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MOLECULAR PLASTICITY

Epigenetics and epilepsy

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Summary

Seizures can give rise to enduring changes that reflect alterations in gene-expression patterns, intracellular and intercellular signaling, and ultimately network alterations that are a hallmark of epilepsy. A growing body of literature suggests that long-term changes in gene transcription associated with epilepsy are mediated via modulation of chromatin structure. One transcription factor in particular, repressor element 1-silencing transcription factor (REST), has received a lot of attention due to the possibility that it may control fundamental transcription patterns that drive circuit excitability, seizures, and epilepsy. REST represses a suite of genes in the nervous system by utilizing nuclear protein complexes that were originally identified as mediators of epigenetic inheritance. Epigenetics has traditionally referred to mechanisms that allow a heritable change in gene expression in the absence of DNA mutation. However a more contemporaneous definition acknowledges that many of the mechanisms used to perpetuate epigenetic traits in dividing cells are utilized by neurons to control activity-dependent gene expression. This review surveys what is currently understood about the role of epigenetic mechanisms in epilepsy. We discuss how REST controls gene expression to affect circuit excitability and neurogenesis in epilepsy. We also discuss how the repressor methyl-CpG-binding protein 2 (MeCP2) and activator cyclic AMP response element binding protein (CREB) regulate neuronal activity and are themselves controlled by activity. Finally we highlight possible future directions in the field of epigenetics and epilepsy.

KEY WORDS: REST, RE1, NRSF, Epilepsy, Epigenetics, Chromatin, Histone, Neurogenesis, MeCP2, CREB, Seizure, Excitability, G9a, CoREST, SIN3, HDAC.

Epigenetic Regulation of Chromatin Structure by REST Controls Transcription

Humans possess approximately 2 meters of DNA per cell, which encodes approximately 23,000 protein-coding genes. Therefore, 3×10^9 bases of DNA have to be packed into a nucleus in such a way that the nuclear machinery can access genes to modulate gene transcription levels. In eukaryotes, this is accomplished by combining the functions of DNA-packaging and gene regulation. DNA is wrapped around clusters of small basic proteins termed histones, and the resultant nucleoprotein polymer is termed chromatin. The wrapping of DNA around histones results in packaging and compaction, which aids in packing the DNA fiber into the nucleus. The repeating unit of chromatin is the nucleosome, which consists of 140 base pairs of DNA wrapped around an octamer of two H3 histones, two H4 histones, two H2A histones, and two H2B histones (Luger et al., 1997). The histone octamer at the heart of a nucleosome is not an inert structure that serves merely to wrap DNA. The nature of histone–DNA interactions is dynamically regulated to control accessibility of genes to the transcriptional apparatus. Histones possess long N-terminal tails that protrude from the nucleosome and are prone to a diverse array of posttranslational modifications such as acetylation, methylation, phosphorylation, and ribosylation. These modifications serve to modulate the nature of histone–DNA interaction and act as docking sites for other protein complexes, which ultimately control the transcription level of genes (Strahl & Allis, 2000 and references therein; Strahl & Allis, 2000; Fig. 1). The pattern of histone modification

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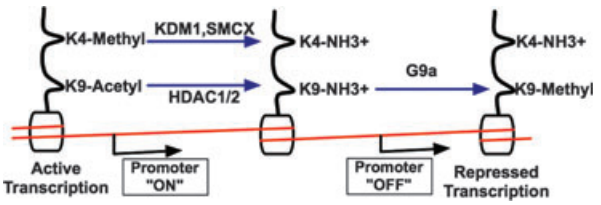

Figure 1.
DNA (red) is wrapped around histones (black barrels) to form nucleosomes. Posttranslational modifications on lysine (K) residues dictate promoter activity on the underlying DNA. These modifications are catalyzed by enzyme complexes, some of which are utilized by REST (indicated).
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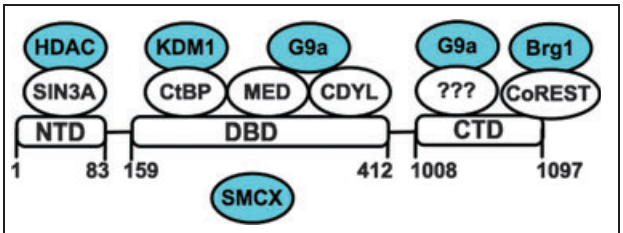

