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Epigenetic regulation of neuronal dendrite and dendritic spine development

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Abstract

Dendrites and the dendritic spines of neurons play key roles in the connectivity of the brain and have been recognized as the locus of long-term synaptic plasticity, which is correlated with learning and memory. The development of dendrites and spines in the mammalian central nervous system is a complex process that requires specific molecular events over a period of time. It has been shown that specific molecules are needed not only at the spine's point of contact, but also at a distance, providing signals that initiate a cascade of events leading to synapse formation. The specific molecules that act to signal neuronal differentiation, dendritic morphology, and synaptogenesis are tightly regulated by genetic and epigenetic programs. It has been shown that the dendritic spine structure and distribution are altered in many diseases, including many forms of mental retardation (MR), and can also be potentiated by neuronal activities and an enriched environment. Because dendritic spine pathologies are found in many types of MR, it has been proposed that an inability to form normal spines leads to the cognitive and motor deficits that are characteristic of MR. Epigenetic mechanisms, including DNA methylation, chromatin remodeling, and the noncoding RNA-mediated process, have profound regulatory roles in mammalian gene expression. The study of epigenetics focuses on cellular effects that result in a heritable pattern of gene expression without changes to genomic encoding. Despite extensive efforts to understand the molecular regulation of dendrite and spine development, epigenetic mechanisms have only recently been considered. In this review, we will focus on epigenetic mechanisms that regulate the development and maturation of dendrites and spines. We will discuss how epigenetic alterations could result in spine abnormalities that lead to MR, such as is seen in fragile X and Rett syndromes. We will also discuss both general methodology and recent technological advances in the study of neuronal dendrites and spines.

Keywords

epigenetics; neurodevelopment; dendritic spine; synapse; microRNA; methyl-CpG binding protein 2 (MeCP2); mental retardation

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1 Introduction: Dendrites and spines in development and diseases

1.1 Introduction to neuronal dendrites and dendritic spines

Santiago Ramon y Cajal, historically the founder of neuroscience and Nobel Prize winner, was the first to propose in the late 19th century that the nervous system is made up of individual neurons that are able to communicate with neighboring cells through long projecting axons and highly branched dendrites. He first described dendritic spines in 1891 as “the tips of charge or points of reception of impulses, their retraction would result in the individualization or disaggregation of neurons. The awake state would correspond to the swelling and lengthening of spines, while the resting state would correspond to the retraction of these appendages” (Cajal, 1891).

Cajal's observations were based on Golgi staining and a light microscope over 100 years ago. To date, significant advances in biology have confirmed that dendritic spines can undergo long-term modifications, such as changes in number and shape, in response to novel experiences, suggesting that dendritic spines are the locus of long-term synaptic plasticity associated with memory storage in the brain (Segal, 2005). In light of advances in genetic and molecular tools, scientists still ponder why dendrites and dendritic spines become grossly impaired in individuals faced with mental retardation and neuropathology.

The brain is composed of a complex network of neurons that communicate with each other through specialized cell junctions called synapses (Fig. 1A, B). Most of these synaptic junctions are chemical synapses, in which a chemical neurotransmitter is released by the axon of the presynaptic neuron. The neurotransmitter can diffuse into the synaptic cleft (space between synaptic contact), where it can act on the corresponding neurotransmitter receptors on the postsynaptic neuron (Fig. 1B). The synapses are typically found on the dendritic shaft, located on stubby spines and filipodia (long, thin dendritic protrusions), and are characterized by the clustering of certain proteins on the pre- and postsynaptic sites of contact. Dendritic spines have been classified into many types based on their shape. The most common types of spines in the central nervous system (CNS), and also the primary focus of pathological studies, are the following: mushroom-like spines with a bulbous head attached to the dendrite by a narrow neck, short and stubby spines with no neck, and long and thin spines with no head enlargement (Fiala et al., 2002). It is believed that long, thin spines are mostly immature spines, and they develop into the more mature mushroom-like spines (Fig. 2). During development, dendrites start out with no spines, and during synaptogenesis and neuronal maturation, filipodia emerge from the dendrites and form nascent synapses with axons (Fiala et al., 1998; Fiala et al., 2002). Mature synapses are gradually formed on mushroom-like spines that have a well-defined head (Harris and Kater, 1994), and the density of mature spines increases along the dendrite as the neuron matures and forms functional connections with the surrounding brain circuitry (Zhao et al., 2006) (Fig. 3). More about the specific molecular machinery found in the mature excitatory synapse will be discussed in the next section.

1.2 Activity-dependent modulation of gene expression controls dendrite and spine development

Glutamatergic neurons in the mammalian brain have elaborate dendrites covered with dendritic spines. These spines function as the primary sites of excitatory synaptic input for the neuron (Alvarez and Sabatini, 2007). The formation of an excitatory synapse in the CNS during development is initiated by the contact between the presynaptic axon and the postsynaptic dendrite, followed by the recruitment of pre- and postsynaptic proteins to the site of contact, and the stabilization of these interactions. Dendritic development and synapse formation are highly influenced by neuronal activities. Spine pruning occurs during early postnatal development, characterized by an experience-dependent loss of spines that selectively maintains spines of active synapses resulting in the appropriate maturation of neuronal circuitry (Grutzendler et al., 2002).

A major area of research in neurodevelopment is how neuronal activity-dependent modulation of gene expression affects dendritic development and synapse formation. It has been shown that dendritic and spine morphogenesis depends on proper neuronal development and activation of glutamate receptors, which maintain appropriate connections between neurons (Parrish et al., 2007b). Activation at the synapse leads to calcium influx into the dendrites of the postsynaptic neuron, which regulates the dendritic outgrowth of postsynaptic neurons. Calcium not only functions locally at the site of entry, but also leads to changes in gene transcription in the nucleus. Calcium influx through the N-methyl-D-aspartate (NMDA) receptor or voltage-sensitive calcium channels (VSCCs) during development can activate many signaling pathways, such as the calcium-sensitive calcium/calmodulin kinases (CaMKs) known to be important for signal transduction in neurons. For example, activated CaMKII regulates the number of α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate (AMPA) receptors at the synapse and the complexity of neuronal dendrites by influencing actin cytoskeleton (Dillon and Goda, 2005). In cultured neurons, CaMK activity initiates signaling to the nucleus, where activation of cAMP response element binding protein (CREB) leads to activity-dependent gene expression and subsequent dendritic morphological changes (Redmond et al., 2002; Wayman et al., 2006). Thus, intrinsic gene expression programs can be modified by neuronal activity to modulate spine morphogenesis and dendritic development (Cohen and Greenberg, 2008).

1.3 Dendritic spine pathology

Because spines are the key sites of synaptic input, altered spine morphology associated with pathological conditions may have a dramatic impact on the properties of the individual neuron, the neural networks, and mental function as a whole. In fact, dendritic spine distribution and structure is abnormal in many diseases and injuries, as well as many forms of mental retardation (MR) (Fig. 4). Thus, it has been proposed that the cognitive and motor deficits observed in MR may result from altered spine development and function. Understanding the effect of altered spines in pathological conditions will provide researchers with a better understanding of how spines contribute to normal synaptic conditions during development and learning in the adult brain.

As summarized in Table 1, morphological dendritic spine abnormalities are found in many types of pathological conditions, including mental retardation such as is seen in autism spectrum disorder (Persico and Bourgeron, 2006), Rett syndrome (Zhou et al., 2006), and fragile X syndrome (Bagni and Greenough, 2005). Spine abnormalities are also found in schizophrenia (Lewis et al., 2003), depression, stress (Pittenger and Duman, 2008), drug addiction (Robinson and Kolb, 2004), epilepsy, and neurodegenerative disorders like Alzheimer's disease, Parkinson's disease (Day et al., 2006), and Huntington's disease (Spires et al., 2004).

