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REVIEW

MicroRNAs in neural development: from master regulators to fine-tuners

Marek Rajman and Gerhard Schratt*

ABSTRACT

The proper formation and function of neuronal networks is required for cognition and behavior. Indeed, pathophysiological states that disrupt neuronal networks can lead to neurodevelopmental disorders such as autism, schizophrenia or intellectual disability. It is well-established that transcriptional programs play major roles in neural circuit development. However, in recent years, post-transcriptional control of gene expression has emerged as an additional, and probably equally important, regulatory layer. In particular, it has been shown that microRNAs (miRNAs), an abundant class of small regulatory RNAs, can regulate neuronal circuit development, maturation and function by controlling, for example, local mRNA translation. It is also becoming clear that miRNAs are frequently dysregulated in neurodevelopmental disorders, suggesting a role for miRNAs in the etiology and/or maintenance of neurological disease states. Here, we provide an overview of the most prominent regulatory miRNAs that control neural development, highlighting how they act as 'master regulators' or 'fine-tuners' of gene expression, depending on context, to influence processes such as cell fate determination, cell migration, neuronal polarization and synapse formation.

KEY WORDS: MicroRNAs, Neurogenesis, Dendrite, Synapse, Neurodevelopmental disorders

Introduction

MicroRNAs (miRNAs) are a class of small non-coding RNAs that were first described in *Caenorhabditis elegans* (Lee et al., 1993; Wightman et al., 1993). They are ∼18-25 nt long and are involved in gene silencing (Lee et al., 1993; Wightman et al., 1993), and as such can regulate a vast array of cellular processes. miRNA genes can exist as single genes or as clusters that give rise to up to 50 different miRNA sequences (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). In the genome, miRNAs are localized either in areas without any known coding potential (Lau et al., 2001) or within the introns of coding genes, their so-called host genes (Baskerville and Bartel, 2005). The expression levels of miRNAs that are localized in introns mostly correlate with those of their host gene, suggesting usage of the same promoter sequence. Moreover, the expression of miRNAs belonging to the same cluster (usually within 50 kb of genomic sequence) is highly correlated, indicating that cluster miRNAs are mostly derived from polycystronic primary transcripts (Baskerville and Bartel, 2005).

The process of miRNA biogenesis has been studied in great detail (reviewed by Ha and Kim, 2014; Krol et al., 2010). Briefly, the vast majority of miRNA genes are transcribed by RNA polymerase II, giving rise to primary transcripts (pri-miRNAs) that are polyA tailed. In the canonical pathway, the pri-miRNA, which can be up to several kilobases long, is processed in the nucleus by the RNase III family enzyme Drosha, in a complex with DGCR8 protein. This processing generates a ∼70-nt-long precursor miRNA (pre-miRNA) that is transported to the cytoplasm by exportin 5. Another RNase III family member, DICER, often in a complex with TRBP (also known as TARBP2), then cleaves the pre-miRNA to generate a ∼20 bp miRNA/miRNA* duplex. Following this, depending on the specific miRNA sequence, one of the strands of this duplex is loaded into the miRNA-induced silencing complex (RISC), whereas the other strand (the passenger strand, or the miRNA*) is usually released and degraded. However, the loading of both strands into different RISCs is also frequently observed. Finally, the active miRNA-containing RISC (miRISC) is recruited to target mRNAs that harbor specific, partially complementary binding sites within their 3′UTRs. In this way, miRNAs suppress the translation and/or promote the degradation of up to a few hundred target genes (Krol et al., 2010). There is still no consensus on the extent to which and the order in which the repression of mRNA translation and the promotion of mRNA decay might contribute to miRNA function, suggesting that miRNA regulation might be highly context dependent. For example, some miRNAs (e.g. those involved in cell fate choices during neurogenesis or gliogenesis) have been shown to induce robust mRNA degradation, whereas others are involved in the local regulation of mRNA translation (e.g. during synapse development), suggesting that the fine-tuning of mRNA translation might be the primary mode of regulation for these miRNAs (Schratt et al., 2006; Siegel et al., 2009).

Soon after their discovery, and based on their ability to regulate gene expression, it was proposed that miRNAs could regulate specific phases of development (given their embryonic- or adult-specific expression patterns) and that they could have tissue-specific functions (based on their cell- and organ-specific expression patterns). This was particularly evident in the nervous system, where individual miRNAs or families of miRNAs were shown to regulate gene expression in specific neuronal cell types, at particular stages of development, and even in particular regions of a cell. In highly polarized neurons, for example, miRNA-dependent regulation of gene expression can occur both at the level of the entire cell or in specific subcellular compartments, such as axons or dendrites. These many studies have revealed that miRNAs are crucially involved in the tight spatiotemporal regulation of neuronal gene expression that is essential for neural differentiation, circuit development and the activity-dependent modification of neuronal networks. Moreover, depending on the specific context, miRNAs can have either protective or disease-promoting effects. The manipulation of miRNAs might therefore offer novel therapeutic opportunities for neurodevelopmental disorders of complex genetic origins.

In this Review, we provide an overview of the most prominent regulatory miRNA interactions that are involved in nervous system

Biochemisch-Pharmakologisches Centrum, Institut für Physiologische Chemie, Philipps-Universität Marburg, Marburg 35043, Germany.

*Author for correspondence (gerhard.schratt@staff.uni-marburg.de)

G.S., 0000-0001-7527-2025

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development, focusing on how miRNA-mediated control of gene expression can modulate cell fate, cell migration and cell polarization during embryonic and early postnatal development, and how miRNAs have been implicated in synapse development and the correct formation of neuronal circuits. In recent years, evidence for an important role of miRNAs in adult synaptic plasticity and cognition is also accumulating. However, these studies are not the focus of this Review, and we refer the reader to recent reviews on this subject (Aksoy-Aksel et al., 2014; Olde Loohuis et al., 2012; Wang et al., 2012; Ye et al., 2016).

Cell fate determination

The development of an organism is a complex process during which thousands of different cell types are generated and subsequently organized into unique tissues or organs. In mammals, cell fate determination occurs throughout development – from embryogenesis through to early postnatal stages and beyond – and depends on complex spatiotemporal regulation of gene expression. The recent literature provides ample evidence that miRNAs are involved in determining the fate of the two major cell types – neurons and glia – that are found in the central nervous system (CNS) as well as in the peripheral nervous system (PNS). Neurons and glia originate from the same type of neuronal precursor cell (NPC). However, whereas neuronal differentiation occurs mainly during embryonic development, glial differentiation continues to take place in the early postnatal nervous system. These processes of neuro- and gliogenesis involve many intermediate cell types; a detailed discussion of these is beyond the scope of this Review but can be found in recent reviews (Paridaen and Huttner, 2014; Taverna et al., 2014). In addition to embryonic neurogenesis, miRNAs have also been associated with the control of adult neurogenesis, a process that is limited to few niches in the adult brain (e.g. the subgranular zone of the hippocampus) and likely plays a role in learning and memory. We refer the reader to excellent recent reviews for a detailed discussion of miRNA function in adult neurogenesis (Luikart et al., 2012; Schouten et al., 2012) and focus our discussion here on embryonic neurogenesis – a period during which many miRNAs are enriched. Indeed, the global monitoring of miRNA expression during neurogenesis *in vivo* has identified time-specific (Barca-Mayo and De Pietri Tonelli, 2014; Lv et al., 2014; Miska et al., 2004; Nielsen et al., 2009; Yao et al., 2012), spatially restricted (ventral midline/midbrain dopaminergic progenitor pool) (Anderegg et al., 2013) or cell type-specific (Paridaen and Huttner, 2014; Ghosh et al., 2014) miRNAs, suggesting that different sets of miRNAs might be involved in neuronal versus glial differentiation. This is supported by the finding that 116 miRNAs (out of 351) are differentially expressed in primary cultures enriched for neurons, astrocytes, oligodendrocytes and microglia (Jovicic et al., 2013). As we discuss below, these and other findings have highlighted key roles for a number of miRNAs during neurogenesis and gliogenesis, and during the specification of particular neuronal cell types (Fig. 1).