Figure 2.
REST enables many histone modifications on its NRSE-containing target genes by recruiting a host of lysine-modifying enzymes via multiple corepressors. Numbers refer to residues on REST. Colored molecules possess enzymatic activity. SMCY homologue, X-linked (SMCX) binds REST but the domain has yet to be determined.
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marks are sometimes referred to as a histone code that is read by histone readers; enzymes that attach or remove modifications ''write'' the code, whereas proteins that bind the modified histone tails ''read'' the code (Strahl & Allis, 2000).

Repressor element 1-silencing transcription factor (REST) is a transcriptional repressor that functions to limit transcription of target genes. REST binds to a 17–33 bp element termed the RE1 site (repressor element-1, also called the NRSE, neuron restrictive silencing element) via a DNA binding domain consisting of a cluster of eight zinc fingers. This sequence element is found in the regulatory regions of about 1,800 genes (Johnson et al., 2007), with many of the genes being expressed preferentially in non-neuronal cells. Upon binding the RE1 site, REST recruits a number of corepressor complexes that cannot themselves directly bind DNA and have no sequence specificity. Corepressor complexes consist of multiple proteins and possess enzymes that catalyze the posttranslational modifications of histone tails as well as the mobilization of whole nucleosomes. REST has three repression domains that mediate corepressor recruitment: The N-terminal domain (NTD), C-terminal domain (CTD), and the DNA binding domain (DBD), which also doubles as a repression domain (Fig. 2).

The NTD binds the SIN3 histone deacetylase complex (HDAC; Huang et al., 1999; Grimes et al., 2000; Roopra et al., 2000). The two mammalian *SIN3* genes encode SIN3A and SIN3B, which share a similar domain structure. SIN3A and SIN3B contain an HDAC interaction domain (HID) that binds the HDAC1/2 enzymes. HDAC1 and HDAC2 catalyze the removal of acetyl groups from lysines on histone tails. Generally, acetylated histones render genes on nearby DNA active. This is partly due to acetyl groups neutralizing the positive charge on lysine amino groups and thus reducing electrostatic interactions between the histone tails and negatively charged DNA backbone and neighboring nucleosomes. The resulting loose DNA–histone and histone–histone interactions allow easy access of RNA polymerase complexes to promoter sequences, thereby making transcription efficient. Acetylated histones also act as docking sites for proteins that possess a bromo domain. Many positive acting coactivator complexes contain this domain such that activation of a gene by acetylation results in a platform for coactivator binding and perpetuation of the activated state. Therefore, the SIN3–HDAC complex acts to ''write'' a histone code—removal of an acetyl group—that is ''read'' by bromo domain–containing proteins. By recruiting the SIN3–HDAC complex to the promoter regions of neuronal genes, REST directs the removal of acetyl marks allowing compaction of chromatin and dismissal of coactivator complexes, and thereby reduces the rate of transcription.

The CTD binds a number of proteins. CoREST was the first REST corepressor to be described (Andres et al., 1999). In the context of REST, CoREST is part of a chromatin remodeling complex containing BRG1 called hSWI/SNF (Battaglioli et al., 2002). Chromatin remodeling, as compared to chromatin modification, describes the ATP-dependent movement of nucleosomes on DNA. It is likely that the CTD recruits CoREST and associated ATPase to shuffle nucleosomes to cover or expose other elements including the promoter to repress transcription.