Aberrant dendritic spine development includes a broad range of changes in spine morphology and structure, such as increases or decreases in spine density, altered spine size or shape, dendritic beading with subsequent loss of spines, and ectopic spines in abnormal locations (Fiala et al., 2002). Since the shape and structure of a spine are closely associated with its function, the presence of abnormal spine morphology in many of these diseases suggests that the resulting cognitive phenotype is a result of dysfunctional spines. Understanding the effect of altered spines in pathological conditions will provide researchers with a better understanding of how spines contribute to normal synaptic conditions during development and learning in the adult brain.

The spine pathogenesis among various types of mental retardation is strikingly similar. These observations suggest that various genetic and epigenetic deficits related to mental retardation may be the result of abnormal dendrites and spines leading to the disruption of neural homeostasis and the ability of the brain to return to a set point following perturbation (Ramocki and Zoghbi, 2008). Interestingly, many of the mental retardation susceptibility genes encode proteins that regulate neuronal dendrite and spine development. This suggests that the molecular factors involved in behavioral and cognitive processes function in a tightly regulated homeostatic fashion. More specifically, single genes or noncoding RNAs do not encode specific cognitive processes, but instead encode biological processes. It is when a particular biological process, such as synaptic transmission, is disrupted during development that we can appreciate the resulting numerous neurological phenotypes.

Despite the fact that several protein pathways have been identified as critical players in spine development and pathology, the molecular pathogenesis of aberrant spine morphology in these diseases has yet to be clearly and comprehensively elucidated. It is apparent that complex programs of gene expression work to shape the developing nervous system. In this review, we will discuss the roles of epigenetic mechanisms in this important process. Revealing the roles of signaling factors and epigenetic gene regulators in dendritic spine pathologies will provide us with a better understanding of dendritic and spine disease and offer new approaches to treating neurodevelopmental disorders.

2 Epigenetic regulations are critical for neuronal dendritic development

2.1 Introduction to epigenetic regulation

In eukaryotic cells, genomic DNA exists in the form of chromatin and is tightly associated with histones and other chromatin proteins. Epigenetic regulation is defined as heritable changes in gene expression that are not coded within the DNA sequence itself. Epigenetic

modulation of the genome involves three interacting systems, DNA methylation, histone modification (Egger et al., 2004; Li and Zhao, 2008), and noncoding RNA-mediated processes (Li and Zhao, 2008) (see Table 2). Recent literature has demonstrated that the phenotype of the cell is not only dependent on the genotype, but also the epigenotype. For example, DNA methylation within promoter regions (e.g., CpG islands) can result in heritable silencing of gene expression and has likely evolved as a host defense mechanism against viral sequences (Bestor and Tycko, 1996; Yoder et al., 1997). This type of epigenetic modulation can imprint dynamic environmental changes on a fixed genome, resulting in a stably transmitted alteration of phenotype, and has long been proposed to be an epigenetic silencing mechanism of fundamental importance (Holliday and Pugh, 1975; Riggs, 1975). The epigenotype shows far greater plasticity than the genotype during normal development, and disruption of these systems can lead to inappropriate expression or silencing of genes, resulting in “epigenetic diseases.” This section will review the epigenetic mechanisms that regulate dendrite and spine development and related diseases.

2.1.1 DNA methylation—DNA methylation involves covalent modification of cytosine at position C5 in CpG dinucleotides. Over 70% of CpG dinucleotides in the mammalian genome are methylated, and most DNA methylation occurs at CpG dinucleotides. The exception is CpG islands, defined as more than 500 base pairs of sequence composed of 55% GC content. CpG islands are found in the promoters and the first exon of about 40% of the mammalian genes, and they are normally kept free of DNA methylation by mechanisms that are still unclear (Takai and Jones, 2002). Methylation of CpG islands is associated with stable heritable transcriptional silencing (Jones and Baylin, 2002). The methylation of CpG dinucleotides is catalyzed by several DNA methyltransferases (DNMTs). The *de novo* establishment of DNA methylation relies on DNMT3a and 3b and cofactor DNMT3L, whereas the maintenance of DNA methylation depends on DNMT1, which specifically recognizes semi-methylated DNA and methylates the remaining strand (Bestor, 2000; Robertson et al., 2000; Jaenisch and Bird, 2003). Mammalian DNA methylation has been implicated in a diverse range of cellular functions, including tissue-specific gene expression, cell differentiation, cell fate determination, genomic imprinting, and X chromosome inactivation (Bird, 2002). Here we focus on the function of DNA methylation in neurodevelopment.

Deletion of DNMT1 in neural progenitor cells leads to hypomethylation in neurons and subsequent abnormalities in synaptic maturation and function (Golshani et al., 2005; Hutnick et al., 2009). Although deletion of DNMT1 in neuronal precursors at E9-E10 leads to normal development through birth, it adversely affects neuronal survival after birth, resulting in death of the mice (Fan et al., 2001). Recently, Feng et al. have shown that DNMT1 and DNMT3a regulate synaptic function in neurons (Feng et al., 2010). Feng's group made use of conditional mutant mice that lacked Dnmt1, Dmnt3a, or both specifically in post-mitotic forebrain neurons of the postnatal mice. Their findings suggest that DNMT1 and DNMT3a are required for synaptic plasticity and learning and memory, likely due to their functions in maintaining DNA methylation and controlling gene expression in post-mitotic neurons. Although these studies provide sufficient evidence that DNMTs and DNA methylation are critical for neuronal development, the function of DNA methylation in

postmitotic neurons of the adult brain is still for the most part unknown. DNMT1 is highly expressed in neurons, and the deletion of DNMT1 from post-mitotic neurons of postnatal brains leads to neuronal death (Fan et al., 2001), suggesting that DNMT1 may have other roles in addition to its methyltransferase activity. In fact, DNMT1 has been shown to form a complex with histone deacetylases (HDACs) and participates in transcriptional repression (Robertson et al., 2000; Jaenisch and Bird, 2003), suggesting that DNMTs may have complex roles in transcriptional regulation. Researchers have yet to identify specific genes affected by Dnmt deficiency, which impacts synaptic function, as well as learning and memory.

Despite the magnitude of literature on DNMTs and DNA methylation, much less is known about DNA demethylation. It has been proposed that the steady-state methylation of a particular gene is a dynamic equilibrium between methylase and demethylase activities. It has been found that methyl-CpG binding protein 2 (MBD2) has DNA demethylase activity (Bhattacharya et al., 1999; Detich et al., 2002). It has also been proposed that Gadd45a erases DNA methylation marks by DNA repair-mediated DNA demethylation (Barreto et al., 2007). More recently, Gadd45b was found to be important for the activity-induced demethylation of promoters and the expression of corresponding genes critical for adult neurogenesis, such as *Bdnf* and *Fgf-1* (Ma et al., 2009). It was also shown that demethylation can take place in the absence of DNMT1 and DNMT3a *in vivo* (Feng et al., 2010). Although researchers have yet to elucidate the mechanisms of active DNA demethylation in the brain, it has been proposed that DNA oxidation and repair are possible mechanisms underlying this process.

DNA methylation represses gene transcription either through directly blocking the access of transcription factors to their binding sites or through indirectly recruiting MBDs. The MBD protein family consists of a growing number of DNA-binding proteins with the ability to recognize methylated CpGs in the genome. The MBD family includes at least MBD1, MBD2, MBD3, MBD4, MECP2, and Kaiso (Klose and Bird, 2006). In a sense, MBDs translate genomic CpG methylation into gene expression changes; therefore, these proteins are the central components of the DNA methylation pathway. DNA methylation is important in mammalian brain development (Chahrour and Zoghbi, 2007). One of the best examples of this occurs in Rett syndrome (RTT), an X-linked dominant pervasive neurodevelopmental disorder caused by *de novo* mutations in methyl-CpG binding protein 2 (MeCP2) (Amir et al., 1999). MeCP2 is thought to be involved in the structural conformation of chromatin. Once MeCP2 is bound to methylated DNA, MeCP2 recruits a complex of chromatin-remolding enzymes that help to condense the DNA surrounding the MeCP2 binding site, and silence transcription (Bird, 2002; Chahrour and Zoghbi, 2007). Mutations in MeCP2 lead to Rett syndrome, a severe neurodevelopmental disorder. As further discussed below, extensive evidence suggest that Mecp2 plays an important role in dendritic development and neuronal maturation.

