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A Tale of Two Epiphenomena: The Complex Interplay of Epigenetics and Epilepsy

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1. Introduction

Epilepsy is a disorder primarily characterized by the spontaneous recurrence of unprovoked seizures. Seizures can be triggered by multiple factors including genetic mutations, head injury, toxins, a fever, high or low blood sugar, a tumor, electrolyte imbalance, drug withdrawal; and are also a core component of developmental and degenerative disorders (Loscher and Brandt 2010). However, not all patients that have seizures go on to develop epilepsy, and the mechanisms of epileptogenesis are still poorly understood. Only a small number of genetic mutations identified in ion channels or proteins associated with these channels have been directly linked to causing epilepsy (Greenberg and Pal 2007). In fact, complex epidemiological studies indicate that the interplay of environmental factors with relatively minor genetic alterations may contribute to the difference between the susceptibility to suffer seizures, and the development of epilepsy (Ottman et al. 1996). Evidence is emerging that epileptogenesis involves changes in the expression patterns of several classes of functionally or genomically-grouped genes that coordinate neural development, homeostasis and stress responses, and neural network formation (Lukasiuk et al. 2006 and references therein). This has led to speculation that minor and modifiable changes outside the open reading frames of affected genes could alter the course of epilepsy. How entire groups of genes may be co-regulated with precision during different stages of neural development and function could be the result of epigenetic changes in histone and chromatin structure and DNA methylation that accompany shifts in neural "state."

Chromatin structure and function can be altered to silence gene expression by DNA methylation leading to the recruitment of methyl-DNA binding proteins and histone deacetylation. Histones can also be modified at their N-terminus by phosphorylation, acetylation, methylation, ubiquitination, ADP ribosylation, carbonylation, SUMOylation, glycosylation and biotinylation. Here we will focus on DNA methylation and histone acetylation, and discuss how these epigenetic modifications could regulate developmental alterations that may contribute to the process of epileptogenesis. We will summarize how epigenetic changes may both regulate and be regulated by activity-dependent synaptic plasticity, and how involvement of common mechanisms underlying glial-neuronal interactions could lead to epileptogenesis. Finally, we will discuss how intervening in epilepsy by treating with widely-used drugs that themselves can alter chromatin state (like Valproic Acid) may further affect ongoing epileptogenesis, and discuss which specific epigenetic modifications may be novel therapeutic targets for the treatment of epilepsy.

2. Epigenetics and development

With limited exceptions, all cells in the human body have an identical genotype, and yet development produces a wide range of differentiated cell types with distinct functions that form highly specialized tissues and organs. This is especially true in the immensely complex and highly structured central nervous system (CNS). Cells differentiate from a stem cell to become increasingly specialized through a process of state-dependent (stage- and lineage-specific) gene activation and gene silencing, as many genomic regions become folded into heterochromatin, and are excluded from transcription (silenced) (MacDonald and Roskams 2009). Thus, progressive cell differentiation results, in part, from the epigenetic regulation of gene expression, which has been operationally defined as the study of heritable changes in gene function that are independent of changes in the underlying DNA sequence (Berger et al. 2009).

2.1 Chromatin and histone modifications

Both DNA and its associated histone proteins are subject to epigenetic modifications that change the overall structure of chromatin and the physical appearance of DNA within the nucleus (Goldberg, Allis and Bernstein 2007) (Figure 1). The fundamental unit of eukaryotic chromatin is the nucleosome, and it is composed of 147 base pairs of DNA wrapped around an octamer of four core histone proteins (H3, H4, H2A and H2B). The core histones are predominantly globular with flexible tails located at the N-terminus (Luger et al. 1997, Strahl and Allis 2000, Wade 2001). These tails vary in length and are composed of amino acids that allow for at least nine distinct types of modifications: phosphorylation, acetylation, methylation, ubiquitination, ADP ribosylation, carbonylation, SUMOylation, glycosylation and biotinylation (reviewed in Kouzarides 2007). Histone modifications correlate with gene activation or repression (Jenuwein and Allis 2001), and recently, histone domains containing both activating and repressive modifications have been identified (Azuara et al. 2006, Bernstein et al. 2005, Bernstein et al. 2006). These modifications can be context-dependent allowing gene transcription or silencing depending on the localization in the coding region versus the regulatory regions flanking the promoter (Kouzarides 2007, Vakoc et al. 2005). In addition to distinct histone modifications, DNA methylation can also silence gene transcription; and there is evidence of significant cross-talk between these processes to dynamically modify chromatin structure in response to external stimuli (Bernstein, Meissner and Lander 2007, Fuks 2005, Goldberg et al. 2007, Kouzarides 2007).

Both DNA and histone proteins are subject to epigenetic modifications like DNA methylation and histone acetylation that change the overall structure of chromatin, and alter the extent to which DNA is wrapped around histones and therefore the availability of genes to be activated.