The CTD also binds the G9a histone methylase (Roopra et al., 2004). G9a catalyzes the addition of two methyl groups to lysine 9 of histone H3 (H3K9; Tachibana et al., 2001). Methylation of H3K9 (to yield dimethylated H5K9—H3K9Me2) generates a binding site for the histone binding protein heterochromatic protein-1 (HP1). HP1 contains a chromodomain that specifically binds methyl-H3K9. HP1 then binds methylated H3K9 and condenses chromatin to limit transcription (Bannister et al., 2001). Again, G9a would be considered a code writer and HP1 would be the histone code reader. Intriguingly G9a

itself contains six ankyrin repeats that were recently shown to form a methyl H3K9 binding motif (Collins et al., 2008). It is tempting to speculate that REST may recruit G9a to generate a local chromatin domain of H3K9Me2 that subsequently acts as a platform for continued G9a presence even if REST disappears from this locus. This would be a mechanistic explanation for epigenetic silencing of a locus.

The DNA-binding domain of REST doubles as a repression domain. A complex of proteins termed Mediator binds the REST zinc finger cluster and independently recruits G9a to the DBD (Ding et al., 2008). Mediator is a highly conserved nuclear complex found in yeast through mammals. It serves as an interface between RNA polymerase and multiple activators and repressors, thus acting as a conduit through which transcription factors can communicate with the transcription apparatus.

Just as methylation of histone H3 lysine 9 is associated with gene repression, methylation of a nearby residue, H3K4, is associated with gene activation (Santos-Rosa et al., 2002). REST recruits the histone demethylase KDM1 (also known as LSD1), which catalyzes the conversion of dim-methylated H3K4 to H3K4 (i.e., H3K4Me2 to H3K4; Shi et al., 2004). KDM1 also binds the DBD, although not via Mediator (Schoenike BS, Roopra A, unpublished data). The H3K4Me2 mark acts as a docking site for the activating ISWI complex, and so removal of methylation at H3K4 prevents ISWI binding (Wysocka et al., 2006). Furthermore, histone tails that are unmethylated at H3K4 bind the repressive nucleosome remodeling domain (NuRD) complex; methylation at K4 prevents NuRD binding (Zegerman et al., 2002). Hence, H3K4 demethylation by REST and KDM1 may repress transcription by the simultaneous shedding of activating complexes and binding of repressive complexes to local chromatin.

REST also utilizes the corepressor C-terminal binding protein (CtBP—so named because it was originally cloned as a protein that interacts with the C-terminus of the viral E1a protein) to repress transcription (Garriga-Canut et al., 2006). As with KDM1 and Mediator, CtBP is bound by the DNA binding domain (Schoenike BS, Roopra A, unpublished data). CtBP exists as two highly similar isoforms CtBP1 and CtBP2, encoded by different genes and their interaction with REST is indistinguishable (Chinnadurai, 2002). CtBP binding to REST, and some other transcription factors, is regulated by energy metabolism (Zhang et al., 2002; Mirnezami et al., 2003). CtBP undergoes an allosteric conformational shift upon binding with reduced nicotinamide adenine dinucleotide (NADH; Kumar et al., 2002). High NADH levels cause CtBP to be dismissed by REST, resulting in de-repression of transcription. Low NADH levels allow CtBP to bind REST and shut down transcription. Because the bulk of nuclear NADH is thought to be in equilibrium with cytoplasmic NADH and is derived in large part through glycolysis (Stryer, 2002), lower rates of glycolysis result in reduced NADH, increased CtBP binding to REST, and more repression of REST target genes. This mechanism was exploited by Garriga-Canut et al. (2006) to control proepileptic gene expression in the kindling model using glycolytic inhibitors (see below).

It should be noted that Humphrey et al. (2001) described a CoREST complex that includes G9a, KDM1, and CtBP1. However, REST recruits G9a, KDM1, and CtBP independently of CoREST. Therefore, G9a binds the CTD in the absence of CoREST binding (Roopra et al., 2004), and CtBP and KDM1 bind the DBD, a region not bound by CoREST (Schoenike BS, Roopra A, unpublished data). In summary, REST utilizes an array of chromatin modifying and remodeling complexes to control transcription of genes that contribute to epilepsy. A detailed molecular understanding of at least one of these (CtBP) has resulted in a new treatment regimen to test in clinical trials. Future advances in chromatin studies are likely to yield further novel approaches to therapeutic regulation of gene expression in epilepsy.

