nature neuroscience

# Dynamic epigenetic regulation in neurons: enzymes, stimuli and signaling pathways

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**The development and function of neurons require the regulated expression of large numbers of very specific gene sets. Epigenetic modifications of both DNA and histone proteins are now emerging as fundamental mechanisms by which neurons adapt their transcriptional response to developmental and environmental cues. In the nervous system, the mechanisms by which extracellular signals regulate the activity of chromatin-modifying enzymes have just begun to be characterized. In this Review, I discuss how extracellular cues, including synaptic activity and neurotrophic factors, influence epigenetic modifications and regulate the neuronal transcriptional response. I also summarize additional mechanisms that induce chromatin remodeling events by combinatorial assembly of multiprotein complexes on neuronal gene promoters.**



Epigenetic changes in general refer to stable and heritable modifications of chromatin—the DNA and its associated histone proteins—that are independent of the underlying DNA sequence and that help to determine the phenotypic traits of cells during development[1]. The core unit of chromatin is the nucleosome, which consists of 147 bp of DNA folded around histone octamers containing two each of the histone proteins H2A, H2B, H3 and H4 (ref. 2). Changes in chromatin structure allow (or forbid) specific multiprotein transcriptional regulator complexes to access DNA sequences. Such changes in chromatin structure are achieved chiefly by three distinct mechanisms: DNA methylation, histone modifications and ATP-dependent chromatin remodeling[3]. Epigenetic modifications of chromatin have generally been considered to be both stable and heritable. However, in post-mitotic cells such as fully differentiated neurons, epigenetic modifications might be highly dynamic, and could thereby support neuronal functions and plasticity[4,5].

Several recent reviews have discussed the molecular mechanisms of epigenetic modification and chromatin remodeling complexes in the nervous system (for example, refs. 4,6). Here I focus on recent evidence that physiological signals and developmental cues influence adaptive transcriptional responses in neurons through the epigenetic modification of chromatin. I give particular emphasis to the regulation of histone acetylation and DNA methylation in response to neural activity and neurotrophin signaling. I also briefly discuss mechanisms that induce chromatin modifications by the combinatorial assembly of multiprotein complexes on neuronal gene promoters.

## Dynamic regulation of histone modifications

Histones are covalently modified at their N-terminal tails to regulate transcriptional activation and repression. In addition to the well-studied examples of acetylation and methylation, histones can undergo numerous other post-translational modifications, including phosphorylation, ADP-ribosylation, sumoylation, ubiquitylation and proline isomerization[2,3]. Moreover, histones are often concurrently modified on several residues. Covalently modified histone residues, either alone or in combination, generate distinct docking sites for the recruitment of multiprotein nuclear complexes that influence the structure and function of chromatin[7].

The nuclear enzymes that regulate the known post-translational modifications of histone have been identified, including those responsible for histone acetylation and deacetylation, arginine methylation, lysine methylation and demethylation, histone phosphorylation and ubiquitylation[3]. The recent discovery of histone deimination[8,9], a process that counteracts arginine methylation by converting arginine to citrulline, suggests that the known epigenetic histone modifications can in principle be regulated dynamically, and thus may contribute to fast adaptive transcriptional responses to extracellular cues. It is conceivable that most, if not all, chromatin-modifying enzymes are targeted by signaling pathways that directly link environmental cues to gene expression. The dynamic turnover of covalent histone modifications and their implications for the cellular adaptive response have been the object of extensive investigation[10]. Nevertheless, the extracellular signals and intracellular pathways that lead to histone modifications in neurons remain poorly understood.

## Chromatin-modifying enzymes

Chromatin-modifying enzymes are usually found in large multiprotein complexes that are recruited to gene promoters by transcription factors that bind to specific DNA sequences and confer target specificity. In neurons, histone acetylases (HATs) and histone deacetylases (HDACs) are the best-characterized chromatin-modifying enzymes.

HATs function enzymatically by transferring an acetyl group from acetyl-coenzyme A to the ε-amino groups of histone lysine residues. This modification usually leads to increased transcription of associated DNA through mechanisms that include stabilizing the binding of other nuclear factors to gene promoters[7,11,12] and changing nucleosome structure by weakening the interaction between the positively charged histone tails and the negatively charged DNA[13–15].

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Published online 26 October 2010; doi:10.1038/nn.2671

The protein p300 and its close homolog CREB binding protein (CBP) are ubiquitously expressed transcriptional coactivators with HAT activity that participate in the activities of numerous transcription factors[16]. This includes many—such as CREB, SRF, MEF2, c-Jun and p53—that have key roles in the development and maintainance of the nervous system. CBP and p300 also interact with components of the basal transcriptional machinery and with other HATs (steroid receptor coactivators SRC1, ACTR and P/CAF)[17]. Analysis of the expression of CBP and p300 in the nervous system has shown that both proteins are broadly distributed throughout the developing neural tube[18]. During the later phases of embryogenesis, their expression becomes more restricted; in the adult CNS they are mostly confined to subpopulations of cortical cells and motor neurons[18]. Transgenic mice that lack CBP die at an early gestational age and are affected by a host of developmental defects including severe nervous system abnormalities[19]. The loss of one CBP allele causes human Rubinstein-Taybi syndrome, which is characterized by mental retardation and other severe developmental defects[20].