2.1.1 DNA methylation

DNA methylation takes place post-synthesis, and is a chief determinant of the stability of gene expression states (Jaenisch and Bird 2003). In vertebrates, and in humans specifically, DNA methylation happens almost exclusively within cytosine–phosphate–guanine (CpG) dinucleotides, and 70-80% of all CpG dinucleotides are methylated (Ehrlich et al. 1982); (reviewed in Bird 2002, Goll and Bestor 2005). DNA methylation is catalyzed by DNA methyltransferases, or DNMTs, which covalently bind a methyl group to position C5 of cytosine residues (Bird 1992). DNA methylation represses transcription directly by

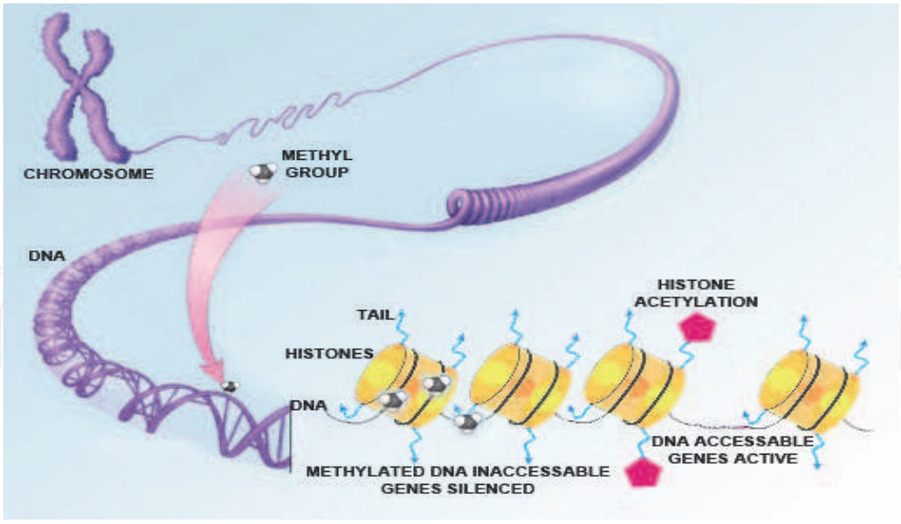

Fig. 1. Epigenetic regulation in the nucleosome (modified from the NIH http://nihroadmap.nih.gov/EPIGENOMICS/images/epigeneticmechanisms.jpg) Both DNA and histone proteins are subject to epigenetic modifications like DNA methylation and histone acetylation that change the overall structure of chromatin, and alter the extent to which DNA is wrapped around histones and therefore the availability of genes to be activated.

inhibiting the binding of specific transcription factors, and indirectly, by recruiting methyl-CpG-binding (MBD) proteins and their associated repressive chromatin remodeling activities (Robertson 2005). DNMT1 is the predominant enzyme in mammals, and is responsible for maintenance methylation (post-replicative restoration of hemi-methylated sites to full methylation), whereas DNMT3A and DNMT3B are thought to be involved primarily in *de novo* methylation (Laird 2003). Methylation is necessary for mammalian development, and mice with DNMT knocked out die during development (Li, Bestor and Jaenisch 1992, Okano et al. 1999). This absolute requirement for DNA methylation in development likely reflects the diverse range of cellular functions and pathologies to which it contributes, including silencing of repetitive and centromeric sequences, tissue-specific gene expression, genomic imprinting, maintenance of X chromosome inactivation, carcinogenesis and aging (Bird 2002, Jaenisch and Bird 2003, Jones and Baylin 2002, Paulsen and Ferguson-Smith 2001).

2.1.2 Histone deacetylation

Histone deacetylases (HDACs) regulate gene expression by removing the acetyl groups of specific lysine residues from histone protein tails, thereby increasing their positive charge, and enhancing their interaction with the negatively charged phosphate groups in the DNA backbone. The functional consequence of deacetylation is to stabilize compacted forms of chromatin, which can restrict binding of transcription factors to promoter regions of genes. This is a reversible reaction, and histone acetyl transferases (HATs) can add acetyl groups to lysine residues, removing the positive charge and loosening the chromatin from the histone core. In general, increased histone acetylation (hyperacetylation) is associated with increased transcriptional activity, whereas decreased acetylation (deacetylation or hypoacetylation) is associated with repression of gene expression (Strahl and Allis 2000, Wade 2001). There are four main classes of HDACs which are grouped into class I, class II,

class III and class IV based on their sequence homology to their yeast orthologues Rpd3, HdaI and Sir2, respectively (de Ruijter et al. 2003, Gregoretti, Lee and Goodson 2004). The classical HDACs are comprised of Class I (HDACs 1, 2, 3, and 8), Class II (HDACs 4, 5, 6, 7, 9, and 10), and Class IV (HDAC 11), and are zinc-dependent enzymes with divergent patterns of expression in the brain (Bjerling et al. 2002, Fischle et al. 2002, Yang and Seto 2008). Class I HDACs have a highly conserved sequence homology and are relatively small proteins (377–488 amino acids) that primarily localize in the nucleus. Class II HDACs are larger proteins (669–1215 amino acids) that are capable of shuttling in and out of the nucleus in response to cellular signals like activity-dependent calcium release (de Ruijter et al. 2003, Yang and Seto 2008). The lone class IV member, HDAC11, shows sequence similarity to both class I and class II HDACs, but phylogenetic analysis reveals that it is divergent enough to warrant a separate class (Gao et al. 2002, Gregoretti et al. 2004). Class III HDACs or sirtuins are nicotinamide adenine dinucleotide (NAD+) dependent and may be important for linking metabolic state in cells to gene expression (Guarente and Picard 2005).

