Mamm Genome (2008) 19:541–551
DOI 10.1007/s00335-008-9129-6

Thinking about RNA? MicroRNAs in the brain

Christian Barbato · Corinna Giorgi · Caterina Catalanotto · Carlo Cogoni

Received: 11 April 2008 / Accepted: 5 June 2008 / Published online: 1 August 2008

Abstract MicroRNAs (miRNAs) are a recently discovered class of small RNA molecules implicated in a wide range of diverse gene regulatory mechanisms. Interestingly, numerous miRNAs are expressed in a spatially and temporally controlled manner in the nervous system. This suggests that gene regulation networks based on miRNA activities may be particularly relevant in neurons. Recent studies show the involvement of RNA-mediated gene silencing in neurogenesis, neural differentiation, synaptic plasticity, and neurologic and psychiatric diseases. This review focuses on the roles of miRNAs in the gene regulation of the nervous system.

Introduction

One of the most fascinating properties of the mammalian brain is its complexity. Neurons respond to numerous stimuli, both functional and developmental, by changing their gene expression patterns. Transcriptional and post-transcriptional gene regulation mechanisms underlying development, plasticity, and networking thus might participate in the establishment and maintenance of such complexity. MicroRNAs (miRNAs) are short regulatory RNAs that modulate gene expression at the post-transcriptional level by inhibiting translation of target mRNAs. Multiple studies suggest that miRNAs are fine-regulators of several biological processes and notably contribute to organisms' complexity. MicroRNAs are expressed at different levels and in a tissue-specific manner. Bioinformatics tools predict hundreds of mRNA targets for each miRNA, suggesting that many genes are subject to miRNA-mediated regulation. This is being confirmed by increasing efforts to identify the specific targets of miRNAs; while before it was thought that around 30% of human genes were targeted by miRNAs, now this number appears to be underestimated. miRNAs are involved in the control of neuronal fate specification and differentiation, dendritic spine development, synaptic plasticity, and local protein synthesis. Possibly as a consequence of these multiple roles, miRNA involvement has been reported also in neurologic diseases such as Parkinson's and Alzheimer's, as well as in several neurologic pathologies, including Tourette and Fragile X syndromes and brain tumors. In this review we discuss the recent studies in this exciting field with respect to development, synaptic plasticity, and nervous system diseases.

C. Barbato (✉) · C. Giorgi · C. Catalanotto · C. Cogoni
European Brain Research Institute (EBRI), Fondazione EBRI, Rita Levi-Montalcini Via del Fosso di Fiorano, 64/65, 00143 Roma, Italy
e-mail: c.barbato@inmm.cnr.it

C. Catalanotto · C. Cogoni
Dipartimento di Biotecnologie Cellulari ed Ematologia, Sezione di Genetica Molecolare, Università di Roma "La Sapienza", Viale Regina Elena, 324, 00161 Roma, Italy

MicroRNA-mediated gene silencing: an overview

MicroRNAs are noncoding single-stranded RNA molecules, approximately 22 nucleotides long, encoded in the genomes of invertebrates, plants, and vertebrates. The first two miRNAs to be discovered were *lin-4* and *let-7* in *Caenorhabditis elegans,* which regulate expression of *lin-14* and *lin-28* mRNAs, respectively, and are required

for larval developmental timing (Lee et al. 1993; Olsen and Ambros 1999; Reinhart et al. 2000). Since then, miRNAs have been observed in many contexts including their function in numerous regulatory pathways, deserving the attention they are now receiving. MicroRNA genes represent about 1–2% of the known eukaryotic genomes. Computational predictions suggest that each miRNA can target more than 200 transcripts and that a single mRNA can be regulated by multiple miRNAs (Lindow and Gorodkin 2007). This suggests that miRNAs and their targets are part of complex regulatory circuitries. With the exception of miRNAs located within Alu-repetitive elements, transcribed by RNA polymerase III, miRNAs are always transcribed by RNA polymerase II (Rodriguez et al. 2004). The initial transcript, or "primary miRNA" (pri-miRNA), can be hundreds to thousands nucleotides long and, as any other PolII transcript, undergoes capping and polyadenylation (Lee et al. 2003). The mature miRNA is part of a 60-80-nucleotide stem-loop structure contained within the pri-miRNA. The first step in miRNA biogenesis occurs in the nucleus and requires the excision of this hairpin structure by a complex called Microprocessor. This complex contains the RNAseIII-like Drosha enzyme and the RNA binding protein DGCR8/Pasha (Gregory et al. 2004). The excised hairpin, now called pre-miRNA, is exported to the cytoplasm by a protein heterodimer consisting of the transport factor Exportin-5 and its cofactor Ran (Bohnsack et al. 2004). Once exported, the pre-miRNA is then processed by another RNaseIII enzyme called Dicer. This endonuclease removes the loop region of the hairpin, releasing the mature miRNA:miRNA* duplex. Dicer interacts with several RNA binding proteins such as TRBP and PACT, which are not essential for its cleavage activity but are important for miRNA stability and loading of the effector complex RISC (RNA-induced silencing complex) (Chendrimada et al. 2005; Lee et al. 2006). During the assembly of the RISC complex with the miRNA, only one strand of the duplex is loaded, whereas the complementary miRNA* strand is removed and degraded (Kim 2005). The mature miRNA is now ready to direct its activity on a target mRNA by binding miRNA responsive elements usually located in the 3′UTR of the transcript. This association may result in either cleavage or translational repression of the target mRNA, depending on the degree of base-pairing between the miRNA and the responsive element. Perfect complementarity generally results in cleavage, whereas imperfect base-pairing leads to translational repression (Meister and Tuschl 2004). These alternative effects might also reflect differences in the biochemical composition of the RISC complex associated to each specific miRNA:mRNA duplex (Hock et al. 2007). The exact composition of the RISC complex is actually unknown. All biochemical purifications performed so far have revealed the presence of at least one member of the Argonaute family of proteins (Ago), suggesting that these proteins are a key component of the ribonucleoprotein complex RISC. Argonaute proteins are highly basic proteins that contain four domains: the N-terminal, PAZ, Mid, and PIWI domains (Hutvagner and Simard 2008). The PAZ domain binds the single-stranded 3′ end of miRNAs. The three-dimensional structure of the PIWI domain resembles that of the RNAse H enzyme. However, only certain Ago proteins such as mammalian Ago2 retain a functional catalytic motif (Asp-Asp-His) and may perform the endonuclease activity. Finally, a motif (named MC) has been identified within the Mid domain of Ago proteins, bearing significant similarity to the translation initiation factor eIF4E cap-binding domain. Recently, this motif has been shown to play a role in miRNA-mediated translational repression (Kiriakidou et al. 2007).

