Cell. Mol. Life Sci. (2012) 69:89–102
DOI 10.1007/s00018-011-0788-1

Cellular and Molecular Life Sciences

REVIEW

MicroRNA networks direct neuronal development and plasticity

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Received: 29 April 2011 / Revised: 24 June 2011 / Accepted: 21 July 2011 / Published online: 11 August 2011

Abstract MicroRNAs (miRNAs) constitute a class of small, non-coding RNAs that act as post-transcriptional regulators of gene expression. In neurons, the functions of individual miRNAs are just beginning to emerge, and recent studies have elucidated roles for neural miRNAs at various stages of neuronal development and maturation, including neurite outgrowth, dendritogenesis, and spine formation. Notably, miRNAs regulate mRNA translation locally in the axosomal and synaptodendritic compartments, and thereby contribute to the dynamic spatial organization of axonal and dendritic structures and their function. Given the critical role for miRNAs in regulating early brain development and in mediating synaptic plasticity later in life, it is tempting to speculate that the pathology of neurological disorders is affected by altered expression or functioning of miRNAs. Here we provide an overview of recently identified mechanisms of neuronal development and plasticity involving miRNAs, and the consequences of miRNA dysregulation.

Keywords MicroRNA · Neuronal development · Synapse · Learning and memory · Neurological diseases

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Introduction

Synaptic development and plasticity are essential processes for fine-tuning brain circuitry during development, and for learning, memory, and cognition. A number of developmental and adult brain disorders are associated with abnormal changes in synaptic connectivity and plasticity, including fragile X syndrome (FXS) and autism [1]. Previous studies have indicated that synaptic plasticity is under tight control via a number of different post-transcriptional mechanisms, such as the ubiquitin–proteasome system [2], translation initiation and elongation [3], and miRNAs [4]. miRNAs have emerged as an important class of small non-coding RNAs, which are highly conserved and involved in a subset of biological processes such as developmental timing, cell proliferation, apoptosis, metabolism, cell differentiation, and morphogenesis [5]. They act to control the expression of sets of genes and entire pathways, and are thus thought of as master regulators of gene expression [6]. A few miRNAs are expressed in mammalian brains specifically, suggesting unique regulatory roles in neuronal development and higher brain function [7]. Recent estimates put the number of human miRNAs at 1100 or more, composing complex regulatory networks that influence the expression of as many as two-thirds of all genes [8]. Such a large family of genes could explain some of the difficulties that neurobiologists generally have encountered in their efforts to link individual miRNA genes to mental disorders. Because they are single molecular entities that dictate the expression of fundamental regulatory pathways, miRNAs represent potential drug targets of unprecedented power. Here, we review recent progress in our understanding of the post-transcriptional mechanisms of miRNAs during neuronal development and plasticity. We will emphasize the role of

miRNAs in regulating pre- and post-synaptic protein synthesis systems essential for long-lasting memory formation in the brain.

miRNA biogenesis and function

Although most miRNAs are encoded in intergenic regions, miRNA genes are also found in introns or exons of non-coding RNA genes, or within introns of protein-coding genes [9]. The miRNA pathway starts in the nucleus with the RNA polymerase II-mediated transcription of primary (pri-) miRNAs which can be several kilobases (kb) in size (Fig. 1). The stem-loop conformation of pri-miRNAs is recognized by the nuclear protein Drosha. With its RNase III activity, it cleaves the pri-miRNA into a precursor (pre-) miRNA of around 70–80 nucleotides (nts). The specific processing by Drosha is directed by DiGeorge syndrome critical region 8 (DGCR8) protein [10, 11]. DGCR8 increases the activity of Drosha eight fold [11]. Similar to Drosha knockdown (KD), the KD of DGCR8 leads to the accumulation of pri-miRNAs and a decrease in the amount of pre-miRNAs [10, 11]. The Drosha/DGCR8 complex generates a pre-miRNA with a 2 nt 3′ overhang [12, 13] and a stem-loop conformation ideally consisting of ~22 nts, which will become the mature miRNA later on in the maturation pathway [13]. The characteristic 3′ overhang and stem-loop conformation are essential for miRNA recognition by exportin-5, which transports the pre-miRNA from the nucleus to the cytoplasm with high efficiency in a Ran/GTP-dependent manner [12]. Although the majority of miRNAs are processed by Drosha, there is also miRNA population that bypasses the Drosha/DGCR8 enzyme complex. One of the miRNA subclasses, known as "miRtrons", is encoded in the introns of protein-coding genes, and mimics the structural features of pre-miRNAs. miRtrons are spliced out to form pre-miRNAs that can be further processed through the common miRNA processing cascade [14–17]. Also, shRNA-derived miRNAs and endogenous hairpin-derived siRNAs are processed in a Drosha-independent manner. The expression patterns as well as the functions of these subclasses of miRNAs and