2.1.2 Histone code—Histones are abundant nuclear proteins. Eukaryotic genomic DNA wraps around histones, which form the basic unit of chromatin, the nucleosome that consists of ∼147 base pairs of DNA wrapped around a core histone octamer (∼1.65 turns). Each histone octomer includes two copies of H2A, H2B, H3, and H4 histones, and all of them can

undergo different types of post-translational modifications, such as acetylation, methylation, and phosphorylation (Lachner et al., 2003). The types and sites of histone modifications, the so-called “histone code,” have a significant impact on chromatin structure and gene expression.

Chromatin is present in two states: heterochromatin and euchromatin. Heterochromatin is in a condensed state that is repressive for gene transcription. When the chromatin is in an open state, called euchromatin, genes can be transcribed. Chromatin remodeling is a mechanism that alters the chromatin structure and functions to modulate DNA-protein interactions and gene activity without changing genomic DNA sequences. The chromatin structure and maintenance are not only critical for gene expression, but also for many cellular processes, such as chromosome segregation during mitosis and X-chromosome inactivation (Grewal and Elgin, 2007).

Recent genome-wide studies have demonstrated that histone modifications, as well as recruitment of other chromatin proteins, can be used as markers for gene expression state. Among histone modifications, lysine acetylation and methylation are the most characterized markers. For example, methylation of a histone at lysine 4 (H3K4), H3K36, or H3K79 is correlated with open chromatin and generally active gene transcription, whereas methylation of H3K9, H3K27, or H4K20 is correlated with condensed chromatin and gene inactivation (Santos-Rosa et al., 2002; Krogan et al., 2003; Schubeler et al., 2004). Additionally, mono-, di-, and trimethylation at the same lysine residues lead to different levels of gene activation or repression and are involved in distinct cellular pathways (Barski et al., 2007). Histone acetylation leads to less condensed chromatin structure and can be used to mark transcriptionally active regions. On the other hand, histone hypoacetylation is associated with more condensed heterochromatin and is used to mark transcriptionally inactive regions (Grewal and Elgin, 2007). Additionally, histones are subject to a number of other modifications, including phosphorylation, ubiqutinylation, etc. (Lachner et al., 2003). Thus, post-translational modifications of histones demonstrate a high degree of diversity and complexity and reflect the importance of the “histone code” in gene expression regulation.

The enzymes that catalyze histone modifications are critical components of the epigenome and play important roles during neurodevelopment. For example, studies of histone acetylation have focused on two opposing enzymes, histone acetyltransferases (HAT) that catalyze acetylation, and histone deacetylases (HDAC) that catalyze deacetylation. Transcription activators can either recruit HATs or utilize their own internal HAT domains (for example CREB binding protein, CBP) to catalyze histone acetylation to promote active chromatin structure. Conversely, transcription repressors can recruit HDACs that lead to histone deacetylation and gene repression. The opposing activities of HATs and HDACs are important for gene transcription regulation and therefore are tightly regulated during development. Alteration of these processes leads to developmental disorders, such as Rubinstein-Taybi syndrome, which results from heterozygote mutations of CBP. It is thus not surprising that HDAC inhibitors, such as valproic acid (VPA), have been developed and used to treat diseases, including psychiatric disorders (Phiel et al., 2001). VPA has been shown to increase neuronal differentiation in hippocampal neural progenitors (Hsieh et al., 2004). More recently, VPA has been shown to alter the morphology of motor cortex neurons

in a rat model of autism (Snow et al., 2008) and affect neurite outgrowth in mouse neuroblastoma cells (Yamauchi et al., 2008; Yamauchi et al., 2009). Another HDAC inhibitor, trichostatin A (TSA), has also been shown to increase neuronal differentiation of neural stem cells (NSCs) and enhance the dendritic length and complexity of NSC-derived neurons (Balasubramaniyan et al., 2006). More recently, it was found that the neuronal abundant HDAC2 suppresses synapse formation and dendritic spine development of hippocampal neurons through its negative regulation of multiple neuronal genes. The authors demonstrated that HDAC2 functions to suppress synaptic plasticity and memory formation (Guan et al., 2009).

Another histone modification enzyme in the spotlight is enhancer of zeste homolog 2 (Ezh2), an H3-K27 histone methyltransferase and a part of the Polycomb group (PcG) protein complexes. PcG proteins and Ezh2 are important for neurogenesis and stem cell function in the brain (O'Carroll et al., 2001; Lee et al., 2006). PcG proteins are known to function in maintaining the bivalent chromatin state in stem cells (Boyer et al., 2006; Lee et al., 2006). For example, genes in embryonic stem cells related to differentiation contain both activating (methylated-H3K4) and repressing (trimethylated-H3K27) chromatin markers (Azuara et al., 2006; Bernstein et al., 2006). This bivalent chromatin state is believed to “prime” genes for expression, but “hold them in check” at the same time, therefore controlling the balance between proliferation and differentiation in embryonic stem cells (Bernstein et al., 2007). Recently PcG proteins have been shown to regulate dendritic arborization in *Drosophila* sensory neurons in a cell-autonomous manner (Parrish et al., 2007a, 2007b). We have recently found that Ezh2 expression is controlled by the crosstalk between MeCP2 and microRNAs (Szulwach et al., 2010), suggesting that aberrant regulation of histone methyltransferase could be involved in the mammalian neuronal development and pathogenesis of neurodevelopmental disorders.

Together, these studies provide evidence that histone modification and chromatin structure modulation play significant roles in the regulation of neuronal development and the formation of dendrites and spines.

2.1.3 Noncoding RNAs—The human genome contains only about 2% coding genes, whereas more than 80% of the genome is transcribed into RNA. Mounting evidence points to important roles for noncoding RNAs in gene expression regulation and cell phenotype determination (Li and Zhao, 2008). Micro-RNAs (miRNAs) are a class of small noncoding RNAs that are transcribed from the genome. Experimental evidence indicates that miRNAs function to modulate gene expression at the post-translational level by partial base-paring with the seed sequence located in the 3′ untranslated region (UTR) of the protein-coding mRNAs, leading to repression of translation efficiency or cleavage of the target mRNA (Filipowicz et al., 2008). miRNAs are expressed in many different tissues, particularly in the brain. The expression levels and patterns of miRNAs in the brain are dynamically regulated, suggesting that they play important roles in neuronal development (Bushati and Cohen, 2007; Barbato et al., 2008). One of the most studied brain specific miRNAs is miR-9. This miRNA has been of particular interest because it plays an important role in neurogenesis (Krichevsky et al., 2006), zebrafish brain patterning (Leucht et al., 2008), and its expression levels increase as neuronal precursors differentiate into the neurons. It was shown that

miR-9 and miR-124 repress BAF53a, a process important for NSC proliferation and post-mitotic dendritic outgrowth (Yoo et al., 2009). Additionally, miR-9 regulates the expression of orphan nuclear receptor (Tlx), a gene important for self renewal of NSCs (Shi et al., 2004). Additionally, Tlx also regulates expression of miR-9, demonstrating a regulatory loop that functions to regulate neurogenesis (Zhao et al., 2009). Tlx is also targeted by *Let-7b* (Zhao et al., 2010), a member of the *let-7* miRNA family. The let-7 miRNA family was one of the first families of miRNAs found to regulate stem cell function (Rybak et al., 2008; Liu and Zhao, 2009; Schwamborn et al., 2009). Additionally, let-7 is known to affect self-renewal and differentiation, and it has been shown to interact with the 3′UTR of high mobility group A2 (HMGA2), a known target of let-7. Together, let-7 and HMGA2 regulate the expression of p16Ink4a partially responsible for aged-dependent decline in self-renewal ability of brain NSCs (Nishino et al., 2008).