The mechanism of miRNA-mediated gene regulation remains contentious. However, artificial tethering of Ago proteins to the 3′UTR of a reporter mRNA is sufficient to induce its translational repression, suggesting that the role of miRNAs is to guide the deposition of the RISC complex on a specific site of the target mRNA (Pillai et al. 2004). Several mechanisms of miRNA-mediated translational regulation have been proposed, including (1) interference with the initiation step of translation, (2) repression at postinitiation steps, and (3) rapid degradation of target mRNAs.

Interference with initiation step of translation

The first evidence suggesting an involvement in the initiation step of translation is the observation that the presence of miRNAs shifts the target mRNAs from active polysomes to lighter sucrose gradient fractions, containing translationally inactive mRNA ribonucleoprotein particles (mRNP) (Pillai et al. 2005). Several strategies have been proposed to explain this effect. The MC domain of Ago proteins may compete with eIF4E in binding to the mRNAs 5′ cap structure, an essential first step in 43S initiation complex assembly (Humphreys et al. 2005; Kiriakidou et al. 2007). Analogous competition between RISC and the cap-binding complex EIF4F was recently reported in an in vitro study using mouse cell-free extracts (Mathonnet et al. 2007). Alternatively, it has been proposed that Ago proteins may directly recruit the translational repressor eIF6 on target mRNAs; this factor competes with the small ribosomal subunit in binding the large subunit, thereby blocking mature ribosome assembly (Chendrimada et al. 2007). Similar inhibition of mature ribosome formation was also observed in a study utilizing a *D. melanogaster* cell-free system (Thermann and Hentze 2007).

Repression at postinitiation steps

Several lines of evidence support instead the idea that miRNA might repress translation subsequently to the initiation step. First, early studies in *C. elegans* and later in mammalian cells showed that both miRNAs and Ago proteins cofractionate with polyribosomes in sucrose gradients (Olsen and Ambros 1999). Moreover, some report that IRES-dependent translational initiation can also be repressed by miRNAs, suggesting that miRNAs inhibit translation at a step downstream of cap recognition. It has been proposed that during elongation, the RISC complex may induce a rapid ribosome "drop-off" from its mRNA target (Maroney et al. 2006; Petersen et al. 2006). Recently, it has also been suggested that the RISC complex may interact with an unknown protease able to degrade the nascent polypeptide chain cotranslationally (Nottrott et al. 2006).

Rapid degradation of target mRNAs

A recent study also supports the hypothesis that miRNAs could regulate target mRNAs' expression by affecting their stability. This could occur via an endonucleolitic pathway in case of perfect complementarity between the miRNA and its target, and also by recruiting deadenylating and decapping enzymes to the mRNAs (Behm-Ansmant et al. 2006; Yekta et al. 2004). It is interesting to note that several proteins required for miRNA activity, such as GW182/TNRC6A, the DCP1/DCP2 decapping complex, CAF1/CCR4/NOT deadenylation complex, and RCK/P54, accumulate in cytoplasmic foci called "processing bodies" (P-bodies) (Behm-Ansmant et al. 2006; Chu and Rana, 2006; Rehwinkel et al. 2005). These are cytoplasmic sites where mRNAs are thought to be either degraded or temporarily stored in a translationally silenced state. The current model is that miRNA-mediated silencing of its mRNA targets may occur by inducing their relocation to the P-bodies (Eulalio et al. 2007). The RISC complex acts to control mRNA molecules' translation within the complex framework of other 3′UTR binding regulatory proteins. The destiny of a transcript therefore will be determined by the coordinated contribution of these multiple effectors (Bhattacharyya et al. 2006; Jing et al. 2005; Kedde et al. 2007; Vasudevan et al. 2007). An example of this crosstalk is offered by the AU-rich-element binding protein HuR. Bhattacharyya et al. (2006) recently showed that under stress conditions HuR binding to the 3′UTR releases the mRNA from miRNA-mediated repression by inducing its relocation from P-bodies to the cytoplasm. miRNA-mediated post-transcriptional regulation is also likely influenced by the presence of tissue-specific factors. In neurons, for example, the RISC complex has been shown to cooperate with FMRP (Fragile X Mental Retardation Protein 1) and to functionally respond to neuronal stimuli (Caudy et al. 2002; Ishizuka et al. 2002; Jin et al. 2004). The interplay between miRNAs' basic mechanism of action and the cellular environment in which they act is therefore extremely important to understanding their function.