3. Epigenetically regulated developmental changes in the brain can lead to epilepsy

As many cell types throughout the brain differentiate, there is a concurrent shift in expression of DNMTs, HATs and HDACs that subsequently alter the compaction of chromatin. Developmental changes in epigenetic state thus underlie distinct shifts in gene expression that ultimately allow for structural and functional organization of the brain through control of neuro- and gliogenesis, and activity-dependent synaptic plasticity. Each stage of neural development carries a signature gene expression pattern, with a progressive restriction in the expression of developmentally regulated genes as maturation proceeds (Schuurmans and Guillemot 2002, Tietjen et al. 2003, Abramova et al. 2005, Lim et al. 2006). How repression or silencing as a result of epigenetic changes in chromatin contributes to these shifts in gene expression is slowly becoming better understood. Perhaps more importantly, improper regulation of each of these steps can lead to a variety of pathologies - apoptosis, alterations in neuro- and gliogenesis, aberrant neuronal migration, ectopic integration of neurons and glia causing structural malformations, and the formation of hyperexcitable circuits, all of which may contribute to seizure activity. Moreover, seizure activity itself can exacerbate many of these pathologies, and further perturb epigenetic factors, resulting in epileptogenesis and cognitive impairment.

3.1 Methylation state modulates neuro- and gliogenesis

In general, the known developmental effects of DNA methylation on gene expression involve long-term silencing of gene expression such as the establishment of parental-specific imprints during meiosis and X-chromosome inactivation (Jaenisch and Bird 2003), but recently much attention has been paid to its role in regulating gene expression during neuro- and gliogenesis. DNMTs are directly involved in neuronal maturation and survival (Fan et al. 2001, MacDonald, Gin and Roskams 2005, Feng et al. 2005). In a study by Fan and colleagues, conditional knock out of DNMT1 in nestin-positive cells is prenatal lethal, but mosaic mice with 30% of their neural cells missing DNMT1 survive into adulthood (Fan et al. 2001). In these animals, DNMT1-deficient neural precursor cells give rise to hypo-methylated progeny cells, including postmitotic neurons. However, within three weeks postnatal, all DNMT1-negative cells are eliminated, suggesting that these neurons were not

stable enough to functionally mature (Fan et al. 2001). The *de novo* DNMTs (DNMT3a and DNMT3b) are sequentially expressed during neurogenesis, and are critical for regulating genes directly implicated in neurogenesis and neural function (Jin et al. 2008, Feng et al. 2005). Dnmt3b may be important for the early phase of neurogenesis, while Dnmt3a regulates prenatal progenitors as well as the maturation of post-mitotic neurons (Feng et al. 2005).

DNMT1 is also a critical cell-intrinsic determinant of astrocyte differentiation. For example, the promoter of glial fibrillary acid protein (GFAP) is methylated in progenitor cells during the neurogenic stages of embryonic development, but at later stages during gliogenesis, the promoter becomes demethylated (Teter et al. 1996). Methylation of the STAT binding element within the GFAP promoter inhibits association of activated STATs with the glial promoter (Takizawa et al. 2001), thereby repressing transcription of GFAP and preventing cells from proceeding down an astroglial lineage during the neurogenic stages of brain development (reviewed in MacDonald and Roskams 2009). However, if DNMT1 is knocked out, precocious astroglial differentiation occurs, presumably through hypomethylation of the GFAP promoter and other genes encoding the core components of the gliogenic JAK-STAT pathway (Fan et al. 2005). Since neurons rely on radial glial cells in order to migrate to the appropriate position during development, one consequence of precocious astrocyte formation could be the loss of radial glial "guide wires" and subsequent structural malformations caused by aberrant neuronal migration. These structural malformations are increasingly being discovered in epileptic patients as brain imaging technology advances (Scaravilli 1998).

MBD proteins are also regulators of neurogenesis, particularly in adult neurogenic niches like the subventricular zone. While MBD1 knockout mice are viable and appear relatively normal, they do have decreased neuronal differentiation of adult stem cells and diminished hippocampal neurogenesis (Zhao et al. 2003). MeCP2 is perhaps the best characterized MBD protein, because mutations within its coding region cause Rett syndrome (Amir et al. 1999, Bienvenu and Chelly 2006). Analysis of MeCP2 knockout mice revealed aberrant regulation of factors responsible for neurotransmitter biosynthesis and for promoting the differentiation and maturation of various neural cell types (Urdinguio et al. 2008), suggesting that MeCP2 regulates genes that are known to be involved in epileptogenesis. Furthermore, because seizures occur in many Rett patients (Glaze, Schultz and Frost 1998), there is strong evidence for a direct relationship between dysregulated MBD proteins and epilepsy.

3.2 Acetylation state modulates neuro- and gliogenesis

HDAC1 and HDAC2 are expressed at distinct stages of neuronal commitment and differentiation during CNS development (MacDonald and Roskams 2008), allowing them to modulate gene expression across neurodevelopmental stages. HDAC1 is enriched in progenitors clustered in neurogenic zones throughout the CNS (MacDonald and Roskams 2008). Neural progenitors that maintain the expression of HDAC1 largely differentiate into glial cells, while those that lose HDAC1 expression and begin to upregulate HDAC2 differentiate into neurons (MacDonald and Roskams 2008). In fact, HDAC1 is also highly expressed in the corpus callosum during oligodendrocyte differentiation, and when HDACs are inhibited, oligodendrocytes fail to differentiate and cause hypomyelination in the corpus callosum of postnatal rats (Shen, Li and Casaccia-Bonnefil 2005). Further to this, an elegant body of work has placed Class 1 HDACs, and HDAC1 in particular, as a critical regulator of the production and differentiation of oligodendrocyte precursor cells (Shen and Casaccia-Bonnefil 2008). HDAC1 can also directly regulate stem cell proliferation, as HDAC1 null animals display a significant reduction in cell proliferation (Lagger et al. 2002). HDAC2, on

the other hand, is necessary to inhibit astrocyte differentiation, while HDAC1 is not (Humphrey et al. 2008). Taken together, HDAC2 may be involved in silencing glial gene expression, while HDAC1 likely silences neuronal genes.

