromosome stability. Several neurological disorders arise in response to mutations in the proteins that are involved in methylating DNA or that are recruited to methylated DNA, suggesting that appropriate DNA methylation within the nervous system is vital to stave off disease. It has been a long-held belief that DNA methylation patterns, once set during development, are maintained throughout the life of the organism and provide a permanent storage mechanism for specific cell lineages. More recent data, however, has provided evidence that suggests DNA methylation may be dynamic and that removal of methylation marks could play an important role in plasticity and normal functioning of the nervous system. Methylated CpG dinucleotides contribute to gene repression by inhibiting the binding of specific transcription factors [83] or recruit proteins that contain methyl-CpG-binding domains and act as transcriptional repressors [84].

Fragile X syndrome, the most common form of inherited mental retardation, affecting 1 in 1000 individuals, is the result of DNA methylation of an expansion of CGG sequence within the 5′ untranslated region of *FMR1* [FMRP (fragile X mental retardation protein) gene]. FMR1 is an RNA-binding protein that plays a role in regulating translation. FMRP associates with polyribosomes and inhibits translation of some mRNAs in neurons [85,86]. DNA methylation of the *FMR1* gene silences its expression, leading to increased translation in areas of the cerebral cortex [87,88]. Mutations in MeCP2, which binds to methylated CpG dinucleotides and represses transcription [89], cause Rett syndrome [90]. This progressive neurodevelopmental disorder that is linked with mental retardation, almost exclusively affects females. In normal development, MeCP2 expression increases as neurons differentiate prior to synaptogenesis [91], whereupon it translocates to the nucleus [92]. Once in the nucleus, MeCP2 represses transcription [93], either by recruiting HDAC (histone deacetylase) activity (via the co-repressor mSin3) [94,95], histone H3 lysine 9 methylase activity [96], interacting directly with chromatin [97] or remodelling chromatin, possibly via its interaction with the Brahma chromatin-remodelling complex [98], resulting in a more compact chromatin structure [99]. Three specific mutations within the MeCP2 gene, which lead to mental retardation, have been shown to disrupt the binding of MeCP2 to ATRX (α-thalassaemia/mental retardation, X-linked) rather than affecting its binding to DNA directly [100]. The precise function of ATRX *in vivo* is unclear, although it can interact with a number of chromatin-binding proteins and, *in vitro*, possesses

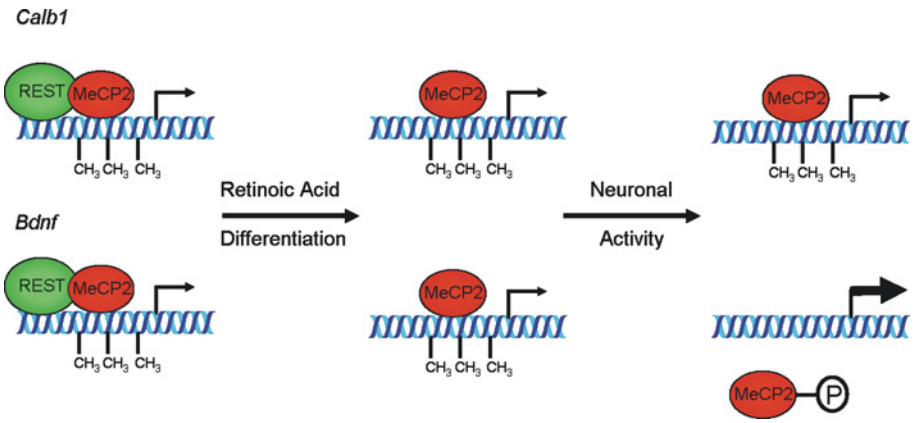

Figure 4 Control of MeCP2 gene regulation

In neuronal progenitor cells *Calb1* and *Bdnf* promoters are repressed by the transcriptional repressor REST. Neuronal differentiation results in the loss of REST expression, but the co-repressors CoREST (not shown) and MeCP2 remain bound at the promoters containing methylated DNA and repression of *Calb1* and *Bdnf* is maintained. Neuronal activity stimulates the expression of *Bdnf* as a result of loss of MeCP2, owing to its phosphorylation, which results in cytoplasmic sequestration of MeCP2 and/or demethylation of DNA, which in turn removes the MeCP2 binding site. It is not clear why neuronal activity can result in loss of MeCP2 from the *Bdnf* promoter, but not the *Calb1* promoter; presumably other, as yet undetermined, factors are also required.

chromatin-remodelling activity [101]. Mutations in the gene also lead to mental retardation, suggesting that there may be a commonality in the mechanism that leads to these neurological defects. Although it is widely expressed throughout the body, deletion of MeCP2 in the brain [102] or specifically in neurons [103] results in the neurological symptoms that mimic Rett syndrome. Encouragingly this neurological disorder can be rescued by artificially induced expression of MeCP2, even in mature animals [104]. However the level of MeCP2 expression is likely to be critical, because just as too little MeCP2 is deleterious, so is too much. Duplication of the MeCP2 gene in human males is associated with progressive neurodevelopmental disorders and mental retardation [105], whereas in mice, a 2-fold increase in expression results in neurological abnormalities [106].

Identifying the specific genes that are affected by MeCP2 abnormalities should provide some understanding of the development of neurological disorders. A genome-wide screen for gene expression changes in the brain due to the loss of MeCP2 resulted in only very modest differences being noted [107]. Several MeCP2 target genes have been uncovered, the best studied of which is BDNF [108,109]. MeCP2 has been shown to be recruited to promoter III of the mouse *Bdnf* gene, where it represses expression of BDNF. Upon membrane depolarization, MeCP2 is phosphorylated by a CaMKII-dependent mechanism and is released from the *Bdnf* promoter, resulting in de-repression of BDNF [22,108,110]. Such findings would suggest that patients with Rett syndrome are likely to have increased expression of BDNF. However, mice lacking MeCP2 show decreased levels of BDNF and overexpression of *Bdnf* in these mice increased locomotor activity and lifespan [111]. As BDNF levels are known to be stimulated by neuronal activity, this apparent discrepancy in BDNF levels in MeCP2-null mice may be a secondary effect due to altered neuronal activity in MeCP2-null mice, perhaps by a compensatory repressive mechanism via other factors such as the complex of SUV39H1, a methyltransferase, with HP1 (heterochromatin protein 1) or a reflection of altered promoter usage. Interestingly MeCP2 is also recruited to the *Calb1* promoter in neural stem cells, though membrane depolarization does not appear to lead to loss of MeCP2 from *Calb1* in neural stem cells even under the same conditions that lead to loss of MeCP2 from the *Bdnf* gene [22]. The selective loss of MeCP2 from the *Bdnf* promoter may be due to a loss of 5-methylcytosine that has been observed at the *Bdnf* promoter in response to membrane depolarization (Figure 4) [109]. *In vivo*, MeCP2 is also recruited to the promoters of *Sgk* (serum glucocorticoid-inducible kinase 1) and *Fkbp5* (FK506-binding protein 5), two genes whose expression is increased in MeCP2-null mice, linking MeCP2 with the stress-response pathway in the brain. Together the results suggest that MeCP2 functions as a modulator of gene expression in response to neuronal activity. Such a model might explain why no significant structural changes are seen in brains of mice lacking MeCP2 compared with normal animals, why Rett syndrome presents as a progressive neurodevelopmental disorder and why only subtle changes in global gene expression profiles have been found.

Transient DNA methylation

DNA methylation mediates long-term storage of gene-expression information throughout the life of the organism. It would make sense that similar mechanisms could be used in the encoding of memories, some of which are stored and are able to be recalled many years later. Traditionally, however, in differentiated tissues, DNA methylation was considered to be a static, rather than dynamic, modification. It was believed that once a DNA methylation pattern was set and the cells differentiated, that methylation pattern would be permanent. However, it has recently come to light that DNA methylation patterns in the developed brain may in fact be dynamically regulated. DNA is methylated by DNMTs (DNA methyltransferases), which catalyse the addition of a methyl group at the C-5 position of cytosine residues in CpG dinucleotides. Consistent with this idea, DNMTs are expressed at high levels in developing tissue but decline during differentiation and are expressed at only low levels in differentiated tissue. The brain, however, is somewhat exceptional in that it continues to express high levels of DNMT mRNA into adulthood, and some previous experiments have shown that expression of DNMT is dynamically regulated and that its function is important for synaptic plasticity [112].