Neurogenesis

The highly neuronal enriched miR-9 is one of the most studied miRNAs in the context of neurogenesis. In the late stages of embryonic CNS development in zebrafish, miR-9 expression restricts the pool of neural progenitors in the midbrain-hindbrain boundary (MHB); gain-of-function experiments *in vivo* show that miR-9 promotes neurogenesis and diminishes the MHB progenitor pool by simultaneously targeting different components of the fibroblast growth factor (FGF) signaling pathway and anti-neurogenic basic helix-loop-helix transcription factors (Leucht et al., 2008). In mice, miR-9 overexpression induces neuronal differentiation via direct inhibition of the nuclear TLX receptor (also known as NR2E1), which is an important regulator of neural stem cell renewal (Zhao et al., 2009). Furthermore, TLX negatively regulates miR-9 expression levels, pointing to sophisticated feedback mechanisms that precisely control the balance between neural stem cell renewal and differentiation (Zhao et al., 2009). To further complicate matters, miR-9 levels are also regulated by another miRNA, miR-107 (Ristori et al., 2015). Indeed, in the zebrafish MHB, miR-107 directly inhibits expression of the miRNA processing factor Dicer. This results in global downregulation of mature miRNAs, with miR-9 showing the strongest response to increased miR-107 activity. *In situ* hybridization studies have further revealed that the localization of miR-107 and Dicer is mutually exclusive along the hindbrain ventricular zone, thereby determining the border between the progenitor pool and differentiated neurons (Ristori et al., 2015).

Besides its function in neural stem cells, miR-9 in combination with miR-124 can convert human fibroblasts into physiologically functional neurons (Yoo et al., 2011). This pro-neurogenic function of miR-9 (and miR-124) involves decreased expression of BAF53a (ACTL6A), a component of the BAF complex that is involved in recruiting the Polycomb repressive complex 2 to chromatin and establishing H3K27me3 repressive marks at thousands of genomic loci (Ho et al., 2009, 2011). This suggests that the neuron-specific transcriptional signature observed upon miR-9/miR-124 expression is a result of extensive epigenetic alterations. Interestingly, however, miR-9 functions in neurogenesis can be context dependent. For example, in the *Xenopus* hindbrain, miR-9 activity increases the number of progenitor cells, whereas in the forebrain, miR-9 promotes progenitor cell apoptosis (Bonev et al., 2011). In addition, miR-9 regulates neuron differentiation in the developing mouse retina (La Torre et al., 2013) and the occurrence of sensory organ precursors (progenitor cells) in *Drosophila* (Li et al., 2006). It also controls the generation of late-born neurons in the zebrafish hindbrain, delaying cell cycle exit by targeting progenitor-promoting genes (Coolen et al., 2012), as well as the number of neurons in the mouse cortex by suppressing pro-glial factors (Zhao et al., 2015). Together, these studies provide strong evidence that miR-9 is a central regulator of neurogenesis in different biological contexts. In the future, it will be interesting to understand the extent to which miR-9 targets overlap with each other, either between different developmental stages or in specific progenitor regions within the CNS or PNS.

Notably, a convergence point for several different neurogenic miRNAs appears to be TLX, a known upstream activator of the Wnt signaling pathway (Qu et al., 2010) that is well known for its role in neuronal progenitor self-renewal. An important downstream effector of Wnt signaling is the cyclin D1 gene (Shtutman et al., 1999), and several miRNAs that target the TLX/Wnt/cyclin D1 pathway have been shown to affect neurogenesis. For example, in addition to being targeted by miR-9, *Tlx* mRNA is directly suppressed by let-7b, which at the same time inhibits cyclin D1 mRNA translation (Zhao et al., 2010a). Furthermore, miR-137, a miRNA implicated in schizophrenia (Kim et al., 2012), interferes with TLX function in the embryonic mouse brain by inhibiting histone lysine-specific demethylase 1 (LSD1; KDM1A) (Sun et al., 2011), a TLX transcriptional co-repressor (Yokoyama et al., 2008). Similar to the miR-9/TLX interaction (Zhao et al., 2009), TLX and miR-137 constitute a negative-feedback loop. Additional miRNAs such as miR-20a/20b and miR-23 also negatively regulate cyclin D1 levels via direct 3′UTR interaction (Ghosh et al., 2014), and

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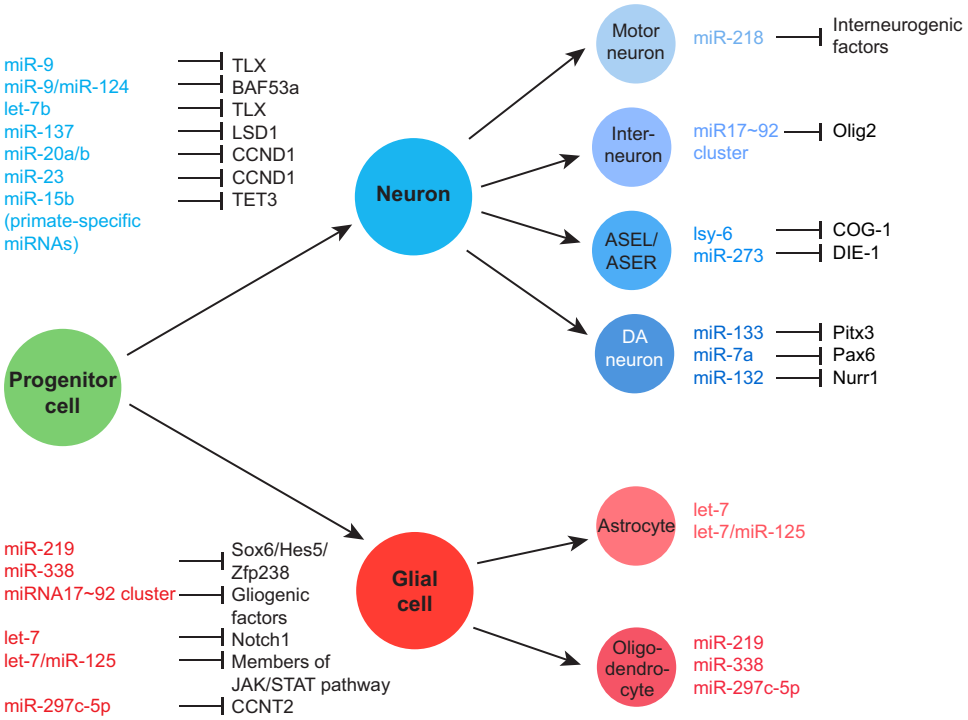