HDAC1 and HDAC2 have been proposed to work in concert through large multi-protein complexes like REST nuclear protein (RE-1 silencing transcription factor, also called NRSF) and Co-REST. Through these complexes, HDAC-mediated acetylation can enable the transcriptional repression of genes containing a repressor element-1 (RE1/NRSE) in their promoter (Huang, Myers and Dingledine 1999). Because many neuron-specific genes that encode ion channels, synaptic vesicle proteins and neurotransmitter receptors contain an RE-1 motif, it has been proposed that REST silences these genes in all other cell types and acts as a master regulator of neurogenesis and neuronal differentiation (Ooi and Wood 2007, Ballas and Mandel 2005, Hsieh and Gage 2005). Intriguingly, REST is responsible for regulating the expression of several genes implicated in epileptogenesis, including growth factors, ion channels, neurotransmitter receptors, gap junctions, and neurosecretory vesicles, as well as those involved in seminal neural developmental processes and adult neurogenesis (reviewed in Qureshi and Mehler 2009, Qureshi and Mehler 2010)

In summary, abnormal activity of epigenetic mediators including DNMTs, MBDs, HDACs and repressor complexes could result in altered neuro- and gliogenesis, aberrant migration of newly born cells, and improper integration of these cells into circuits, thereby causing hyper-excitable circuits and seizures (Figure 2).

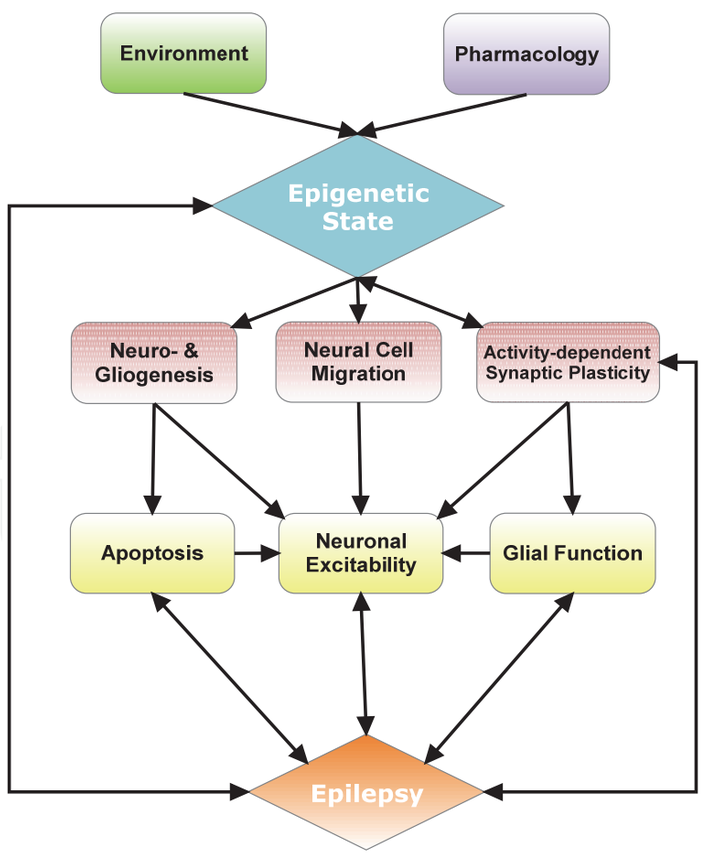

Fig. 2. Epigenetic gene regulation can dynamically impact numerous physiological processes in the nervous system.

Epigenetic state can be altered by extrinsic factors such as environmental stimulation and pharmacological manipulation, and internal factors that regulate neuronal activity. Since epigenetic factors such as DNMTs, MBDs, HDACs and repressor complexes modulate processes that include neuro- and gliogenesis, neural cell migration, and activity-dependent synaptic plasticity; dysregulation of these epigenetic factors can lead to apoptosis, neuronal hyperexcitability, impaired glial function, and ultimately can contribute to seizure activity, epileptogenesis, and epilepsy. Conversely, the hyperexcitability associated with epilepsy can also impact the expression of genes that regulate epigenetic state.

4. Epilepsy modulates epigenetic state and affects brain morphology

Clearly a dysregulation of epigenetic factors during development causes changes in neuro- and gliogenesis that may increase the likelihood of developing seizures, but the reversed scenario can also occur. It is now clear that seizures themselves can regulate epigenetic factors that then affect neurogenesis, neuronal migration, synapse formation, and neural activity.

It is well established that seizures cause alterations in neurogenesis in animal models and humans, but there is an age-dependent paradoxical effect in how seizures alter cell genesis in the CNS. For example, there is a postictal decrease in dentate granule cell birth in one week old rats subjected multiple brief seizures (Houser 1990, McCabe et al. 2001, Liu et al. 2003). This postictal decrease is age-related because a similar seizure paradigm performed in adult rats causes an increase in dentate granule cell birth (Houser 1990, McCabe et al. 2001, Liu et al. 2003). Furthermore, these effects depend on the frequency and severity of the seizures. Acute seizure activity causes a proliferative surge in neural stem cells of the subgranular zone of the hippocampus leading to increased production of new neurons during the first few weeks after the seizure episode, while chronic epilepsy is associated with declined hippocampal neurogenesis (Houser 1990, Parent et al. 1997, Scharfman, Goodman and Sollas 2000, McCabe et al. 2001, Porter 2008, Kuruba, Hattiangady and Shetty 2009). Surgically resected hippocampi from children with extra-hippocampal seizures also reveal a significant decrease in hippocampal neurons (Mathern et al. 1994), suggesting that the rate of cell death is exceeding the rate of neurogenesis and potential cell replacement.