It has been shown previously that miRNAs are important for a number of cellular processes, such as differentiation, apoptosis, metabolism, and dendritic development (Brennecke et al., 2003; Johnston and Hobert, 2003; Chang et al., 2004; Chen et al., 2004; Poy et al., 2004). Loss of important components of the miRNA pathway, such as Dicer and DGCR8, can alter the proliferation and differentiation of stem cells (Kanellopoulou et al., 2005; Wang et al., 2007). Additionally, miRNAs have been demonstrated to play a role in the modulation of proliferation and differentiation of different stem cell types (Ivey et al., 2008; Szulwach et al., 2010).

One of the most exciting recent discoveries in neuroscience is that miRNAs have a role in synaptic plasticity. Learning and memory in the brain requires the synapse to undergo long-term modifications. It is believed that these changes require the local translation of factors important for synaptic function (Sutton and Schuman, 2005). Local protein synthesis at the dendritic spine involves transport of mRNAs to the dendritic compartment; however, we know little about how translation at the synaptic compartment is regulated. In *Drosophila melanogaster*, it has been shown that mRNAs important for synaptic plasticity are targets of the miRNA pathway (Ashraf et al., 2006). The authors have analyzed the brains of RISC mutants and demonstrated that protein translation of neuronal CaMKII is increased in dicer-, armitage-, or aubergine-mutant brains. At the same time, Schratt et al. were able to directly link a specific miRNA to dendrite and spine development in mammalian neurons (Schratt et al., 2006). First, they showed that miR-134 is localized in the dendrites of cultured mouse hippocampal neurons and is in dendritic spines that are apposed to synapsin-positive presynaptic terminals. Next, they found that overexpression of miR-134 leads to decreased spine size, whereas inhibition of endogenous miR-134 leads to increased spine size. Therefore miR-134 may act as a negative regulator of dendritic spine maturation. Recently, the same group found that miR-138 is important for dendritic patterning and spine morphogenesis. miR-138 is highly enriched in the brain and is localized in the dendrites. The authors revealed that miR-138 negatively regulates dendritic spine size in rat hippocampal neurons by controlling the protein translation of APT1 (Siegel et al., 2009). In addition, brain-specific miR-124 is localized at presynaptic terminals of Aplysia and regulates synaptic plasticity by regulating the transcription factor CREB (Rajasethupathy et al., 2009). Recently, a neuronal activity-dependent miRNA, miR-132, was shown to regulate

dendritic development by targeting a Rho family GTPase-activating protein, p250GAP (Wayman et al., 2008). We have demonstrated that neuron-enriched miR-137 has a significant impact *in vivo* and *in vitro* on the maturation and dendritic morphogenesis of young hippocampal neurons by regulating the translation of Mib1, a ubiquitin ligase known to be important for neurogenesis and neurodevelopment (Smrt et al., 2010). Overall, these reports demonstrate that miRNAs have the capacity to modulate the expression of mRNAs important for dendrite and spine development (Fig. 5). The identification of these specific miRNAs that are important for dendritic development and synaptic plasticity are critical for our understanding of miRNAs in the CNS. New methods for detecting and quantifying miRNA at dendritic spines and synapses will help untangle the role of miRNAs in dendritic spine development and synaptic plasticity (see review Schratt, 2009).

2.2 Epigenetic diseases that affect dendritic spine development

Cognitive functions like learning and memory are extremely complex and require a precise balance of epigenetic and regulatory mechanisms. Extensive data support the link between epigenetic dysregulation of gene expression and neurodevelopmental disorders (Table 1). In this section, we will focus on two of the best-characterized epigenetic disorders with known dendritic pathology, Rett syndrome and fragile X syndrome.

2.2.1 Rett syndrome—Rett syndrome (RTT) is a neurodevelopmental disorder and an autism spectrum disorder. RTT is one of the most common forms of mental retardation in young females. RTT patients develop normally until 6–18 months of age, and thereafter experience a myriad of neurological deficits, including seizures, ataxia, and stereotypical hand movements. RTT is caused by loss-of-function mutations in *MECP2*, an X-linked gene encoding the MeCP2 protein, one of the MBDs. MeCP2 is a central player in epigenetic regulation by binding methylated DNA and recruiting factors, such as histone deacetylases, leading to the repression of gene expression (Amir et al., 1999; Bird, 2002) (Fig. 5).

Extensive evidence supports the role of MeCP2 in neuronal maturation (Hagberg et al., 1983; Smrt et al., 2007). The neurological symptoms of RTT appear after a seemingly normal embryonic development, suggesting that MeCP2 is not required for early developmental neurogenesis. However, MeCP2 expression coincides with a period of synaptogenesis (Akbarian et al., 2001; Shahbazian and Zoghbi, 2002; Zoghbi, 2003), suggesting that Mecp2-dependent epigenetic modulation of gene expression is important for the maturation and maintenance of neurons during brain development. Several mouse models have contributed to our understanding of the function of Mecp2 in neuronal and dendritic spine development (Bienvenu and Chelly, 2006; Chahrour and Zoghbi, 2007; Smrt et al., 2007). Expression studies in these animal models indicate that MeCP2 expression steadily increases during neuronal maturation, and is low or absent in immature neurons (Kishi and Macklis, 2004). Both RTT patients and *Mecp2* mutant mice have excess immature neurons in the olfactory epithelium and reduced transition of immature neurons into mature neurons (Matarazzo et al., 2004; Smrt et al., 2007). Additionally, human postmortem tissues show less complex dendritic arborization, smaller soma size, decreased dendritic spine density (Fig. 4B), and lowered levels of the dendritic cytoskeletal protein microtubule-associated protein 2 (MAP2) in RTT brains (Kaufmann and Moser, 2000;

Armstrong, 2002). On the other hand, exogenous Mecp2 expression leads to increased neurite complexity in cultured neurons (Jugloff et al., 2005; Smrt et al., 2007). Consistent with human pathology, adult *Mecp2* mutant mice have smaller soma sizes and less complex dendrites in layer II/III pyramidal neurons in the cortex (Kishi and Macklis, 2004). Using retroviral labeling of new cells, we have shown that *Mecp2*-deficient mice have reduced dendritic spine density and exhibit delayed maturation of newborn dentate granule neurons (Smrt et al., 2007). On the other hand, both we and others have shown that exogenous MeCP2 expression can lead to increased neurite complexity in cultured neurons (Jugloff et al., 2005; Smrt et al., 2010), further suggesting an important role for MeCP2 in dendritic development.

Extensive efforts have been devoted to the identification of downstream effectors of MeCP2, but only a handful of them have been verified, including *BDNF*, *DLX5*, and *DLX6* (Tudor et al., 2002; Chahrour and Zoghbi, 2007). Since these known targets cannot fully explain the deficits associated with MeCP2 deficiency, the identification of additional MeCP2 effector genes is needed to paint a full picture of the pathogenesis of RTT. Recently, epigenetic regulation of noncoding RNAs by MeCP2 has been suggested as a novel mechanism for understanding the pathogenesis of Rett syndrome. It was found that the miR-184 transcript is imprinted and exclusively expressed on the paternal allele and that MeCP2 binds upstream of miR-184 before neuronal depolarization, and releases after depolarization in an activity-dependent manner (Nomura et al., 2008). However, the miR-184 expression level is decreased in *Mecp2* KO brains, which contradicts the authors' finding. It is possible that miR-184 is indirectly regulated by MeCP2. In fact, we have found that miR-184 is directly regulated by MBD1 (Liu et al., 2010). We have shown that MeCP2 can directly regulate the expression of a subset of miRNAs in primary neural progenitor cells isolated from postnatal brains. One of these miRNAs, miR-137, modulates neural progenitor cell (NPC) proliferation and differentiation by regulating protein translation of *Ezh2*, a histone H3K27 methyltransferease (Szulwach et al., 2010). In developing neurons, miR-137 represses dendrite and spine morphogenesis by targeting another neurodevelopmental factor, Mib-1 (Smrt et al., 2010). Therefore, it is conceivable that MeCP2 regulates neuronal dendrite and spine development through noncoding RNAs. Functional crosstalk between the DNA methylation pathway and small regulatory RNA could be an important and novel mechanism regulating mammalian neurodevelopment (Fig. 5).