MicroRNAs in brain: neuronal development and differentiation

The bewildering diversity of neurons, including their distribution in specific functional areas and complex synaptic circuitry, is determined during development and differentiation and is achieved by multiple levels of gene regulation. In the last few years, microRNAs have emerged as important players in post-transcriptional regulation in the brain. A major advancement in understanding how miRNAs are involved in this phenomenon comes from studies on miRNA expression profiles. Several analyses have shown spatially and/or temporally restricted distribution of miRNAs, suggesting that they might be fine-tuning neuronal gene expression (Bak et al. 2008; Hohjoh and Fukushima 2007; Johnston and Hobert 2003; Kim et al. 2004; Krichevsky et al. 2003, 2006; Lagos-Quintana et al. 2002; Miska et al. 2004; Sempere et al. 2004; Smirnova et al. 2005; Wheeler et al. 2006; Wienholds et al. 2005). Genetic analyses in zebrafish that addressed the role of the Dicer enzyme have suggested the engagement of miRNAs in neural development. Maternal-zygotic *dicer* mutant embryos showed altered patterns of brain morphogenesis and neuronal differentiation caused by a failure in the production of mature microRNAs. These developmental brain defects were partially rescued by injection of *miR-430* into the mutant fish, a miRNA family expressed at the onset of zygotic transcription and required for embryonic morphogenesis and clearance of maternal mRNAs (Giraldez et al. 2005). This suggests that the developmental defect is mainly due to the impairment in *miR*-430 biosynthesis. Is Dicer involved in neurodevelopment also in mammals? To date, this question remains unresolved given that Dicer-deficient mice die at embryonic day 7.5, before neurulation (Bernstein et al. 2003). However, when Dicer activity was conditionally knocked down in mouse Purkinje cells (Schaefer et al. 2007) and dopaminergic neurons (Kim et al. 2007), progressive death of neuronal cells was observed. In addition, it has been shown that during maturation of cultured rat cerebellar granule neurons, Dicer expression was upregulated and that Dicer levels increase during differentiation of hippocampal neurons in vitro (Barbato et al. 2007). These data suggest that miRNAs are involved in differentiation and maintenance of neuronal identity in the mature nervous system.

In *C. elegans*, specific miRNA involvement in neuronal fate specification was described for two chemosensory neurons, ASE left (ASEL) and ASE right (ASER), in which the expression of different chemoreceptors gives rise to functional diversity between the two neuronal populations. The neuronal identity of ASEL and ASER and the organization of left-right asymmetry in the ASE neurons (Chang et al. 2004; Johnston and Hobert 2003; Johnston et al. 2005) is maintained by a feedback loop involving miRNAs and transcription factors. In particular, the miRNAs *lsy-6* and *miR-273* downregulate translation of the transcriptional repressor *Cog-1* and the transcriptional activator *Die-1*, respectively. Concomitantly, *Die-1* induces expression of the *lsy6* miRNA. ASEL-specific gene expression patterns are due to *lsy6*-mediated repression of *Cog-1* expression. In ASER neurons, activity of *miR-273* blocks expression of *Die-1*, and consequently transcription of *lsy6*, thereby allowing *Cog-1* expression. Overall, the asymmetry in ASE neurons reflects differential expression of miRNAs, which in turn drives *Cog-1* expression in opposite directions, determining expression of genes specific for the left-right asymmetry (Hobert 2006).