A Role for REST in Epilepsy

The prolonged seizures of generalized status epilepticus (SE) in humans and rodents trigger a series of molecular and cellular events that eventually culminate in the appearance of spontaneous seizures, that is, epilepsy. These events include selective neuronal degeneration, inflammatory reactions involving reactive microglia and astroglia, selective axonal sprouting with new synapse formation, neurogenesis, and a myriad of changes in synaptic efficacy in the hippocampus. The breadth of the phenotypic consequences of SE, and their elaboration over days and weeks, raises the possibility that one or more broad regulators of gene expression could mediate some or even many of these consequences.

As described above and schematized in Fig. 2, REST is a transcriptional repressor that recruits histone deacetylases, demethylases, and methyltransferases to cause epigenetic remodeling of chromatin architecture around the REST target genes (Huang et al., 1999; Roopra et al., 2000, 2004; Garriga-Canut et al., 2006; Tahiliani et al., 2007; Ding et al., 2008; Mulligan et al., 2008). The most prominent modifications occur on H3K4 and H3K9 (Fig. 1; Zheng et al., 2009).

REST is strongly induced in hippocampal pyramidal and dentate granule neurons after SE induced by kainate (Palm et al., 1998) or pilocarpine (Fig. 3). More than 1,300 genes, or approximately 5% of the protein-encoding genome, are confirmed REST targets (Bruce et al., 2004; Johnson et al., 2006), including many genes known to be involved in neuronal excitability (Roopra et al., 2001), making REST an excellent candidate transcription factor

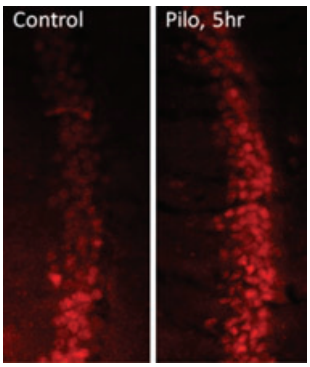

Figure 3.
Rapid induction of REST protein in dentate granule neurons 5 h after pilocarpine-induced SE (Hsieh J, Dingledine R, unpublished data).

to mediate seizure-induced widespread changes in gene expression. REST target genes are highly overrepresented among the differentially expressed genes after SE (Dingledine R, Hsieh J, unpublished data), consistent with a wide scope of REST's influence.

Cooperation among the multiple histone-modifying enzymes recruited by REST suggests that the ultimate effect on transcription can be context and cell dependent. For example, although REST is best known as a transcriptional repressor, in some cases there is credible evidence that REST or its truncated splice variant, REST4, might act as an activator of gene expression (Kuwabara et al., 2004; Abramovitz et al., 2008). The picture is thus emerging of a dynamic set of REST protein complexes working together to determine cell-specific and activity-dependent gene expression profiles that, in turn, drive incredibly diverse biologic processes. REST recruits at least three classes of epigenetic modifier enzymes (Fig. 1), some of which might have a net effect of promoting and others opposing epileptogenesis. In this respect, whether the net effect of REST-mediated changes in gene expression promotes or opposes epileptogenesis is controversial at this point.

Some ion channel genes are both epigenetically regulated and repressed after seizures including Gria2, which encodes GluA2 (Myers et al., 1998; Huang et al., 1999), and HCN1(McClelland et al., 2011). HCN1 (hyperpolarization-activated cyclic nucleotide-regulated cation channel) typically functions to dampen excitability in cortical neurons. Mice lacking HCN1 in the forebrain exhibit more seizures and higher mortality in both kindling and pilocarpine models (Santoro et al., 2010). This finding suggests that HCN1 down-regulation following severe seizures may contribute to disease progression. McClelland et al. (2011) showed that REST binding to the RE1 element in the HCN1 promoter in the hippocampus was augmented 2 days after kainate-induced status epilepticus, and that intraventricular administration of oligodeoxynucleotides targeted to the HCN1-RE1 both disrupted REST binding to the HCN1 promoter and prevented down-regulation of HCN1 protein. It is important to note that rescue of HCN1 protein levels was accompanied by full restoration of the I_h current amplitude in CA1 pyramidal cell dendrites and by fewer spontaneous seizures in the chronic epilepsy phase. The experimental design did not rule out roles for REST target genes other than HCN1, but this study does strongly reinforce the notion that REST induction after SE contributes to the development of epilepsy.