In acutely dissociated hippocampal slices from mouse brain, pharmacological inhibition of DNMTs using either 5-aza-2-deoxycytidine or zebularine resulted in an increase in the level of unmethylated DNA in a CpG-rich region of the *Reelin*

promoter [113]. The effect was rapid, being observed after a 40 min incubation, and correlating with this loss of methylated DNA was a concomitant loss of LTP [113]. Other groups have shown that incubation of dissociated mouse hippocampal neurons with DNMT inhibitors results in a decrease in the frequency of mEPSCs (miniature excitatory postsynaptic currents). Such currents are important for controlling neuronal excitability and a decrease in mEPSCs would be predicted to reduce neuronal network activity [114]. In the absence of DNMT inhibitors, synaptic activity alone through NMDA receptors was sufficient to reduce DNA methylation and frequency of mEPSCs [114], suggesting a negative feedback loop and homoeostatic control of synaptic activity. Furthermore the decrease in mEPSCs required MeCP2, as DNMT inhibition had no effect in neurons from MeCP2-knockout mice, which show constitutively low frequency of mEPSCs [115]. Together these results suggest that one function of MeCP2 *in vivo* is to limit synaptic transmission by repressing genes that show increased DNA methylation in response to synaptic activity. In rats, DNMT mRNA is up-regulated in the hippocampus in a behavioural model of learning and memory known as fear conditioning [116]. Inhibition of DNMT during this process using either 5-aza-2-deoxycytidine or zebularine blocked memory formation, suggesting that an increase in DNA methylation (and thus an increase in the repression of specific genes) is important in this process [116]. In response to fear conditioning, methylation of the PP1 (protein phosphatase 1) gene, whose protein product is know to suppress memory formation [117], was increased and the mRNA levels of PP1 declined. Inhibition of DNMT prevented both the increased PP1 methylation in response to fear conditioning and the decrease in PP1 mRNA levels, consistent with a role for DNA methylation in repressing gene expression [116]. In contrast with the PP1 gene, Reelin (which encodes a protein that enhances synaptic plasticity [118]) showed a reduction in the level of methylated DNA and an increase in *Reelin* mRNA in the CA1 hippocampal region, with both of these effects being potentiated by the DNMT inhibitor 5-aza-2-deoxycytidine. As in the *in vitro* hippocampal-slice model, DNA methylation changes in the adult rat brain are very rapid and changes in DNA methylation levels and the effects of DNMT inhibition are seen within 1 h of the fear-conditioning response [116].

The existence of an activity that demethylates DNA is still somewhat controversial and, at least for some, a convincing demonstration of its molecular identity is still eagerly anticipated. The observations that high levels of DNMT mRNA are present in the brain and that synaptic activity correlates with increased levels of methylated DNA and reduced mRNA of some genes, coupled with the ability of at least two DNMT inhibitors to prevent appropriate memory formation, provides some compelling evidence that *de novo* DNA methylation is important in synaptic plasticity. If the addition of methyl groups to the DNA at some genes is an important mechanism in learning and memory, then there must be some mechanism to remove these marks, otherwise there would be a lifetime's accumulation of methylated DNA in our brains, eventually leading to a loss of plasticity in response to altered synaptic input. Several candidates for a DNA demethylase have been proposed. Initially, MBD2 (methyl-CpG-binding domain protein 2) was identified as having DNA demethylase activity [119] via an oxidative demethylation mechanism based on GC–MS of the components in a DNA demethylase assay [120]. However, other groups have struggled to replicate this demethylase activity of MBD2 [121,122]. In *Xenopus laevis* the DNA repair protein Gadd45a (growth arrest and DNA-damage-inducible 45 alpha) was proposed to stimulate DNA demethylation by a DNA-repair process [123]. However, such activity may be species-specific, since the human Gadd45a does not appear to promote DNA demethylation in HEK-293 cells [124]. Most recently a mechanism involving deamination of methylated cytosine residues by DNMT3a/b followed by DNA repair by TDG (thymine-DNA glycosylase) has been proposed [125]. This last set of results suggests that DNMTs may promote both DNA methylation and demethylation. One prediction from this hypothesis would be that DNMT inhibitors would inhibit DNA methylation and demethylation. However, to date, only reduction in DNA methylation has been observed following DNMT inhibition. Though each of these studies has examined DNA demethylation in non-neuronal proliferating cells, it is likely that mechanisms of DNA demethylation would be the same in neurons. In fact, as proliferating cells would be expected to have relatively high levels of DNMT expression, the relatively high levels of DNMT in the nervous system would make this an attractive candidate for a DNA demethylase. Given the current interest and recent progress in this area, a more consensual opinion should hopefully emerge in the near future.

Histone modifications

In eukaryotes, DNA is wrapped around histone octamers, consisting of two copies each of of the histones H2A, H2B, H3 and H4 to form chromatin. Though originally thought to be a means of packaging a large amount of DNA into a relatively small nucleus, it has become clear that changes in the post-translational modifications of histones are also important in regulating transcription. The modifications include acetylation, methylation, phosphorylation and ubiquitination. Enzymes that add or remove these modifications play important roles in regulating gene expression, and the addition and removal of all of these marks is dynamic. Furthermore, multiple states of methylation can occur on either arginine or lysine residues (mono- or di- for arginine and mono-, di- or tri- for lysine). Given the huge potential for different combinations of histone modifications, it has been proposed that the pattern of modifications could function as a code that could provide regulatory information for the genes encoded within the surrounding DNA [126]. Although such a code, if it exists, has yet to be deciphered, much has been learnt recently regarding the association of specific histone modifications with gene-regulatory events.

Acetylation

High levels of histone acetylation are associated with transcriptionally active DNA, whereas hypoacetylated histones are associated with transcriptionally repressed DNA [127,128]. Histones are acetylated by HATs such as CBP and p300 [39] and the acetyl groups removed by HDACs such as HDAC1 [129]. Mutations in the histone acetyltransferase CBP are the cause of Rubenstein–Taybi syndrome, a condition with clinical features that include facial abnormalities and mental retardation, implicating HAT activity as vital for normal cognitive function [130]. In the adult brain, regulation of acetylation and deacetylation of histones plays an important role in synaptic plasticity and in the response to epileptic insults, ischaemia and anti-psychotic drugs [131–133].

Kainic acid-induced seizures in rats lead to increased expression of the transcriptional repressor REST in hippocampal neurons [134]. REST represses transcription by recruiting multiple chromatin-modifying enzymes, including HDACs [30], and increased expression of REST in response to pilocarpine-induced seizures in rats correlates with a reduction in the acetylation levels of histone H4 at the *Gria2* (glutamate receptor, ionotropic, AMPA 2) promoter and decreased expression of

GluR2 (glutamate receptor subunit 2) [131]. Reduction in *Gria2* mRNA could be prevented by pre-administration of the HDAC inhibitor trichostatin A, indicating that the observed deacetylation is required for gene repression. Changes in histone acetylation levels in response to seizures are promoter-specific and can increase as well as decrease. BDNF is transcribed from four independent promoters (I, II, III and IV), and expression of *Bndf* increases in response to seizure activity [135]. Like *Gria2*, *Bndf* is also repressed by REST via an RE1 (repressor element 1) sequence within promoter II [136]. Seizure activity results in increased H4 acetylation levels at promoter I and decreased H4 acetylation levels at promoter IV [131], though the sum of these changes are an overall increase in *Bndf* mRNA [135]. REST expression is also increased in the hippocampus in response to global ischaemia [132] and results in repression of *Gria2* mRNA in one type of hippocampal neuron (the CA1 pyramidal neuron), which subsequently die, owing to the increased calcium entry via GluR2 lacking AMPA receptors [132]. Increased REST levels also repress the expression of *Mor1* (μ opioid receptor gene), which is highly expressed in basket cells and interneurons within the CA1 hippocampal region [137]. Repression of the *Mor1* gene is associated with deacetylation of histones H3 and H4 and dimethylation of H3 lysine 9 at the *Mor1* promoter, presumably a result of the recruitment of HDACs and the H3 lysine 9 methyltransferase G9a by REST [137]. Unlike repression of GluR2, which promotes neuronal cell death, repression of *Mor1* promotes cell survival. *Mor1* activation inhibits the release of the inhibitory neurotransmitter GABA by interneurons. Reduction of *Mor1* would therefore be predicted to result in increased GABA release, reduced synaptic activity and would be opposed to excitotoxicity mediated by excessive glutamate release. Additonally, anti-psychotic drugs such as cocaine stimulate acetylation of H3 and H4 in the striatum [133].

Histone deacetylation has been implicated in the pathogenesis of several neuronal diseases, and inhibitors of HDACs have been successfully used to reverse gene repression in animal models of human diseases such as Huntington's disease [138], neurodegeneration [139] and Rubinstein–Taybi syndrome [140]. Despite the fact that these HDAC inhibitors are non-specific and will inhibit all HDAC activity within the brain, these inhibitors do not appear to increase expression of all genes, nor do they have widespread deleterious side-effects. Thus these inhibitors are currently very promising for use in the treatment of a range of human disorders that result in impaired cognition (for a discussion on therapeutic use of HDAC inhibition, see [141]).

Methylation

Unlike histone acetylation, the effects of histone methylation are dependent on the specific residue that is modified. For example, methylation of H3K4 (H3 Lys^4) is associated with gene activation, while methylation of H3K9 and H3K27 (H3 Lys^9 and Lys^{27}) are associated with gene repression. Initially, methylation of lysine residues on histones was thought to be irreversible. However, in 2004, Yang Shi's laboratory identified the first lysine-specific demethylase, LSD1 [142], and soon other LSDs were identified [143]. Several independent observations have highlighted the importance of histone methylation in the nervous system.