In mammals, many miRNAs specifically expressed or enriched in brain tissues have been identified. During differentiation of P19 embryonic carcinoma cells into neurons after retinoic acid treatment, several miRNAs such as *let-7a*, *miR-218*, and *miR-125* were upregulated (Sempere et al. 2004). *miR-125* was later shown to modulate the stability and translation of Lin28 mRNA, an important transcription factor involved in P19 differentiation (Wu and Belasco 2005). Other noteworthy examples of a role of miRNAs in establishing and maintaining cell identity originate from studies in which mouse embryonic stem (ES) cell neural differentiation was induced (Krichevsky et al. 2006). The expression profile of miRNAs in these neural precursors revealed that several miRNAs are coinduced during differentiation of neuronal cell and astrocytes. The miRNA profile obtained with these cellular models of neurogenesis in vitro correlated visibly with embryonic neurogenesis in vivo, suggesting that specific neuronal miRNAs are important players in mammalian brain development (Krichevsky et al. 2006). In particular, *miR-9* and *miR-124* affect the neuron-to-astroglia ratio during neural lineage differentiation. Transfections of miRNA duplexes (*miR-124*, *miR-9*, *miR-125*, and *miR-22*) in progenitor neurons decrease the number of cells expressing glia-specific markers (glial fibrillary acidic protein, GFAP) while increasing the number of neurons. Conversely, inhibition of miRNAs by use of 2′-O-methyl antisense oligonucleotides induces reduction of cells expressing neuron-specific marker Tuj1 (Krichevsky et al. 2006). Finally, profiling experiments were performed to define temporal expression of miRNAs in mouse brain. The neuron-specific *miR-124*, *miR-125*, and *miR-128* accumulated in parallel to neuronal maturation, whereas *miR-23* and *miR-29* were expressed in astrocytes and at low levels during embryonic development (Smirnova et al. 2005). Following these studies, experiments have been performed to address the specific function of these neuronal miRNAs during development and differentiation of the nervous system. In this framework, the best studied neuronal miRNA is *miR-124*, which constitutes 25–50% of total miRNAs from brain tissue (Johnston and Hobert 2003) and is extensively involved in silencing non-neuronal genes, thereby controlling the neuronal identity. Overexpression of *miR-124* in HeLa cells induces downregulation of more than 100 non-neuronal mRNAs, producing a neuron-like expression profile and suggesting that it plays a role in neuronal differentiation (Conaco et al. 2006; Lim et al. 2005). In cortical neurons, several non-neuronal mRNA transcripts increase upon *miR-124* knockdown (Conaco et al. 2006), and during chicken spinal cord development *miR-124* is necessary for the preservation of neuronal identity (Visvanathan et al. 2007). REST (RE1 silencing transcription factor) is a transcriptional repressor of neural genes in non-neuronal tissues, including *miR-124* (Conaco et al. 2006). Decreased level of REST together with increased *miR-124* expression leads to the terminal differentiation of neuronal cells (Visvanathan et al. 2007). Moreover, *miR-124* downregulates REST activity by silencing non-neuronal mRNAs, including the small C-terminal domain phosphatase-1 (SCP1), an activator of REST, involved in the antineural REST pathway (Visvanathan et al. 2007). Overall, in non-neuronal cells and neuronal precursors, REST and SCP1 repress the expression of *miR-124* and other neuronal genes, which are derepressed during neural differentiation (Visvanathan et al. 2007; Wu and Xie 2006). Another gene controlled by *miR-124* is the polypyrimidine tract-binding protein *PTB*, an important splicing regulator. During neuronal differentiation *PTB* expression is substituted by that of its neuronal homolog *nPTB*. This switch has substantial consequences on the splicing patterns of genes involved in neuronal functions. *MiR-124* inhibits *PTB* expression, thus favoring the neuronal-specific splicing activity of *nPTB* (Makeyev et al. 2007). The connection between RNA silencing (*miR-124*) and splicing (*PTB/nPTB*) was confirmed by reciprocal expression analyses of these genes in several differentiated and nondifferentiated neuronal areas (Makeyev et al. 2007), shedding light on a new chapter of neuronal gene expression regulation. On the other hand, *nPTB* is regulated directly by *miR-133*, a muscle-enriched miRNA, during muscle development, mirroring the example of *miR-124* in the nervous system (Boutz et al. 2007).

Recently, a miRNA-guided RNA silencing pathway was linked to differentiation of dopaminergic neurons (DN). *MiR-133b*, specifically expressed in mammalian midbrain

DN, regulates their maturation by modulating the transcription factor *Pitx3* through a negative feedback circuit in which *Pitx3* induces the transcription of *miR-133b* (Kim et al. 2007). Overexpression of *miR-133b* in primary embryonic rat midbrain cultures represses dopaminergic differentiation, whereas its inhibition by 2′-O-methyl antisense oligonucleotides leads to an increase in DNs. Moreover, reduced expression of *miR-133b* is associated with neurodegeneration of DN in Parkinson's disease, as described below.

The data reported here seem to indicate that miRNAs contribute to define specific pathways of neuronal differentiation and development by coordinating the appropriate gene expression patterns. However, the complex composition of the brain and the nervous system, containing several neuronal and glial istotypes, while representing its main biological characteristic is also the principal obstacle for a correct tissue dissection. This is especially true in postmortem human brains. For these reasons, the source of neural tissues, together with the microrarray platforms and the RNA isolation techniques used, needs to be carefully considered in the interpretation of these data. The identification of miRNAs' physiologic target genes should therefore be a primary approach to reveal the specific contribution of microRNAs to neural development, differentiation, and function.

Potential roles of miRNAs in synaptic plasticity

Enduring changes in neural circuits are at the core of brain activities such as learning and memory and rely on long-lasting changes in synaptic efficacy. It is now believed that long-term modification of synapses requires local translation of key molecular synaptic constituents (Sutton and Schuman 2005). Synaptically localized protein synthesis makes necessary the corresponding mRNAs to be transported in the dendritic compartment and for their translational activation to be site specific. While little is known of how translation at synapses is regulated, it is generally thought that dendritic mRNAs are transported in a translationally silenced state within multiribonucleoprotein complexes called " neuronal RNA granules" (Kiebler and Bassell 2006).