Recently, more direct evidence for the roles of neuronal REST expression in epilepsy has been acquired following the creation of mice in which the REST gene had been conditionally deleted in glutamatergic forebrain neurons (Hu et al., 2011a) or all neurons (Liu et al., 2012). In the initial studies, a conditional REST knockout (nREST cKO-CAMKII) was created by crossing a mouse line bearing a floxed REST gene with a mouse line expressing cre recombinase under the CAMKII promoter. In the kindling model, nREST cKO-CAMKII mice exhibited dramatically accelerated seizure progression and prolonged afterdischarge duration compared with control mice (Hu et al., 2011a). This finding suggests that in the kindling model, REST may function to oppose epileptogenesis. Quite a different result was obtained in the pentylenetetrazol (PTZ) model of acute seizures, using nREST cKO-NSE mice created using neuron-specific enolase cre, which ablates the REST gene from most if not all neurons. Although the initial clonic convulsions caused by PTZ were not different between nREST cKO-NSE and control mice, tonic convulsions and death required a higher PTZ dose in nREST cKO-NSE mice (Liu et al., 2012). These findings, considered alone, would suggest that REST might contribute to seizure initiation or generalization. Taken together with the findings from the nREST cKO-CAMKII mice, however, it is unclear whether the opposing conclusions are due to the animal model (kindling vs. PTZ), the subset of cells from which the REST gene had been ablated, or some unknown, laboratory-specific factor.

A potential role for REST in the ketogenic diet therapy for epilepsy has been reported by two groups. Garriga-Canut et al. (2006) reasoned that, because the ketogenic diet is high fat plus low carbohydrate, glycolytic inhibition itself by 2-deoxyglucose (2-DG) might replicate the anticonvulsant effect of the diet. They tested this hypothesis with the kindling model and found that systemic administration of 2-DG could retard the progression of seizure intensity during kindling. Moreover, they found that 2-DG treatment caused decreased expression of brain-derived neurotrophic factor (BDNF) and tyrosine kinase receptor B (TrkB), both REST target genes. These results are consistent with the observation that knockout of TrkB or

BDNF showed reduced or zero epileptogenesis in the kindling model of temporal lobe epilepsy (He et al., 2004). Reduction in BDNF and TrkB was accompanied by deacetylation and methylation of lysine 9 on H3 associated with the BDNF RE1 element that is the binding site for REST. The transcriptional corepressor CtBP (Fig. 2), which is allosterically regulated by NADH (see above), was shown to mediate the formation of the repressive chromatin environment in 2-DG treated animals. Following this study, the antiepileptic effect of 2-DG in the kindling model was found to be abolished in nREST-cKO-CAMKII mice (Hu et al., 2011b), indicating that REST expression in forebrain glutamatergic neurons could be required for the antiepileptic effect of 2-DG. Of interest, the antiepileptic effect of the ketogenic diet itself was maintained in nREST-cKO-CAMKII mice, pointing to a benefit of the high fat diet that is independent of glycolytic inhibition.