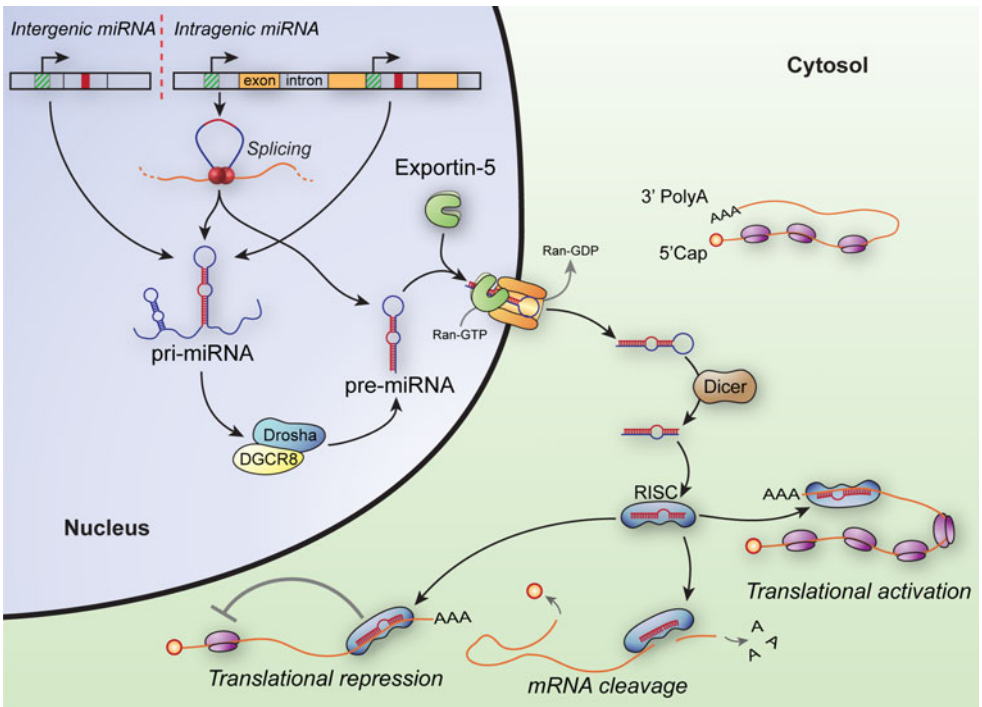

Fig. 1 The biogenesis of miRNAs. Pri-miRNAs are transcribed from intergenic genes and later capped and polyadenylated. Alternatively, miRNAs transcription can occur from genes coded within introns of coding or non-coding genes. Pri-miRNA transcripts are processed into a pre-miRNA by the Drosha/DGCR8 complex, creating a ~70–80 nts, hairpin-looped molecule, which is then shuttled out of the nucleus via the exportin-5 mediated transport. Some intronic-encoded miRNAs evade nuclear processing prior to nuclear export. Cytoplasmatic digestion of the pre-miRNA is facilitated by Dicer, resulting in double-stranded mature miRNAs. Mature miRNAs modulate gene expression by associating with Argonaute-containing complexes to form the RNA induced silencing complex (RISC). By imperfectly or near-perfectly base pairing with sequences in the 3′ UTRs of target mRNAs, miRNAs modulate gene expression through transcript destabilization and translational attenuation. Alternatively, mRNA deadenylation and decapping precedes mRNA cleavage, a process depending on the degree of base-pairing [126]. Some miRNAs can enhance translation upregulation of target mRNAs on cell cycle arrest, while repressing translation in proliferating cells [127]

siRNAs in mammalian somatic tissues is a yet unexplored area [14]. Moreover, different subclasses of small nucleolar RNAs (snoRNAs), which fulfill miRNA-related functions outside of the nucleolus, are found to be processed independently of Drosha.

In the cytoplasm, pre-miRNAs are recognized and processed by the RNase III Dicer. Dicer KO in *Caenorhabditis elegans* [18, 19] and mammalian cell lines [20] resulted in the accumulation of cytoplasmatic pre-miRNA. This was first demonstrated for let-7, a miRNA highly conserved among many species, with an essential role in developmental timing in *C. elegans* and *Drosophila* [18–21], and prominently involved in many forms of cancer [21]. Fragile X mental retardation protein (FMRP) interacts with RNAs of around 80 nts and with Dicer in vivo. The complex immunoprecipitated with anti-FMRP antibodies is able to process the long double-stranded pre-miRNAs into mature miRNAs of around 22 nts [23]. An exception on this Dicer-dependent miRNA processing is miR-451, which instead uses AGO2 slicer catalytic activity in order to be processed into a mature miRNA. The use of this alternative processing pathway is accomplished by the structural conformation of miR-451 [25]. Once processed into their mature form, miRNAs enter the miRNA silencing complex (miRISC) to degrade or silence mRNAs. During this process, the miRISC complex is supported by a set of proteins, among which Argonaute (AGO)1, AGO2, Pumilio2 (Pum2), and Moloney leukemia virus (MOV10). Within this miRISC complex, AGO1 directly associates with miRNAs [22], and in addition interacts with FMRP [23]. Similarly, AGO2 interacts with mature miRNAs captured in the miRISC complex, and suppression of AGO2 reduced the ability of cells to silence exogenous reporters [24].

Each miRISC-incorporated mature miRNA can act on the expression levels of many mRNAs and conversely individual mRNAs can be targeted by a number of miRNAs. This allows for vast combinatorial complexity that could partly account for the genetic complexity associated with a multitude of essential biological processes. While mRNA targeting and regulation in plants requires a near-perfect complementarity between mature miRNA and the target mRNAs, in animals miRNAs regulate gene expression by imperfect or near-perfect base pairing to mostly the 3′ untranslated region (UTR) of target mRNAs, thereby inhibiting protein synthesis or causing mRNA degradation [26]. miRNA-mediated translational inhibition or mRNA degradation in addition depends on the 5′ cap region of the target mRNAs, since it was shown that miRNAs are able to inhibit the translation of m7G-capped mRNAs but not of mRNAs with an internal ribosome entry site or with a non-functional ApppN cap [27, 28]. In eukaryotic cells, mRNAs are circularized by initiation factors, among which eIF4E and poly(A)-binding protein 1 [29]. AGO proteins stimulate miRNA-dependent inhibition of translation by competing with eIF4E for the 5′ cap binding site, thereby preventing circularization of the mRNA and thus lowering the initiation efficiency and at the same time sensitizing the poly(A) tail for exonuclease [28].