Other HATs are also likely to have key roles in dynamic neuronal functions. For example, the HAT activity of CLOCK is necessary for the expression of core circadian clock genes in the suprachiasmatic nucleus of the hypothalamus, and for the maintenance of circadian rhythmicity[21,22]. SRCs 1–3 are another family of HATs that are expressed in the nervous system; mice lacking SRC-1 have motor neuron and cerebellar dysfunctions[23].

The counterplayers of the HATs, HDACs, are found in all eukaryotic organisms and are essential for cellular proliferation, differentiation and homeostasis[24,25]. Alteration of HDAC activity has been implicated in the onset and progression of a host of human diseases, including neurodegenerative disorders, cardiovascular disease and cancer[26,27]. HDACs inhibit transcription through two distinct features, their intrinsic deacetylase activity and their interaction with co-repressors. Importantly, HDACs can deacetylate both histone and non-histone substrates, and they do not directly bind DNA[25]. Their transcriptional repressor function therefore depends on interaction with co-repressors that target them to specific chromosomal loci, often concurrently enhancing their enzymatic activity.

Mammalian HDACs are divided into four classes on the basis of their amino acid sequence and structure. Class I HDACs are ubiquitously expressed, with predominantly nuclear localization. They share a large, highly conserved deacetylase domain that contains an active site with two adjacent histidines and a $Zn^{2+}$ ion[27]. By contrast, class II and IV HDACs are more selectively expressed and can be found in both the nucleus and the cytoplasm; they can undergo stimulus-dependent nucleo-cytoplasmic shuttling[25]. Sirtuins, or class III HDACs, share no homology with class I, II or IV HDACs; their deacetylase activity depends on the co-factor NAD+ and they are found in all subcellular compartments[28].

All HDACs that have been identified are expressed in the nervous system, often in a developmentally regulated manner[29]. The highly homologous class I HDACs, for example, are expressed at different stages in neuronal development; HDAC1 is confined to neural stem cells and glia, whereas HDAC2 is mostly found in postmitotic neuroblasts and differentiated neurons[30]. Interestingly, HDACs can regulate their own transcription. Disruption of HDAC1 in embryonic stem cells induces the expression of other class I HDACs[31]. However, increasing the level of one or more HDACs does not necessarily result in functional compensation, even for the highly homologous HDAC1 and HDAC2. For example, upregulation of HDAC1 was not sufficient to rescue the morphological and electrophysiological defects in mice that lack HDAC2 in the hippocampus, indicating that, at least in adult neurons, HDAC1 and HDAC2 have non-redundant and specifically regulated functions[32].

Under normal conditions, the availability and enzymatic activities of HATs and HDACs are maintained in a balance, with the degree of chromatin acetylation contributing to either transcriptional repression or transcriptional activation. It has generally been assumed that the activation of HATs and the activation of HDACs have mutually exclusive effects on gene expression. However, a recent genome-wide analysis of promotor occupancy in human lymphocytes has revealed that both types of enzyme are found at transcriptionally active genes associated with acetylated histones and phosphorylated RNA polymerase II (PolII) (ref. 33). Genes that were transcriptionally inactive but were poised for activation (bearing the epigenetic H3K4 histone methylation mark[34]) were maintained in an inactive state by dynamic and transient cycles of histone acetylation and deacetylation. Pharmacological inhibition of HDAC activity correlated with increased binding of PolII to gene promoters, suggesting that the main mechanism by which HDACs maintain poised genes in an inactive state might be by inhibiting the recruitment of PolII. By contrast, silent genes, which had little or no H3K4 methylation, were not bound by HDACs and HATs and remained repressed when exposed to extracellular stimuli[33]. These findings suggest that HATs and HDACs can collaborate in certain scenarios, and also that stimulus-dependent activation of one class of enzyme does not necessarily need to be coupled with the inhibition of its functional counterparts.

## Synaptic activity–dependent histone modifications

In neurons, the transcription factor CREB is essential for activity-induced gene expression[35]. The HAT CBP is an important co-factor for CREB-dependent transcription. CBP itself can be phosphorylated by a number of extracellular signal–responsive serine/threonine kinases, including $Ca^{2+}$/calmodulin-dependent kinase-IV (CamK-IV)[36,37], ribosomal protein S6 kinase-2 (RSK2)[38] and p42/p44 mitogen-activated protein kinase (MAPK)[39,40]. CBP is recruited to CREB-regulated genes through a mechanism that requires an increase in nuclear calcium and CamKIV-dependent phosphorylation of CBP[36,37,41]. Interestingly, the route of calcium entry (either through L-type $Ca^{2+}$ channels or through NMDA receptors in response to stimulation with glutamate or KCl) differentially controls the association of CBP with neuronal gene promoters[37] (**Fig. 1**). CamKIV activity influenced the transcriptional activity of CBP only after activation of L-type $Ca^{2+}$ channels, suggesting that the mode of $Ca^{2+}$ influx elicits distinct signal transduction pathways that might account for transcriptional specificity. Although it has been proposed that CamKIV-dependent phosphorylation of CBP is involved in the activation of CREB-regulated genes, it remains unclear how this post-translational modification affects the functions of CBP.