Fig. 1. miRNA function in neuronal and glial cell fate determination. Specific miRNA-target interactions involved in the differentiation of progenitor cells into neuronal or glial cells, and the further specialization of neuronal cells [e.g. into motor neurons, interneurons, ASEL/ASER bilateral taste receptor neurons, or dopaminergic (DA) neurons] and glial cells (e.g. into astrocytes or oligodendrocytes), are shown. Neurogenic miRNAs are indicated in blue, gliogenic miRNAs in red, and target genes in black. Note that, in some cases, miRNAs (e.g. the miR17~92 cluster) can induce different differentiation programs (i.e. neurogenic versus gliogenic) depending on cellular competence.

increased cyclin D1 activity leads to elevated miR-23 and decreased miR-20a/b expression levels; importantly, the inhibition of each of these miRNAs interferes with proper neuronal differentiation (Ghosh et al., 2014). Finally, it has been shown that another miRNA, miR-15b, inhibits cyclin D1 expression by regulating the methylation status of the cyclin D1 promoter via suppression of Tet methylcytosine dioxygenase 3 (TET3) mRNA translation (Lv et al., 2014). In summary, the Wnt signaling pathway provides an excellent example of a module that is concomitantly regulated by multiple miRNAs during neurogenesis. In-built regulatory feedback mechanisms help to sharpen expression domains, in this case the establishment of borders between progenitor cell pools and differentiated neurons.

These emerging roles for miRNAs during neurogenesis also have implications for our understanding of nervous system evolution. For instance, there is a strong positive correlation between the number of miRNA genes an organism possesses and its complexity, in particular with regard to cognitive abilities that are cortex dependent. This finding led to the hypothesis that recently evolved miRNAs might play a role in the development of higher cognitive functions in primates via the regulation of neurogenesis, in particular in the context of cortical development (corticogenesis). Corticogenesis in primates differs from that in non-primates in a number of ways, including the extent of cortical expansion, the identity of precursor lineages, and the emergence of neurogenic niches, so-called germinal zones (GZs) (Dehay et al., 2015). A recent study (Arcila et al., 2014) analyzed and compared miRNA expression profiles of a laser-dissected GZ (divided into an internal and external outer subventricular zone, the latter of which is specific for primates) and the cortical plate of the macaque visual cortex at embryonic day 80. Intriguingly, this analysis revealed that primate-specific miRNAs are amongst the most differentially regulated miRNAs between the regions analyzed (Arcila et al., 2014). This finding implies that newly evolved miRNAs could contribute to the emergence of primate-specific cortical features and could be involved in higher cognitive functions unique to primates.

Gliogenesis

In the nervous system, different types of glial cells (astrocytes, oligodendrocytes and microglia in the CNS; Schwann cells in the PNS) are generated from neuronal precursor cells during late neurogenesis or during early postnatal development (Kriegstein and Alvarez-Buylla, 2009). Recent studies support an important role for miRNAs in the development of these different glial lineages.

Two seminal papers (Dugas et al., 2010; Zhao et al., 2010b) revealed the importance of a functional miRNA pathway in oligodendrocyte differentiation. They both demonstrated that ablation of the *Dicer1* gene specifically in oligodendrocyte precursor cells (OPCs) interferes with oligodendrocyte differentiation. By comparing knockout (KO) and wild-type mice, the authors found that miR-219, miR-338 and miR-138 are enriched in OPCs and are highly expressed upon birth. Importantly, the re-introduction of miR-219 and miR-338 could partially rescue oligodendrocyte differentiation in *Dicer1* KO animals, demonstrating their functional importance. These studies further showed that the function of miR-219 and miR-338 is mediated by suppression of the translation of oligodendrocyte differentiation inhibitors (e.g. Sox6, Hes5) and pro-neuronal genes (e.g. Zfp238; also known as Zbtb18). This important role for Dicer1 during oligodendrocyte differentiation was also confirmed in an independent study (Zheng et al., 2010). It should be noted, however, that the interpretation of results obtained from different *Dicer1* KO models is often complicated by the massive apoptosis and tissue disorganization that results from the complete lack of Dicer1-dependent small RNAs in these animals (see Box 1).

Surprisingly, miRNAs that have important roles in neuronal fate determination can also positively regulate glial differentiation. For example, specific ablation of the miR-17~92 cluster, which includes six different miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b and miR-92a), interferes with oligodendrocyte differentiation (Budde et al., 2010). It has also been shown that miR-92a is required for astrocyte differentiation downstream of the transcription factor NANOG (Selvi et al., 2015). In addition,

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Box 1. Insights from Dicer conditional knockouts

The RNAse III enzyme Dicer1 is crucial for the biogenesis of most cellular small RNAs, including miRNAs, and Dicer loss-of-function models have thus been widely used for the investigation of miRNA function in neural development in intact animals (Choi et al., 2008; Giraldez et al., 2005; Huang et al., 2010; Kim et al., 2007; McLoughlin et al., 2012; Schaefer et al., 2007). However, there are several caveats that have to be considered when using Dicer-deficient animals. First, Dicer1 deficiency has been associated with cell death in multiple studies, which can hamper the interpretation of potential phenotypes. The systemic knockout of *Dicer1*, for example, leads to early embryonic death due to death of differentiating cells (Bernstein et al., 2003). Even when *Dicer1* deletion is performed at later stages of neuronal development or in specific brain tissues, increased cell death is repeatedly observed (Davis et al., 2008; De Pietri Tonelli et al., 2008; Huang et al., 2010; McLoughlin et al., 2012; Schaefer et al., 2007), narrowing the time window in which specific biological questions (except cell survival) can be addressed. This might also explain why slightly different strategies for conditional Dicer knockout during embryonic development, such as using either Foxg1-Cre (Nowakowski et al., 2011) or Emx1-Cre (De Pietri Tonelli et al., 2008), have very different effects on NPC specification. Second, Dicer1 has been shown to control post-transcriptional gene expression independent of miRNAs (Rybak-Wolf et al., 2014), raising the possibility that some aspects of the phenotype observed in Dicer1-deficient animals might be unrelated to the loss of miRNAs. Third, not all miRNAs are dependent on Dicer1 for their biogenesis (Yang and Lai, 2011), suggesting that functionally important miRNAs might be missed in the Dicer1 models. The observation that the neural phenotypes of different knockout models for miRNA biogenesis factors (e.g. Dicer1, Drosha, Dgcr8) are distinct is in agreement with additional, non-canonical functions of these factors (Babiarz et al., 2011; Burger and Gullerova, 2015; Marinaro et al., 2017). Finally, even if one assumes that the loss of specific miRNAs underlies a phenotype in the *Dicer1* KO brain, teasing apart the contribution of individual miRNAs is extremely challenging. Interfering with individual miRNAs, miRNA families or clusters will likely provide more easily interpretable data that might also be of clinical value.

miRNA-23a, which promotes neurogenesis (as discussed above), positively regulates oligodendrocyte differentiation and myelin synthesis (Lin et al., 2013). This raises the question of how the same miRNA is able to determine two different cell fates from the same precursor. However, it is important to consider that before glial cells are generated, neural stem cells undergo a switch from neurogenic to gliogenic competence, meaning that their ability to respond to neurogenic or gliogenic signals changes. miR-17 and its paralog miR-106 are able to prevent this switch during early development (Naka-Kaneda et al., 2014), and gliogenic factors are only expressed when the expression of these miRNAs gradually decreases during development, and this is then followed by the acquisition of gliogenic competence and glial differentiation (Naka-Kaneda et al., 2014). Furthermore, miR-153, which is highly expressed during early embryonic development, suppresses gliogenesis-inducing factors [nuclear factor I (NFI) A and B] and prevents gliogenesis (Tsuyama et al., 2015). Once miR-153 levels are decreased, NFIA/B accumulates and NPCs acquire gliogenic competence (Tsuyama et al., 2015). Additional miRNAs that are important in glial cell lineage decisions include let-7 family members (Gökbuget et al., 2015; Patterson et al., 2014; Shenoy et al., 2015).