Loss of the transcriptional repressor REST is an important step in the differentiation of neural stem cells to neurons [22,24,25]. Two of the co-repressors recruited by REST, G9a and LSD1, alter histone methylation levels [29,142]. LSD1 demethylates H3K4, thus removing a chromatin mark associated with active transcription, while G9a dimethylates H3K9 and H3K27 [144], thus adding chromatin marks associated with gene repression. One of the co-repressors present in the complexes associated with REST is the chromatin-associated high-mobility-group protein BRAF35 (BRCA2-associated factor 35) [145]. BRAF35 is highly expressed in proliferating cells but not in differentiated neurons. During differentiation of neural progenitor cells, expression of the BRAF35 family member iBRAF (inhibitor of BRAF35) is induced and inhibits REST-mediated repression, at least in part, by recruitment of the H3K4 methylase MLL (mixed-lineage leukaemia) [146]. Importantly, MLL is able to trimethylate H3K4 and, once trimethylated, H3K4 is resistant to demethylation by LSD1, which, because of its mechanism of catalysis, is only able to use mono- or di-methylated H3K4 as a substrate [142].

JARID1C [Jumonji, AT-rich interactive domain 1C; also known as SMCX (Smcy homologue, X-linked)] is a histone demethylase specific for H3K4 that will demethylate tri- or di-methylated H3K4 to monomethylated H3K4 [147]. JARID1C is highly expressed in the brain of mice and mutations in JARID1C result in X-linked mental retardation in humans [148]. In zebrafish (*Danio rerio*) brains, loss of JARID1C results in increased neuronal cell death, whereas in rodent neurons knockdown of JARID1C decreases dendrite length [147], suggesting that JARID1C mutations in humans may result in reduced neuronal numbers and/or altered neuronal morphogenesis. Huntington's disease is also associated with altered neuronal histone methylation patterns. Post-mortem brains from Huntington's-disease patients show increased levels of H3K9 methylation, and a mouse model of Huntington's disease shows elevated H3K9 methylation and increased levels of the H3K9 methyl transferase ESET [ERG (ets-related gene)-associated protein with SET (suppressor of variegation, enhancer of zest and trithorax) domain] [149]. The increased ESET expression observed in these mutant mice may be the result of increased Sp1 and/or Sp3 activity or sequestration of CBP by the mutant Huntingtin protein. In support of the latter hypothesis, mice lacking one allele of the CBP gene show increased expression of the histone methyltransferase ESET, due to increased activity of Ets-2, and high levels of H3K9 methylation in neurons [150].

Phosphorylation

Histone H3 can be phosphorylated on Ser^{10} and Ser^{28}. Phosphorlyation of H3 has been associated with mitotic chromatin condensation and also with highly active gene transcription [151,152]. In neurons, phosphorylation of H3 is clearly not associated with mitosis, but does seem to be an important component of responses in gene expression evoked by synaptic activity. Stimulation of neuronal activity by injection of either dopamine, muscarinic or glutamate receptor agonists into mouse brains resulted in a transient H3 Ser^{10} phosphorylation in the dentate gyrus and CA3 regions of the hippocampus, which peaked within 1 h and was lost after 3 h [153]. Phosphorylation of H3 occurred in the same neurons as phosphorylation of ERK, which is thought to be at least partly responsible for inducing H3 phosphorylation. Furthermore the time course of H3 phosphorylation correlated with the induction of expression of the immediate early genes c-*fos* and MKP-1 and MKP-3 [MAPK (mitogen-activated protein kinase) phosphatases 1 and 3] [153]. Glutamate treatment of cultured striatal neurons induces c-*fos* and c-*jun* mRNA expression via induction of ERK and its downstream target mitogen- and stress-activated kinase-1 (MSK1) [154]. MSK1 directly phosphorylates histone H3 Ser^{10} and Ser^{28} [155] and inhibition of this phosphorylation is sufficient to prevent induction of c-*fos* expression by glutamate, suggesting that this is the regulatory step [154]. In a

mouse model of Huntington's disease, striatal neurons were found to express low levels of c-*fos* mRNA, lack MSK1 activity, and phosphorylation of histone H3 at the c-*fos* promoter did not occur in response to activation of the ERK pathway [156]. Analysis of post-mortem brain samples from Huntington's patients showed a reduced level of MSK-1 in the striatum, but not the cerebral cortex, suggesting that the findings in the mouse model are relevant to the human disease [156]. As described above, increased acetylation of histones is correlated with transcriptional activation. It has been suggested that phosphorylation of H3 Ser^{10} enhances acetylation of H3, and several histone acetyltransferases have a higher affinity for a phosphorylated H3 template [157]. It is not clear how H3 phosphorylation mediates transcriptional activation, though its ability to promote H3 acetylation would be one potential mechanism. However, stimulation of neuronal activity did not appear to increase the global level of acetylated H3 Lys^9 or Lys^{14}, nor the levels at the *c-fos* promoter, which were already high in hippocampal neurons [153]. This suggests that, although phosphorylation may stimulate acetylation of H3, there must be another additional mechanism by which phosphorylated H3 activates transcription. Potentially, phosphorylated H3 could stimulate acetylation of histone H4, or the phospho-acetylated H3 tail may provide a platform to recruit other transcriptional activator proteins.

There still remains much to be uncovered regarding the role of histone modifications in regulating neuronal gene expression. Owing to technical limitations, most of what we know about the role of histone modification has come from studying homogeneous cell populations, usually yeast or immortalized mammalian cell lines. The advantage of these systems is that histone modifications can be easily perturbed using chemical treatments that inhibit specific histone-modifying enzymes and thus allow the preparation of large amounts of chromatin for biochemical analysis. By contrast, the brain not only contains a heterogeneous mix of cell types, but the neurons themselves are a phenotypically diverse population with individual gene-expression profiles. Recent advances in techniques such as ChIP, mean that fewer cells than ever before are required in order to analyse histone modification. For this reason, it will now be possible to interrogate histone modification changes in neuronal specific subtypes, and these kinds of studies are expected to yield interesting new data in the near future.

MicroRNAs and neuronal gene expression

MicroRNAs are short RNAs approx. 22 nucleotides in length that down-regulate expression of proteins by either inhibiting translation or promoting mRNA degradation. (While the consensus view is that microRNAs result in down-regulation of mRNA and/or protein levels of their targets, recent evidence suggests that at least some microRNAs which repress expression in proliferating HeLa and HEK-293 cells can activate expression of their target mRNAs upon cell-cycle arrest [158]. This has led to the suggestion that microRNA function is cell-cycle-dependent. However, whether some microRNAs can also activate expression in neurons has not been tested, and to date all of the experimental evidence supports a role for repression only by microRNAs in neurons.) The biogenesis and function of microRNAs in the brain was recently reviewed [159], and here we will focus on some recent examples which provide some insight into the role of microRNAs in controlling neuronal function by contributing to specific gene-regulatory networks.

The microRNA mir-124 was originally identified as a microRNA specifically expressed in the brain [160,161] and later was shown to negatively regulate the expression of

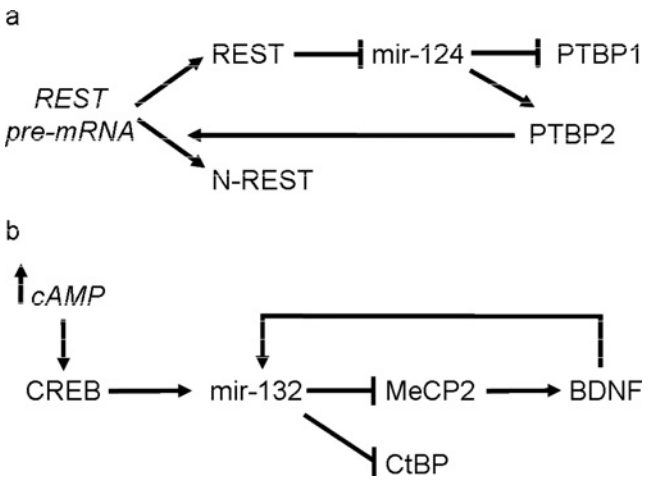

Figure 5 microRNA feedback loops in neuronal gene expression

(**a**) REST can be alternatively spliced, producing a full length-protein (REST) or C-terminal truncated protein found exclusively in neurons (N-REST) that is non-functional. In non-neuronal cells and neural stem cells, REST represses the microRNA mir-124. Loss or inhibition of REST results in increased expression of mir-124, which inhibits the expression of the splicing regulator PTBP1, resulting in increased expression of the related protein PTBP2. PTBP2 promotes splicing of neuronal-specific isoforms, which would lead to increased levels of N-REST and diminished levels of REST, forming a double-negative-feedback loop driving neuronal differentiation. (**b**) One of the target genes of CREB is the microRNA mir-132 whose expression is elevated in response to activation of the CREB signalling pathway. mir-132 inhibits expression of MeCP2 so activation of CREB would result in reduced MeCP2. Paradoxically, reduced MeCP2 levels result in reduced levels of BDNF. Like activated CREB, BDNF also activates expression of mir-132 so reduced BDNF leads to reduced mir-132. This network produces a homoestatic loop of mir-132 - MeCP2 and BDNF expression levels which is regulated by CREB signalling. Most likely such regulatory loops as this exist because it allows a fast response in expression levels of the components in response to an external stimulus, in this case CREB activation.