2.2.2 Fragile X syndrome—Fragile X syndrome (FXS) is the most common cause of inherited mental retardation and is attributed to mutations in the X-linked *FMR1* gene. Epigenetic mechanisms have been implicated in the molecular mechanisms of the disease (Graff and Mansuy, 2009). Typically, fragile X syndrome is caused by the expansion of a polymorphic CGG repeat in the 5′ UTR of the gene. If the CGG expansion reaches more than 200 repeats in female carriers, it is considered a full mutation. As a result, the repeat, the upstream CpG island, and the surrounding sequence become hypermethylated, and the gene is silenced (Oberle et al., 1991). One distinct characteristic of patients with FXS is that they have many more dendritic spines than normal individuals (Fig. 4C), and their spines are longer and thinner, resembling immature spines (Hinton et al., 1991; Irwin et al., 2001). It has been suggested this is due to misregulated development and elimination. This phenotype

is also seen in the mouse model of FXS (Hinton et al., 1991; Comery et al., 1997; Greenough et al., 2001; Irwin et al., 2001; Nimchinsky et al., 2001). During dendritic spine development, synapses may be formed in excess numbers. Therefore, maturation and pruning are needed to establish the final synaptic pattern. In FXS, the activity-dependent events that lead to removal of excess or inappropriately placed synapses do not occur (Galvez et al., 2003).

Fragile X mental retardation protein (FMRP) is an RNA-binding protein, part of the heterogeneous nuclear ribonucleoproteins (hnRNPs), which function in many aspects of mRNA metabolism and biology, including nuclear export of mRNA and subcellular localization (Van de Bor and Davis, 2004). FMRP is part of a large protein complex that is involved in the transportation and translation of mRNA in neurons. Studies have shown that abnormal spines in FXS are associated with impaired neuronal plasticity; therefore, it has been suggested that FMRP may function to transport coding and noncoding RNA to the synapse and participate in local protein synthesis in dendrites (Fig. 5). Local protein synthesis in dendrites modulated by FMRP can potentially influence biochemical pathways or signaling cascades involved in spine morphogenesis, such as Rac1, MAP1B, CamKII, calbindin, and cadherins (Grossman et al., 2006; Penagarikano et al., 2007). The mechanism by which FMRP regulates translation of synaptic factors at the dendritic spine is still under investigation. It has also been shown that the bidirectional transport of the FMRP-mRNA complex between the soma to the dendrites and spines is driven by neuronal activity (Antar et al., 2004; Ling et al., 2004; Antar et al., 2005), indicative of multiple roles for FMRP in the activity-dependent regulation of gene expression at the dendritic spine.

It has been suggested that FMRP can associate with miRNAs and regulate the expression of a subset of target synaptic mRNAs (Jin et al., 2004; Vanderklish and Edelman, 2005; Penagarikano et al., 2007; Fiore et al., 2008) (Fig. 5). Recently, it was shown that a number of microRNAs, including miR125b and miR-132, can associate with FMRP in the mouse brain. While these miRNAs have opposing effects on dendritic spine morphology, knockdown of FMRP ameliorates the effects of overexpressing these microRNAs on spine morphology. The FMRP-associated miRNA 125b was found to target glutamate receptor subunit NR2A, suggesting FMRP-associated miRNAs may have a profound impact on synaptic plasticity, as well as on the pathophysiology of FXS (Edbauer et al., 2010). Although there is supporting evidence that FMRP collaborates with miRNAs to suppress the expression of genes important for dendritic spine morphology and synaptic plasticity, the details of how miRNA and FMRP interact are unclear. One proposed interaction is that miRNA and mRNA could act as the “kissing complex” RNA structure that has previously been proposed to bind the KH2 domain of FMRP (Bassell and Warren, 2008).

3 Methods for studying the epigenetic regulation of dendrite and spine development

3.1 Genetic and molecular methods

Significant advances in genetics and molecular biology have made a profound impact on our understanding of neurodevelopment and disease. Here we will summarize a few important methods.

3.1.1 Drosophila genetics—*Drosophila* neurogenetics was born in Seymour Benzer's lab at Caltech in the mid-1960s, when he proposed that genes can control behaviors in the fruit fly (Benzer, 1967). Benzer's trainees went on to isolate the shaker (sk) gene, which is a member of the transient receptor potential (trp) ion channel family (Papazian et al., 1987). A number of classical studies of brain functional genetics involved the use of loss-of-function approaches in *Drosophila* (Hotta and Benzer, 1970; Lin et al., 1998; Lush et al., 1998; Min and Benzer, 1999).

Many neurologic as well as other human diseases can be modeled using *Drosophila* to characterize genetic, epigenetic, and cellular pathways that lead to the disease state. *Drosophila* genetics are appealing to biologists because the tools of this trade are relatively simple and behavioral paradigms are well characterized, providing an extremely powerful method to go from mutant phenotypes to genotypes (forward genetics). Thus, the use of *Drosophila* models of human disease has grown rapidly and significantly contributed to our understanding of the human pathogenesis. The molecular and cellular pathways in *Drosophila* are generally considered to be highly conserved with vertebrates; approximately 75% of human genes known to be associated with disease correspond to a *Drosophila* ortholog (Reiter et al., 2001). *Drosophila* can be used to conduct assays ranging from genetic screens to drug target validation. For example, genetic modifier screens can identify proteins and genes that interact with pathways associated with pathology (Egger et al., 2004). Additionally, flies are excellent subjects for high-throughput drug screening libraries and are responsible for the identification of mGluR5 as a drug target for treating fragile X syndrome (Marsh and Thompson, 2006; Chang et al., 2008).

Drosophila has been used to understand the genetic pathways implicated in fragile X syndrome. The *Drosophila* genome contains the gene *Drosophila* fragile X-related (*dfxr* or *dfmr1*), which is homologous to FMR1 (Wan et al., 2000) and contains all the functional motifs related to FMRP. It was found that *Drosophila* genetics could be used to show dFXR plays a role in neuronal development, including synaptic formation, axonal growth, and dendritic development (Zhang et al., 2001; Dockendorff et al., 2002; Morales et al., 2002; Lee et al., 2003; Michel et al., 2004).

Drosophila has been used for studying epigenetic regulation related to histones and miRNAs. For example, dFMR1 is important in the miRNA pathway (Caudy et al., 2002; Ishizuka et al., 2002; Yang et al., 2007) and has been shown to modulate miR-124a levels, which are important for dendritic branching in *Drosophila* (Xu et al., 2008). However, CpG

methylation is essentially absent in *Drosophila*, making it an invalid model for studying mammalian DNA methylation.

3.1.2 Genetic mouse models—Genetic manipulation of the mouse genome along with the characterization of mutant phenotypes has made a profound impact on our understanding of the pathophysiology of many human diseases. Mice and humans share 99% of their genes and have similar physiological and biochemical features, making genetic mouse models a powerful tool to study human disease (Rosenthal and Brown, 2007). This section will briefly describe the most common mouse models used to study Rett syndrome and fragile X syndrome, both implicated in abnormal spine development.

3.1.2.1 The MeCP2 mouse: To model Rett syndrome and related disorders in mice, three mouse models were generated with different *Mecp2* genetic alterations. One of these models is the *Mecp2* conditional KO mouse, which lacks either exon 3 or both exons 3 and 4 of the *Mecp2* gene (Chen et al., 2001; Guy et al., 2001). These conditional knockout mice develop normally during the first 3–6 weeks of life, and thereafter develop motor dysfunction, hind limb clasping, and breathing irregularities. Mutant brains show reduced brain weight and more densely packed neurons, but do not show neuroanatomical abnormalities. The female mice, which are heterozygous for the *Mecp2* mutation (*Mecp2* +/−), show behavioral phenotypes that are less severe and with a later onset.

Another mouse model was generated by truncating *Mecp2* at amino acid 308. This truncation resulted in a hypomorphic allele that contains a truncated C-terminal region, reminiscent of Rett syndrome patients found with C-terminal deletions (Shahbazian et al., 2002). These mice have many similarities with the conditional knockout model described above, but have a less severe phenotype that leads to longer longevity.