Together, these studies highlight that the same miRNA can indeed induce different differentiation programs (i.e. neurogenic versus gliogenic) depending on cellular competence. Similar to neurogenesis, miRNA functions in gliogenesis are highly context dependent, i.e. regulating different aspects in embryonic versus adult gliogenesis.

Neuronal cell type determination

In addition to regulating major cell fate decisions (e.g. neuronal versus glial differentiation), miRNAs are involved in the determination of more specific glial or neural cell types, for example in determining whether a neuron becomes a motor neuron versus an interneuron, or whether a glial cell becomes an astrocyte or an oligodendrocyte (Fig. 1).

In a series of elegant studies, the Hobert laboratory established a fundamental role for miRNA-dependent regulatory circuits in determining specific neural cell fates in *C. elegans*. First, the miRNA lsy-6 was identified as the master regulator of the fate of two morphologically bilateral taste receptor neurons – the ASE left (ASEL) and ASE right (ASER) neurons (Chang et al., 2004; Johnston and Hobert, 2003, 2005); it was shown that *lsy-6* expression is restricted to fewer than ten ASEL neurons, and that *lsy-6* mutants exhibit loss of the ASEL-specific chemoreceptor expression profile with a concomitant gain of the ASER-specific profile. lsy-6 exerts its action by suppressing the expression of COG-1, an Nkx-type homeobox transcription factor that controls ASE-specific chemoreceptor expression profiles (Chang et al., 2003). Furthermore, the expression of *lsy-6* itself is controlled by the transcription factor DIE-1, which is expressed specifically in ASEL neurons. By contrast, *die-1* expression in ASER neurons is blocked by another miRNA, miR-273, expression of which is strongly biased in favor of ASER neurons (Chang et al., 2004). It was also shown that, after differentiation, ASEL neurons are still able to switch to an ASER fate. This effect is mediated by another transcription factor, LSY-2 (a C2H2 zinc finger), which is specifically expressed in ASEL neurons and prevents loss of their identity by maintaining lsy-6 expression levels (Johnston and Hobert, 2005). Together, these studies provided one of the first examples that miRNAs, through the regulation of key transcription factors, can trigger highly specific neuronal cell fates.

More recently, it has been demonstrated that miRNAs can also regulate neuronal subtype determination in higher organisms. For example, a miR-133b/Pitx3 regulatory loop has been shown to control the differentiation of mammalian dopaminergic neurons (Kim et al., 2007), which play a central role in different behaviors (e.g. sociability, addiction, motor coordination) and are lost in Parkinson's disease. miR-133b positively regulates dopaminergic neuron numbers in mouse primary midbrain cultures by downregulating Pitx3 – a transcription factor that is a known regulator of midbrain dopaminergic neuron differentiation and maintenance. Interestingly, it has been reported that miR-133b levels are decreased in the brains of individuals with Parkinson's disease compared with healthy controls (Kim et al., 2007), suggesting that an interaction between Pitx3 and miR-133b is necessary to maintain a stable population of dopaminergic neurons. In contrast, a separate study using systemic *Mir133b* KO in mice did not find any defects in dopaminergic neuron development and maintenance *in vivo* (Heyer et al., 2012), casting some doubt on the physiological relevance of the miR-133b/Pitx3 pathway in dopaminergic neuron differentiation. One possible explanation for these disparate findings is that the systemic loss of miR-133b during development causes compensatory mechanisms to be engaged in the KO mice. In the future, the use of conditional mice lacking miR-133b specifically in the dopaminergic lineage could thus help to resolve this issue. In another example, the site-specific generation of dopaminergic neurons in the mouse forebrain was shown to be under the control of miR-7a (de Chevigny et al., 2012).

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Within the forebrain, the lateral wall of the ventricle contains a mosaic of spatially separated neural stem cells that generate defined types of olfactory bulb neurons, including dopaminergic neurons. It was shown that miR-7a inhibits the expression of Pax6, a key transcription factor that controls dopaminergic neuron differentiation, along the entire ventricle wall except for the most dorsal part. There, the absence of miR-7a expression allows Pax6 expression and hence dopaminergic neuron differentiation to occur (de Chevigny et al., 2012). Both of these examples suggest that miRNA/transcription factor loops could be a common theme in dopaminergic neuron differentiation. This hypothesis is further supported by the discovery of a functional interaction between miR-132 and yet another transcription factor important for differentiation of dopaminergic neurons, Nurr1 (Nr4a2) (Yang et al., 2012).

An important role for specific miRNAs in interneuron specification (Fig. 1) has also been identified, as highlighted by two examples from the spinal cord. First, expression of the miR17∼92 cluster was shown to inhibit the expression of Olig2 – a transcription factor that is enriched in progenitors of spinal motor neurons – to promote interneuron generation (Chen et al., 2011). Second, it was shown that the motor neuron-enriched miR-218 suppresses the development of an interneuron phenotype by selectively targeting interneuron-specific genes (Thiebes et al., 2015).

In summary, the examples discussed above highlight that some specific miRNAs can play a causal role in the determination of different neuron populations. Moreover, an unbiased screen in mouse (He et al., 2012) has discovered hundreds of miRNAs that are differentially enriched in different brain regions (e.g. the cortex versus the cerebellum), in excitatory versus inhibitory neurons, and even in different subtypes of inhibitory neurons. In the future, single-cell RNA sequencing should thus further expand the repertoire of miRNAs that are selectively expressed in different neuronal cell types. This will provide a rich source for functional studies that will undoubtedly reveal additional miRNAs that are relevant for the specification of neural cell identity in the mammalian brain.

Migration

Following their specification, the correct migration of newborn neurons to specific locations within the nervous system is a prerequisite for the establishment and maintenance of neural circuitry. Indeed, defects in neuronal migration can lead to severe neurodevelopmental disorders. A number of recent publications have documented the importance of miRNA-dependent gene control in neuronal migration (Fig. 2A,B).