Signaling pathways have been described in dendrites that control general synaptic protein synthesis at the level of translation initiation and elongation via phosphorylation of the cap binding protein eIF4E and of the elongation factor eEF2. However, how is differential expression of specific dendritic mRNA achieved? Few proteins have been identified that could confer such specificity. One is the zipcode-binding-protein ZBP1, required for both dendritic targeting and translational repression of β-actin mRNA (Huttelmaier et al. 2005).

Another prominent example of a translational repressor involved in dendritic mRNA expression is the Fragile X protein, FMRP. Silencing of the *Fmr1* gene leads to the Fragile X syndrome (FX), one of the most common forms of mental retardation. The disease leads to a broad set of neurologic symptoms and is characterized by abnormalities in the structural development of dendritic spines (Penagarikano et al. 2007). The current view is that FMRP acts as translational repressor of a subset of dendritic mRNAs and that the disease is caused by deregulated expression of its target mRNAs at synapses, as shown by translational profiles of FX patients and of *Fmr1* knockout mice (Vanderklish and Edelman 2005). Indeed, transcripts that are controlled by FMRP activity have been identified by microarray analyses and encode factors that are required for synaptic plasticity and development. The mechanism underlying the translational control exerted by FMRP is yet unclear and recent reports seem to indicate that it might act in concert with miRNAs to specifically target and silence dendritic mRNAs (Fiore et al. 2008).

Evidence for the participation of miRNAs in synaptic expression of mRNAs was first observed in *Drosophila melanogaster*. Two initial studies (Caudy et al. 2002; Ishizuka et al. 2002) found that *dFmr1*, the *Drosophila* ortholog of mammalian fragile X protein, interacts with the RNA interference machinery, in particular with Argonaute 2. Following this lead, the Warren laboratory investigated further the interactors of FMRP in mammalian cells finding that it associates with endogenous miRNAs and with mammalian *AGO1* (Jin et al. 2004). They then performed genetic studies in *Drosophila* to find that *Ago1* depletion suppresses *dFrm1* overexpression phenotype, and that a trans-heterozygote for both *AGO1* and *dFmrp1* shows an even more pronounced synaptic overgrowth phenotype than the *dFmr1* null mutant. This indicates that *AGO1* is possibly a limiting factor in *dFmr1* function in synaptogenesis and suggests that microRNAs might mediate the *FMR1* role in the silencing of neuronal mRNAs.

A recent study addressing the composition of neuronal granules in *Drosophila* also points in this same direction. Microscopy analyses of endogenously and exogenously expressed proteins in *Drosophila* motor neurons reveal an interesting overlap in the composition of neuronal granules and P-bodies, the somatic sites of mRNA degradation, and the function of miRNAs (Barbee et al. 2006; Hillebrand et al. 2007). The authors also show how a core P-body factor, Me31B, participates in the translational repression driven by FMRP and miRNAs.

The presence of translational repressors characteristic of somatic P-bodies in FMRP-containing neuronal granules suggests that these classes of granules are similar not only in composition but also in function (Anderson and Kedersha 2006; Kiebler and Bassell 2006). It also implies

that in concert with FMRP-mediated translational control, expression of mRNAs transported in neuronal granules might be modulated via additional mechanisms characteristic of P-bodies, such as microRNAs and mRNA degradation pathways like the Nonsense Mediated Decay (NMD). Indeed, essential NMD factors such as eIF4AIII and Upf1 were found to colocalize with FMRP in the somatodendritic compartment of mammalian neurons, and to regulate stability and expression of Arc mRNA, an important dendritic transcript involved in synaptic plasticity (Giorgi et al. 2007).

More direct evidence of a link between miRNA function and synaptic plasticity in *Drosophila* was recently reported by Kunes' laboratory (Ashraf et al. 2006). In this study, the authors started by showing that the 3′UTR of CaMKII mRNA, an mRNA involved in synaptic plasticity, is sufficient to drive the activity-dependent dendritic localization and synaptic translation of a reporter EYFP construct, under conditions that produce long-term memory. Putative miRNA binding sites are present in the 3′UTR of CaMKII, as well as in the 3′UTR of the transcripts coding for Staufen and Kinesin-Heavy Chain, two dendritic granule-associated proteins. To test whether these mRNAs are the targets of miRNA-silencing activity, their expression levels were assayed in brains from mutants of the RISC pathway. The results were that synaptic translation of CaMKII increases in *dicer*-, *armitage*-, and *aubergine*-mutant brains, indicating that this mRNA is indeed subject to a RISC-mediated translational repression. *Armitage* also seems to affect the expression levels of both Staufen and Kinesin-Heavy Chain. Furthermore, this study suggests an elegant modulation of *armitage*-driven repression of CaMKII expression in response to neural activity. Synaptic activation induces a decrease in the levels or Armitage protein and a correspondent increase in CaMKII abundance. The decrease in Armitage protein is shown to be due to the activity of the proteasome which is known to act at the synaptic level to contribute in modulating synaptic protein content (Bingol and Schuman 2005, 2006). Overall, Ashraf et al. (2006) propose a novel and intriguing regulatory mechanism whereby CaMKII translational repression is driven by miRNAs and in turn is relieved by activity-dependent proteasome-mediated degradation of Armitage.