The third mouse model involves a two-fold over-expression of human MeCP2 that leads to an initial increase in synaptic plasticity and contextual learning; however, by 20 weeks of age the mice develop progressive neurological phenotypes, including motor abnormalities, hind limb clasping, and seizures, and they die by 1 year of age (Collins et al., 2004). This is also seen in the clinic, where duplication of *MECP2* in human male patients causes severe mental retardation. Moreover, selective overexpression of MeCP2 in post-mitotic neurons, under the tau promoter, leads to a progressive neurological phenotype in mice (Luikenhuis et al., 2004).

In yet another mouse model, deletion of *Mecp2* specifically in post-mitotic neurons using a CaMKII Cre transgene results in a phenotype resembling the *Mecp2*$^{-/y}$ knockout. This suggests that dysfunction of brain-specific MeCP2 leads to the neurological phenotype observed in Rett syndrome (Chen et al., 2001; Gemelli et al., 2006). Additionally, when *Mecp2* is expressed in post-mitotic neurons under the tau promoter in null mice, the Rett phenotype is rescued, further supporting this idea (Luikenhuis et al., 2004). Taken together, these studies demonstrate that perturbations in the homeostatic balance of MeCP2 expression can result in aberrant neurological functioning.

3.1.2.2 The fragile X mouse: FMR1 is highly conserved between humans and mice (Ashley et al., 1993). The FMR1 knockout mouse was generated by disrupting exon 5 of the FMR1 gene by homologous recombination. This resulted in the absence of normal FMRP protein (Bakker et al., 1994). These mice show cognitive and anatomical impairments that are related to those seen in human patients, making the fragile X knockout mouse an important model to study the function of FMRP. The fragile X knockout mouse displays distinct phenotypes, such as hyperactivity, special learning deficits, macroorchidism, and altered dendritic spines (Bakker et al., 1994; Kooy, 2003). Among the similarities between the human pathology and the mouse model is cognitive function. It has been found that FMR1 KO mice have special learning deficits when challenged with the Morris water maze and radial arm maze (Bakker et al., 1994; Kooy et al., 1996; Mineur et al., 2002; Kooy, 2003), two tests generally considered to reflect problems in hippocampal functioning (Morris et al., 1982; Logue et al., 1997). We found that FMR1 KO mice exhibit impaired hippocampal neurogenesis, which may contribute to the deficits in hippocampus-dependent learning (Luo et al., 2010). Among structural abnormalities in the mouse model, FMR1 KO mice show macroorchidism due to increased Sertoli cell proliferation (Slegtenhorst-Eegdeman et al., 1998). No gross neuroanatomical differences are observed in human patients or fragile X mouse brains (Bakker et al., 1994); however, it is well appreciated that patients and mice have long, thin immature spines with increased spine density in dendrites of the cortex (Comery et al., 1997; Irwin et al., 2001). FRM1 KO mice also show a propensity for epileptic seizures. Seizures occur in fragile X patients (Hull and Hagerman, 1993) and can be elicited by auditory stimuli in knockout mice (Chen and Toth, 2001). Epileptic seizures in fragile X are likely due to dendritic spine abnormalities in mice and patients. Fragile X mice have been used for developing therapeutic treatments, though the results are still controversial. Pharmacological approaches have been proposed to compensate for the loss of FMRP (Kooy, 2003; Penagarikano et al., 2007). More work is required for a better understanding of the mechanism of how FMRP regulates mRNA translation, and how FMRP functions in activity-dependent local protein synthesis in the dendrite.

3.1.3 Isolation and RNA content analysis of synaptoneurosomes—

Synaptoneurosomes are small vesicle structures that are prepared by subcellular fractionation of brain homogenate. These structures are known to be enriched in dendritic spines (Lugli et al., 2008). It has been shown that polyribosomes, as well as many mRNAs, are present in dendrites and are recruited into dendritic spines (Ostroff et al., 2002; Bourne et al., 2007). Gene chip analysis of postsynaptic density (PSD) fraction-associated mRNAs shows that mRNAs encoding many postsynaptic proteins are highly concentrated in PSD fractions (Suzuki et al., 2007). In addition, synaptoneurosomes also contain a large number of miRNAs. To characterize microRNA expression at the synapse, Lugli and collaborators isolated synaptic fractions from the mouse forebrain and analyzed microRNA expression using microarrays; enriched microRNAs were subsequently confirmed by quantitative RT-PCR (qRT-PCR) (Lugli et al., 2008). Lugli et al. found that a number of microRNAs were highly enriched in synaptoneurosomes and are predominately associated with PSDs. Their study further supports the role of miRNAs in protein synthesis at the synapse. It is likely that synaptosomes contain other types of RNAs and perhaps even DNA. With the development

of next-generation sequencing, more synaptosome RNAs will be revealed, providing a better picture of the regulatory network governing dendrite and spine development.

3.2 Histological methods

3.2.1 The Golgi method—The Golgi method was discovered by Camillo Golgi and published in 1873 (Golgi, 1873); however, it was popularized by Ramon e Cajal, who modified the method to study a myriad of species and tissues during many developmental periods, including the brain (De Carlos and Borrell, 2007). Since then, the application of the Golgi technique in neurosciences has been used to study morphological abnormalities in dendrites that are typical in diseases associated with mental retardation. The pioneers of Golgi impregnations to study dendrites in MR were Huttenlocher (Huttenlocher, 1970, 1974) and Purpura (Purpura, 1975), who relied on post-mortem and biopsy material. Using the Golgi method, Parpura found cortical pyramidal neurons to have shorter and less complex dendritic branches in individuals with unclassified MR (Purpura, 1974). This dendritic “dysgenesis” was also observed in individuals with chromosomopathies and genetic disorders associated with MR (Marin-Padilla, 1972; Ferrer et al., 1984) (see Fig. 4). Golgi impregnation in Rett syndrome post-mortem tissue showed a reduction in the dendritic arborization of cortical neurons throughout life in RTT patients (Armstrong et al., 1995). Similar results were obtained using this method in an animal model of RTT lacking MeCP2 (Kishi and Macklis, 2004). In fragile X syndrome, Golgi preparations showed long dendritic spines (Hinton et al., 1991), and the *FMR1* knockout mouse also shows spine dysgenesis (Comery et al., 1997).

3.2.2 Lipophilic dye—Lipophilic dyes are fluorescent substances that can be microinjected into a single cell and visualized using a confocal microscope to study neuronal morphology in both animals and humans (Belichenko et al., 1994). This method offers advantages over the Golgi method because that experimenters can select the neuron(s) that are to be infused with the dye and perform digital 3-D reconstruction of dendritic morphology with laser confocal imaging, instead of the traditional light microscope with Camera-Lucida method. Belichenco's group used the dye Lucifer Yellow and confocal microscopy to reconstruct the 3-D morphology of neurons in the prefrontal, motor, and temporal areas of RTT brain tissue. They showed reduced apical dendritic morphology and spine density, as well as dendritic segments lacking spines, which they called “naked dendrites,” in pyramidal neurons of the frontal cortex (Belichenko et al., 1994; Belichenko et al., 1997). Belichenko and his colleagues used this method to study the morphology of neurons in a number of diseases and injuries associated with spine pathology (Belichenko et al., 1994; Belichenko et al., 1997; Johansson and Belichenko, 2002; Belichenko et al., 2004).