Neuronal localization of miRNAs

The expression of miRNAs in the brain can be ubiquitous or restricted to a specific brain region, e.g., the hippocampus, cortex, or amygdala [30–32]. This regional specificity in miRNA expression reflects the differences in mRNA composition of cells within various brain tissues and their need for a selection of miRNAs. This notion offers the possibility to establish cell- and tissue-specific miRNA functions by identifying their target mRNAs. Several recent studies have shown subcellular localization of miRNAs within neuronal dendrites of the forebrain [33] and the hippocampus [34]. In synaptoneurosomes, representing the dendritic spines of the neuronal cells, pre-miRNA levels are high in comparison to the levels of mature miRNAs. In synaptosomes consisting mainly of axon terminals and the adherent post-synaptic densities, pre-miRNAs are enriched as well. miRNAs are thus delivered at the synapse predominantly in their premature forms, destined to be processed locally in correspondence with neuronal conditions [33]. miR-26a was found to be one of the miRNAs most enriched in the dendrites. Sequence-specific miRNA KD identified microtubule-associated protein 2(MAP2) mRNA as a target for miR-26a. This miRNA reduces microtubule assembly during synaptic plasticity through inhibition of MAP2 mRNA translation [34].

miRNAs have been shown to be present in the axonal compartments where they can actively regulate local mRNA translation. Key proteins involved in the RNAi/miRNA pathway, i.e., miRISC complexes, can assemble and function in developing axons [35]. Recently, Natera–Naranjo [36] and colleagues employed compartmentalized Campenot cell culture chambers to obtain a pure axonal RNA fraction of superior cervical ganglia (SCG) neurons, and determined the miRNA expression levels in these subcellular structural domains. Their data revealed stable expression of a number of mature miRNAs that were enriched in the axons and at presynaptic nerve terminals. Among the 130 miRNAs identified in the axon, miR-15b, miR-16, miR-204, and miR-221 were found to be highly abundant in distal axons as compared to the cell bodies. Interestingly, a number of miRNAs encoded by a common pri-miRNA transcript were differentially expressed in the distal axons, suggesting that there is a differential subcellular transport of miRNAs derived from the same coding

region of the genome. In addition, Aschrafi et al. [37] used the same compartmentalized cell culture setting and identified miR-338 as a novel modulator of the intra-axonal translation of cytochrome c oxidase IV (COXIV) mRNA, a nuclear-encoded mitochondrial protein that plays a rate-limiting role in the assembly of the mitochondrial enzyme complex IV and in oxidative phosphorylation. Regulation of axonally localized COXIV mRNA by miR-338 resulted in a decrease in mitochondrial activity, as measured by the reduction of cellular oxygen consumption and ATP levels. Despite increasing evidence for the existence of miRNAs in axons obtained from cell culture experiments, the in vivo relevance of axonal miRNAs in local translation is still unknown. Moreover, little is known about the mechanism of selective miRNA transport into neuronal endings [38].

Molecular basis of local synaptic translation

While the majority of neuronal mRNAs are translated in the neuronal soma, increasing evidence from more than three decades of neurobiological research suggests that a fraction of the neuronal transcriptome is selectively transported to distal domains of the neuron. The local translation of these mRNAs has a key role in neuronal development, including neuronal survival [39], navigation of the axon [40–45], axonal regeneration [46, 47], synapse formation [48], and activity-dependent synaptic plasticity [49–52]. Notably, a fundamental observation in the field of learning and memory is that new proteins must be synthesized locally at the synapse in order to consolidate newly formed memories [53]. In addition, several Mendelian forms of intellectual disability, such as FXS are caused by changes in synaptic plasticity as a major cellular correlate of their neuropathological phenotypes [54]. As with memory, the stabilization of several distinct forms of activity-dependent synaptic plasticity requires a wave of post-synaptic protein synthesis in the dendrites of neurons. Each form of synaptic plasticity has very distinct characteristics, such as the activation of specific signaling cascades and receptor internalization or surface expression, suggesting that different sets of proteins are required to consolidate the different forms of plasticity [55]. mRNA transport and subsequent protein synthesis at specific synaptic sites allow regulation at the level of individual synapses, a process required for proper neuronal and behavioral plasticity [56–61]. The local distinction between synapses that undergo long-term potentiation (LTP) and synapses that do not, lies within the ability of a synapse to use the proteins made upon neuronal stimulation: only synapses that receive neuronal stimuli will be able to use the proteins made by the activity-induced transcription [59, 62]. The advantage of site-specific translation is that only a few mRNA copies have to be transported to the synapses which in turn can be translated multiple times, whereas multiple proteins produced in the soma from the same mRNA would have to be transported to the synapses [63]. Regulation of local mRNA translation alters the synaptic protein content upon neuronal stimulation by changing the amount of proteins, by changing the entire synaptic proteome through alterations in protein abundance relative to each other, or by the expression of protein isoforms [56, 62].

Many studies have identified a number of mRNAs localized in the dendrites of neurons and thus representing candidates being linked to synaptic plasticity [34, 64–66]. These dendritic mRNAs encode proteins involved in multiple cellular processes regulating spine activity, including signal transduction, mRNA translation, protein transport and degradation, cytoskeleton regulation, and RNA binding and silencing. Among the locally synthesized signaling pathway components are the mitogen-activated protein kinase (MAPK), calcium/calmodulin-dependent protein kinase (CaMK) and protein kinase C (PKC) pathways, which are needed for the induction of morphological changes in synaptic connections and for the maintenance of LTP [65]. Importantly, mRNAs encoding proteins that are essential for translation are also dendritically localized, such as mRNAs coding for the eukaryotic initiation factors eIF4γ2, eIF3 and eIF5 [64], and elongation factors EF1α, EF2 [64] and eEF1A [65], suggesting that local translation is tightly regulated at multiple levels. Furthermore, mRNAs encoding proteins that participate in neuronal outgrowth and spine formation were detected at the spines, among which FMRP, Pum2, and activity-regulated cytoskeleton-associated protein (Arc). FMRP is required for activity-dependent translation [60], Pum2 regulates eIF4E-dependent mRNA translation, dendritic morphology and neuronal excitability, and Arc is involved in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) internalization. For neurite outgrowth, also cytoskeletal remodeling is required, a process regulated by the locally translated MAP2 [65]. Furthermore, CaMKII and post-synaptic density 95 (PSD-95), fulfilling a scaffolding function at post-synaptic spines, were found to be locally expressed [34].