many non-neuronal genes [162] and play an important role in neuronal differentiation [163]. One of the targets for mir-124 is *PTBP1* (polypyrimidine-tract-binding protein 1), a regulator of mRNA splicing that represses production of neuronal specific mRNA isoforms. By inhibiting *PTBP1*, mir-124 enhances the production of neuronal-specific transcript isoforms and promotes neuronal differentiation [164,165]. Interestingly expression of mir-124 is repressed by the transcriptional repressor REST [163,166]. REST is expressed at high levels in non-neuronal cells, and down-regulation of REST is a requirement for neuronal differentiation [22]. At least some neurons do express low levels of REST, and an alternatively spliced form of REST is present in neurons, the function of which is unclear (see [30] for a discussion on the alternatively spliced form of REST). Given that mir-124 promotes splicing of neuronal-specific transcripts, then induction of mir-124 could inhibit the function of REST by promoting the incorporation of the neuronal-specific exon of REST, giving rise to a truncated protein that shows diminished repressing ability. This would thus provide a double negative-feedback loop between REST and mir-124 (Figure 5a). Such a loop would produce a bistable switch with the potential to provide for robust changes in mir-124 expression levels in response to a modest initiation signal (Figure 5a). Other microRNAs important for neuronal development include mir-133b, which regulates the maturation and function of midbrain dopaminergic neurons as part of a negative feedback loop with the transcription factor Pitx3 (paired-like homeodomain transcription factor 3) [167].

Not only are microRNAs important during development, but they also have a function in the mature nervous system and contribute to the changes in gene expression that underlie neuronal plasticity. One such microRNA is mir-132, which is expressed

in neurons and whose levels are increased via activation of the CREB signalling pathway [168]. One of the targets of mir-132 is MeCP2, and inhibition of mir-132 in cortical neurons using an antisense oligonucleotide resulted in increased MeCP2 protein levels, but had no effect on MeCP2 mRNA levels [169]. Despite MeCP2 being a well-characterized repressor of gene expression, surprisingly, its increased level is accompanied by an increase in BDNF expression (as observed in MeCP2 null mice; see 'Epigenetic regulation' subsection above). Whether the increase in BDNF is a direct or indirect effect of MeCP2 or a secondary effect of mir-132 on another target gene is not known. Increases in BDNF have been shown to result in increased expression of mir-132 [168], suggesting that mir-132, MeCP2 and BDNF form a homoeostatic loop where increases in BDNF would lead to increased mir-132 levels that reduce MeCP2 protein, leading to reduced BDNF (Figure 5b). BDNF antagonizes the function of another microRNA, mir-134, which is expressed in neurons and localized specifically to dendritic spines [170]. mir-134 decreases the size of dendritic spines by inhibiting translation of the Lim (Lin11, Isl-1 and Mec-3)-domain-containing protein Lmtk1 (lemur tyrosine kinase 1). Stimulation of the neurons with BDNF results in the movement of Lmtk1 mRNA to the polysomes and relieves inhibition by mir-134. Although the exact mechanism of relief by BDNF is unclear, intriguingly it does not seem to require the dissociation of mir-134 from the Lmtk1 3′-untranslated region [170].

CONCLUSIONS

Not only is the brain a mixture of cell types, namely neurons, astrocytes and glia, but, additionally, the neurons themselves encompass a wide range of phenotypes, each of which has a unique gene-expression profile. As a further layer of complexity, the gene-expression profiles in each neuron are very dynamic and exquisitely responsive to synaptic activity. Thus each neuron within the brain has the potential to possess a unique set of chromatin modifications and gene-expression profile. Currently many of the tools available to study the control of gene expression require starting material from a large number of ideally homogeneous cells. Thus, although great strides have been made in elucidating mechanisms which involve post-translational modifications of chromatin proteins in non-neuronal cells, providing such detailed information with regard to neurons has thus far been technically challenging. Recent advances in ChIP for relatively small numbers of cells [171,172] should help progress in this area, perhaps in combination with methods to tag and isolate specific subpopulations of neurons [173,174].

REFERENCES

1 Lein, E. S., Hawrylycz, M. J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Boe, A. F., Boguski, M. S., Brockway, K. S., Byrnes, E. J. et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. Nature **445**, 168–176

2 Jaenisch, R. and Young, R. (2008) Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell **132**, 567–582

3 Chambers, I., Silva, J., Colby, D., Nichols, J., Nijmeijer, B., Robertson, M., Vrana, J., Jones, K., Grotewold, L. and Smith, A. (2007) Nanog safeguards pluripotency and mediates germline development. Nature **450**, 1230–1234

4 Masui, S., Nakatake, Y., Toyooka, Y., Shimosato, D., Yagi, R., Takahashi, K., Okochi, H., Okuda, A., Matoba, R., Sharov, A. A. et al. (2007) Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat. Cell Biol. **9**, 625–635

5 Guillemot, F. (2005) Cellular and molecular control of neurogenesis in the mammalian telencephalon. Curr. Opin. Cell Biol. **17**, 639–647

6 Bertrand, N., Castro, D. S. and Guillemot, F. (2002) Proneural genes and the specification of neural cell types. Nat. Rev. Neurosci. **3**, 517–530

7 Sugimori, M., Nagao, M., Bertrand, N., Parras, C. M., Guillemot, F. and Nakafuku, M. (2007) Combinatorial actions of patterning and HLH transcription factors in the spatiotemporal control of neurogenesis and gliogenesis in the developing spinal cord. Development **134**, 1617–1629

8 Berninger, B., Guillemot, F. and Gotz, M. (2007) Directing neurotransmitter identity of neurones derived from expanded adult neural stem cells. Eur. J. Neurosci. **25**, 2581–2590

9 Hori, K., Cholewa-Waclaw, J., Nakada, Y., Glasgow, S. M., Masui, T., Henke, R. M., Wildner, H., Martarelli, B., Beres, T. M., Epstein, J. A. et al. (2008) A nonclassical bHLH Rbpj transcription factor complex is required for specification of GABAergic neurons independent of Notch signaling. Genes Dev. **22**, 166–178

10 Hoshino, M., Nakamura, S., Mori, K., Kawauchi, T., Terao, M., Nishimura, Y. V., Fukuda, A., Fuse, T., Matsuo, N., Sone, M. et al. (2005) Ptf1a, a bHLH transcriptional gene, defines GABAergic neuronal fates in cerebellum. Neuron **47**, 201–213

11 Cheng, L., Arata, A., Mizuguchi, R., Qian, Y., Karunaratne, A., Gray, P. A., Arata, S., Shirasawa, S., Bouchard, M., Luo, P. et al. (2004) Tlx3 and Tlx1 are post-mitotic selector genes determining glutamatergic over GABAergic cell fates. Nat. Neurosci. **7**, 510–517

12 Cheng, L., Samad, O. A., Xu, Y., Mizuguchi, R., Luo, P., Shirasawa, S., Goulding, M. and Ma, Q. (2005) Lbx1 and Tlx3 are opposing switches in determining GABAergic versus glutamatergic transmitter phenotypes. Nat. Neurosci. **8**, 1510–1515

13 Parras, C. M., Schuurmans, C., Scardigli, R., Kim, J., Anderson, D. J. and Guillemot, F. (2002) Divergent functions of the proneural genes Mash1 and Ngn2 in the specification of neuronal subtype identity. Genes Dev. **16**, 324–338

14 Mizuguchi, R., Sugimori, M., Takebayashi, H., Kosako, H., Nagao, M., Yoshida, S., Nabeshima, Y., Shimamura, K. and Nakafuku, M. (2001) Combinatorial roles of olig2 and neurogenin2 in the coordinated induction of pan-neuronal and subtype-specific properties of motoneurons. Neuron **31**, 757–771

15 Petryniak, M. A., Potter, G. B., Rowitch, D. H. and Rubenstein, J. L. (2007) Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. Neuron **55**, 417–433

16 Briscoe, J., Pierani, A., Jessell, T. M. and Ericson, J. (2000) A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. Cell **101**, 435–445

17 Novitch, B. G., Chen, A. I. and Jessell, T. M. (2001) Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. Neuron **31**, 773–789

18 Hack, M. A., Saghatelyan, A., de Chevigny, A., Pfeifer, A., Ashery-Padan, R., Lledo, P. M. and Gotz, M. (2005) Neuronal fate determinants of adult olfactory bulb neurogenesis. Nat. Neurosci. **8**, 865–872

19 Ding, Y. Q., Marklund, U., Yuan, W., Yin, J., Wegman, L., Ericson, J., Deneris, E., Johnson, R. L. and Chen, Z. F. (2003) Lmx1b is essential for the development of serotonergic neurons. Nat. Neurosci. **6**, 933–938

20 Zhou, Q. and Anderson, D. J. (2002) The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. Cell **109**, 61–73

21 Jacob, J., Ferri, A. L., Milton, C., Prin, F., Pla, P., Lin, W., Gavalas, A., Ang, S. L. and Briscoe, J. (2007) Transcriptional repression coordinates the temporal switch from motor to serotonergic neurogenesis. Nat. Neurosci. **10**, 1433–1439