Around the same time, an important step forward was made in linking miRNA function to synaptic development and plasticity in mammalian neurons (Schratt et al. 2006). Schratt et al. found that the microRNA *miR-134* is localized in dendrites of mouse cultured hippocampal neurons, close to synapsin-positive puncta. Its overexpression causes a significant decrease in dendritic spine size, and conversely its depletion via 2′-O-methyl antisense oligonucleotides leads to a small increase in spine volume. This indicates that *miR-134* plays a role in spine morphogenesis and potentially in plasticity. Still, what are the downstream effectors that mediate such activity? Scanning for potential targets of *miR-134*, the authors found corresponding binding sites in the 3′UTR of the mRNA encoding the Lim-domain-containing protein kinase 1 (Limk1). This protein is a particularly good candidate in mediating *miR-134* activity since it is controlled by BDNF and is involved in actin filament dynamics, a key step in the cytoskeletal modification of spines associated with plasticity. Colocalization experiments with exogenously expressed *miR-134* and *Limk1* mRNA 3′UTR successfully showed that this miRNA and its putative target are present in the same dendritic granules and their association requires an intact binding site for *miR-134*. In addition, expression of a luciferase reporter gene fused to *Limk1* 3′UTR is downregulated by *miR-134* overexpression and upregulated upon its depletion mediated by 2′-O-methyl antisense oligonucleotides. The positive effect of *miR-134* depletion on *Limk1* mRNA expression was also observed on the endogenous transcript, suggesting that *Limk1* mRNA is indeed silenced by *miR-134* in vivo. The authors then tested whether the spine size defects caused by *miR-134* overexpression were due to deregulation of *Limk1* expression. If this were the case, then coexpression of a *Limk1* mRNA with a mutated binding site for *miR-134* should be able to rescue the defect in spine morphology. Indeed, a rescue was observed when the *miR-134*-insensitive mutant *Limk1* mRNA was transfected in cells overexpressing *miR-134*, while a much milder rescue resulted from expression of wild-type *Limk1* transcript. Similar to what was indicated by Kunes' laboratory (Ashraf et al. 2006), in this case neuronal activation also intervenes to put a brake on miRNA-mediated silencing. Schratt et al. (2006) show how *miR-134* repression of *Limk1* translation is relieved upon BDNF stimulation of synaptic activity. This was demonstrated by monitoring the effects of BDNF treatment on the translation of a *Limk1* 3′UTR luciferase reporter mRNA carrying a wild-type or mutated *miR-134* responsive site in cortical neurons. In transfected neurons with wild-type *Limk1* 3′UTR, BDNF induces the translation of the reporter mRNA. When cortical neurons were transfected with mutated *Limk1* 3′UTR, BDNF treatment did not affect the reporter gene translation.

The studies reported here, while preliminary, indicate that miRNAs have the potential to contribute to the fine-tuning of synaptic protein synthesis and plasticity by modulating expression of dendritic mRNAs (Fig. 1). Evidence for direct involvement of specific miRNAs and the identification of their target mRNAs is still very limited, and further investigation is needed to demonstrate a contribution of miRNA activity in establishing long-term synaptic modifications in mammals. However, miRNAs can represent an important mechanism for the modulation