Genome-wide studies of the occupancy of CBP and p300 on DNA showed that the interaction of these coactivators with chromatin is not restricted to gene promoters[42,43]. CBP and p300 have been found in association with lysine 4 monomethylation of histone H3 (H3K4me1), an epigenetic mark that is associated with enhancer functions. In the brain, sites where CBP or p300 binds are enriched at highly conserved noncoding regions near activated genes, which might indicate that the binding of CBP or p300 to these sequences determines the activation of neighboring genes[43]. Indeed, genome-wide screens of mouse cortical neurons using chromatin immunoprecipitation sequencing (ChIP-seq) have shown that the recruitment of CBP to enhancers is markedly increased after membrane depolarization[44] (**Fig. 1**). CREB, and to a lesser extent SRF, also becomes associated with activity-regulated enhancers after stimulation of cortical neurons with KCl[44]. Because

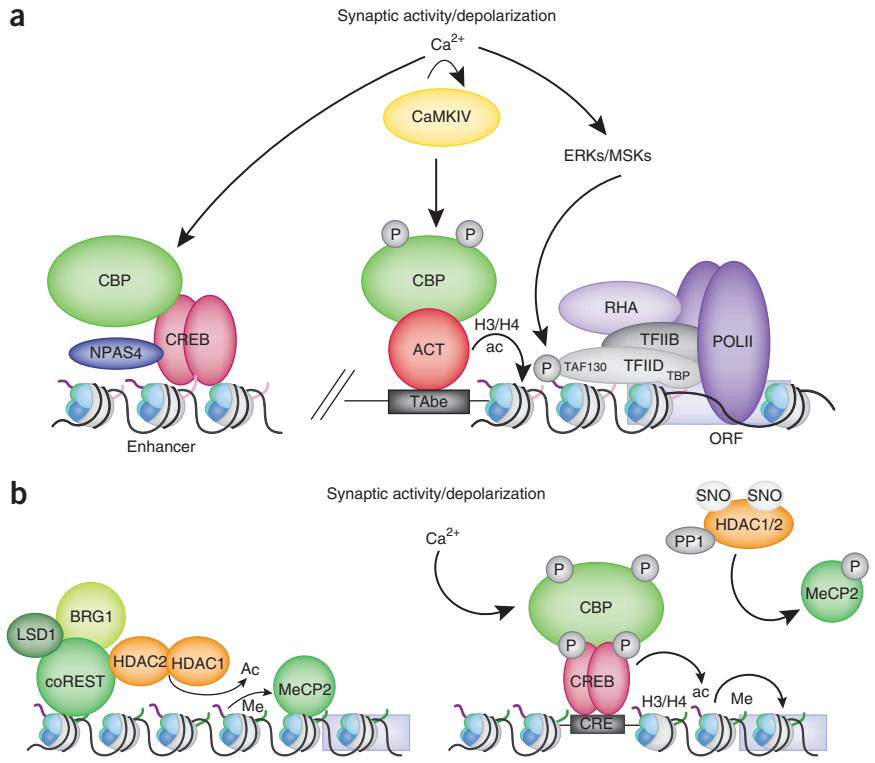


**Figure 1** Activity-dependent epigenetic regulation. (**a**) Synaptic activity and depolarizing stimuli phosphorylate CBP and influence transcription by increasing intracellular $Ca^{2+}$ through both NMDA receptors and L-type $Ca^{2+}$ channels, although activation of CaMKIV is required only for the latter. The concomitant phosphorylation of histone H3 on serine-10 cooperates with histone acetylation (ac) to induce chromatin infolding and gene expression. Neural activity also induces the recruitment of CBP, CREB and the cofactor NPAS4 to transcriptional enhancers of synaptic activity-dependent genes. (**b**) In differentiated neurons, certain neuronal genes such as *Bdnf* are maintained in a repressed state through a mechanism that includes the recruitment of coREST, HDAC1, HDAC2 and MeCP2. After synaptic stimulation, HDAC2, and possibly HDAC1, are S-nitrosylated (SNO), whereas MeCP2 is phosphorylated, resulting in the dissociation of the co-repressor complex from gene regulatory regions and the recruitment of co-activators. ACT, activator; ORF, open reading frame; PP1, protein phosphatase 1; RHA, RNA helicase A; TAbe, transcriptional activator binding element.

activity-dependent binding of CREB to gene promoters depends on the acetylation state of the chromatin that surrounds CRE sequences and is mediated by calcium signaling and nitric oxide[45,46], the recruitment of both CBP and CREB to chromatin may share similar molecular mechanisms and signaling pathways.