In *C. elegans*, the miR-9 homolog miR-79 was shown to interfere with proteoglycan synthesis and thereby prevent hermaphrodite-specific neuron (HSN) migration (Pedersen et al., 2013). In mice, global miRNA reduction (via Nestin-Cre-mediated *Dicer1* deletion) in late-born embryonic neurons impairs the migration of neurons into upper cortical layers (Kawase-Koga et al., 2009). In contrast, when *Dicer1* is deleted early postnatally using the Camk2a-Cre system, neuronal migration is not affected (Davis et al., 2008), suggesting that the miRNA pathway regulates neuronal migration during a critical developmental window. It was also demonstrated that, by regulating the Meis2-Pax6 transcription factor cascade, miR-9 controls the tangential migration of interneurons (Shibata et al., 2011) (Fig. 2B). In addition to these targets, miR-9 in combination with miR-132 suppresses the expression of Foxp2, a transcription factor known to regulate radial migration of cortical projection neurons (Clovis et al., 2012).

miRNAs can also regulate the expression of doublecortin (*Dcx*) – one of the most studied genes in the context of neuronal migration. *Dcx* encodes a microtubule-associated protein, and its mutation leads to lissencephaly in humans (Pilz et al., 1998). miRNAs can regulate Dcx levels either directly (e.g. in the case of miR-134; Gaughwin et al., 2011) or indirectly by targeting members of the CoREST/REST transcriptional repressor complex (e.g. in the case of miR-22 and miR-124; Volvert et al., 2014). In these studies, elevated levels of miR-134, miR-22 or miR-124 attenuated neuronal migration by downregulating Dcx expression. Notably, miR-134 is located within the largest known mammalian-specific miRNA cluster – the miR-379∼410 cluster, which comprises 39 different miRNA genes. However, in contrast to miR-134, other members of the miR-379∼410 cluster (miR369-3p, miR-496 and miR-543) were shown to promote neuronal migration by reducing expression of the neuronal adhesion molecule N-cadherin (cadherin 2) (Rago et al., 2014). For this effect, a combinatorial action of several cluster miRNAs is likely to be required, as the manipulation of individual miRNAs alone does not have a strong effect on neuronal migration. Together, these studies provide support for the notion that miRNAs originating from the same cluster might regulate similar biological processes, either through the regulation of different targets that together contribute to this process or through the combinatorial regulation of key targets, such as Dcx or N-cadherin.

Neuronal polarization, axon pathfinding and dendritogenesis

Neuronal polarization refers to the process that ultimately leads to functional separation of the neuron into axonal and dendritic compartments. Neurons are able to change their polarity during migration. During cortex development in rat, for example, a bipolar-to-multipolar transformation of neural progenitor cells occurs when they reach the intermediate zone; another transformation – back to a bipolar morphology – is required for the subsequent glia-guided locomotion of these cells in the cortical plate (Noctor et al., 2004). Notably, *Dicer1* deletion at this stage inhibits the conversion of neurons from a multipolar to a bipolar shape during migration (Volvert et al., 2014), suggesting an involvement of miRNAs in neuronal polarization (Fig. 2C). In addition, the re-introduction of miR-22 and miR-214 restores the multipolar-to-bipolar conversion, arguing for a particularly important role for these two specific miRNAs in polarization (Volvert et al., 2014).

Upon proper localization, neurons start to grow axons and dendrites to establish functional connections. Axons represent the presynaptic compartment and are important for information transmission over long distances. In humans, axons in the peripheral nerve system can reach up to 1 m in length. Unbiased miRNA screens have shown that miRNAs are present in axons, with specific miRNAs even being enriched in axons in comparison with the neuronal cell body (Natera-Naranjo et al., 2010; Sasaki et al., 2014). These findings suggest that miRNAs reach axons not by mere diffusion, but by active transportation into this compartment. They further raise the possibility that miRNAs can participate in the local regulation of axonal protein synthesis, thereby controlling processes such as axonal branching and guidance (Campbell and Holt, 2001; Jung et al., 2012) (Fig. 3A). In support of this, it has been reported that brain-derived neurotrophic factor (BDNF)-induced axonal branching in the developing mouse retina depends on increased levels of miR-132, which promotes axonal branching by inhibiting the translation of its known target Rho family GTPase-activating protein, p250GAP (Arhgap32) (Marler et al., 2014). Increased levels of other miRNAs, such as miR-124 in hippocampal neurons (Franke et al., 2012) and miR-29a in cortical neurons (Li et al., 2014), can also induce axonal branching.

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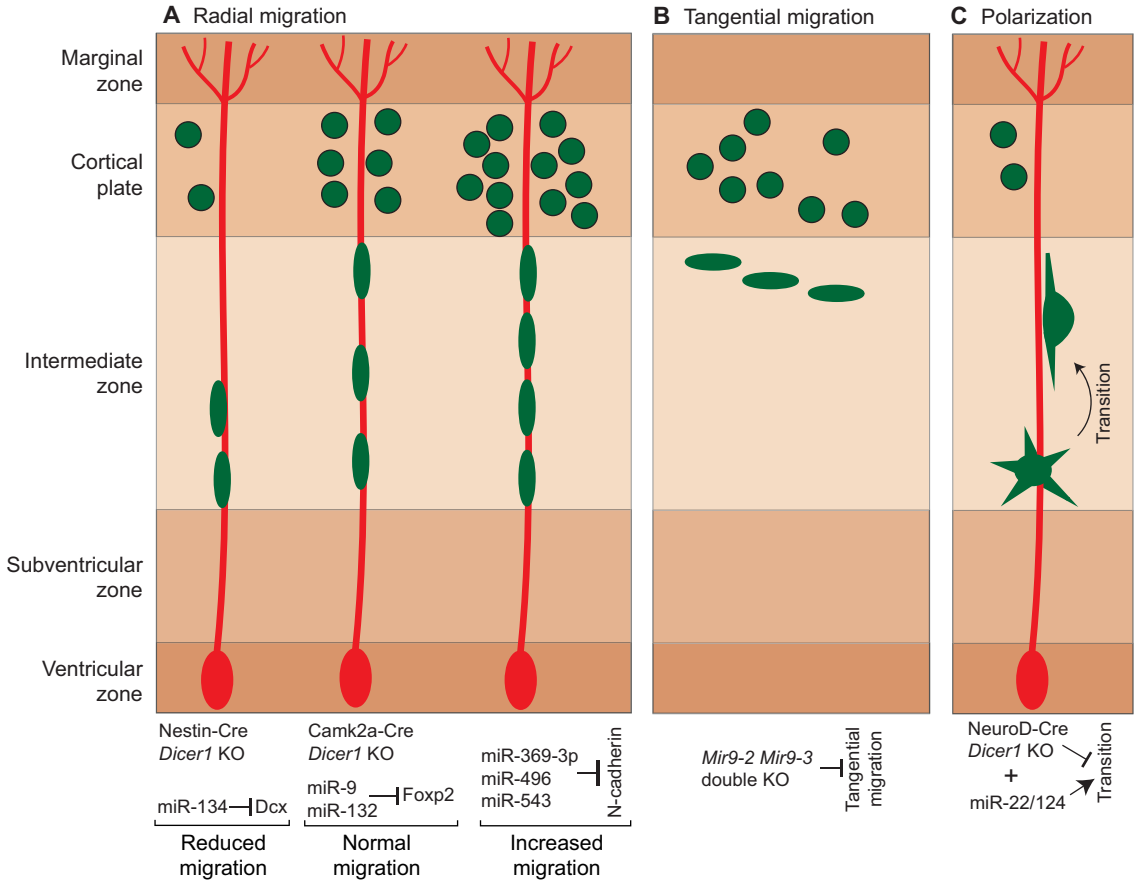

Fig. 2. miRNA function in neuronal migration and polarization. Specific miRNA-target interactions involved in neuronal migration and polarization are indicated. (A) miRNAs can have negative (e.g. miR-134), protective (e.g. miR-9/132) or positive (e.g. miR-369/496/543) effects on neuronal migration, controlling the radial migration of various neurons (green) along radial glial cells (red) through distinct cortical layers. In addition, the global (i.e. Nestin-Cre-mediated) but not conditional (i.e. Camka2-Cre based) knockout of *Dicer1* decreases neuronal migration, suggesting that the miRNA pathway regulates radial neuronal migration during a critical developmental window. (B) The double KO of *Mir9-2* and *Mir9-3* interferes with tangential migration, inhibiting the migration of cortical interneurons. (C) Transition from a multipolar to a bipolar neuronal morphology during migration is positively controlled by miR-22/124.