Other pathologies associated with epilepsy-induced dysregulation of epigenetic factors include altered neuronal migration and increased mossy fiber (MF) sprouting. Examination of surgically resected hippocampal tissue from patients with temporal lobe epilepsy (TLE) confirms aberrant supragranular inner molecular layer MF sprouting, and a pathology known as granule cell dispersion (GCD), which has been linked to decreased expression of the glycoprotein Reelin (Haas et al. 2002, Haas and Frotscher 2010, Mathern et al. 1994). Reelin is critical for mediating neuronal migration throughout the CNS, and hippocampal patterning during brain development (Stanfield and Cowan 1979, Forster, Zhao and Frotscher 2006), and its expression is regulated directly by promoter methylation (Levenson, Qiu and Weeber 2008, Kobow et al. 2009). Recently, hippocampal tissue samples from TLE patients revealed increased promoter methylation in TLE specimens compared to controls, and this was significantly correlated with GCD (Kobow et al. 2009), implying that chronic epilepsy can cause epigenetic changes that may exacerbate disease pathology. In fact, seizure activity can perturb the migration of newly born neurons postnatally, resulting in their ectopic location in the hilus (and even as far as CA3), aberrant synapse formation, and consequently enhanced excitability (Scharfman et al. 2000).

Seizures can induce histone modifications for a number of genes involved in neuronal plasticity and synapse formation, including the neurotrophin brain-derived neurotrophic factor (BDNF) and the glutamate receptor GluR2 (Tsankova, Kumar and Nestler 2004, Huang, Doherty and Dingledine 2002). Seizure activity induces acetylation of the BDNF promoter, thereby up-regulating BDNF (Tsankova et al. 2004). BDNF upregulation following seizure activity is thought to contribute to epileptogenesis, and BDNF infusion in epileptic animals can trigger seizure-like events (Scharfman, Goodman and Sollas 1999). In addition, transgenic mice that overexpress BDNF display heightened seizure susceptibility, spontaneous seizures and hyperexcitability of the hippocampus (Croll et al. 1999). Furthermore, BDNF has been shown to attenuate γ-aminobutyric acid (GABA)ergic inhibitory neurotransmission (Tanaka, Saito and Matsuki 1997), which can lead to an imbalance in neuronal excitatory transmission. At the same time, seizure activity leads to deacetylation of histones at the GluR2 promoter, and reduced expression of the receptor, resulting in enhanced AMPA receptor-mediated epileptogenesis (Sanchez et al. 2001). Thus, seizure activity can induce epigenetic changes that contribute to epileptogenesis, but why does this occur? It is highly likely that seizure activity harnesses much of the same molecular machinery involved in activity-dependent synapse formation and learning and memory.

5. Epigenetic factors can both regulate, and be regulated by, synaptic plasticity

Repeated patterns of synaptic transmission lead to diverse forms of synaptic plasticity at excitatory and inhibitory synapses, including long-term potentiation (LTP) and long-term depression (LTD), whereby the strength of synaptic transmission is increased or decreased respectively (reviewed in Malenka and Bear 2004). Certain forms of LTP and LTD are long lived and are dependent on lasting changes in gene expression (Borrelli et al. 2008). Based on the critical role that epigenetics plays in mediating lasting alterations in gene expression, DNA and histone modifiers are poised to provide a mechanism that both encodes and stabilizes these changes in synaptic strength. This is already the case in mice who over-express HDAC2 in neurons, and exhibit decreased dendritic spine density, synapse number, synaptic plasticity and memory formation (Guan et al. 2009). These results suggest that deacetylation may cause transcriptional repression of the neuronal genes involved in forming and maintaining functional synapses. Since seizures result in synchronized neuronal firing, it is possible that they trigger the same types of epigenetic responses as LTP, and that aberrant stabilization of hyper-excitable circuits could lead to progressive epileptogenesis. Theoretically, specific treatment of the area of seizure-genesis with targeted HDAC2 inhibition could thus interrupt this cycle, and may be a promising therapeutic strategy.

DNA methylation can also be dynamically regulated by synaptic activity. For example, neuronal activity and learning can produce DNA methylation of distinct genomic sites in the human brain (Siegmund et al. 2007), and this methylation signature varies by brain region (Ladd-Acosta et al. 2007). These observations are consistent with the recent findings that DNA methylation can occur rapidly and reversibly in the nervous system (Levenson et al. 2006) in contrast to the previous dogma that methylation state is permanent. BDNF exemplifies this principle, and is demethylated upon neuronal activity. It is proposed that neural activity via increases in cellular Ca^{2+} levels and activation of Ca^{2+}/calmodulin kinases leads to the phosphorylation of MeCP2, and its release from the CoREST complexes

on the BDNF promoter (Ballas et al. 2005), resulting in increased BDNF expression (reviewed in Borrelli et al. 2008). Increased BDNF expression is associated with alterations in GABA receptor subunit composition which can lead to reduced neuronal inhibition (Lagrange, Botzolakis and Macdonald 2007), and increased excitatory neurotransmission by enhancing presynaptic glutamate release and phosphorylating NMDA receptors (Takei et al. 1997, Lin et al. 1998). Thus, DNA methylation is regulated by neuronal activity, and in turn can also influence neural plasticity through the regulation of activity-dependent neuronal genes. Collectively, this neuronal excitability can play a modulatory role in learning and memory, but in the context of disease, it can feed-forward into the cycle of epileptogenesis.