3.2.3 The single-cell genetic approach—The use of retrovirus-mediated gene delivery, also known as the single-cell genetic approach, has made it possible to study the morphology and functional properties of newborn neurons throughout their lifetime (Gage, 2002; van Praag et al., 2002). Newborn neurons in the adult hippocampus have been used as a model to study neuronal development, because they recapitulate many features of embryonic hippocampal development (Song et al., 2005). When using the retroviral

approach, newborn cells in the hippocampus are infected with retrovirus, causing them to express a live reporter, such as green fluorescent protein (GFP), throughout the cell, including the dendritic processes spines. This allows researchers to conduct a temporal analysis of the dendrite and spine development of newborn neurons by direct visualization of living newborn cells (Zhao et al., 2006). We used the single-cell genetic approach in an animal model of RTT to investigate the role of Mecp2 in developing neurons of the adult hippocampus (Smrt et al., 2007). We found that four-week-old new neurons in adult *Mecp2* knockout animals showed reduced dendritic spine density, a characteristic feature of immature neurons suggesting that newborn dentate granule neurons lacking Mecp2 have impaired maturation. Using a similar approach, we demonstrate that miR-137 overexpression inhibits dendritic morphogenesis (Smrt et al., 2010). These data are consistent with similar findings using other methods described in this section to study dendritic spines under pathological conditions and support the idea that abnormal dendrite and spine development is the common point of vulnerability leading to the neurological deficits in diseases associated with mental retardation. The retrovirus-labeled GFP+ neurons can be further analyzed using immuno-electron microscopy to reveal synapse formation and structure (Duan et al., 2007; Toni et al., 2008).

4 Conclusions

In this review, we have discussed the role of epigenetics in dendritic and spine morphogenesis. Since Cajal's epic contributions to neuroscience in the late 19th and early 20th centuries, contemporary science and advances in genetic and molecular tools have enabled researchers to look deeper into the underlying mechanisms of dendritic and synaptic development. The advent of epigenetic research has provided much new knowledge and also opened up more questions regarding the role of gene expression regulation in neurodevelopment. Our focus as researchers is to use our growing knowledge of molecular biology and genetics to understand how dendrites and dendritic spines become grossly impaired in individuals faced with mental retardation and neuropathology.

Recent studies have shown that mutations in the epigenetic machinery lead to many forms of mental retardation disorders. Synaptic plasticity, as well as learning and memory, is believed to depend on proper neuron-neuron communication, and identification of the genes involved in synaptic development is crucial to understanding these human disorders.

The important challenge for both scientists and clinicians is to identify the molecular basis for cognitive and behavioral symptoms in order to design adequate pharmacological treatments. A number of epigenetic-based therapeutic methods have been proposed as treatments for epigenetic-associated diseases. Many pharmacological agents have been discovered that alter methylation patterns on DNA and histones, many of which are being extensively tested *in vitro* and in clinical trials (Egger et al., 2004). However, epigenetic reagents, such as HDAC or DNA methylation inhibitors, have met with concerns about the specificity of the drug's action. MicroRNAs have recently been identified as promising candidates for the treatment of diseases, such as heart disease and cancers. The challenge is to identify the specific miRNA(s) that could be considered treatment targets and to deliver

that miRNA or miRNA inhibitor precisely to the target cells with high efficiency and without side effects.

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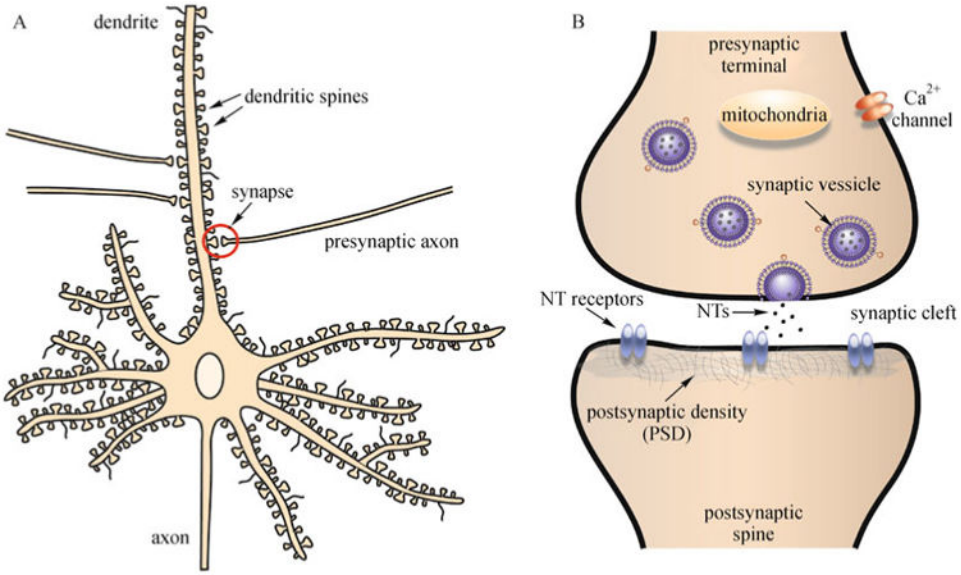

Fig. 1. Schematic diagram of a mature neuron and synapse

A: A mature neuron has elaborate processes that are composed of multiple dendrites and one axon. Dendrites contain a large number of dendritic spines that form contacts, or synapses, with other neurons. The synapses are typically found on the dendritic shaft, located on stubby spines and filipodia. B: A neuronal synapse is the point of contact between two neurons, a presynaptic neuron and a postsynaptic neuron. It is characterized by specific synaptic proteins located on the pre- and postsynaptic sites.

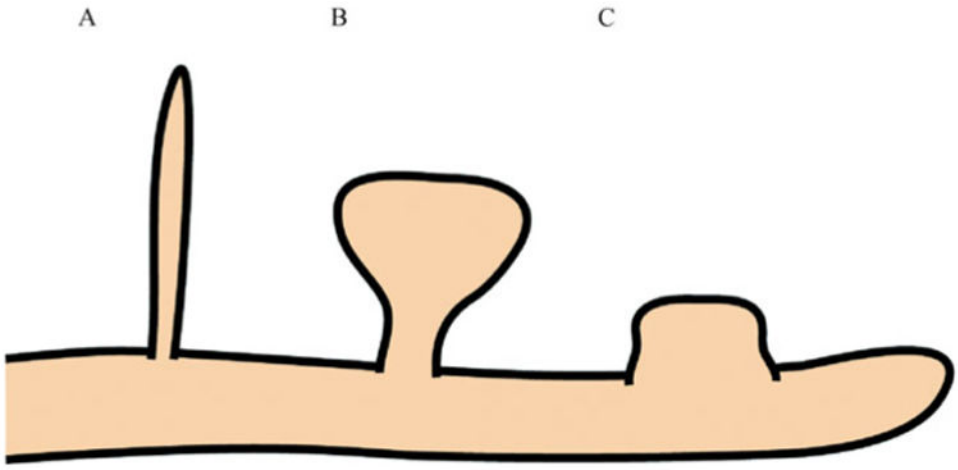

Fig. 2. Common dendritic spine types in the CNS

Three types of dendritic spines are commonly found in the CNS. A: the long and thin filipodia spine with no head enlargement; B: the mushroom-like spine with a bulbous head attached to the dendrite by a narrow neck; and C: the short and stubby spine with no neck. It is believed that long, thin spines are mostly immature spines, and they develop into the more mature mushroom-like spines. CNS: central nervous system.