miRNAs are involved in synaptic maturation and plasticity

Several studies address the importance of miRNAs in neuronal differentiation and synaptic functionality using KO models of Dicer or DGCR8. Dicer ablation in mice causes impaired neuronal differentiation, reduction in neuronal size, loss of branching and disturbed axonal pathfinding [67–69]. DGCR8 KO mice display deficits in

synaptic connectivity due to a reduction in the number and size of dendritic spines, reduced synaptic complexity and impaired synaptic transmission [70]. However, by knocking out Dicer or DGCR8 the major part of miRNA processing is blocked, giving an indication of the functions of miRNAs in general. In contrast, studies using single miRNA KD provide a more specific way to investigate the role of individual miRNAs throughout neuronal differentiation steps or during synaptic activation.

Recent findings from studies using single RNA KD also indicate that miRNAs may be involved in modulating local translation and consequently long-term synaptic plasticity (Table 1). The miRISC protein MOV10 regulates miRISC-associated synaptic mRNAs like CaMKII, lim-domain-containing protein kinase 1 (Limk1) and lysophospholipase 1 (Lypla1). Synaptosomal preparation studies have shown that upon N-methyl-D-aspartate receptor (NMDAR) activation, MOV10 is degraded at the synapses, allowing the translation of synaptic mRNAs that are important for synaptic plasticity [71]. Mature and precursor miRNAs are also located in the soluble component of biochemically isolated synaptic fractions [33]. This finding raises the possibility that miRNAs are formed locally via the processing of pre-miRNAs within dendritic spines.

Upon induction of LTP or long-term depression (LTD), several miRNAs are upregulated in the adult mouse hippocampus. Most miRNAs are rapidly upregulated within 15 min after chemical LTP or metabotropic glutamate receptor (mGluR)-dependent LTD induction. While the majority of the miRNAs have been shown to be upregulated by both LTP and LTD, temporal expression patterns seem to differ between these two forms of plasticity [72]. Following high frequency stimulation in the dentate gyrus (DG), miR-132 and miR-212 are upregulated [73]. In the adult DG, transcription of pri-miR-132 and pri-miR-212 strongly depends on mGluR, rather than NMDAR activation. Recently, Goodman and associates created a conditional KO mouse lacking miR-132 and/or miR-212, and examined the involvement of these miRNAs in the maturation of dendrites of newborn neurons in the adult hippocampus. Simultaneous deletion of the miR-132/212 genes caused a dramatic decrease in dendritic length, arborization, and spine density. Out of the miRNAs generated from this locus, miR-132 is proposed to be the miRNA responsible for normal dendrite maturation in newborn hippocampal neurons. Also, ablation of miR-132/212 has similar effects on dendritic development as the loss of cAMP response element-binding (CREB), and miR-132 inhibitors can block the effects of CREB on dendritic maturation, suggesting that miR-132 is involved in CREB signaling [74].

KO studies targeting miR-124 [75], miR-125b [76], miR-132 [77], miR-134 [78, 79], miR-137 [80], and miR-138 [81], convincingly revealed the roles of these miRNAs in synaptic development and dendritic arborization. miR-124 is the most abundant miRNA in the mouse brain as it accounts for 25–48% of all brain-expressed miRNAs [82]. Transfection of mouse neuronal stem cells with miR-124 stimulates neuron-like differentiation by promoting neuron-specific neuron-specific class III beta-tublin 1 (Tuj1) and MAP2 expression, and causing G0/G1 cell cycle arrest [83]. In differentiating mouse P19 cells as well as in primary cortical neurons, miR-124 targets the Rho GTPase family. By targeting cell division cycle 42 (Cdc42) and deactivating ras-related C3 botulinum toxin substrate 1 (Rac1) of the Rho GTPase family, miR-124 diminishes F-actin density and stimulates tubulin acetylation and possibly regulates cytoskeletal reorganization, resulting in the stimulations of neuronal outgrowth (Fig. 2) [84]. In vivo experiments in the mouse subventricular zone (SVZ) have demonstrated that miR-124 downregulates SRY-box containing gene 9 (Sox9) and thereby inhibits precursor division and stimulates differentiation [75]. Overall, miR-124 appears to play a major role in neuronal differentiation by downregulating genes essential for precursor proliferation, while stimulating neuron-specific genes and cytoskeletal rearrangements.

Recently, miR-132 was shown to repress translation of p250GAP, an NMDAR receptor-associated Rho GTPase-activating protein (GAP), which regulates spine morphogenesis by modulating Rac1 and/or RhoA (Fig. 2). miR-132 expression in rat hippocampus is low during the first postnatal week but accelerates between day 7 and 21, correlating with a period of active synaptogenesis. Conversely, p250GAP expression is high early in development and decreases during maturation. In organotypical hippocampal neurons, inhibition of miR-132 attenuates activity-induced spine formation and decreases spine head size, whereas introduction of miR-132 was sufficient to increase spine formation. Likewise, KD of p250GAP increases spine density and spine size, and furthermore increased both mEPSC frequency and the number of GluR1-positive spines. Additionally, miR-132/p250GAP signaling regulates Rac1 activity and spine formation by modulating synapse-specific kalirin7/Rac1/Pak signaling [85]. Notably, miR-132 together with miR-125b were recently shown to form a functional interaction with FMRP [76] (see below). Overexpression of miR-132 led to an increase in mean mEPSC amplitude and frequency, a phenotype that was prevented by the KD of FMRP. These data suggest that, through the regulation of p250GAP and Rac1 signaling, FMRP and miRNA-132 can cooperate to control synaptic structure and function and potentially explain the spine and behavioral phenotype observed in FXS patients. Indeed, a few reports have linked FMRP's effect on spine morphology to the Rac1 GTPase signaling pathway. The mRNA encoding Rac1 was found to be present in