6. Environmental and nutritional factors that influence epigenetic state and vulnerability to epilepsy

In mammalian development, the prenatal and postnatal periods are characterized by dynamic structural and functional re-organization of the brain, as its pathways become shaped by stimulation and experience. During this highly plastic period, environmental experiences influence neural structure, synaptic strength, and consequently our behavior. There is increasing evidence that epigenetic factors are at the interface of environment and gene regulation, and that changes in epigenetic state are stable enough to be heritable, but not static, thereby allowing future experience to modify them. A particularly good example of this is maternal diet, which can cause heritable changes in epigenetic state that then alter gene expression and behavior in offspring. Mechanistically, protein-restricted diets can inhibit DNMTs and cause hypomethylation of specific gene promoters in the offspring (Lillycrop et al. 2007). In addition, folate and vitamin B12 are essential cofactors for the methylation cycle, thus deficiencies in these vitamins also inhibit DNMTs and DNA methylation, which can cause oxidative stress and neuronal cell death (Kruman et al. 2002, Duan et al. 2002, Seshadri et al. 2002, Shea, Lyons-Weiler and Rogers 2002). Another B-vitamin, biotin, modulates chromatin regulation through histone biotinylation (Hassan and Zempleni 2006). Moreover, biotin deficiency can cause epilepsy as well as other clinical features including hypotonia, ataxia, mental retardation, and fetal malformations (Zempleni et al. 2008). These studies suggest that eating a diet with plenty of protein and foods rich in B vitamins may help lower seizure vulnerability through epigenetic regulation of gene expression. In fact, a ketogenic diet (high fat, adequate protein, low carbohydrate) has been used for decades to control refractory seizures in children (Lefevre and Aronson 2000, Stafstrom 2004), and a recent study by Garriga-Canut et al. provides a potential epigenetic mechanism for the antiepileptic properties of the ketogenic diet and of a potentially new treatment for epilepsy, 2-deoxy-D-glucose (2DG) (Garriga-Canut et al. 2006). Metabolic intermediates including NADH can modulate co-activators and co-repressors, thereby linking energy availability to chromatin structure and transcriptional output (Guarente and Picard 2005). Since 2DG is a glycolytic inhibitor, it may act as a small molecule regulator of the NAD+ HDAC III sirtuins, thereby repressing transcription of genes that contribute to epileptogenesis. In fact, Garriga-Canut and colleagues (2006) show that 2DG can reduce the progression of epileptogenesis in kindled rats by raising the after-discharge threshold, reinforcing that diet can induce changes in epigenetic state that impact the development of epilepsy.

Prenatal and postnatal stress can also influence epigenetic state and neurodevelopment, subsequently changing our behavior patterns. Early-life stress can cause epigenetic changes in the methylation status of certain promoters including the glucocorticoid receptor (GR).

Stress can increase levels of DNA methylation in the NGFI-A binding site of the GR 17 promoter and decrease histone H3-K9 acetylation (a marker of transcriptional activation), the functional consequence of which is a heightened stress response (Weaver et al. 2004, Weaver et al. 2007). Severe early-life stress can increase excitotoxic cell death of hippocampal neurons (Brunson et al. 2003), whereas prenatal stress contributes to the susceptibility for febrile convulsions, afebrile seizures, and cerebral palsy (Greenwood et al. 1998, Weinstock 2001). Perhaps the most striking finding is that maternal stress in the latter half of pregnancy lowers the seizure threshold, potentially increasing seizure susceptibility in the unborn offspring (Edwards et al. 2002). Thus the nervous system is particularly sensitive to alterations in epigenetic regulation, probably because of the fine balance needed to maintain heritable cellular memory while still being capable of adapting to changing environmental conditions.

7. DNA methylation inhibitors and HDAC inhibitors are potential epigenetic targets for epilepsy treatment

Since epigenetic factors play such an important role in regulating gene expression during neuro- and gliogenesis and key phases of synaptic plasticity and learning, pharmacological manipulation of these factors in targeted time windows in the etiology of disease holds enormous therapeutic potential. This is particularly important for a complex disease like epilepsy which involves multiple genes and downstream effectors. Recent evidence has emerged that modifying chromatin structure can indeed alter the disease course of epilepsy, and serves as a fertile ground for exploring targeted remodeling of chromatin as a potential therapeutic strategy in epilepsy (summarized in Table 1).

7.1 DNA methylation inhibitors and treatment of epilepsy

In humans, a variety of mental retardation syndromes with an epileptic phenotype including Rett syndrome, Fragile-X, Rubinstein–Taybi, Prader-Willi and Angelman syndromes, have all been linked to mutations or disruptions in methylation factors (Egger et al. 2004), suggesting that methylation inhibitors may be a novel therapeutic target for epilepsy. Current generation DNMT inhibitors are methylcytosine analogues that reduce DNA methylation through covalent sequestering of DNMTs as opposed to the direct removal of methyl groups from DNA (Juttermann, Li and Jaenisch 1994). These inhibitors are widely used clinically for their anti-cancer efficacy (reviewed in Das and Singal 2004), but have not been systematically evaluated to treat epilepsy. The DNMT inhibitors 5-azacytidine (5azaC) and zebularine can effectively inhibit DNA methylation in neurons and block LTP and memory formation (Levenson et al. 2006, Miller, Campbell and Sweatt 2008, Miller and Sweatt 2007), suggesting that they are good candidate drugs to evaluate in epilepsy paradigms. When both agents were applied in a Rett syndrome mouse model, treatment facilitated a significant decrease in frequency of miniature excitatory post-synaptic currents (mEPSCs) and rate of spontaneous synaptic vesicle fusion (Nelson et al. 2008). Since decreasing mEPSCs can reduce neuronal excitability, methylation inhibitors may possess anti-seizure properties, and should thus be tested in more classic epilepsy models. In addition to methylcytosine analogs, DNA methylation can be reversed pharmacologically by increasing histone acetylation through the use of HDAC inhibitors (Cervoni and Szyf 2001, Milutinovic et al. 2007), underscoring that multiple epigenetic factors work in a concerted manner to regulate gene expression and careful consideration of