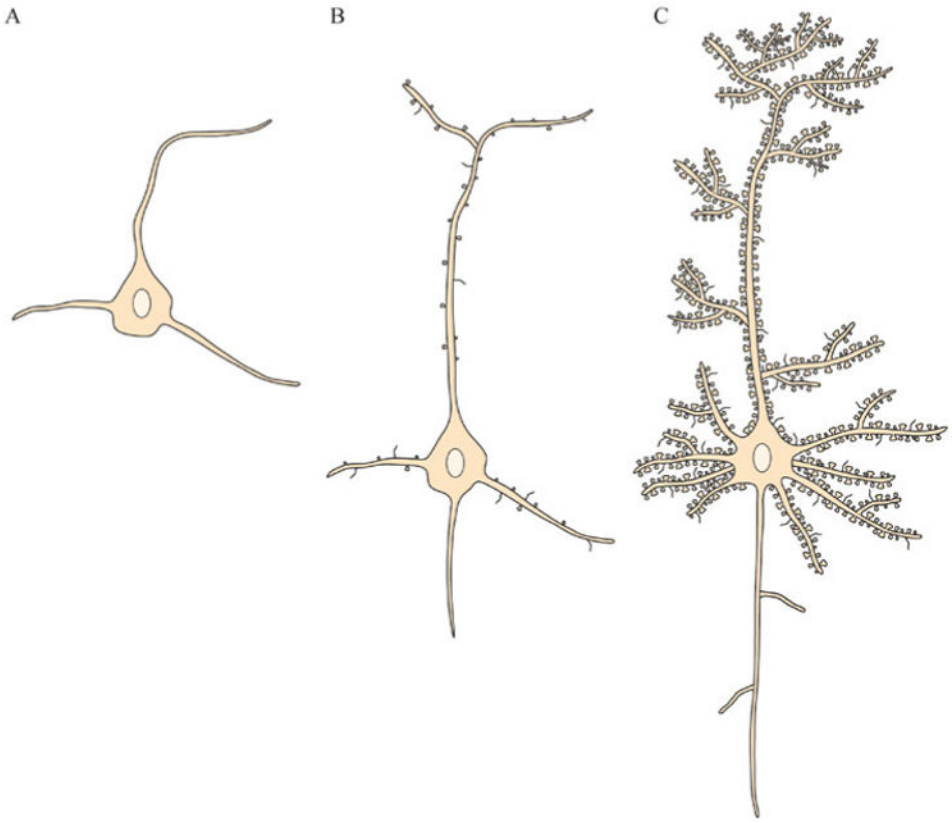

Fig. 3. The stages of morphological development of dendrites and dendritic spines

A: Immature neurons have shorter dendrites, and these dendrites have no spines. B: During synaptogenesis and neuronal maturation, neurons develop a more complex dendritic arbor, and these dendrites begin to form spines. Some of these spines start to receive input from the axons of other neurons. C: Mature neurons have elaborate dendritic trees and a high density of mature spines. These neurons form functional connections with other neurons and participate in the brain circuitry.

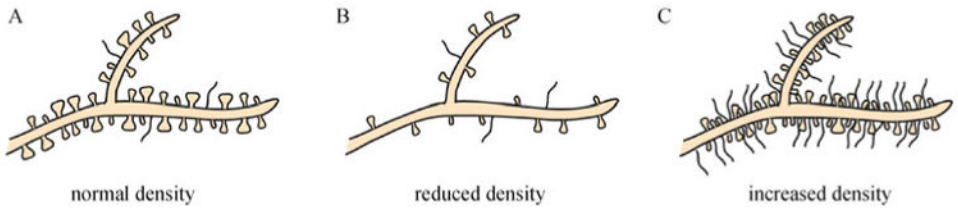

Fig. 4. Pathological changes in spine density. Common pathological alterations in dendritic spines are altered spine density and abnormal spine shapes

A: Healthy neuronal dendrites contain spines with a typical variation of spine types. B: Reduced spine density is common in many cognitive and developmental disorders, such as Rett syndrome. C: Increased spine density with abnormally increased long and thin types of spines is commonly found in patients with fragile X syndrome.

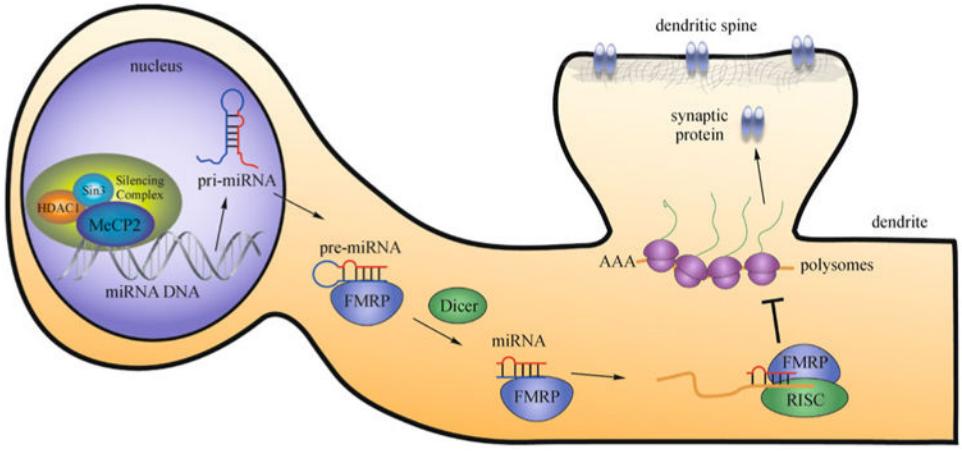

Fig. 5. Epigenetic regulation of biogenesis and functions of miRNA in the neuron

Transcription of miRNA, which can be regulated by epigenetic machinery, leads to the production of miRNA transcripts (pri-miRNAs), which are cleaved in the nucleus and exported into the cytoplasm as pre-miRNAs. The pre-miRNA is processed by the RNase Dicer and is likely associated with other accessory proteins, such as fragile X mental retardation protein (FMRP), to form an intermediate miRNA duplex. The leading strand of the miRNA duplex can then associate with the miRNA-induced silencing complex (miRISC), and then pair with sequences located on the 3′UTR of target mRNAs. This leads to translational repression of the target mRNA. Recent evidence suggests that RNA-binding proteins like FMRP interact with miRNAs to modulate mRNA translation. This example illustrates the effect of miRNA-mediated translational repression of proteins important for dendritic spine function.

Table 1
Spine pathology related to genetic mutations and mental retardation

disorder	genetic abnormality	spine pathology	reference
Rett syndrome	MECP2 mutation	reduced spine density, reduced dendritic complexity and maturation	Belichenko et al., 1994; Amir et al., 1999; Moretti et al., 2006; Smrt et al., 2007
fragile X syndrome	FMR1 protein deficiency	increased spine density, abnormal spine morphology	Hinton et al., 1991; Wisniewski et al., 1991; Comery et al., 1997; Irwin et al., 2001
Angelman syndrome	chromosome 15q11–q13, UBE3A	reduced spine density, abnormal spines, small RNA pathway	Miura et al., 2002; Lalande and Calciano, 2007; Ding et al., 2008
Prader–Willi syndrome	chromosome 15q11–q13	autism, reduced cognitive ability	Battaglia, 2005; Sahoo et al., 2008
Down's syndrome	chromosome 21 trisomy	reduced spine density, reduced dendritic complexity	Marin-Padilla, 1972, 1976; Suetsugu and Mehraein, 1980; Takashima et al., 1981; Ferrer and Gullotta, 1990; Takashima et al., 1994
Lafora disease	EPM2 mutation	reduced spine density	Busardz et al., 1987
Patau syndrome	chromosome 13 trisomy	abnormal spine morphology	Marin-Padilla, 1972
tuberous sclerosis	mutations of TSC1 or TCS2 genes	reduced spine density, abnormal spine morphology	Machado-Salas, 1984
Niemann–Pick disease	deficiency of sphingomyelinase	reduced spine density, reduced dendritic complexity	Walkley and Baker, 1984; Higashi et al., 1993; Sarna et al., 2003
Potocki–Lupski syndrome	duplications of 17p11.2	autism	Potocki et al., 2007
Smith–Magenis syndrome	chromosome 17p11.2, *RA11* mutations	autism, abnormal brain anatomy	Slager et al., 2003; Elsea and Girirajan, 2008
Williams–Beuren syndrome	7q11.23 (CYLN2, LIMK1, FZD9)	reduced brain volume, altered spine morphology	Hoogenraad et al., 2002; Meng et al., 2002; Zhao et al., 2005; Berg et al., 2007; Lim et al., 2007
22q11.2 deletion syndrome and DiGeorge syndrome	deletion of 22q11.2, DGCR8	miRNA pathway, autism, smaller spines, smaller dendrites	Lee and Lupski, 2006; Gothelf et al., 2007; Kobrynski and Sullivan, 2007; Stark et al., 2008

Table 2

Summary of epigenetic modifications and their direct effects on gene expression

epigenetic modification	target	direct effect on gene expression
DNA methylation	CpG dinucleotides	repression
	H3 (K4, K36, K79)	activation
histone methylation	H3 (K9, K27), H4 (K20)	repression
	H3 (R17, R23), H4 (R3)	activation
histone acetylation	H3 (K9, K14, K18, K56), H4 (K5, K8, K13, K16)	activation
microRNA	mRNA	repression