miRNAs are also involved in the directional growth of axons, a process known as axon guidance. Studies from multiple model organisms have shown that correct axon guidance is regulated by guidance cues secreted by targets and depends on local protein synthesis (Brittis et al., 2002; Campbell and Holt, 2001), thereby implicating miRNAs in this process. In a fish model of axonal growth, the knockdown of miR-204 leads to misguided growth of retinal ganglion cell axons into retinal layers; downregulation of miR-204, which targets ephrin type receptor B 2 (Ephb2) and ephrin B3 (Efnb3), both of which are important signaling molecules in axon guidance, rescues these defects (Conte et al., 2014). In *C. elegans*, the miR-125a/b homolog lin-4 reduces axonal growth induced by the axon guidance factor UNC-6 (a Netrin homolog) via inhibition of the transcription factor LIN-14 (Zou et al., 2012). In contrast, it has been shown that increased LIN-14 activity induces axonal initiation in *C. elegans* HSN neurons independently of external guiding cues (e.g. UNC-40/DCC, SAX-3/Robo receptors) by targeting LIN-14 and the 'stemness' factor LIN-28 (Olsson-Carter and Slack, 2010). In primary rat cortical neurons, two axonally localized miRNAs (miR-338, miR-181c) attenuate axonal outgrowth by modulating the expression of transcripts involved in the axon guidance machinery (Kos et al., 2016a,b). Together, these results suggest that miRNAs can regulate both intrinsic axon growth programs as well as axonal growth stimulated by specific guidance cues. More recent findings suggest that miRNAs might also be involved in mediating the spatiotemporal effects of such guidance/growth factors during axonal growth. For instance, the expression of microtubule-associated protein 1B (MAP1B), which plays an important role in axonal outgrowth and branching (Bouquet et al., 2004), can be locally regulated by miR-9 in mouse cortical neurons (Dajas-Bailador et al., 2012) and by miR-181 in mouse peripheral sensory neurons (Wang et al., 2015). Importantly, it was shown that the levels of miR-9 and miR-181 in these contexts, and hence the translation of *Map1b* mRNA, are regulated by BDNF (Dajas-Bailador et al., 2012) and nerve growth factor (Wang et al., 2015), respectively. Thus, these studies could help to explain the spatiotemporal dependence of neurotrophin action in axon guidance.

Several unbiased screens have shown that miRNAs can also be specifically localized to the synapto-dendritic compartment (Kye et al., 2007; Sambandan et al., 2017; Siegel et al., 2009), and follow-up studies have indeed demonstrated the functional importance of individual miRNAs in dendritogenesis (Fig. 3B). For example, miR-132 was shown to regulate the dendritic growth and branching of mouse and chick young hippocampal neurons *in vitro* and *in vivo* by repressing p250GAP (Magill et al., 2010; Marler et al., 2014; Remenyi et al., 2013). Another activity-regulated miRNA, miR-134, was shown not be involved in dendritogenesis under normal growth conditions, but is specifically required for the activity-induced dendritic growth of cultured rat hippocampal neurons, acting by targeting the RNA-binding protein Pum2 in dendrites (Fiore et al.,

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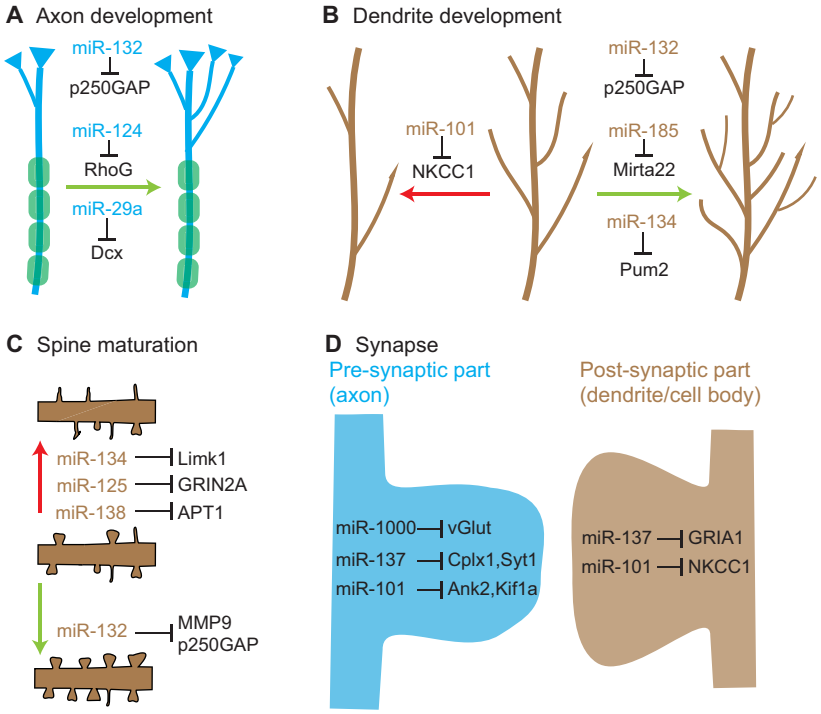

Fig. 3. miRNA function in neural circuit development. (A-C) miRNAs can regulate the development of neuronal circuits at the levels of axon development (A), dendrite development (B), spine maturation (C) and synaptic transmission (D). (A) Intrinsic and activity-induced axonal branching are regulated by miR-29/miR-124 and miR-132, respectively. (B) Dendritogenesis is inhibited (red arrow) or activated (green arrow) by the indicated miRNA-target interactions. Note that miR-134 is selectively required for activity-induced dendritic growth. (C) Dendritic spine maturation is inhibited (red arrow) or promoted (green arrow) by the indicated miRNA-target interactions. (D) Multiple miRNA-target interactions control synaptic transmission. miR-137 (or its *Drosophila* ortholog miR-1000) and miR-101 are examples of miRNAs that coordinate synapse function by repressing different sets of targets in the pre- and post-synaptic compartments.

2009). Following on from this, miR-134 overexpression was shown to reduce cortical pyramidal neuron dendritogenesis in the mouse brain *in vivo* (Christensen et al., 2010). In addition, miR-9 was shown to be necessary for proper dendrite development in the mouse brain (Giusti et al., 2014) and in *Drosophila melanogaster* sensory neurons (Wang et al., 2016), suggesting that the function of miR-9 in dendrite development is conserved.

The importance of these roles for miRNAs in controlling neuronal morphogenesis is highlighted by recent studies of disease states. For example, in the 22q11.2 microdeletion mouse model of schizophrenia, in which one copy of the *Mir185* gene is deleted along with other genes (Karayiorgou et al., 1995; Xu et al., 2008), reduced miR-185 levels are accompanied by a severe reduction in the dendritic complexity of pyramidal neurons. It was further shown that miR-185 regulates dendritic complexity by suppressing Mirta22 (now known as Emc10), a previously unknown gene product that localizes to the Golgi apparatus (Xu et al., 2013). In a more recent study, the transient inhibition of miR-101 using antagomirs induced dendritic growth in the CA1 and CA3 region of the hippocampus by repressing the sodium transporter NKCC1 (SLC12A2). Intriguingly, this transient inhibition of specific miRNAs such as miR-101 in young mice was sufficient to cause cognitive impairments in adulthood (Lippi et al., 2016). Therefore, this study provides evidence that miRNA-dependent control of neuronal morphogenesis during developmental stages has an important impact on the function of neural circuits and cognitive abilities in the adult.