Drug name	Epigenetic target	(Deutsch et al. 2008)	Outcome	Reference
Sodium Butyrate	Inhibits class 1 and II HDACs except HDAC6 and HDAC10	IECS to produce tonic hindlimb extension	Increased H3 and H4 acetylation in hippocampus and cortex and improved anti-seizure efficacy of MK-801	(Deutsch et al. 2008)
VPA	Inhibits class I HDACs and to a lesser extent class II HDACs	IBO injection (excitotoxin) to kill GABAergic and cholinergic neurons	Increased H3 acetylation in cortex and NBM and significant protection of both cholinergic and GABAergic neurons present in the injected area	(Eleuteri et al. 2009)
		Kainic Acid induced SE	No significant effect on seizure strength or frequency but inhibited seizure-induced neurogenesis in hippocampus and prevented memory impairment	(Jessberger et al. 2007)
		PTZ induced seizures	Increased thresholds to all seizure types	(Hoffmann, Czapp and Loscher 2008)
TSA	Inhibits class I and II HDACs	Kainic Acid induced SE	Prevented the down-regulation of GluR2 in hippocampus	(Jessberger et al. 2007)
		PTZ induced seizures	No dose-dependent anticonvulsant effects	(Hoffmann et al. 2008)
		Pilocarpine induced seizures	Prevented the down-regulation of GluR2 in hippocampus	(Huang et al. 2002)
		Kainic Acid induced seizures	Histone hyperacetylation of IEGs c-jun and c-fos	(Sng, Taniura and Yoneda 2005)
Zebularine	Inhibits DNMTs	Rett Syndrome (MeCP2 KO mice)	Deficits in spontaneous synaptic transmission	(Nelson, Kavalali and Monteggia 2008)

Abbreviations: HDAC=histone deacetylase. SE=status epilepticus. IECS=incremental electroconvulsive shock. VPA=valproic acid. IBO=ibotenic acid. NBM=nucleus basalis magnocellularis. TSA=trichostatin A. GluR2=glutamate receptor 2. PTZ=pentylenetetrazole. IEG=immediate early genes. DNMT=DNA methyltransferase. KO=knock out

Table 1. Epigenetic therapy in epilepsy models

undesired secondary effects of such compounds must be taken when designing a therapeutic approach.

7.2 HDAC inhibitors and treatment of epilepsy

HDAC inhibitors (HDACi) are classified into six groups based on their chemical structures: (1) hydroxamic acids, including TSA and SAHA; (2) small-molecular-weight carboxylates, including sodium butyrate, valproic acid, and sodium phenylbutyrate; (3) benzamides, including MS-275 and CI-994; (4) epoxyketones, including AOE and trapoxin B; (5) cyclic peptides, including depsipeptide and apicidin; and (6) hybrid molecules, such as CHAP31 and CHAP50 (Drummond et al. 2005 and references there in). Most HDACi are broad-spectrum within the classical HDAC family, and isoform-specific inhibitors have been difficult to design due to the high sequence homology within the catalytically-active sites of HDACs (Bieliauskas and Pflum 2008). Therefore, the majority of published studies have employed HDAC inhibitors that have multiple secondary targets within and beyond the CNS, but collectively, a compelling argument can be made to explore their efficacy in most epilepsy paradigms. Hydroxamic acids encompass the broadest set of HDACi, and primarily inhibit class I/II HDACs when used in the nanomolar range (Bieliauskas and Pflum 2008), however side effects in patients have been reported, particularly with TSA (Villar-Garea and Esteller 2004). In contrast, the carboxylates are very well tolerated in animals and humans, and have been used in dose escalating studies in the clinic (Atmaca et al. 2007). HDAC inhibitors have been extensively studied in models of neurodegenerative disease (Hahnen et al. 2008), and some agents–such as valproic acid (VPA), sodium butyrate and LBH589–are now being tested in clinical trials in patients with spinal muscular atrophy (SMA), Huntington's disease (HD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS).

The most widely used HDAC inhibitor, Valproate (VPA), has been used as an anti-epileptic therapeutic agent for over 40 years, and its effects in the nervous system have recently been reviewed in detail elsewhere (MacDonald and Roskams 2009, Loscher 1999). However, VPA has primarily been used for its multitude of effects on CNS excitation pathways that have not taken into account its HDAC inhibition activity. Briefly, VPA can (1) increase the level of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), (2) reduce sodium conductance through voltage-gated sodium channels, (3) suppress N-methyl-D-aspartate (NMDA) receptor-mediated excitation, (4) have teratogenic activity in both humans and mouse models.