22 Ballas, N., Grunseich, C., Lu, D. D., Speh, J. C. and Mandel, G. (2005) REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. Cell **121**, 645–657

23 Singh, S. K., Kagalwala, M. N., Parker-Thornburg, J., Adams, H. and Majumder, S. (2008) REST maintains self-renewal and pluripotency of embryonic stem cells. Nature **453**, 223–227

24 Ekici, M., Hohl, M., Schuit, F., Martinez-Serrano, A. and Thiel, G. (2008) Transcription of genes encoding synaptic vesicle proteins in human neural stem cells: chromatin accessibility, histone methylation pattern, and the essential role of REST. J. Biol. Chem. **283**, 9257–9268

25 Su, X., Gopalakrishnan, V., Stearns, D., Aldape, K., Lang, F. F., Fuller, G., Snyder, E., Eberhart, C. G. and Majumder, S. (2006) Abnormal expression of REST/NRSF and Myc in neural stem/progenitor cells causes cerebellar tumors by blocking neuronal differentiation. Mol. Cell Biol. **26**, 1666–1678

26 Westbrook, T. F., Hu, G., Ang, X. L., Mulligan, P., Pavlova, N. N., Liang, A., Leng, Y., Maehr, R., Shi, Y., Harper, J. W. et al. (2008) SCFβ–TRCP controls oncogenic transformation and neural differentiation through REST degradation. Nature **452**, 370–374

27 Roopra, A., Sharling, L., Wood, I. C., Briggs, T., Bachfischer, U., Paquette, A. J. and Buckley, N. J. (2000) Transcriptional repression by neuron-restrictive silencer factor is mediated via the Sin3–histone deacetylase complex. Mol. Cell Biol. **20**, 2147–2157

28 Andres, M. E., Burger, C., Peral-Rubio, M. J., Battaglioli, E., Anderson, M. E., Grimes, J., Dallman, J., Ballas, N. and Mandel, G. (1999) CoREST: a functional corepressor required for regulation of neural-specific gene expression. Proc. Natl. Acad. Sci. U.S.A. **96**, 9873–9878

29 Roopra, A., Qazi, R., Schoenike, B., Daley, T. J. and Morrison, J. F. (2004) Localized domains of G9a-mediated histone methylation are required for silencing of neuronal genes. Mol. Cell **14**, 727–738

30 Ooi, L. and Wood, I. C. (2007) Chromatin crosstalk in development and disease: lessons from REST. Nat. Rev. Genet. **8**, 544–554

31 Johnson, R., Gamblin, R. J., Ooi, L., Bruce, A. W., Donaldson, I. J., Westhead, D. R., Wood, I. C., Jackson, R. M. and Buckley, N. J. (2006) Identification of the REST regulon reveals extensive transposable element-mediated binding site duplication. Nucleic Acids Res. **34**, 3862–3877

32 Johnson, D. S., Mortazavi, A., Myers, R. M. and Wold, B. (2007) Genome-wide mapping of *in vivo* protein–DNA interactions. Science **316**, 1497–1502

33 Bruce, A. W., Krejci, A., Ooi, L., Deuchars, J., Wood, I. C., Dolezal, V. and Buckley, N. J. (2006) The transcriptional repressor REST is a critical regulator of the neurosecretory phenotype. J. Neurochem. **98**, 1828–1840

34 Bruce, A. W., Donaldson, I. J., Wood, I. C., Yerbury, S. A., Sadowski, M. I., Chapman, M., Gottgens, B. and Buckley, N. J. (2004) Genome-wide analysis of repressor element 1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) target genes. Proc. Natl. Acad. Sci. U.S.A. **101**, 10458–10463

35 Pance, A., Livesey, F. J. and Jackson, A. P. (2006) A role for the transcriptional repressor REST in maintaining the phenotype of neurosecretory-deficient PC12 cells. J. Neurochem. **99**, 1435–1444

36 Paquette, A. J., Perez, S. E. and Anderson, D. J. (2000) Constitutive expression of the neuron-restrictive silencer factor (NRSF)/REST in differentiating neurons disrupts neuronal gene expression and causes axon pathfinding errors *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **97**, 12318–12323

37 Impey, S., Mark, M., Villacres, E. C., Poser, S., Chavkin, C. and Storm, D. R. (1996) Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron **16**, 973–982

38 Chrivia, J. C., Kwok, R. P., Lamb, N., Hagiwara, M., Montminy, M. R. and Goodman, R. H. (1993) Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature **365**, 855–859

39 Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H. and Nakatani, Y. (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell **87**, 953–959

40 Zhou, Y., Wu, H., Li, S., Chen, Q., Cheng, X. W., Zheng, J., Takemori, H. and Xiong, Z. Q. (2006) Requirement of TORC1 for late-phase long-term potentiation in the hippocampus. PLoS ONE **1**, e16

41 Kovacs, K. A., Steullet, P., Steinmann, M., Do, K. Q., Magistretti, P. J., Halfon, O. and Cardinaux, J. R. (2007) TORC1 is a calcium- and cAMP-sensitive coincidence detector involved in hippocampal long-term synaptic plasticity. Proc. Natl. Acad. Sci. U.S.A. **104**, 4700–4705

42 Tao, X., Finkbeiner, S., Arnold, D. B., Shaywitz, A. J. and Greenberg, M. E. (1998) Ca^{2+} influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. Neuron **20**, 709–726

43 Impey, S., McCorkle, S. R., Cha-Molstad, H., Dwyer, J. M., Yochum, G. S., Boss, J. M., McWeeney, S., Dunn, J. J., Mandel, G. and Goodman, R. H. (2004) Defining the CREB regulon: a genome-wide analysis of transcription factor regulatory regions. Cell **119**, 1041–1054

44 Zhang, X., Odom, D. T., Koo, S. H., Conkright, M. D., Canettieri, G., Best, J., Chen, H., Jenner, R., Herbolsheimer, E., Jacobsen, E. et al. (2005) Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc. Natl. Acad. Sci. U.S.A. **102**, 4459–4464

45 Gomez-Ospina, N., Tsuruta, F., Barreto-Chang, O., Hu, L. and Dolmetsch, R. (2006) The C terminus of the L-type voltage-gated calcium channel Ca(V)1.2 encodes a transcription factor. Cell **127**, 591–606

46 Kubodera, T., Yokota, T., Ohwada, K., Ishikawa, K., Miura, H., Matsuoka, T. and Mizusawa, H. (2003) Proteolytic cleavage and cellular toxicity of the human α1A calcium channel in spinocerebellar ataxia type 6. Neurosci. Lett. **341**, 74–78

47 Kordasiewicz, H. B., Thompson, R. M., Clark, H. B. and Gomez, C. M. (2006) C-termini of P/Q-type Ca^{2+} channel α1A subunits translocate to nuclei and promote polyglutamine-mediated toxicity. Hum. Mol. Genet. **15**, 1587–1599

48 Lam, Y. C., Bowman, A. B., Jafar-Nejad, P., Lim, J., Richman, R., Fryer, J. D., Hyun, E. D., Duvick, L. A., Orr, H. T., Botas, J. et al. (2006) ATAXIN-1 interacts with the repressor Capicua in its native complex to cause SCA1 neuropathology. Cell **127**, 1335–1347

49 Bowman, A. B., Lam, Y. C., Jafar-Nejad, P., Chen, H. K., Richman, R., Samaco, R. C., Fryer, J. D., Kahle, J. J., Orr, H. T. and Zoghbi, H. Y. (2007) Duplication of Atxn1l suppresses SCA1 neuropathology by decreasing incorporation of polyglutamine-expanded ataxin-1 into native complexes. Nat. Genet. **39**, 373–379

50 Steffan, J. S., Bodai, L., Pallos, J., Poelman, M., McCampbell, A., Apostol, B. L., Kazantsev, A., Schmidt, E., Zhu, Y. Z., Greenwald, M. et al. (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. Nature **413**, 739–743

51 Hell, J. W., Westenbroek, R. E., Breeze, L. J., Wang, K. K., Chavkin, C. and Catterall, W. A. (1996) *N*-Methyl-D-aspartate receptor-induced proteolytic conversion of postsynaptic class C L-type calcium channels in hippocampal neurons. Proc. Natl. Acad. Sci. U.S.A. **93**, 3362–3367

52 Chauvet, V., Tian, X., Husson, H., Grimm, D. H., Wang, T., Hiesberger, T., Igarashi, P., Bennett, A. M., Ibraghimov-Beskrovnaya, O., Somlo, S. et al. (2004) Mechanical stimuli induce cleavage and nuclear translocation of the polycystin-1 C terminus. J. Clin. Invest. **114**, 1433–1443

53 von Rotz, R. C., Kohli, B. M., Bosset, J., Meier, M., Suzuki, T., Nitsch, R. M. and Konietzko, U. (2004) The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor. J. Cell Sci. **117**, 4435–4448

54 Shaw, J. P., Utz, P. J., Durand, D. B., Toole, J. J., Emmel, E. A. and Crabtree, G. R. (1988) Identification of a putative regulator of early T cell activation genes. Science **241**, 202–205