Neural activity can also modulate chromatin acetylation by regulating the intracellular distribution of HDAC. The class II HDACs can shuttle in and out of the nucleus, and this shuttling can be regulated by extracellular cues. In hippocampal neurons, for example, nuclear export of HDAC4 is induced by spontaneous electrical activity, whereas HDAC5 export depends on $Ca^{2+}$ influx mediated by the activation of NMDA receptors[47].

In addition to acetylation, synaptic activity also influences changes in chromatin structure by inducing other histone modifications, such as histone H3 phosphorylation. Inducible histone H3 phosphorylation on serine 10 was first demonstrated in human fibroblasts in response to stimulation with epidermal growth factor (EGF) and activation of RSK2 (ref. 48). More recently, this epigenetic mark has been detected in a number of neuronal cell types and in response to multiple signaling pathways. For example, in the hippocampus activation of both the MAPK-ERK (extracellular signal–related kinase) and MSK (mitogen and stress–activated kinase) pathways results in histone H3 phosphorylation[49–51]. Interestingly, histone H3 phosphorylation induced by synaptic activity is associated with a change in nuclear structure[51], which might suggest that nuclear geometry and transcriptional response are functionally linked.

## Neurotrophin-dependent epigenetic changes

The neurotrophins are a family of peptide growth factors that promote the growth and survival of neurons by activating the Trk family of receptor tyrosine kinases. The binding of neurotrophin to Trk receptors triggers the activation of several signaling cascades, most prominently the RAS-MAPK, $InsP_3$ kinase-AKT and phospholipase-Cγ (PLCγ) pathways[52]. The transcriptional response to neurotrophins is mediated by the activation of a host of nuclear factors, including CREB, SRF, c-Fos, c-Jun, MEF2D and FOXO1 (ref. 35). Neurotrophins also directly induce epigenetic changes by activating CBP through the p42/44 MAPK pathway[39] (**Fig. 2**). In the nerve growth factor (NGF)-responsive PC12 cell line, CBP is constitutively associated with p42/44 MAPK. After exposure to NGF (which differentiates PC12 cells into sympathetic-like neurons), CBP becomes phosphorylated and transcriptionally active. Although the amino acid residue(s) that are targeted by p42/44 MAPK have not been identified, they are likely to be located within the growth factor (GF) box (amino acids 312–440), a small region of CBP that is required for growth factor-dependent activation of CBP-regulated genes[53]. Activation of CBP by NGF depends on multiple intracellular pathways. In PC12 cells, NGF induces the association of S6 kinase pp90RSK with CBP through a mechanism that requires the Ras pathway[53] (**Fig. 2**). Although assembly of the pp90RSK–CBP complex has been shown to mediate the expression of Ras-responsive genes in PC12 cells exposed to NGF, it is unclear whether this pathway is also linked to transcription activation by neurotrophic factors in primary neurons.

An additional mechanism by which neurotrophins regulate the functions of CBP and p300 relies on the nuclear translocation of the transcriptional coactivator and acetyl transferase pCAF[54]. In PC12 cells, NGF causes pCAF to dissociate from the PP1–PP2 protein phosphatases complex, to become phosphorylated and to accumulate in the nucleus. A number of kinases that are activated by NGF signaling, including phosphoinositide-3-kinase and the $Ca^{2+}$-dependent kinases RSK2 and MSK1, are required for nuclear translocation of pCAF[54]. pCAF-dependent acetylation of nuclear substrates, including the transcription factor p53 and possibly histones, are required for NGF-dependent neuronal differentiation.

Neurotrophins and other stimuli that increase intracellular calcium, including synaptic activity, can directly affect epigenetic events by inducing the accumulation of nuclear nitric oxide (NO) and *S*-nitrosylation of nuclear factors[46]. The main source of NO in the nervous system is the neuron-specific, $Ca^{2+}$-dependent isoform of NO synthase (nNOS), whose expression in the brain is tightly