Synapse development

One of the basic functions of the nervous system is to store and transmit information. Within its extensive neural circuits, information between different neurons is transmitted via specialized junctions known as synapses. A typical chemical synapse consists of a presynaptic part (provided by the axon terminal) and a postsynaptic part (represented by the dendrite). Synapses are very dynamic structures that can bi-directionally adjust their strength in response to external stimuli, a process known as synaptic plasticity. Dendritic spines, the protrusions on which the majority of excitatory synapses terminate, change their morphology according to synaptic activity and are therefore often used as a correlative measure for synaptic strength. Given that dendritic spine morphogenesis depends on the local synthesis of proteins, it was thought that miRNAs might also be important regulators of postsynaptic development and function (Fig. 3C). Indeed, over 10 years ago, the dendritic miRNA miR-134 was shown to negatively regulate dendritic spine size by inhibiting the local synthesis of Limk1, a kinase that promotes actin polymerization in spines (Schratt et al., 2006). Subsequent studies confirmed the importance of miRNA-dependent gene regulation in spine morphogenesis, suggesting regulation of the actin cytoskeleton as a common endpoint of miRNA function. In addition to miR-134, miR-132 was shown to regulate the Rac signaling pathway, but in a positive manner by inhibiting expression of the Rac-GAP p250GAP (Edbauer et al., 2010; Vo et al., 2005). More recently, matrix metalloproteinase 9 (MMP9) was identified as an additional target involved in the spine growth-promoting function of miR-132 (Jasińska et al., 2016). In contrast, miR-138 activates the RhoA signaling pathway by targeting the Gα12/13 depalmitoylase LYPLA1 (Siegel et al., 2009), thereby inducing spine shrinkage.

A number of miRNAs have also been implicated in synaptic transmission and synaptogenesis. One of the most studied of these is miR-137. Interest in miR-137 was sparked by results from a large-scale genome-wide association study of schizophrenia patients, which revealed that single nucleotide polymorphisms (SNPs) located in the *MIR137* gene were among the most significant SNPs associated with the disease [Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011]. Later, by expressing sequences with specific SNPs in either SH-SY5Y stable cell lines or in neurons induced from human fibroblasts, it was shown that individual SNPs within or in close vicinity of the *MIR137* gene could either decrease (Strazisar et al., 2015) or increase (Siegert et al., 2015) miR-137 levels, suggesting that tight regulation of miR-137 is crucial for correct brain function.

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Moreover, it was reported that decreased levels of miR-137 lead to increased expression of hundreds of genes associated with synaptic transmission and synaptogenesis (Strazisar et al., 2015), suggesting excessive synaptic function as a possible result of *MIR137* mutation. Accordingly, miR-137 gain of function in multiple cell lines was shown in another study to lead to decreased mRNA translation of numerous mRNAs encoding presynaptic proteins (Siegert et al., 2015), which in turn resulted in impaired presynaptic function due to a decreased number of neurotransmitter vesicles close to the synaptic cleft (Siegert et al., 2015). Intriguingly, miR-137 function is not restricted to the presynapse: miR-137 was also shown to target mRNA encoding the AMPA-type glutamate receptor subunit GluA1 (Olde Loohuis et al., 2015). A postsynaptic role of this interaction is supported by the finding that downregulation of miR-137 selectively enhances AMPA receptor-mediated synaptic transmission and converts silent synapses to active synapses. In addition, the virus-directed overexpression of miR-137 selectively in postsynaptic CA1 pyramidal cells has no effect on the paired-pulse ratio, a classical parameter of presynaptic function, further invoking an additional postsynaptic role of miR-137 (Olde Loohuis et al., 2015). It should also be noted that miR-137 function at the postsynapse is not limited to basal synaptic transmission: activity-dependent regulation is also required for long-term depression, a form of long-term synaptic plasticity (Olde Loohuis et al., 2015). In *D. melanogaster*, miR-1000, which harbors a very similar seed to mammalian miR-137, targets the vesicular glutamate transporter (vGlut), a protein responsible for glutamate loading into presynaptic vesicles (Verma et al., 2015). In line with this, *mir-1000* mutants display increased neuronal apoptosis probably due to toxic effects of the resulting excessively high glutamate levels. In addition, miR-1000 expression levels can be regulated by activity, suggesting a role for miR-1000 in synaptic plasticity (Verma et al., 2015), in agreement with studies of its mammalian counterpart miR-137. In conclusion, miR-137 and related miRNAs apparently fulfill important functions in activity-dependent pre- and postsynaptic physiology.

Another miRNA that apparently coordinates pre- and postsynaptic functions during neural circuit development is miR-101. Transient inhibition of miR-101 activity by antagomir injection into the dorsal hippocampus of mice soon after birth was shown to change the balance between excitatory and inhibitory synaptic transmission (E/I balance) in adult animals (Lippi et al., 2016). This study also elegantly showed that a single miRNA can simultaneously regulate pre- and postsynaptic development by suppressing different sets of targets. The activity-dependent, coordinated control of pre- and postsynaptic function by miRNAs such as miR-101 and miR-137 could ensure network homeostasis during development and in the adult (Fig. 3D), and impaired function of these miRNAs could lead to neurodevelopmental defects, as observed in schizophrenia, autism and intellectual disability (Table 1). It will be interesting to learn whether this coordinated action at the synapse also applies to other miRNAs that have been primarily studied in postsynaptic spines.

Neuronal circuits are shaped by experience within ‘critical’ or ‘sensitive’ periods during early postnatal life. One of the most extensively studied models of experience-dependent network maturation is the primary visual cortex. When sensory input into the primary visual cortex is blocked during a critical period (approximately postnatal day 25), e.g. by eye suture, networks within the visual cortex are not properly developed and sight is lost (Hensch, 2005). Two groups have independently shown that the miR-132/212 cluster is required for the proper maturation of the mouse visual cortex (Mellios et al., 2011; Tognini et al., 2011). These studies revealed that miR-132/212 expression is induced at the transcriptional level by elevated sensory input during eye opening. Importantly, the inhibition of miR-132 activity prevents ocular dominant plasticity (i.e. the relative anatomical or physiological strength of connections from either eye to individual cells in the primary visual cortex) and affects the maturation of dendritic spines (Mellios et al., 2011; Tognini et al., 2011). Thus, these studies showed for the first time a function of

Table 1. miRNAs implicated in neurological disorders

Disease	Implicated miRNA(s)	References
22q11.2 deletion syndrome (high susceptibility for schizophrenia and autism)	26 miRNAs (e.g. miR-134)	(Stark et al., 2008)
	miR-338-3p	(Chun et al., 2017)
	miR-185	(Xu et al., 2013)
Schizophrenia	miR-137	[Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011] (Wright et al., 2016; Siegert et al., 2015; Lett et al., 2013)
Idiopathic autism	Multiple miRNAs	(Wu et al., 2016; Mor et al., 2015; Mundalil Vasu et al., 2014)
Fragile-X syndrome	miR-9, miR-124	(Xu et al., 2011)
	let-7b/c, miR-125a, miR-181a, miR-296, miR-342	(Wan et al., 2016)
	miR-125b, miR-132	(Edbauer et al., 2010)
	miR-125a	(Muddashetty et al., 2011)
	miR-181d	(Wang et al., 2015)
Rett syndrome	miR379-410 cluster and other miRNAs	(Wu et al., 2010)
	let-7f	(Mellios et al., 2014)
	miR132-212 cluster	(Im et al., 2010)
	377 miRNAs (e.g. miR-134, miR-383, miR-382, miR-182)	(Cheng et al., 2014)
	miR-199a	(Tsujimura et al., 2015)

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miRNAs in the activity-dependent maturation of neural circuits, a form of developmental synaptic plasticity, in the mammalian brain *in vivo*. Further evidence for a role of miRNAs in synaptic plasticity, both during development and in adults, has been provided in recent years (reviewed by Aksoy-Aksel et al., 2014; Olde Loohuis et al., 2012; Wang et al., 2012; Ye et al., 2016), underscoring the importance of miRNAs in correct neural circuit formation, maturation and function.