55 Rossow, C. F., Dilly, K. W. and Santana, L. F. (2006) Differential calcineurin/NFATc3 activity contributes to the Ito transmural gradient in the mouse heart. Circ. Res. **98**, 1306–1313

56 Okamura, H., Garcia-Rodriguez, C., Martinson, H., Qin, J., Virshup, D. M. and Rao, A. (2004) A conserved docking motif for CK1 binding controls the nuclear localization of NFAT1. Mol. Cell Biol. **24**, 4184–4195

57 Graef, I. A., Mermelstein, P. G., Stankunas, K., Neilson, J. R., Deisseroth, K., Tsien, R. W. and Crabtree, G. R. (1999) L-type calcium channels and GSK-3 regulate the activity of NF-ATc4 in hippocampal neurons. Nature **401**, 703–708

58 Hernandez-Ochoa, E. O., Contreras, M., Cseresnyes, Z. and Schneider, M. F. (2007) Ca^{2+} signal summation and NFATc1 nuclear translocation in sympathetic ganglion neurons during repetitive action potentials. Cell Calcium **41**, 559–571

59 Seybold, V. S., Coicou, L. G., Groth, R. D. and Mermelstein, P. G. (2006) Substance P initiates NFAT-dependent gene expression in spinal neurons. J. Neurochem. **97**, 397–407

60 Groth, R. D. and Mermelstein, P. G. (2003) Brain-derived neurotrophic factor activation of NFAT (nuclear factor of activated T-cells)-dependent transcription: a role for the transcription factor NFATc4 in neurotrophin-mediated gene expression. J. Neurosci. **23**, 8125–8134

61 Luoma, J. I. and Zirpel, L. (2008) Deafferentation-induced activation of NFAT (nuclear factor of activated T-cells) in cochlear nucleus neurons during a developmental critical period: a role for NFATc4-dependent apoptosis in the CNS. J. Neurosci. **28**, 3159–3169

62 Groth, R. D., Weick, J. P., Bradley, K. C., Luoma, J. I., Aravamudan, B., Klug, J. R., Thomas, M. J. and Mermelstein, P. G. (2008) D1 dopamine receptor activation of NFAT-mediated striatal gene expression. Eur. J. Neurosci. **27**, 31–42

63 Groth, R. D., Coicou, L. G., Mermelstein, P. G. and Seybold, V. S. (2007) Neurotrophin activation of NFAT-dependent transcription contributes to the regulation of pro-nociceptive genes. J. Neurochem. **102**, 1162–1174

64 Hyman, S. E., Malenka, R. C. and Nestler, E. J. (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu. Rev. Neurosci. **29**, 565–598

65 Jung, H. and Miller, R. J. (2008) Activation of the nuclear factor of activated T-cells (NFAT) mediates upregulation of CCR2 chemokine receptors in dorsal root ganglion (DRG) neurons: a possible mechanism for activity-dependent transcription in DRG neurons in association with neuropathic pain. Mol. Cell. Neurosci. **37**, 170–177

66 Abbadie, C., Lindia, J. A., Cumiskey, A. M., Peterson, L. B., Mudgett, J. S., Bayne, E. K., DeMartino, J. A., MacIntyre, D. E. and Forrest, M. J. (2003) Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. Proc. Natl. Acad. Sci. U.S.A. **100**, 7947–7952

67 Fuentes, J. J., Genesca, L., Kingsbury, T. J., Cunningham, K. W., Perez-Riba, M., Estivill, X. and de la Luna, S. (2000) DSCR1, overexpressed in Down syndrome, is an inhibitor of calcineurin-mediated signaling pathways. Hum. Mol. Genet. **9**, 1681–1690

68 Gwack, Y., Sharma, S., Nardone, J., Tanasa, B., Iuga, A., Srikanth, S., Okamura, H., Bolton, D., Feske, S., Hogan, P. G. et al. (2006) A genome-wide *Drosophila* RNAi screen identifies DYRK-family kinases as regulators of NFAT. Nature **441**, 646–650

69 Arron, J. R., Winslow, M. M., Polleri, A., Chang, C. P., Wu, H., Gao, X., Neilson, J. R., Chen, L., Heit, J. J., Kim, S. K. et al. (2006) NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. Nature **441**, 595–600

70 Crabtree, G. R. and Olson, E. N. (2002) NFAT signaling: choreographing the social lives of cells. Cell **109** (Suppl.), S67–S79

71 Carrion, A. M., Link, W. A., Ledo, F., Mellstrom, B. and Naranjo, J. R. (1999) DREAM is a Ca^{2+}-regulated transcriptional repressor. Nature **398**, 80–84

72 Spreafico, F., Barski, J. J., Farina, C. and Meyer, M. (2001) Mouse DREAM/calsenilin/KChIP3: gene structure, coding potential, and expression. Mol. Cell. Neurosci. **17**, 1–16

73 Sanz, C., Mellstrom, B., Link, W. A., Naranjo, J. R. and Fernandez-Luna, J. L. (2001) Interleukin 3-dependent activation of DREAM is involved in transcriptional silencing of the apoptotic Hrk gene in hematopoietic progenitor cells. EMBO J. **20**, 2286–2292

74 Edling, Y., Ingelman-Sundberg, M. and Simi, A. (2007) Glutamate activates c-fos in glial cells via a novel mechanism involving the glutamate receptor subtype mGlu5 and the transcriptional repressor DREAM. Glia **55**, 328–340

75 Ledo, F., Kremer, L., Mellstrom, B. and Naranjo, J. R. (2002) Ca^{2+}-dependent block of CREB–CBP transcription by repressor DREAM. EMBO J. **21**, 4583–4592

76 Ledo, F., Carrion, A. M., Link, W. A., Mellstrom, B. and Naranjo, J. R. (2000) DREAM–αCREM interaction via leucine-charged domains derepresses downstream regulatory element-dependent transcription. Mol. Cell Biol. **20**, 9120–9126

77 Link, W. A., Ledo, F., Torres, B., Palczewska, M., Madsen, T. M., Savignac, M., Albar, J. P., Mellstrom, B. and Naranjo, J. R. (2004) Day–night changes in downstream regulatory element antagonist modulator/potassium channel interacting protein activity contribute to circadian gene expression in pineal gland. J. Neurosci. **24**, 5346–5355

78 Papadia, S. and Hardingham, G. E. (2007) The dichotomy of NMDA receptor signaling. Neuroscientist **13**, 572–579

79 Yuan, Z., Becker, E. B., Merlo, P., Yamada, T., DiBacco, S., Konishi, Y., Schaefer, E. M. and Bonni, A. (2008) Activation of FOXO1 by Cdk1 in cycling cells and postmitotic neurons. Science **319**, 1665–1668

80 Rena, G., Prescott, A. R., Guo, S., Cohen, P. and Unterman, T. G. (2001) Roles of the forkhead in rhabdomyosarcoma (FKHR) phosphorylation sites in regulating 14-3-3 binding, transactivation and nuclear targetting. Biochem. J. **354**, 605–612

81 Wang, X. T., Pei, D. S., Xu, J., Guan, Q. H., Sun, Y. F., Liu, X. M. and Zhang, G. Y. (2007) Opposing effects of Bad phosphorylation at two distinct sites by Akt1 and JNK1/2 on ischemic brain injury. Cell Signal. **19**, 1844–1856

82 Dhandapani, K. M., Wade, F. M., Wakade, C., Mahesh, V. B. and Brann, D. W. (2005) Neuroprotection by stem cell factor in rat cortical neurons involves AKT and NFκB. J. Neurochem. **95**, 9–19

83 Prendergast, G. C. and Ziff, E. B. (1991) Methylation-sensitive sequence-specific DNA binding by the c-Myc basic region. Science **251**, 186–189

84 Miranda, T. B. and Jones, P. A. (2007) DNA methylation: the nuts and bolts of repression. J. Cell. Physiol. **213**, 384–390

85 Feng, Y., Absher, D., Eberhart, D. E., Brown, V., Malter, H. E. and Warren, S. T. (1997) FMRP associates with polyribosomes as an mRNP, and the I304N mutation of severe fragile X syndrome abolishes this association. Mol. Cell **1**, 109–118

86 Li, Z., Zhang, Y., Ku, L., Wilkinson, K. D., Warren, S. T. and Feng, Y. (2001) The fragile X mental retardation protein inhibits translation via interacting with mRNA. Nucleic Acids Res. **29**, 2276–2283

87 Sutcliffe, J. S., Nelson, D. L., Zhang, F., Pieretti, M., Caskey, C. T., Saxe, D. and Warren, S. T. (1992) DNA methylation represses FMR-1 transcription in fragile X syndrome. Hum. Mol. Genet. **1**, 397–400

88 Qin, M., Kang, J., Burlin, T. V., Jiang, C. and Smith, C. B. (2005) Postadolescent changes in regional cerebral protein synthesis: an *in vivo* study in the FMR1 null mouse. J. Neurosci. **25**, 5087–5095

89 Meehan, R. R., Lewis, J. D. and Bird, A. P. (1992) Characterization of MeCP2, a vertebrate DNA binding protein with affinity for methylated DNA. Nucleic Acids Res **20**, 5085–5092