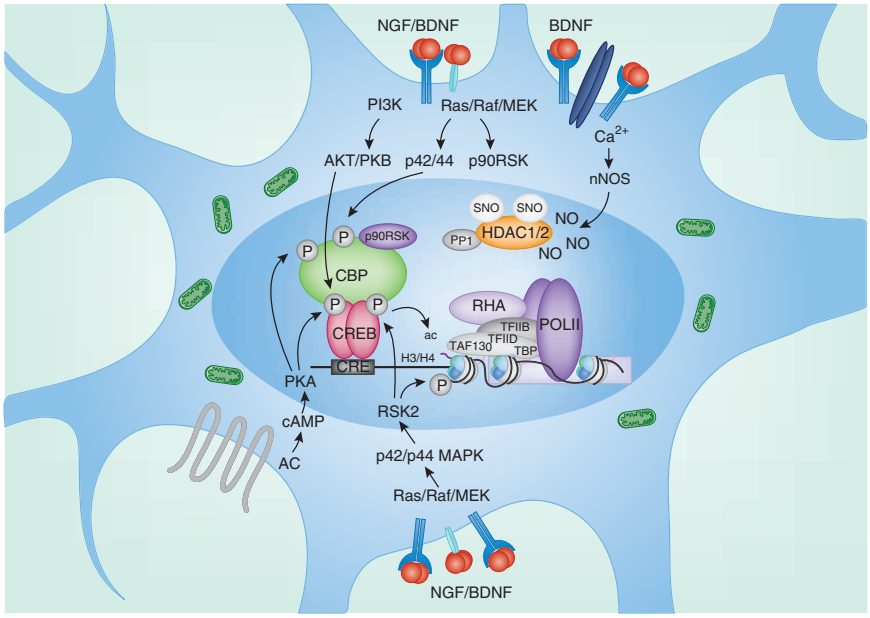


**Figure 2** Epigenetic regulation by neurotrophins. Binding of neurotrophins to Trk receptors initiates a number of signaling pathways that induce phosphorylation of CREB and CBP. BDNF-dependent activation of nNOS increases nuclear NO and triggers *S*-nitrosylation (SNO) of several nuclear proteins, including HDAC2. The dissociation of *S*-nitrosylated HDAC2 and the recruitment of phosphorylated CBP and transcription factors such as CREB lead to transcriptional activation. Both phosphorylation of histone H3 through RSK2 and CBP-dependent histone acetylation (ac) contribute to gene activation. AC, adenylyl cyclase; ac, acetyl.

regulated both temporally and spatially[55]. NO signaling modulates gene expression through the activation of multiple transcription factors, including CREB, N-Myc, NF-κB, p53 and egr-1 (refs. 45,56). *S*-nitrosylation is the covalent and reversible addition of a NO group to cysteine thiols[57]. *S*-nitrosylation regulates the activity of several proteins by affecting their enzymatic activity, protein-protein interactions or subcellular localization[58]. *S*-nitrosylation triggered by inflammatory stimuli directly regulates the activity of various zinc finger–containing transcription factors, including NF-κB[56]. A recent protein-DNA screen has shown that NO donors increase the binding of many transcription factors to their regulatory *cis*-elements, which suggests that NO, or NO-mediated post-translational modifications, might influence gene expression in a more general and profound manner[59].

In cortical neurons that are exposed to brain-derived neurotrophic factor (BDNF), *S*-nitrosylation of HDAC2 occurs at two residues (Cys262 and Cys274) and does not affect its deacetylase activity (**Fig. 2**). Instead, *S*-nitrosylation of HDAC2 induces its dissociation from BDNF-regulated gene promoters, thereby increasing histone acetylation and gene transcription at specific promoter regions[46]. When Cys262 and Cys274 were changed to alanines (which cannot be *S*-nitrosylated), BDNF-dependent histone acetylation in cortical neurons was inhibited and transcription was strongly repressed. HDAC1 is probably nitrosylated on the same cysteine residues, but it does not seem to be associated with the same promoters as HDAC2.

NO-dependent inhibition of HDAC2 activity has also been found in a mouse model of muscular dystrophy[60]. In muscle cells, *S*-nitrosylation of HDAC2 on an unidentified cysteine residue decreases its deacetylase activity, which in neurons remains unchanged. This discrepancy might reflect a difference in the cysteine(s) that are targeted by NO in muscle cells and in neurons; in theory, *S*-nitrosylation of HDAC2 could also occur at Cys152, which is located within the deacetylase active site. In addition, the transcriptional profile of muscle cells and neurons differs greatly, possibly because HDAC2 might be recruited to repressive complexes of cell-type specific genes through distinct mechanisms.

## Regulation of DNA methylation in neurons

Neuronal activity influences DNA methylation in the regulatory regions of genes that are regulated by synaptic activity. DNA methylation was considered for a long time to be a stable and irreversible modification that marked transcriptionally inactive genes. However, there is increasing evidence that in the brain, DNA methylation can be relieved by conditions that either reduce the activity of DNA methyl transferases (DNMTs) or induce the dissociation of nuclear proteins, such as methyl-CpG binding protein 2 (MeCP2), from CpG methylated sequences[5]. For example, activity-dependent changes in DNA methylation correlate with transcriptional repression of genes that mediate the neuronal response that is associated with memory formation[61]. After contextual fear conditioning, *DNMT1* mRNA was increased during a hippocampus-dependent associative memory paradigm. Interestingly, methylation of a CpG island in the promoter of the gene for protein phosphatase 1 (PP1), which is known to suppress memory, was also increased, whereas the gene that encodes reelin, which promotes memory formation[62], was rapidly demethylated.

Although DNA methylation has traditionally been linked to transcriptional inhibition, a genome-wide analysis of the occupancy of the DNA methyl transferase DNMT3-a in neural stem cells (NSCs) has shown that DNA methylation is not restricted to promoter regions of genes that are repressed during postnatal neurogenesis[63]. In NSCs, DNMT3-a was also associated with methylated non-proximal promoter regions of genes that are transcribed during neurogenesis. There was binding of DNMT3-a at genomic regions that were characterized by high levels of H3K4me3, an epigenetic mark that is associated with transcriptional activation, and low levels of H3K273me, a modification that correlates with transcriptional repression. Importantly, DNMT3-a mediated gene expression by counteracting the activation of polycomb repression complex 2 (PRC2), a nuclear repressor complex that is recruited to chromatin by a number of mechanisms, including specific interaction with H3K273me (refs. 63,64).