In a related study, global ischemia elevated REST RNA and protein levels in CA1 pyramidal neurons and in dentate granule neurons of rats (Calderone et al., 2003). Notably, acute knockdown of REST expression prevented down-regulation of the REST target gene, GluA2, which correlated with an attenuation of ischemia-induced cell death of CA1 neurons. In a follow-up study, Noh et al. (2012) demonstrated that local depletion of REST by intrahippocampal injection of lentiviruses expressing either REST RNAi or a dominant negative REST construct could virtually abolish the appearance of FluoroJade-stained, injured neurons in hippocampal CA1 measured 6 days after global ischemia (Noh et al., 2012). Systemic administration of the broad-spectrum HDAC inhibitor, trichostatin A, replicated the effects of REST knockdown. These studies indicate that REST expression and HDAC activity seem critical for ischemia-induced neurodegeneration. It will be important to extend these studies to epilepsy models.

Seizure-Induced Neurogenesis and the Role of REST

In addition to the myriad of cellular changes triggered by SE, seizure activity also rapidly and potently increases the production of new neurons, in both hippocampal subgranular zone (SGZ) and lateral ventricles of subventricular zone (SVZ) of the adult rodent brain (Parent et al., 1997; Parent & Lowenstein, 2002). In the first few days after SE there is marked cell death in the hilar region and molecular layer of the hippocampus. This is followed by robust proliferation of transit-amplifying progenitors (type 2 cells) and increased proliferative activity of doublecortin-positive cells in the SGZ by 1 week after SE (Parent et al., 1997; Jessberger et al., 2005). Between 7 and 21 days after SE, seizure-induced neurons display features of ''aberrant'' neurogenesis, such as mossy fiber sprouting, hilar basal dendrites, and ectopic granule cells, which may contribute to spontaneous recurrent seizures (Parent et al., 1997, 2006; Parent & Lowenstein, 2002; Kuruba & Shetty, 2007; Scharfman et al., 2007; Kuruba et al., 2009). Because adult-generated granule neurons are essential for memory and mood control (Aimone et al., 2011; Sahay et al., 2011; Snyder et al., 2011; Petrik et al., 2012), it is possible that seizure-induced neurogenesis contributes to the cognitive deficits in hippocampal learning and memory that are associated with SE (Stafstrom et al., 1993; Holmes, 1997; Parent & Lowenstein, 2002; Parent et al., 2007).

The extent to which adult-generated neurons are altered if they undergo differentiation in a pathologic environment and contribute to the development of epilepsy is not known. This knowledge is necessary to evaluate their suitability for cell replacement strategies after injury—whether new neurons compromise or contribute to functional recovery in the diseased brain. Therefore, uncovering the molecular mechanisms involved in seizure-generated neurogenesis may assist understanding of the pathophysiology underlying epilepsy development. To fully understand the role seizure-induced neurons may play in epileptogenesis, it is imperative to understand the regulatory cascade controlling adult neural stem cells (NSCs).

As mentioned earlier, REST transcripts are rapidly induced in the hippocampus after seizures (Palm et al., 1998). In addition to its role in mature neurons, REST also plays a key role in the stem/progenitor cell compartment (Ballas et al., 2005; Gao et al., 2011). During development, a dual regulatory mechanism of REST, at the level of mRNA and protein, is important in the transition between embryonic stem (ES) cells and neural progenitors The REST gene is actively transcribed in ES and neural progenitor cells; however, when ES cells exit the cell cycle and differentiate into mature neurons, REST becomes repressed by the unliganded retinoic acid receptor (RAR) repressor complex (Ballas et al., 2005). REST is also posttranslationally degraded when ES cells transition into neural progenitor cells (Ballas et al., 2005; Westbrook et al., 2008). During cortical neurogenesis, release of REST and its corepressor complex from neuronal chromatin is associated with progenitor differentiation into cortical neurons, and RE1-containing target genes become activated, consistent with REST being a transcriptional repressor (Ballas et al., 2005).

Recent work from our laboratory demonstrated an essential role for REST in adult hippocampal neurogenesis (Gao et al., 2011). Conditional deletion of REST in nestin-expressing stem cells (type 1 cells) and their progeny resulted in a transient increase in adult hippocampal neurogenesis, followed by an eventual depletion of proliferating type 1 cells over time. Mechanistically, REST and its corepressors CoREST and mSin3A are recruited to

control stage-specific neuronal gene expression such as *Ascl1* and *NeuroD1*, which restrains the neurogenic program. These results suggest that REST is not only important in preventing precocious neuronal differentiation, but is also required for maintaining a population of quiescent adult NSCs, which could have potential implications in brain repair after injury or during aging.