While VPA is well tolerated and can attenuate seizure activity, evidence suggests that it is not truly antiepileptic. In other words, VPA can acutely and chronically reduce the risk of recurrent seizures, but treatment does not alter the development of epilepsy (Shinnar and Berg 1996, Haut and Shinnar 2008). One possible explanation for this dichotomy is that even though VPA augments presynaptic GABA release (reviewed in Loscher 1999), thereby conferring antiseizure properties, VPA also increases BDNF expression (Fukumoto et al. 2001) and can reduce $GABA_A$ receptor γ2 subunit, GAD65, GAD67, and KCC2 expression (Fukuchi et al., 2009), thus impairing GABAergic function over time and potentially shifting the balance of neurotransmission to a more hyperexcitable state. Furthermore, GABAergic signaling regulates neurogenesis and neuronal differentiation of immature neurons (Ben-Ari 2002), so VPA-mediated alterations in GABAergic neurotransmission could impair neurogenesis and subsequently cause cognitive impairment, particularly in children and adolescents where brain development is still very actively occurring. In fact, mice lacking MeCP2 from GABA-releasing neurons consequently have impaired GABA function, and

display many of the characteristic symptoms of Rett syndrome and autism (Chao et al.). Thus, if inhibitory neurogenesis is indeed perturbed as a secondary action of this compound, treating patients with VPA to control seizures could inadvertently hasten the progression of epilepsy. However, in a recent study, treatment with VPA following kainic acid-induced seizures did inhibit hippocampal neurogenesis, but surprisingly improved cognitive impairment compared with controls who received kainic acid alone (Jessberger et al. 2007). This contradiction supports the hypothesis that seizure-induced neurogenesis can be detrimental, but the consequences of chronically inhibiting neurogenesis are likely to be harmful.

A final potential use for HDAC inhibitors in treating epilepsy is in enhancing the survival of neurons in existing circuits that might otherwise succumb to excitotoxic cell death due to hyper-excitation and the resulting ionic imbalances. A growing body of evidence places HDAC inhibition capable of mediating survival signaling, cytoskeletal stabilization in neurons, and CREB-mediated signaling following ischemia and oxidative stress (reviewed in Sleiman et al. 2009). With the development and testing of new generations of specific HDAC inhibitors that are not only CNS-permeable, but usable in short-term pulses in animal models of appropriate neurological diseases, it is possible that HDACi-mediated neuroprotection and stabilization of existing brain circuitry is a potential mechanism for preventing the spread or progression of epilepsy, and limiting the accompanying cognitive deficits that result from poorly controlled seizures in epileptic patients.

8. Conclusions

After sequencing the human genome – and the genomes of numerous other species, it is apparent that organisms with a higher order of complexity within their CNS have acquired a more complex non-coding genome. The majority of this sequence is comprised of regulatory elements that contextually modulate protein expression and function. Although a large part of development is due to the complex transcriptional regulation required of multiple members of the same gene family, it is likely that analysis of the structural organization, the regulation of the non-coding genome, and the role of epigenetics in modulating these phenomena will reveal a more in-depth understanding of our own human phenotype. Within these unique DNA modifications are clues to the origin, susceptibility, and progression of neurological disease, including epilepsy.

Epigenetic processes are certainly involved in the development and progression of epilepsy, and epilepsy, in turn, can change the epigenetic landscape of the CNS (summarized in Fig 2). Regardless of whether seizures begin early or later in life; the processes outlined above (synaptic plasticity, neuro-gliogenesis, and neuroprotection) are ongoing developmental processes that are all mediated by epigenetic changes in chromatin structure. Those same changes in chromatin--based on environment, stimulation, and developmental programs--drive the individual biology within distinct CNS cells. Thus epigenetics provides a mechanism whereby CNS cells can react to internal and external stimuli, and record the experience in both a modifiable and heritable manner. The emergence of genomic diagnostics, coupled with high resolution imaging to pinpoint functionally "normal" versus "aberrant" cellular responses to these stimuli, will allow us to identify the aberrant circuitry that leads to the progression of epilepsy and its underlying pathology.

Concurrent with this, advances in combinatorial chemistry and high throughput screening approaches will allow for the ongoing and rapid development of subtype-specific HDAC

inhibitors (and drugs to modify other histone marks such as methylation and phosphorylation). Because the pharmacological manipulation of epigenetic factors is a growing target for many diseases (notably cancer), the future holds great promise in being able to evaluate the therapeutic efficacy of these agents in neurological diseases, and in particular, for the beneficial effects they may afford to patients with epilepsy.

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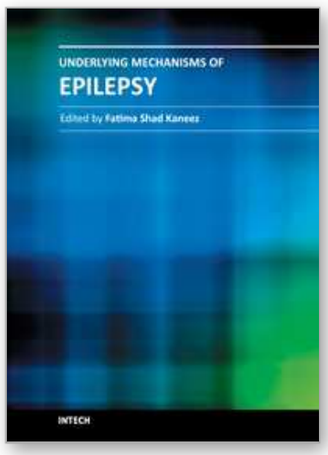

Underlying Mechanisms of Epilepsy

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This book is a very provocative and interesting addition to the literature on Epilepsy. It offers a lot of appealing and stimulating work to offer food of thought to the readers from different disciplines. Around 5% of the total world population have seizures but only 0.9% is diagnosed with epilepsy, so it is very important to understand the differences between seizures and epilepsy, and also to identify the factors responsible for its etiology so as to have more effective therapeutic regime. In this book we have twenty chapters ranging from causes and underlying mechanisms to the treatment and side effects of epilepsy. This book contains a variety of chapters which will stimulate the readers to think about the complex interplay of epigenetics and epilepsy.

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