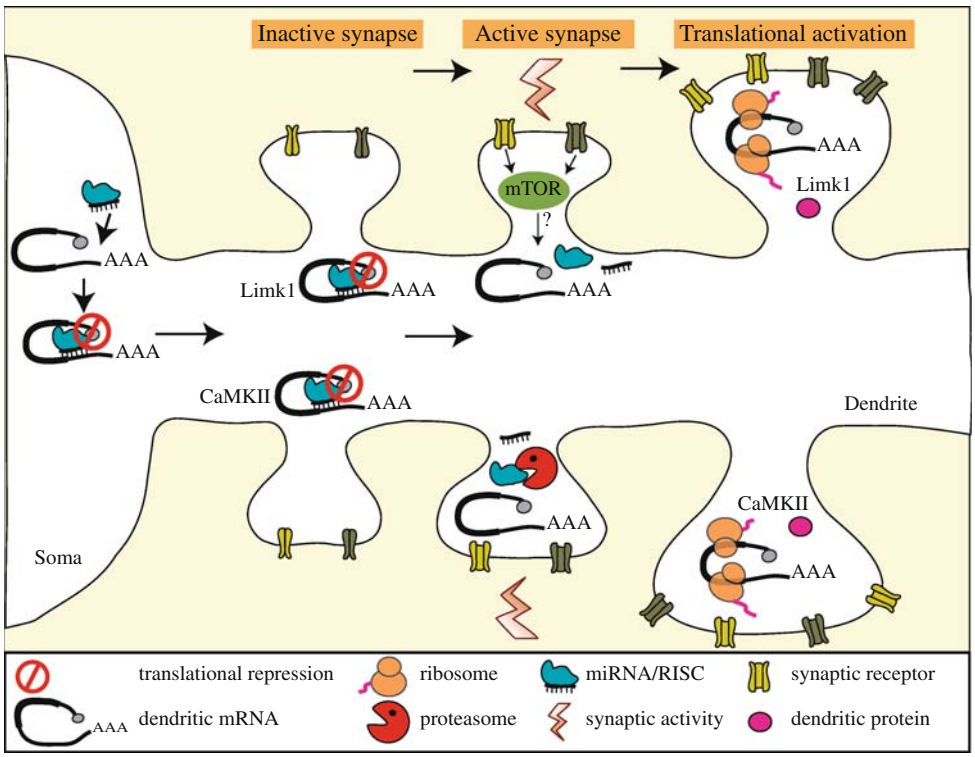

Fig. 1 Model of the role of miRNAs in synaptic plasticity. The model depicted here summarizes two pathways recently proposed where miRNAs modulate synaptic protein synthesis and plasticity. The mechanism unveiled by Schratt et al. (2006) in mammalian neurons is illustrated in the top part of the neuronal dendrite and spines. Here, *Limk1* mRNA is subject to *miR-134*-mediated repression. Conceivably, this repression might begin in the soma prior to dendritic transport. Upon synaptic activation, induced by BDNF treatment, the authors observe translational derepression of *Limk1* mRNA and an increase in spine size. How synaptic activity might release *Limk1* mRNA from *miR-134* repression is unclear. Possibly, BDNF could induce translational activation via the mTOR pathway and inactivation of the miRNA/RISC complex. The mechanism proposed by Ashraf et al. (2006) in *Drosophila* neurons is depicted in the bottom part of the neuronal dendrite and spines. Here, miRNAs are required for the translational silencing of several neuronal mRNAs important for synaptic protein synthesis, including mRNAs encoding CaMKII, Staufen, and Kinesin Heavy Chain. Patterns of synaptic activity that induce long-term memory trigger localized proteasome-mediated degradation of Armitage (RISC) and consequent release of the translational repression guided by miRNAs at the synapse

of synaptic plasticity, given the specificity they offer in regulating the target mRNAs. An interesting aspect arising from these initial studies is the likely interplay between miRNA-mediated regulation and other mechanisms of gene expression that act at the synapse. In addition to their involvement in FMRP translational control, it will be interesting to study potential coupling of the function of miRNAs with other aspects of dendritic mRNA regulation, such as mRNA localization, decay, and protein degradation, and how these different pathways coordinate in orchestrating protein expression at synapses.

MicroRNA and nervous system diseases

MicroRNAs have been implicated in several neurologic disorders, including gliomas, polyglutamine expansions, Parkinson's and Alzheimer's diseases, and the Tourette and Fragile X syndromes. From a biomedical point of view, the discovery of the association between miRNA deregulation and nervous system diseases has generated a novel area of research, mainly on mental and psychiatric illnesses.

Recently, an intriguing mechanism of miRNA involvement in human diseases has emerged from a study on single-nucleotide polymorphisms (SNP). Sequence variants of a candidate gene on chromosome 13q31.1, named *SLITRK1* (Slit and Trk-like 1), were identified in patients with Tourette syndrome (TS), a neurologic disorder that leads to behavioral abnormalities and characterized by motor and vocal tics. The TS variant named var321, found in two unrelated patients, is located in the 3′UTR of *SLITRK1* mRNA, within the binding site for *miR-189* (Abelson et al. 2005). This mutation positively affects the interaction of *SLITRK1* mRNA and *miR-189*, leading to a more stringent repression of *SLITRK1* expression. Involvement of miRNA-mediated repression in TS is further supported by the observed coincident expression profile of *SLITRK1* mRNA and *miR-189* in brain areas

affected in TS patients carrying var321 (Abelson et al. 2005).

The biochemical mechanisms underlying the Fragile X syndrome have been mentioned previously. Recent analyses in *Drosophila* and mammals indicate that FMRP and miRNAs cooperate in repressing dendritic mRNA expression, as discussed above. Considering that translational deregulation of dendritic proteins is suggested to be the underlying defect in the insurgence of this disorder, FMRP-associated miRNAs might likely play a role in FX. Nonetheless, evidence of direct participation of miRNAs in the insurgence or development of the FX syndrome is still lacking. Huntington's disease, as well as many spinocerebellar ataxias, is a neurodegenerative disease caused by a CAG trinucleotide repeat expansion. It was demonstrated that the upregulation of the miRNA bantam modulates the degeneration induced by polyQ protein Ataxin-3, a gene mutated in spinocerebellar ataxia type 3 (SCA3) (Bilen et al. 2006). Moreover, mutations in the Dicer enzyme, which prevent the maturation of all miRNAs, increase the polyQ toxicity in both *Drosophila* and HeLa cells, conferring a neuroprotective role to miRNAs in these neurodegenerative diseases (Bilen et al. 2006). In another study, conditional Purkinje (PK) cell-specific ablation of Dicer leads to PK cell death. Deficiency in Dicer is associated with progressive loss of miRNAs, followed by neurodegeneration and cell death, indicating an involvement of miRNAs in cerebellar neurodegeneration (Schaefer et al. 2007).