One key question is whether REST plays a role in seizure-induced neurogenesis, as it does in basal neurogenesis. In addition, what distinguishes seizure-induced neurogenesis from physiologic neurogenesis? To examine this, we previously reported that the antiepileptic valproic acid (VPA) blocked seizure-induced aberrant neurogenesis, which appeared to be mediated by inhibiting histone deacetylases (HDACs) and normalizing REST-regulated gene expression within the epileptic dentate gyrus (Jessberger et al., 2007). Moreover, VPA treatment potently protected epileptic rats from hippocampus-dependent cognitive impairment after kainic acid–induced seizures. Given these results, it will be interesting in future studies to assess the functional requirement of REST in seizure-induced aberrant neurogenesis using conditional knockout approaches.

Epilepsy and Epigenetics—MeCP2 and CREB Control of BDNF

From these studies, one theme that emerges is that neuronal activity (via Ca^{2+} influx) triggers transcriptional and epigenetic changes, which is a critical aspect in development and in nervous system function (Ernfors et al., 1991; West et al., 2001, 2002). Notwithstanding, neuronal activity induced by seizures also leads to rapid expression of activity-induced genes, such as BDNF(Zafra et al., 1990; Dugich-Djordjevic et al., 1992; Lin et al., 2008). BDNF is a small, secreted protein that binds to its receptors TrkB and p75 and is well studied for its central roles in synaptic plasticity, neuronal survival, and differentiation (Poo, 2001). One question is how BDNF mRNA expression is spatially and temporally controlled by neuronal activity? One upstream regulator of BDNF is the transcriptional repressor methyl-CpG binding protein 2 (MeCP2) which, when mutated, causes Rett syndrome, a major autism-spectrum neurologic disorder (Amir et al., 1999).

Previous reports from multiple groups (Chen et al., 2003; Martinowich et al., 2003; Ballas & Mandel, 2005) have suggested that dynamic regulation of MeCP2 by Ca^{2+} influx plays a pivotal role in regulating specific programs of activity-dependent gene transcription important for nervous system function. Strikingly, seizures induced by kainic acid or metrazole are a potent mediator of MeCP2 phosphorylation selectively in the brain (Zhou et al., 2006). Conceivably, disruptions of gene programs regulated by neuronal activity may underlie the pathology of Rett syndrome and epilepsy.

Using tandem mass spectrometry, two groups showed that membrane depolarization of neurons led to the production of a slow-migrating, phosphorylated form of MeCP2, which exhibited reduced binding to methylated DNA and correlated with the transcriptional induction of an activity-regulated gene, *Bdnf* (Chen et al., 2003; Zhou et al., 2006; Tao et al., 2009). In one study, neuronal activity was reported to induce phosphorylation of MeCP2 at S421, which was thought to control activity-dependent gene expression and neuronal spine maturation (Zhou et al., 2006). A follow-up study added that, in addition to phosphorylation at S421 (and S424), neuronal activity triggered dephosphorylation at S80 of MeCP2 from normal and epileptic brains (Tao et al., 2009). The study went on to show that the dephosphorylation of MeCP2 at S80 contributes to its decreased association with some of its target chromatin regions. Of interest, $MeCP2^{S80A}$ knock-in mice showed decreased locomotor activity, whereas $MeCP2^{S421A;\ S424A}$ knock-in mice showed increased locomotor activity, suggesting opposite regulation of S421 and S80 phosphorylation in response to neuronal activity. The distinct phenotypes of the S80 and S421 knock-in mice (as well as data from in vitro experiments) is consistent with a role of S80 phosphorylation in resting neurons, whereas S421 phosphorylation may be critical in activity-induced neurons.