In the hippocampus, neural activity raises intracellular Gadd45b, which has been shown to possess DNA demethylase activity *in vitro*[65]. Mice that lack Gadd45b show a remarkable decrease in activity-dependent adult neurogenesis *in vivo* and their neurons show a marked reduction in dendritic growth *in vitro*. Importantly, exposure of newborn neurons to depolarizing conditions induced Gadd45b-dependent demethylation of the promotors for *Bdnf* and fibroblast growth factor-1 (*Fgf-1*) promoters. Although these findings suggest an alternative mechanism by which neural activity triggers DNA demethylation and gene transcription, the signaling mechanisms that link physiological synaptic activation with Gadd45b-dependent DNA demethylation remain unclear.

Finally, the discovery that DNA from the nuclei of cerebellar Purkinje cells is surprisingly enriched in 5-hydroxylmethyl cytosine (hmdC)[66] suggests that DNA demethylation in the brain might be regulated in a unique manner. Interestingly, the methyl-CpG binding motif of MeCP2 has low affinity for DNA sequences that contain hmdC[67]. This finding might point toward situations in which changes in DNA methylation in neurons could consist predominantly of oxidative demethylation of hmdC rather than conventional DNA demethylation.

In most cases, MeCP2 bound to methylated DNA acts as a repressor of transcription[68,69] (see ref. 70 for possible exceptions). MeCP2 is also subject to dynamic regulation. Its activity-dependent phosphorylation in cortical neurons enhances *Bdnf* gene expression and dendritic growth through a mechanism that requires elevated intracellular $Ca^{2+}$ and activation of calmodulin-dependent kinases[71,72] (**Fig. 1b**). Interestingly, the phosphorylation of MeCP2 on serine 421 by CaMKII is found only in the brain[73]. In agreement with its essential role for normal brain function, MeCP2 is extraordinarily abundant in mature neurons[74]. Genome-wide analyses of MeCP2 occupancy[74,75] revealed that in the adult brain, MeCP2 binds to methylated DNA throughout the genome, and is not restricted to discrete gene promoters, as had been assumed. The brains of mice that lacked MeCP2 showed a widespread increase in both histone H3 acetylation and histone H1 levels[74], suggesting that MeCP2 might influence global changes in chromatin state perhaps by competing with histone H1 for binding to methylated DNA. These findings also suggest that MeCP2 may recruit co-repressor complexes that depress histone acetylation in regions that are rich in methylated DNA.

These and other studies indicate that dynamic changes in DNA methylation have a key role in regulating many neuronal functions, from dendritic growth to neurogenesis and memory formation. DNA methylases, some of which might be undiscovered, might therefore represent novel therapeutic targets for certain neurodegenerative disorders.

## Regulation of epigenetic state during neuronal development

The fine tuning of epigenetic changes that dictate the transcriptional response during neuronal development is achieved, at least in part, by the assembly of large, functionally malleable multiprotein complexes that can switch genes on and off by changing their subunit composition. In neurons, the REST nuclear protein (RE-1 silencing transcription factor, also called NRSF) provides one of the best examples of cooperation between co-repressors to establish epigenetic modifications that influence the transcription of neuron-specific genes. REST was originally identified as a nuclear factor that binds the repressor element 1 (RE-1)[76,77]. Because the promoters of many neuron-specific genes that encode ion channels, synaptic vesicle proteins and neurotransmitter receptors contain an RE-1 motif[78,79], it was proposed that REST acts as a master regulator of neurogenesis and neuronal differentiation. An initial hypothesis therefore suggested that the main role of REST was to silence neuronal genes in non-neuronal cells. Analysis of the REST mutant mouse that REST is required for the correct development of the nervous system[80], but the lack of widespread expression of neuronal genes in non-neuronal tissues of these mice indicated that additional mechanisms are involved in inhibiting the ectopic expression of neuron-specific genes.

REST-mediated gene repression requires the recruitment of two separate co-repressor complexes, mSin3 and CoREST. Despite the fact that mSin3 and CoREST bind distinct domains of REST (the N-terminal and C-terminal domain, respectively), they mostly cooperate to ensure robust repression of target genes[78,79]. HDAC1 and HDAC2 are recruited by both repressors and are at the core of a dynamic multiprotein complex that includes other nuclear factors, such as the ATP-dependent chromatin-remodeling enzyme BRG1 (ref. 81), the histone demethylase LSD1 (ref. 82) and, in the presence of high nuclear levels of NADH, the NADH-dependent repressor CtBP[83,84]. The orchestrated action of these co-repressors ensures that modifications that are associated with gene activation, such as histone H3K9 acetylation and histone H3K4 methylation, are removed, and that others associated with transcriptional repression are added, resulting in a profound remodeling of REST-bound chromatin. HDAC1 and HDAC2 make a key contribution to the initial phase of this process by deacetylating histones H3 and H4 and thereby enhancing LSD1 demethylase activity[81].