Conclusions

Work over the last decade or so has firmly established that miRNA-dependent control of protein synthesis serves as an important post-transcriptional regulatory layer in basically every aspect of nervous system development. Depending on its target spectrum, a miRNA can either promote or inhibit developmental processes. As the target spectrum for a given miRNA can change as a function of time and space, miRNA activity is often context specific, as nicely exemplified by findings that the same miRNAs can be involved in different cell fate decisions (e.g. neuron versus glia) or developmental stages (e.g. axon pathfinding versus synapse formation). In the future, detailed mapping of the spatiotemporal expression of miRNAs and their targets by single-cell sequencing will be required to further interrogate miRNA function within specific neural circuits.

Mechanistically, miRNA function is intertwined with other gene regulatory processes, in particular mRNA transcription, splicing and stability, providing the system with robustness. A long-standing question in the field, and one that is not limited to neural development, is to what extent miRNAs act as 'master regulators' or 'switches' as opposed to 'fine-tuners' of gene expression. As far as the nervous system is concerned, it is becoming clear that there is not just one answer to this question, but that the modus operandi of miRNAs is dictated by spatiotemporal context. Therefore, whereas some miRNAs that are highly expressed in early neurogenesis (e.g. miR-124, miR-9) can be classified as switch genes that control cell fate, more modestly expressed miRNAs involved at later stages of neural development appear to instead fine-tune gene expression in response to the activity state of the network. Similar differences can be observed at the level of target gene regulation. Whereas some miRNAs have a few crucial targets regulation of which is sufficient to elicit a specific phenotype, other miRNAs contribute to the regulation of up to a few hundred different targets, often in combination with other co-expressed miRNAs (e.g. those that are derived from a common genomic miRNA cluster). The complexity of such combinatorial regulation by different miRNAs is just beginning to be disentangled, and owing to the high degree of redundancy, particular biological roles have been hard to assign.

However, although miRNAs are known to regulate intrinsic gene expression programs during cellular differentiation, it is also becoming evident that they can participate in experience-dependent processes that sculpt neuronal circuits during crucial developmental periods. In fact, the complexity of the underlying mechanisms that regulate the processing, stability and activity of miRNAs themselves in an activity-dependent manner is only just beginning to emerge (see Box 2). Recently developed techniques to capture RNA modifications (editing, methylation, 3′UTR remodeling) at a transcriptome-wide level will shed more light on the complexity of this activity-dependent post-transcriptional regulation. Furthermore, although cell culture models have provided much insight into the role of miRNAs in neural development, animal models that examine miRNA function at the organismic level are still scarce. Nevertheless, first results from miRNA KO models are highly encouraging and suggest that the loss of specific miRNAs can have rather profound consequences for the development of neural circuits and animal behavior (Amin et al., 2015; Tan et al., 2013). Applying CRISPR-Cas technology to analyze miRNA function in the brain will no doubt accelerate efforts to investigate the physiological function of specific miRNA-target interactions. In addition, given that miRNAs might play an important role in neurodevelopmental processes that are associated with the emergence of a highly complex brain (Hu et al., 2011; Somel et al., 2011), the use of human induced pluripotent stem cell-derived neurons might represent a promising experimental system to test the functional relevance of interesting primate- or even human-specific miRNAs at the cellular level. This might also open new possibilities for the investigation of human neurodevelopmental disorders, such as schizophrenia and autism, for which animal models are still of rather limited value. Finally, although miRNA-based therapeutics in the brain are still in their infancy, in large part due to the difficulty of delivering miRNA-targeting oligonucleotides into the brain, some advances have been made. A notable example is that of epilepsy, for which injection of several miRNA antagomirs has proven to be beneficial for the reduction of seizures and associated neurodegeneration in the hippocampus (Gross et al., 2016; Jimenez-Mateos et al., 2012; Rajman et al., 2017). In addition, the virus-directed expression of miR-223 has been shown to be neuroprotective following transient global ischemia and excitotoxic injury (Harraz et al., 2012). With the future development of non-invasive delivery routes, and continued research into miRNA function in the nervous system,

Box 2. The regulation of miRNA processing, stability and repressive activity during neuronal development

Although a number of studies discussed in this Review have highlighted roles for individual miRNAs during neural development, it should also be noted that the more global regulation of miRNA activity – at multiple stages along the miRNA biogenesis and effector pathways – can have a major impact on nervous system development. Examples of how and when the activities of miRNAs themselves can be regulated during neural development include: (1) Regulation of pri-miRNA transcription. For example, pri-miR-184 expression is inhibited by MBD1 via epigenetic silencing (Liu et al., 2010). (2) Regulation of pri-miRNA processing and stability. Examples include amyloid precursor protein (APP), which inhibits miR-547 expression in the developing cerebral cortex by inducing pri-miR-547 degradation (Zhang et al., 2014); ADAR1, which blocks pri-miR-302 processing (Chen et al., 2015); and MeCP2, which interferes with the processing of several neuronal miRNAs (e.g. miR-134, miR-383) by sequestering the microprocessor co-factor Dgcr8 (Cheng et al., 2014). (3) Pre-miRNA stability. For example, expression of the pro-neural miRNA miR-9 is inhibited by Lin28-dependent degradation of pre-miR-9 (Nowak et al., 2014). (4) Regulation by miRNA-sequestering RNAs (Salmena et al., 2011; Tay et al., 2014) also known as 'miRNA sponges' or 'competing endogenous RNAs'. Different RNA classes can function as miRNA sponges, e.g. the 3′ UTR of mRNAs such as Ube3a-1 (Valluy et al., 2015), long non-coding RNAs (lncRNAs) such as LncND (lncRNA termed neurodevelopment) (Rani et al., 2016) or circular RNAs (circRNAs) such as ciRS-7 (also known as CDR1as), which is highly and selectively expressed in hippocampal and neocortical neurons and contains >70 binding sites for the neuronal miR-7 and one perfectly complementary site for miR-671 (Hansen et al., 2013; Memczak et al., 2013). (5) Regulation of the composition and activity of the neuronal miRISC. This includes the activity-dependent control of the phosphorylation of Ago2 (Patranabis and Bhattacharyya, 2016) or its interacting partner FMRP (also known as FMR1) (Muddashetty et al., 2011), as well as the activity-regulated degradation of the miRISC protein MOV10 (Banerjee et al., 2009).

such miRNA therapeutics might also prove to be applicable to neurodevelopmental disorders for which no cure is currently available.

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Competing interests
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