Table 1 An overview of selected miRNAs involved in neuronal growth and plasticity

miRNA	Neuronal function	mRNA target	Reference
miR-9	Directs ES cells towards a neuronal state	Phospho-STAT3[a]	[91]
miR-9	Enhances differentiation of neuronal stem cells	TLX	[92]
	Attenuates neural proliferation		
	Promotes neuronal migration in the developing cortex		
miR-9	Promotes Cajal-Retzius cell differentiation	Foxg1	[93, 94]
	Regulates neurogenesis	Gsh2	
	miR-9 KO mice show impaired brain development		
miR-124	Negative constraint on serotonin-induced long-term facilitation	CREB1	[115]
	Converts short-term to long-term synaptic facilitation		
miR-124	Increases the number of primary neurites in cortical neurons	Cdc42[a]	[84]
		Rac1[a]	
miR-124	Promotes neuronal differentiation of neuronal stem cells	CDK6	[83]
	Stimulates neurite branching		
	Increases the level of neuronal marker Tuj1		
miR-125b	FMRP-associated	NR2A	[76]
	Stimulates dendritic branching	EphA4	
	Reduces spine width		
	Weakens synaptic transmission		
miR-128	Promotes neuronal differentiation	UPF1	[95]
	Enhances dendrite outgrowth	MLN51	
miR-132	Induces neural outgrowth		[77]
miR-132	Expression is regulated by CREB		[74]
	Increases dendritic length, branching, and spine density		
	Neuronal development and maturation in vivo		
miR-132	FMRP-associated		[76]
	Stimulates dendritic complexity		
	Increases spine width		
	Strengthens synaptic transmission		
miR-132	Induces activity-dependent dendritic growth	p250GAP	[85]
	Triggers de novo spine formation	Rac1[a]	
	Stimulates synaptic transmission	Kalirin-7[a]	
miR-132	Expression is upregulated by BDNF	Upregulation of glutamate receptors NR2A[a], NR2B[a], and GluR1[a]	[116]
miR-134	Modulates cortical development in a stage-specific manner	Chrdl-1	[117]
		Dcx	
miR-134	Decreases spine size	LIMK1	[78]
miR-134	Expression is modulated by SIRT1	Inhibits translation of CREB	[79]
	Impairs synaptic plasticity/LTP		
miR-137	Increases the level of neuronal marker Tuj1	CDK6	[83]
miR-137	Inhibits spine development and phenotypic maturation	Mib1 ubiquitin ligase	[80]
miR-138	Regulates spine size negatively	APT1	[71, 81]
	Decreases the amplitude of post-synaptic currents		
	Decreases in AMPAR cluster size		
miR-219	Expression is downregulated by an NMDAR antagonist	CaMKII	[108]
miR-375	Represses the density of dendrites	HuD	[118]
	Diminishes the appearance of neurites after BDNF stimulation		
miR-379/410 cluster	Transcribed by activation of Mef2	Pumilio2 (by miR-134)	[89]
	Inhibits dendritic outgrowth		

[a] No direct expression regulation via the identified miRNA shown

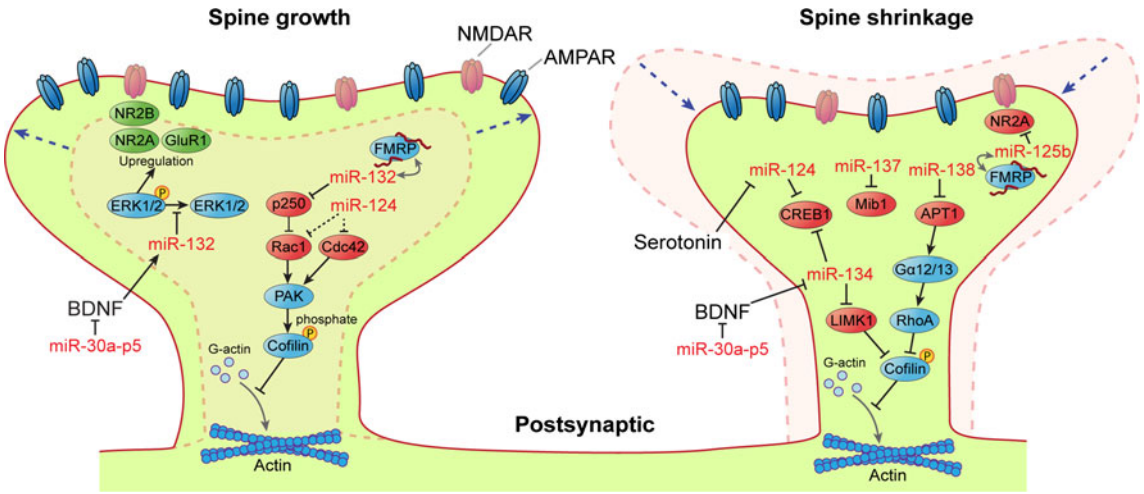

Fig. 2 Overview of miRNAs involved in post-synaptic spine growth or shrinkage with several implicated downstream pathways. The indicated miRNAs can modulate the spine morphologies by targeting components of the actin cytoskeleton, regulate protein expression by targeting transcription factors or (indirectly) targeting components of synaptic ion channels. The molecules involved have been color-coded, and where *red proteins* are direct targets of miRNAs, *blue proteins* depict downstream molecules within the pathways and *green proteins* are upregulated by miRNAs