The subunit composition of the REST complex also regulates the transition from temporary transcriptional repression in stem cells to long-term silencing of RE-1–containing genes in non-neuronal, terminally differentiated cells[85]. In embryonic stem cells, and presumably in neuronal progenitors, HDAC1 and HDAC2 are recruited to the promoters of neuron-specific genes through REST and its co-repressors mSin3 and CoREST. Even if histones are deacetylated and transcription is repressed, the lack of DNA methylation in the chromatin that surrounds the RE-1 sites ensures that the genes are kept in a poised status[85]. Similarly, a family of small PolII C-terminal-domain phosphatases (SCOs) are bound to the 5′ untranslated regions of RE-1 promoters, which keeps neuronal genes inactive but in a permissive state that is ready for subsequent activation[79,86]. By contrast, in terminally differentiated, non-neuronal cells, long-term repression of neuron-specific genes is achieved through the recruitment of the DNA methylase DNMT1 to RE-1 sites[78,79,87]. This event is associated with DNA methylation and MeCP2 binding[88].

Some aspects of the regulatory mechanisms that underlie REST-dependent gene repression remain unclear, in particular the regulation of the levels of REST mRNA and protein. These are modulated in response to several stimuli and are associated with the switch from short-term to long-term gene inactivation[78]. REST is lost during neuronal differentiation, but recruitment of HDAC1 and HDAC2 through CoREST on several gene promoters ensures the transcriptional inhibition of REST-regulated genes that are expressed at low levels but are poised for activation[79]. In differentiated neurons, synaptic activity increases the levels of *Bdnf* transcript at least in part by the selective release of MeCP2 from CoREST complexes on the BDNF promoter[85].

Finally, the binding of other cofactors to the REST complex can also be dynamically regulated. For example, the co-repressor CtBP is recruited to REST complexes under conditions that affect the redox state of neurons, including epilepsy and brain ischemia[84]. Further regulated interactions between the REST–CoREST complex and additional co-factors, including HDAC1 and HDAC2, might influence the functions of REST, perhaps also in response to growth factor stimulation or synaptic activity.

## Regulation of chromatin remodeling by subunit switch

In eukaryotic cells, changes in chromatin structure are achieved by at least three distinct mechanisms: DNA methylation, histone modifications and ATP-dependent chromatin remodeling. Chromatin remodeling refers to dynamic structural changes that involve the disruption and reformation of DNA–histone complexes[89]. As a result of this process, nucleosomes 'slide' along the DNA molecule, facilitating the access of nuclear transcriptional complexes to previously inaccessible DNA. The activation of ATP-dependent chromatin remodeling factors is associated with both transcriptional activation and repression.

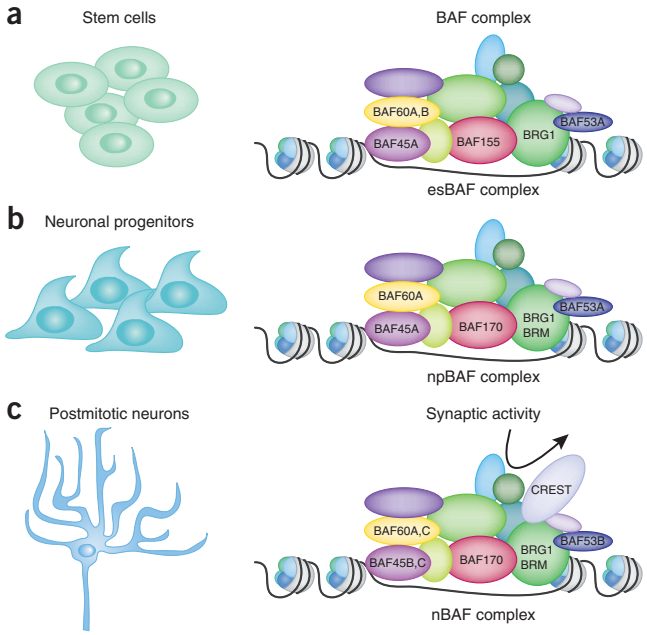


**Figure 3** Composition of BAF complexes at different stages of neuronal differentiation. A number of subunits of the BAF complex are switched during neuronal differentiation (**a**,**b**). The subunits labeled undergo developmentally regulated switches, and most of them have been shown not to be exchangeable under various experimental conditions. (**c**) In postmitotic neurons, the association of BRG1 with the calcium-responsive factor CREST mediates synaptic activity–dependent dendrite outgrowth.

In yeast, the ATP-dependent remodeling complex SWI–SNF functions mainly as a transcriptional activator, whereas the homologous mammalian BAF complex can act as either a repressor or an activator; it can switch between the two modes on one promoter[90,91]. The BAF complex includes a helicase-like subunit that uses the energy derived from ATP hydrolysis to induce a twist in nucleosomal DNA, thereby displacing the histone octamer relative to the DNA[89,92]. In mammalian cells, distinct families of genes encode the ten core subunits of BAF. Subunits that belong to homologous gene families assume mutually exclusive occupancy in the complex, allowing the combinatorial assembly of functionally diverse remodeling complexes with similar subunit compositions[92].