90 Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U. and Zoghbi, H. Y. (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat. Genet. **23**, 185–188

91 Cohen, D. R., Matarazzo, V., Palmer, A. M., Tu, Y., Jeon, O. H., Pevsner, J. and Ronnett, G. V. (2003) Expression of MeCP2 in olfactory receptor neurons is developmentally regulated and occurs before synaptogenesis. Mol. Cell Neurosci. **22**, 417–429

92 Miyake, K. and Nagai, K. (2007) Phosphorylation of methyl-CpG binding protein 2 (MeCP2) regulates the intracellular localization during neuronal cell differentiation. Neurochem. Int. **50**, 264–270

93 Nan, X., Campoy, F. J. and Bird, A. (1997) MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell **88**, 471–481

94 Nan, X., Ng, H. H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N. and Bird, A. (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature **393**, 386–389

95 Jones, P. L., Veenstra, G. J., Wade, P. A., Vermaak, D., Kass, S. U., Landsberger, N., Strouboulis, J. and Wolffe, A. P. (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat. Genet. **19**, 187–191

96 Fuks, F., Hurd, P. J., Wolf, D., Nan, X., Bird, A. P. and Kouzarides, T. (2003) The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. J. Biol. Chem. **278**, 4035–4040

97 Nikitina, T., Shi, X., Ghosh, R. P., Horowitz-Scherer, R. A., Hansen, J. C. and Woodcock, C. L. (2007) Multiple modes of interaction between the methylated DNA binding protein MeCP2 and chromatin. Mol. Cell. Biol. **27**, 864–877

98 Harikrishnan, K. N., Chow, M. Z., Baker, E. K., Pal, S., Bassal, S., Brasacchio, D., Wang, L., Craig, J. M., Jones, P. L., Sif, S. et al. (2005) Brahma links the SWI/SNF chromatin-remodeling complex with MeCP2-dependent transcriptional silencing. Nat. Genet. **37**, 254–264

99 Nikitina, T., Ghosh, R. P., Horowitz-Scherer, R. A., Hansen, J. C., Grigoryev, S. A. and Woodcock, C. L. (2007) MeCP2-chromatin interactions include the formation of chromatosome-like structures and are altered in mutations causing Rett syndrome. J. Biol. Chem. **282**, 28237–28245

100 Nan, X., Hou, J., Maclean, A., Nasir, J., Lafuente, M. J., Shu, X., Kriaucionis, S. and Bird, A. (2007) Interaction between chromatin proteins MECP2 and ATRX is disrupted by mutations that cause inherited mental retardation. Proc. Natl. Acad. Sci. U.S.A. **104**, 2709–2714

101 Xue, Y., Gibbons, R., Yan, Z., Yang, D., McDowell, T. L., Sechi, S., Qin, J., Zhou, S., Higgs, D. and Wang, W. (2003) The ATRX syndrome protein forms a chromatin-remodeling complex with Daxx and localizes in promyelocytic leukemia nuclear bodies. Proc. Natl. Acad. Sci. U.S.A. **100**, 10635–10640

102 Guy, J., Hendrich, B., Holmes, M., Martin, J. E. and Bird, A. (2001) A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. Nat. Genet. **27**, 322–326

103 Gemelli, T., Berton, O., Nelson, E. D., Perrotti, L. I., Jaenisch, R. and Monteggia, L. M. (2006) Postnatal loss of methyl-CpG binding protein 2 in the forebrain is sufficient to mediate behavioral aspects of Rett syndrome in mice. Biol. Psychiatr. **59**, 468–476

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

105 Villard, L. (2007) MECP2 mutations in males. J. Med. Genet. **44**, 417–423

106 Collins, A. L., Levenson, J. M., Vilaythong, A. P., Richman, R., Armstrong, D. L., Noebels, J. L., David Sweatt, J. and Zoghbi, H. Y. (2004) Mild overexpression of MeCP2 causes a progressive neurological disorder in mice. Hum. Mol. Genet. **13**, 2679–2689

107 Tudor, M., Akbarian, S., Chen, R. Z. and Jaenisch, R. (2002) Transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain. Proc. Natl. Acad. Sci. U.S.A. **99**, 15536–15541

108 Chen, W. G., Chang, Q., Lin, Y., Meissner, A., West, A. E., Griffith, E. C., Jaenisch, R. and Greenberg, M. E. (2003) Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. Science **302**, 885–889

109 Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., Fan, G. and Sun, Y. E. (2003) DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. Science **302**, 890–893

110 Zhou, Z., Hong, E. J., Cohen, S., Zhao, W. N., Ho, H. Y., Schmidt, L., Chen, W. G., Lin, Y., Savner, E., Griffith, E. C., et al. (2006) Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth and spine maturation. Neuron **52**, 255–269

111 Chang, Q., Khare, G., Dani, V., Nelson, S. and Jaenisch, R. (2006) The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. Neuron **49**, 341–348

112 Goto, K., Numata, M., Komura, J. I., Ono, T., Bestor, T. H. and Kondo, H. (1994) Expression of DNA methyltransferase gene in mature and immature neurons as well as proliferating cells in mice. Differentiation **56**, 39–44

113 Levenson, J. M., Roth, T. L., Lubin, F. D., Miller, C. A., Huang, I. C., Desai, P., Malone, L. M. and Sweatt, J. D. (2006) Evidence that DNA (cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. J. Biol. Chem. **281**, 15763–15773

114 Nelson, E. D., Kavalali, E. T. and Monteggia, L. M. (2008) Activity-dependent suppression of miniature neurotransmission through the regulation of DNA methylation. J. Neurosci. **28**, 395–406

115 Nelson, E. D., Kavalali, E. T. and Monteggia, L. M. (2006) MeCP2-dependent transcriptional repression regulates excitatory neurotransmission. Curr. Biol. **16**, 710–716

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

117 Blitzer, R. D., Connor, J. H., Brown, G. P., Wong, T., Shenolikar, S., Iyengar, R. and Landau, E. M. (1998) Gating of CaMKII by cAMP-regulated protein phosphatase activity during LTP. Science **280**, 1940–1942

118 Weeber, E. J., Beffert, U., Jones, C., Christian, J. M., Forster, E., Sweatt, J. D. and Herz, J. (2002) Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. J. Biol. Chem. **277**, 39944–39952

119 Bhattacharya, S. K., Ramchandani, S., Cervoni, N. and Szyf, M. (1999) A mammalian protein with specific demethylase activity for mCpG DNA. Nature **397**, 579–583

120 Hamm, S., Just, G., Lacoste, N., Moitessier, N., Szyf, M. and Mamer, O. (2008) On the mechanism of demethylation of 5-methylcytosine in DNA. Bioorg. Med. Chem. Lett **18**, 1046–1049

121 Ng, H. H., Zhang, Y., Hendrich, B., Johnson, C. A., Turner, B. M., Erdjument-Bromage, H., Tempst, P., Reinberg, D. and Bird, A. (1999) MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. Nat. Genet. **23**, 58–61

122 Wade, P. A., Gegonne, A., Jones, P. L., Ballestar, E., Aubry, F. and Wolffe, A. P. (1999) Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. Nat. Genet. **23**, 62–66

123 Barreto, G., Schafer, A., Marhold, J., Stach, D., Swaminathan, S. K., Handa, V., Doderlein, G., Maltry, N., Wu, W., Lyko, F. et al. (2007) Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature **445**, 671–675

124 Jin, S. G., Guo, C. and Pfeifer, G. P. (2008) GADD45A does not promote DNA demethylation. PLoS Genet. **4**, e1000013

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

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

127 Pokholok, D. K., Harbison, C. T., Levine, S., Cole, M., Hannett, N. M., Lee, T. I., Bell, G. W., Walker, K., Rolfe, P. A., Herbolsheimer, E. et al. (2005) Genome-wide map of nucleosome acetylation and methylation in yeast. Cell **122**, 517–527

128 Bernstein, B. E., Kamal, M., Lindblad-Toh, K., Bekiranov, S., Bailey, D. K., Huebert, D. J., McMahon, S., Karlsson, E. K., Kulbokas, 3rd, E. J., Gingeras, T. R. et al. (2005) Genomic maps and comparative analysis of histone modifications in human and mouse. Cell **120**, 169–181

129 Taunton, J., Hassig, C. A. and Schreiber, S. L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science **272**, 408–411

130 Petrij, F., Giles, R. H., Dauwerse, H. G., Saris, J. J., Hennekam, R. C., Masuno, M., Tommerup, N., van Ommen, G. J., Goodman, R. H., Peters, D. J. et al. (1995) Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature **376**, 348–351

131 Huang, Y., Doherty, J. J. and Dingledine, R. (2002) Altered histone acetylation at glutamate receptor 2 and brain-derived neurotrophic factor genes is an early event triggered by status epilepticus. J. Neurosci. **22**, 8422–8428

132 Calderone, A., Jover, T., Noh, K. M., Tanaka, H., Yokota, H., Lin, Y., Grooms, S. Y., Regis, R., Bennett, M. V. and Zukin, R. S. (2003) Ischemic insults derepress the gene silencer REST in neurons destined to die. J. Neurosci. **23**, 2112–2121