fmr1-messenger ribonucleoprotein complexes [86], and *fmr1* and Rac1 genetically interact in *Drosophila*. The *fmr1*-interacting proteins cytoplasmic *fmr1* interacting protein (dCYFIP) and dRac1 [87] biochemically and genetically interact, these three proteins converge into common pathways controlling axonogenesis and synaptogenesis. Evidence was further presented that Rac1 negatively regulates CYFIP, which in turn negatively regulates *fmr1*, with the net result that dRac1 positively regulates dFMR1 action on neuronal morphogenesis [87]. Since FMRP-mediated repression of translation requires an interaction with CYFIP1 and the interaction of CYFIP1 with the translation initiation factor eIF4E [87, 88] it is tempting to speculate that the Rac1/FMRP/CYFIP/eIF4E signaling provides a bridge between activity-dependent cytoskeleton remodeling and translation. This is of interest since neuronal plasticity requires both actin cytoskeleton remodeling and local protein translation in response to extracellular signals. Also of interest is the observation that brain-derived neurotrophic factor (BDNF)-induced miR-132 upregulates glutamate receptors at the synapse. Thus, further supporting the notion that miR-132 is central in controlling synaptic structure and function.

Through a functional screen Siegel et al. recently identified a novel miRNA that is important for spine morphogenesis. miR-138 is localized in dendrites of rat hippocampal neurons [31, 32] and alters synaptic excitability by decreasing the size of GluR2-containing AMPAR clusters and AMPAR-dependent synaptic transmission. Furthermore, miR-138 inhibits spine growth through local suppression of acyl protein thioesterase 1 (APT1), which in turn catalyzes depalmitoylation of proteins, thereby modulating their membrane association. One possible mechanism is that miR-138 activates the Rho signaling pathway by promoting membrane association of Gα12/13, one of the targets of APT1 (Fig. 2). Although a direct link between miR-138 and Rho has not been established yet, through membrane association the small G protein subunit Gα13 has been shown to be involved in Rho-dependent signaling [81].

In the rat hippocampus, miR-134 levels increase during development, reaching maximum expression levels at P13, when synaptic maturation occurs. Neurogenic differentiation factor 2 (NeuroD2), disks large homolog 2 (DLG2), and Limk1 contain target sequences for miR-134. Limk1 regulates actin filament dynamics, thereby controlling cytoskeletal reorganizations essential in spine outgrowth. While targeting of Limk1 by miR-134 inhibits spine growth, BDNF increases the synthesis of the Limk1 protein by relieving the miR-134 suppression via the TrkB/mTOR pathway (Fig. 2). KD with a miR-134 antisense oligonucleotide increases spine volume and width in primary rat hippocampal neurons [78]. Thus, an interesting theme is emerging in which miRNAs play an important role in synapse development and plasticity by controlling actin cytoskeleton remodeling through Rho GTPase signaling [89]. Since both miR-132 and miR-134 are regulated by activity at the transcriptional level, these data also indicate that Rho signaling can be fine tuned in an activity-dependent manner. Since only a small number of miRNA targets have been validated and shown to be functionally important in vivo so far [90], it will thus be interesting to assess if more Rho signaling proteins are controlled by miRNAs at the level of (local) translation.

It was recently demonstrated that NAD-dependent histone deacetylase sirtuin 1 (SIRT1) has the capacity to modulate synaptic plasticity through miRNA-134-dependent regulation of the CREB protein [79]. Previously, SIRT1 was shown to have a number of functions linked to cell metabolism and cellular stress responses. Tsai and associates generated transgenic mice that lack SIRT1 deacetylase activity in the brain. These mice exhibited impaired LTP in CA1 neurons and deficits in memory performance. SIRT1 binds to regulatory DNA sequences upstream of the miR-134 locus and inhibits miR-134 expression upon binding. Furthermore, loss-of-function mutants of SIRT1 inhibited CREB activity in vitro. Addition of miR-134-inhibiting oligonucleotides restored CREB activity, indicating that SIRT1 loss-of-function impairs CREB activity through a miR-134-mediated mechanism. The authors overexpressed miR-134 in CA1 neurons to assess the role of miR-134 in synaptic plasticity. miR-134 overexpression impaired hippocampus-dependent memory formation in wild-type mice and LTP induction in hippocampal slice cultures, suggesting that overexpression of this miRNA in CA1 neurons mimics the effects of SIRT1 loss-of-function. The results of this elegant study indicate that SIRT1 has a key role in synaptic plasticity and memory formation by regulating the protein levels of CREB through miR-134-mediated repression [79].

An example of a miRNA involved in development is miR-9, which was shown to be upregulated during differentiation of mouse embryonic stem (ES) cells towards a neural precursor state, suggesting a role for this miRNA in neuronal differentiation. Overexpression of miR-9 directed ES cells towards a neuronal state versus astroglial cells [91]. Zhao and colleagues provided further evidence for the involvement of miR-9 in differentiation by utilizing in utero electroporation to overexpress miR-9 in the developing brain. miR-9 introduction altered migration and premature differentiation of embryonic neural stem cells lining the ventricular zone. Furthermore, miR-9 was shown to regulate tailless gene (TLX), a gene involved in the division of neural stem cells. Inhibition of TLX by miR-9 attenuated neural stem cell proliferation while enhancing differentiation. Interestingly, TLX was found to inhibit miR-9-1 expression by binding to a 3′ genomic sequence, effectively forming a feedback loop regulation during neuronal differentiation [92]. Besides differentiation of ES cells, miR-9 was found to promote generation of Cajal-Retzius cells in the medial pallium of the developing telencephalon through negative regulation of the forkhead box protein G1 (*foxg1*) gene [93]. These findings were later confirmed and extended by the generation of a miR-9 KO mouse model [94]. Together, these findings suggest miR-9 as a central regulator of early developmental processes, during which this miRNA modulates the differentiation and migration of ES cells leading to proper formation of specific brain areas.