A number of BAF subunits have been identified in screens for genes that are involved in neuronal development[6]. Mice that lack the core ATPase subunit Brg1, for example, show severe defects of the neural tube and die early in embryogenesis[93]. Later during neuronal development, BAF complexes undergo a subunit switch that is essential for the transition from proliferating neuronal precursors to committed neuronal lineages (**Fig. 3**). The BAF45 and BAF53 subunits undergo developmental switches in the mouse brain[94]. Extending the expression of the progenitor-specific subunits BAF43A and BAF53A in chick embryos inhibited neuronal differentiation. These and other findings suggest that in stem cells, neuronal progenitors and differentiated neurons, specific subunit switches in BAF complexes are likely to coordinate the activation of specific transcriptional programs.

As well as mediating long-lasting changes in gene expression associated with neuronal development, BAF complexes can also respond to synaptic activation by recruiting cofactors that are regulated by neural activity. In postmitotic neurons, BAF complexes recruit the $Ca^{2+}$-responsive transactivator CREST, and this association is required for dendritic outgrowth[95,96] (**Fig. 3c**). CREST then can recruit CBP to gene promoters in response to neural activity. Interestingly, CREST and Brg1 have opposing effects on calcium-regulated gene expression, suggesting that CREST might act as a switch between activity-dependent transcriptional activation and repression[96].

## Conclusions

Chromatin is a highly dynamic and plastic structure that can integrate multiple extracellular signals to generate the coordinated transcriptional response that is necessary for the maintenance of cellular homeostasis. Epigenetic changes in both histones and DNA can have lasting effects on neural development and plasticity. Although much is known about the nature of the enzymes that dictate epigenetic changes in neurons, neurobiologists have just begun to investigate their regulation and the interplay among the signaling pathways that regulate their activity.

Many challenging tasks lie ahead. First, for many known post-translational modifications that affect the functions of chromatin regulatory enzymes, the signaling mechanisms that link them to environmental cues remain obscure. For example, class I HDACs are subject to nitrosylation, phosphorylation, acetylation, sumoylation and ubiquitination, and they specifically interact with sphingosine 1 phosphate[97,98]. Whereas all of these modifications can affect HDAC functions, they have rarely been linked to physiological stimuli. The identification of the signaling pathways that regulate the activity of histone-modifying enzymes will greatly enhance our understanding of how neuronal transcriptional programs respond to environmental changes.

Second, it is vital to understand the interplay between signal transduction pathways that lead to changes in the epigenetic landscape. This is not an easy task, especially because distinct signals might affect the duration rather than the nature of epigenetic marks. However, evidence of cooperation between signaling pathways is already emerging. One example is provided by CBP, which is phosphorylated by several kinases in response to both neural activity and neurotrophins. It is tempting to speculate that the coordination of multiple phosphorylation events might be crucial to orchestrate finely tuned activation of CBP, and that this could induce a graded transcriptional response to environmental cues. Moreover, the cooperation between distinct signaling pathways is likely to be at the core of the transition between the short- and long-term epigenetic changes that are required for the proper development of the nervous system. Stable chromatin modifications might be necessary to ensure that genes that need to be expressed only at early developmental stages remain inactive in differentiated neurons and throughout adulthood. By contrast, rapid changes in epigenetic marks are required for a fast transcriptional response to synaptic activation. Both the REST–CoREST and BAF complexes provide remarkable examples of how ductile chromatin remodeling complexes can, in principle, switch between long-lasting developmentally regulated transcriptional changes and more short-term effects in response to neuronal activity.

Finally, a higher level of transcriptional regulation in neurons relies on stimulus-dependent modification of chromatin architecture and nuclear structure. It is becoming increasingly clear that the three-dimensional organization of the genome influences the recruitment of *cis*-acting regulatory elements and chromatin-modifying complexes to distinct transcriptionally active hot spots, also called 'transcriptional factories'[99]. In the nervous system, nuclear architecture has been shown to undergo substantial changes during the different stages of differentiation, presumably in response to developmental cues[100]. Three-dimensional image reconstruction analyses showed that the nuclei of hippocampal neurons undergo infolding and

changes in chromatin organization after a short-term burst of synaptic activation[51]. The increase in nuclear infolding, correlated with transcriptional activation, was initiated by stimulation of intrasynaptic NMDA receptors, and was mediated by an increase in intracellular $Ca^{2+}$ and ERK-MAPK signaling. These findings suggest that changes in nuclear architecture might be required not only for long-lasting expression of specific genes in terminally differentiated cells but also for more rapid transcriptional responses. Although the analysis of chromatin architecture adds yet another layer of complexity to an already intricate field, deciphering epigenetic regulation in its entirety will be necessary if we are to fully understand neuronal functions, and remains one of the most exciting challenges in modern biology.

**ACKNOWLEDGMENTS**

The author is grateful to L. Crepaldi and A. Nott for reading the manuscript. A.R. is the recipient of an MRC Senior Non Clinical Fellowship.

**COMPETING FINANCIAL INTERESTS**

The author declares no competing financial interests.

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