133 Brami-Cherrier, K., Valjent, E., Herve, D., Darragh, J., Corvol, J. C., Pages, C., Arthur, S. J., Girault, J. A. and Caboche, J. (2005) Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. J. Neurosci. **25**, 11444–11454

134 Palm, K., Belluardo, N., Metsis, M. and Timmusk, T. (1998) Neuronal expression of zinc finger transcription factor REST/NRSF/XBR gene. J. Neurosci. **18**, 1280–1296

135 Timmusk, T., Palm, K., Metsis, M., Reintam, T., Paalme, V., Saarma, M. and Persson, H. (1993) Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron **10**, 475–489

136 Timmusk, T., Palm, K., Lendahl, U. and Metsis, M. (1999) Brain-derived neurotrophic factor expression *in vivo* is under the control of neuron-restrictive silencer element. J. Biol. Chem. **274**, 1078–1084

137 Formisano, L., Noh, K. M., Miyawaki, T., Mashiko, T., Bennett, M. V. and Zukin, R. S. (2007) Ischemic insults promote epigenetic reprogramming of μ opioid receptor expression in hippocampal neurons. Proc. Natl. Acad. Sci. U.S.A. **104**, 4170–4175

138 Ferrante, R. J., Kubilus, J. K., Lee, J., Ryu, H., Beesen, A., Zucker, B., Smith, K., Kowall, N. W., Ratan, R. R., Luthi-Carter, R. et al. (2003) Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. J. Neurosci. **23**, 9418–9427

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

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

141 Abel, T. and Zukin, R. S. (2008) Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. Curr. Opin. Pharmacol. **8**, 57–64

142 Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A. and Shi, Y. (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell **119**, 941–953

143 Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M. E., Borchers, C. H., Tempst, P. and Zhang, Y. (2006) Histone demethylation by a family of JmjC domain-containing proteins. Nature **439**, 811–816

144 Tachibana, M., Sugimoto, K., Fukushima, T. and Shinkai, Y. (2001) Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. J. Biol. Chem. **276**, 25309–25317

145 Hakimi, M. A., Bochar, D. A., Chenoweth, J., Lane, W. S., Mandel, G. and Shiekhattar, R. (2002) A core-BRAF35 complex containing histone deacetylase mediates repression of neuronal-specific genes. Proc. Natl. Acad. Sci. U.S.A. **99**, 7420–7425

146 Wynder, C., Hakimi, M. A., Epstein, J. A., Shilatifard, A. and Shiekhattar, R. (2005) Recruitment of MLL by HMG-domain protein iBRAF promotes neural differentiation. Nat. Cell Biol. **7**, 1113–1117

147 Iwase, S., Lan, F., Bayliss, P., de la Torre-Ubieta, L., Huarte, M., Qi, H. H., Whetstine, J. R., Bonni, A., Roberts, T. M. and Shi, Y. (2007) The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. Cell **128**, 1077–1088

148 Jensen, L. R., Amende, M., Gurok, U., Moser, B., Gimmel, V., Tzschach, A., Janecke, A. R., Tariverdian, G., Chelly, J., Fryns, J. P. et al. (2005) Mutations in the JARID1C gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. Am. J. Hum. Genet. **76**, 227–236

149 Ryu, H., Lee, J., Hagerty, S. W., Soh, B. Y., McAlpin, S. E., Cormier, K. A., Smith, K. M. and Ferrante, R. J. (2006) ESET/SETDB1 gene expression and histone H3 (K9) trimethylation in Huntington's disease. Proc. Natl. Acad. Sci. U.S.A. **103**, 19176–19181

150 Lee, J., Hagerty, S., Cormier, K. A., Ferrante, R. J., Kung, A. L. and Ryu, H. (2008) Monoallele deletion of CBP leads to pericentromeric heterochromatin condensation through ESET expression and histone H3 (K9) methylation, Hum. Mol. Genet. **17**, 1774–1782

151 Johansen, K. M. and Johansen, J. (2006) Regulation of chromatin structure by histone H3S10 phosphorylation. Chromosome Res. **14**, 393–404

152 Dong, Z. and Bode, A. M. (2006) The role of histone H3 phosphorylation (Ser10 and Ser28) in cell growth and cell transformation. Mol. Carcinog. **45**, 416–421

153 Crosio, C., Heitz, E., Allis, C. D., Borrelli, E. and Sassone-Corsi, P. (2003) Chromatin remodeling and neuronal response: multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. J. Cell Sci. **116**, 4905–4914

154 Brami-Cherrier, K., Lavaur, J., Pages, C., Arthur, J. S. and Caboche, J. (2007) Glutamate induces histone H3 phosphorylation but not acetylation in striatal neurons: role of mitogen- and stress-activated kinase-1. J. Neurochem. **101**, 697–708

155 Kim, H. G., Lee, K. W., Cho, Y. Y., Kang, N. J., Oh, S. M., Bode, A. M. and Dong, Z. (2008) Mitogen- and stress-activated kinase 1-mediated histone H3 phosphorylation is crucial for cell transformation. Cancer Res. **68**, 2538–2547

156 Roze, E., Betuing, S., Deyts, C., Marcon, E., Brami-Cherrier, K., Pages, C., Humbert, S., Merienne, K. and Caboche, J. (2008) Mitogen- and stress-activated protein kinase-1 deficiency is involved in expanded-huntingtin-induced transcriptional dysregulation and striatal death. FASEB J. **22**, 1083–1093

157 Cheung, P., Tanner, K. G., Cheung, W. L., Sassone-Corsi, P., Denu, J. M. and Allis, C. D. (2000) Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. Mol. Cell **5**, 905–915

158 Vasudevan, S., Tong, Y. and Steitz, J. A. (2007) Switching from repression to activation: microRNAs can up-regulate translation. Science **318**, 1931–1934

159 Kosik, K. S. (2006) The neuronal microRNA system. Nat. Rev. Neurosci. **7**, 911–920

160 Lagos-Quintana, M., Rauhut, R., Yalcin, A., Meyer, J., Lendeckel, W. and Tuschl, T. (2002) Identification of tissue-specific microRNAs from mouse. Curr. Biol. **12**, 735–739

161 Sempere, L. F., Freemantle, S., Pitha-Rowe, I., Moss, E., Dmitrovsky, E. and Ambros, V. (2004) Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol. **5**, R13

162 Lim, L. P., Lau, N. C., Garrett-Engele, P., Grimson, A., Schelter, J. M., Castle, J., Bartel, D. P., Linsley, P. S. and Johnson, J. M. (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature **433**, 769–773

163 Conaco, C., Otto, S., Han, J. J. and Mandel, G. (2006) Reciprocal actions of REST and a microRNA promote neuronal identity. Proc. Natl. Acad. Sci. U.S.A. **103**, 2422–2427

164 Makeyev, E. V., Zhang, J., Carrasco, M. A. and Maniatis, T. (2007) The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol. Cell **27**, 435–448

165 Makeyev, E. V. and Maniatis, T. (2008) Multilevel regulation of gene expression by microRNAs. Science **319**, 1789–1790

166 Johnson, R., Zuccato, C., Belyaev, N. D., Guest, D. J., Cattaneo, E. and Buckley, N. J. (2008) A microRNA-based gene dysregulation pathway in Huntington's disease. Neurobiol. Dis. **29**, 438–445

167 Kim, J., Inoue, K., Ishii, J., Vanti, W. B., Voronov, S. V., Murchison, E., Hannon, G. and Abeliovich, A. (2007) A MicroRNA feedback circuit in midbrain dopamine neurons. Science **317**, 1220–1224

168 Vo, N., Klein, M. E., Varlamova, O., Keller, D. M., Yamamoto, T., Goodman, R. H. and Impey, S. (2005) A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. Proc. Natl. Acad. Sci. U.S.A. **102**, 16426–16431

169 Klein, M. E., Lioy, D. T., Ma, L., Impey, S., Mandel, G. and Goodman, R. H. (2007) Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat. Neurosci. **10**, 1513–1514
170 Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kiebler, M. and Greenberg, M. E. (2006) A brain-specific microRNA regulates dendritic spine development. Nature **439**, 283–289
171 O'Neill, L. P., VerMilyea, M. D. and Turner, B. M. (2006) Epigenetic characterization of the early embryo with a chromatin immunoprecipitation protocol applicable to small cell populations. Nat. Genet. **38**, 835–841
172 Dahl, J. A. and Collas, P. (2008) MicroChIP – a rapid micro chromatin immunoprecipitation assay for small cell samples and biopsies. Nucleic Acids Res. **36**, e15
173 Hempel, C. M., Sugino, K. and Nelson, S. B. (2007) A manual method for the purification of fluorescently labeled neurons from the mammalian brain. Nat. Protoc. **2**, 2924–2929
174 Lin, D. M., Loveall, B., Ewer, J., Deitcher, D. L. and Sucher, N. J. (2007) Characterization of mRNA expression in single neurons. Methods Mol. Biol. **399**, 133–152

Received 16 May 2008/18 June 2008; accepted 19 June 2008
Published on the Internet 27 August 2008, doi:10.1042/BJ20080963