Recently, Bruno and associates revealed that overexpression of miR-128 activates the expression of genes relevant for neurogenesis and neuronal differentiation through disruption of the non-sense-mediated RNA decay (NMD) machinery. miR-128 has the capacity to modulate neuronal differentiation through upregulation of a battery of NMD target genes, by specifically diminishing the levels of the NMD proteins RNA helicase Regulator of Non-sense Transcripts 1 (UPF1), and metastatic lymph node 51 (MLN51) [95].

miRNA expression is linked to learning behavior in rodents

Parsons and colleagues gave a first indication of the involvement of specific miRNAs in learning and memory, exploration, and anxiety behavior [96]. Learning and memory is associated with the expression of a number of miRNAs, such as miR-34c, miR-323, miR-378, and miR-451. Exploration behavior is correlated with the expression of the same set of miRNAs, except for involvement of the miR-34a-form instead of the c-form. Anxiety behavior is associated with miR-34c and miR-323 expression. Finally, miR-212 expression correlates with grooming duration in anxiety tasks [96].

In another study, hippocampal miRNA expression changes were assessed in mice subjected to an olfactory learning paradigm. When the levels of individual miRNAs were analyzed following training, expression of 14 miRNAs were elevated. Their mRNA targets were linked to processes that included LTD, transcription, RNA binding, and miRNA biogenesis pathways. Since whole hippocampi were used for miRNA profiling, local synaptic miRNA changes of trained animals could not be discriminated [97]. The studies summarized here indicate that miRNAs expressed by hippocampal neurons are possibly involved in hippocampus-dependent learning. Recent findings suggest that in mice learning and memory is enhanced by the loss of miRNAs in the brain. DCGR8 KO mice showed wild-type behavior in the open field test, but deficits when subjected to a prepulse inhibition test. In cognitive tasks, these KO mice showed normal freezing in the contextual and cued fear-conditioning. However, in the spatial working memory-dependent learning task DGCR8 KO mice performance was impaired, thus indicating the involvement of miRNAs in working memory function [70]. Konopka and associates deactivated the gene for Dicer specifically in forebrain neurons of adult mice. At 12 weeks of age, Dicer KO mice showed improved learning and memory in behavioral tests, and mutant neurons exhibited increased

numbers of a type of dendritic spine that is associated with learning [98]. In vivo KD studies of miRNAs followed by behavioral experiments would be an additional sophisticated approach to delineate the roles of individual miRNAs during experience-dependent plasticity.

miRNAs in neuronal maturation defects and neurological diseases

Multiple lines of evidence suggest that altered neuronal plasticity and morphology as seen in neurodevelopmental disorders may result from disruption of a common post-translational process that is under tight regulation by miRNAs [99]. Several intellectual disability (ID) syndromes, such as FXS, Rett syndrome and Down syndrome have been linked to the miRNA pathway [23, 100, 101]. Increasing evidence suggests that miRNAs which act as regulators of neuronal gene circuitries are associated with complex neuropsychiatric disorders involving abnormalities in synaptic plasticity, as well as neurodegenerative diseases. Kosik and coworkers profiled human miRNAs in autism and control post-mortem brains, and identified 28 miRNAs to be differentially expressed in autistic as compared to healthy individuals [102]. Recently, a valproic acid (VPA) rat model of autism was employed to assess the molecular role of miRNAs in the chemically induced, neuroplastic changes observed in autism spectrum disorders (ASD). Chronic VPA treatment of adult rats induced alterations of hippocampal miRNA levels. Levels of let-7, miR-128a, miR-24a, miR-30c, miR-34a, miR-221 and miR-144 were significantly altered, and the predicted effectors of these miRNAs are involved in neurite outgrowth, neurogenesis, signaling of PTEN, ERK, and Wnt/beta-catenin pathways [103].

Trinucleotide expansions in the 5′ UTR of the *fmr1* gene leading to reduced FMRP protein levels are associated with FXS, characterized by intellectual disability and autistic features. FMRP is involved in the miRNA pathway through interaction with miRNA biogenesis components or through association with miRNAs directly [104]. Interestingly, Edbauer et al. showed that miR-125b and miR-132, as well as several other miRNAs, are associated with FMRP in mouse brain. More specifically, miR-125b and miR-132 had largely opposing effects on dendritic spine morphology and synaptic physiology in hippocampal neurons. FMRP KD ameliorates the effect of miRNA overexpression on spine morphology, and NMDAR subunit NR2A was identified as a target of miR-125b [76]. FMRP controls pre-miR-124 processing into mature miR-124 [105]. Since miR-124 stimulates neuronal differentiation [75, 83, 84], its link with FMRP may suggest roles for this miRNA in FXS patients.

In schizophrenia patients, miR-16b, 30b [106] and 181b are significantly upregulated in the prefrontal cortex [107]. miR-181b downregulates protein levels of the AMPA glutamate receptor subunit GluR2 and of the RNA binding protein visinin-like 1 (VSNL1). GluR2 stimulates excitatory neurotransmission and has direct effects on synaptic plasticity by increasing growth and density of dendritic spines. VSNL1 is stimulated by glutamate and targets the mBDNF receptor TrkB thereby promoting neuronal maturation. Upregulation of miR-181b in schizophrenia patients thus causes a decrease in neuronal outgrowth by targeting GluR2 and VSNL1 [107]. Recent research has identified miR-219 as a key molecule in the behavioral manifestations associated with NMDAR hypofunction, which has been implicated in the pathophysiology of schizophrenia [108], since acute treatment of mice with the NMDAR antagonist dizocilpine resulted in downregulation of miR-219 in mouse prefrontal cortex. Moreover, treatment of mice with the antipsychotic drugs haloperidol or clozapine attenuated the hyperactivity and prevented the reduction in miR-219 in the prefrontal cortex. Notably, infusion of mouse brain with the antisense oligonucleotides (so-called antagomiRs [109]) against miR-219 both attenuated the locomotor and stereotypic behaviors caused by dizocilpine and increased the expression of miR-219 target CaMKII in the prefrontal cortex. This study suggests that altered miR-219 levels could be a significant factor in the dysregulation of cortical gene expression in schizophrenia.