Recently, Li et al. (2011) separately generated $MeCP2^{S421A;\ S424A/y}$ knock-in mice and performed artificial depolarization, high-frequency electrical stimulation in the hippocampus, and behavioral training. It is notable that their work revealed that loss of activity-induced phosphorylation of MeCP2 enhanced excitatory synaptogenesis, hippocampal long-term potentiation, and spatial memory. However, exploratory activity was not significantly altered between $MeCP2^{S421A;\ S424A/y}$ mice and wild-type littermates, in contrast to the decreased locomotor activity observed by Tao et al. (2009). These differences might be explained by differences in the activity test (dark cycle running wheel test vs. open field test). Together, these studies highlight the importance of MeCP2 phosphorylation as a molecular switch to regulate BDNF gene transcription and other activity-induced genes.

In addition to MeCP2, several other regulators of Ca^{2+}-dependent up-regulation of BDNF promoter IV have been identified, including classic studies describing the cAMP response element binding protein (CREB) binding to a cAMP/Ca^{2+} response element (CRE; Sheng et al., 1991; Shieh et al., 1998; Tao et al., 1998, 2002). More recently, one group generated several mutant mouse models with impaired activity-dependent *Bdnf* expression (Hong et al., 2008). Mutation of one of the CREB-response elements CaRE3/CRE (CREm) at endogenous *Bdnf* promoter IV

resulted in mice in which the neuronal activity-dependent component of *Bdnf* transcription in the cortex is specifically disrupted. CREm knock-in mice displayed a reduction in the number of inhibitory synapses from cortical neurons in vitro and in vivo. Taken together, these studies highlight the importance of neuronal activity-mediated epigenetic mechanisms in regulating the development of inhibition, which may be important for appropriate excitatory–inhibitory balance critical for normal brain physiology and function.

Looking Forward

Epigenetic regulation of gene expression in the nervous system represents an exciting area for future basic as well as translational research (see Lubin, 2012 for an interesting overview). Much of the work to date has focused on the role that individual transcription factors such as REST or MeCP2 play in the regulation of a few genes. Whole genome expression analysis will allow a more integrated approach to understanding how multiple transcription factors coordinate the expression of suites of genes under both physiologic and pathologic conditions. Genome-wide analysis will allow us to understand how programs of genes are controlled. Such network level analysis will force a shift from looking at the role of individual genes and chromatin marks in disease to looking at how network topologies differ in the pathologic and physiologic condition. A more complete understanding of the molecular mechanisms utilized by REST, MeCP2, CREB, and other chromatin modifiers will surely facilitate this transition.

In terms of REST itself, a number of pressing questions remain to be answered. REST exists as two main splice variants (Palm et al., 1999). Although full-length REST has been extensively studied, the role of the truncated variant REST4 remains enigmatic, with reports suggesting it is a repressor, activator, or a null molecule with no function (Magin et al., 2002; Abramovitz et al., 2008). In many cases, splicing of a transcript to generate a truncated protein is a mechanism to induce nonsense mediated decay (NMD) of mRNA and is widely utilized to control mRNA availability (Maquat, 2004). Why splicing of REST to generate a small, truncated isoform via insertion of a small exon, the very conditions that normally trigger NMD, fails to target REST mRNA is totally unexplored.

REST function seems to be protective in the electrical kindling model of temporal lobe epilepsy (Garriga-Canut et al., 2006; Hu et al., 2011b) and yet detrimental in other models (Hu et al., 2011a; McClelland et al., 2011). Finally, dysregulation of REST has also been implicated in the pathogenesis of Alzheimer's disease (Okazaki et al., 1995), Huntington's disease (Zuccato et al., 2003), X-linked mental retardation (Tahiliani et al., 2007), and breast cancer (Wagoner et al., 2010; Gunsalus et al., 2012). Therefore, there is a growing list of diseases dependent on REST dysregulation, ensuring a rich albeit technologically challenging field for years to come.

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Disclosure

The authors declare no conflict of interest. The authors confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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