Extensively studied neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) also display altered miRNA expression profiles. The expression of the β-site of APP cleaving enzyme (BACE1) protein stimulates the formation of amyloid-β-peptide plaques in the brains of AD patients. BACE1 was shown to be targeted by miR-485-5p. A natural antisense transcript, BACE-antisense, however, blocks the binding site for miR-458-5p thereby preventing BACE1-downregulation. BACE-antisense and miR-458-5p compete for the binding to BACE1-mRNA. In AD patients, BACE-antisense appeared to be upregulated while miR-485-5p was downregulated in the entorhinal cortex and the hippocampus, thus increasing the amount of BACE1 protein and thereby amyloid-β formation in these brain regions [110]. Additional miRNAs that are dysregulated in AD are miR-9, miR-138 and miR-125b, the levels of which are increased in the CA1 region of the hippocampus [111].

The maturation of dopaminergic neurons is under the control of miRNAs as well. In mice, induced deletion of Dicer specifically in post-mitotic dopamine neurons yields a phenotype comparable to that of PD patients, namely loss of dopaminergic neurons and reduced locomotion. A specific miRNA involved in dopaminergic maturation is miR-133b and its level is increased in PD [112]. miR133b

Table 2 An overview of selected miRNAs associated with neurological disorders

miRNA	Disease	Dysregulation	Target	Consequence of miRNA dysregulation	Reference
miR-9/9*	Huntington disease	Downregulated	REST (miR-9)	Excessive amount of REST and CoREST in the nucleus	[119]
			CoREST (miR-9*)	Inactivation of neuron-specific genes	
miR-29a	AD	Downregulated	NAV3	NAV3 coexpressed tau neurofibrillary tangles in pyramidal neurons	[120]
miR-29a,-29b-1	Sporadic AD	Downregulated	BACE1	Increase of amyloid-β	[121]
miR-106b	AD	Downregulated	APP	Increase of amyloid-β	[111]
miR-107	AD	Downregulated	BACE1	Increase of amyloid-β	[122]
miR-124	FXS	Downregulated		Decreased neuronal maturation	[105]
miR-133b	PD	Upregulated	Pitx3	Repression of neuronal maturation	[112]
miR-146a/b	Rett syndrome	Downregulated	Irak1	An increase in the amount of Irak1 puts the brain into an inflammatory state	[123]
miR-181b	Schizophrenia	Upregulated in prefrontal cortex	GRIA2	Decrease in neuronal outgrowth	[107]
			VSNL1		
miR-298 and -328	AD	Unknown	BACE1	Increase of amyloid-β	[124]
miR-342-3p	Prion disease	Upregulated			[125]
miR-485-5p	AD	Downregulated in entorhinal cortex and hippocampus	BACE1	Increase of amyloid-β	[110]

targets the transcription factor pituitary homeobox 3 (Pitx3), which is normally induced during neuronal development. Pitx3 in addition upregulates miR-133b expression, thus generating a feedback circuit. Overall, upregulation of miR-133b expression in PD represses neuronal maturation through the inhibition of Pitx3.

From these studies on neurological diseases, it thus appears that miRNAs are essential in neuronal differentiation, dendritic outgrowth, and spine formation. The dysregulation of miRNAs could ultimately underlie some of the clinical features observed in these diseases through their effects on synaptic plasticity. An overview of miRNAs implicated in neurological disorders is provided in Table 2.

Mutations in miRNA genes or gene copy number changes due to genomic deletions and duplications are likely to be involved in neurological disorders. So far, there are no published examples to substantiate this notion, but mutations in miR-96 have been linked to deafness in mice [113]. Moreover, heterozygous mutations in the human SLITRK1 gene have been associated with a variety of neuropsychiatric features including Tourette syndrome, ADHD, and compulsive hair pulling. In Tourette syndrome a single mutation in the 3′ UTR of SLITRK1 disrupts the recognition site of miR-189 [114]. The same mutation was also found in other families with Tourette syndrome and obsessive compulsive disorder, but the mutation was not fully segregated with the disorder, suggesting additional genetic and/or environmental contributions to the disease. Nonetheless, since mutation analysis in human disorders have so far been largely restricted to coding sequences it is to be expected that the rapidly emerging whole-genome sequencing projects will reveal mutations in miRNA genes or their target sequences in human neurodevelopmental disorders.

Future directions

The unique feature of miRNAs is to modulate complex physiological or disease phenotypes by regulating entire genetic circuitries. The findings reviewed here support the hypothesis that miRNAs are ideally positioned to play a central role in regulating complex gene networks involved in synaptic development and plasticity. This characteristic makes miRNAs attractive and novel therapeutic and diagnostic targets for the treatment and detection of complex intellectual disorders. Ultimately, further identification of individual miRNAs in synaptic function and maintenance will generate original insights into the molecular basis of synaptic plasticity. In the long term, the outcome from these investigations will provide a novel avenue for the identification and development of miRNAs as "molecular tools", with the potential to identify novel molecular pathways involved in synaptic maintenance and generate new molecular-based therapies to treat diseases of the central nervous system.

Acknowledgments The research of the authors is supported by grants from the "Donders Center for Neuroscience fellowship award of the Radboud University Nijmegen Medical Center" [to A.A. and N.N.K.]; the "FP7-Marie Curie International Reintegartion Grant" [to A.A. and N.N.K.]; and GENCODYS, an EU FP7 large-scale integrating project grant [Grant number 241995] [to HvB].

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