MicroRNAs are also thought to be involved in other neurodegenerative diseases such as Parkinson's and Alzheimer's. Reduced expression of *miR-133b* in dopaminergic neurons in Parkinson's patients has been previously mentioned (Kim et al. 2007). However, to define the *miR-133b* mechanism of action, it will be necessary to identify relevant mRNA targets in these neuronal cell types.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of dense plaques and neurofibrillary tangles in the brain, resulting in progressive dementia. In AD brains, a role for miRNAs has not been established yet. Recently, the expression profiles of a subset of 12 miRNAs in the AD hippocampus were compared with those of nondemented controls and fetal brain. The results indicate that *miR-9* is upregulated in both fetal and AD hippocampus, *miR-128a* increases in AD but not in fetus or non-emented controls, and *miR-125b* shows a tendency to accumulate in AD (Lukiw 2007). A more recent work on miRNAs and AD indicates that *miR-107* could be a regulator of β-site amyloid precursor protein cleaving enzyme 1 (*BACE1*). *BACE1* mRNA levels have a tendency to increase as *miR-107* levels decrease in the progression of AD. The coordinated application of miRNA profiling, microarrays, bioinformatics predictions, in situ hybridization, and biochemical validations indicate that *miR-107* may be involved in accelerated disease progression through regulation of *BACE1* (Wang et al. 2008). These studies suggest that miRNAs may be important for neuronal survival in the context of human neurodegenerative diseases. They also represent an incentive to identify the miRNAs involved in such pathologies and the biological pathways affected, thus shedding light on the molecular and cellular bases of human pathology.

Importantly, the severe psychiatric disorder schizophrenia might be associated with altered miRNA expression profiles. In postmortem prefrontal cortex from schizophrenia patients, several miRNAs were found expressed at lower levels (Perkins et al. 2007). Interestingly, genetic variants of the brain-expressed *miR-206* and *miR-198* were associated with the schizophrenia disorder (Hansen et al. 2007).

Several studies on the involvement of miRNAs in cancers have unveiled an important role of miRNA in this pathology, including brain tumors. MicroRNAs can act both as tumor suppressors and oncogenes (Esquela-Kerscher and Slack 2006). Glioblastomas (GBM) are the most malignant and lethal of all brain tumors. *MiR-21* was highly expressed in GBMs, functioning as an oncogenic and anti-apoptotic factor (Chan et al. 2005), while it was expressed at basal levels in other brain tumors and in normal brains. *MiR-21* silencing in a glioblastoma context increases the apoptotic versus viable cells ratio, suggesting an anti-apoptotic role for this miRNA (Chan et al. 2005). In several tumors *miR-21* overexpression was demonstrated and it was included in the microRNA expression signature of human solid tumors (Volinia et al. 2006), becoming a potential target in glioblastoma therapy (Mathupala et al. 2006).

Conclusions

In the past few years, it has become clear that miRNAs are important players in the regulation of different aspects of neuronal cell biology, including development, neurogenesis, and synaptic plasticity. The increasing efficiency and sensitivity of miRNA quantification methods such as deep-sequencing techniques is expected to produce detailed miRNA expression maps for specific brain regions and neuron subtypes. This will certainly constitute the basis of identifying candidate miRNAs involved in distinct neuronal phenomena and also in neurologic diseases (Table 1). However, a proper functional characterization of these miRNA candidates will likely require a massive effort, using loss-of-function approaches.

Although the elucidation of the role of individual miRNAs is surely important in understanding the role of miRNAs in processes like synaptic plasticity, it also appears crucial to define the biochemical mechanisms that modulate the activity of the RISC complex itself. For

Table 1 MicroRNAs in the nervous system and human brain diseases

miRNA	Function/disease	mRNA target	References
miR-21	Antiapoptotic/human glioblastoma	Unknown	Chan et al. (2005)
miR-107	Alzheimer's disease	*BACE1*	Wang et al. (2008)
miR-29a/b-1	Alzheimer's disease	*BACE1*	Hébert et al. (2008)
miR-133b	Maturation of dopaminergic neurons/Parkinson's disease	*Pitx3*	Kim et al. (2007)
miR-124	Control of neuronal identity and differentiation	Laminin gamma 1; Integrin beta 1	Cao et al. (2007)
		SCP1	Visvanathan et al. (2007)
		PTBP1	Makeyev et al. (2007)
miR-134	Dendritic spine development	Limk1	Schratt et al. (2006)
miR-132	Neurite outgrowth	P250GAP	Vo et al. (2005)
miR-9; *miR*-125	Neuroblastoma proliferation	Tropomyosin-related kinase C	Laneve et al. (2007)

instance, it has been proposed that synaptic activation may release RISC-mediated translational repression. However, as yet, very little is known about the transduction pathway that links the synaptic activity and RISC functionality. Another essential but not widely appreciated aspect is how the miRNA machinery interacts with translational "environments" that are cell-specific. For instance, the presence of particular translation modulators may dramatically alter the activity of RISC, as in the case of FXR1 (Vasudevan et al. 2007) which appears to turn RISC from a repressor into an activator of mRNA translation.

The computational identification of microRNA targets and the validation of miRNA-target interactions represent fundamental steps tin revealing the contribution of microRNAs to neural functions. To date, the prediction of miRNA targets by computational approaches is based mainly on miRNAs' complementarity to their target mRNAs, and several web-based or non-web-based computer software programs are used to this purpose (Lindow and Gorodkin 2007; Sethupathy et al. 2006; Yoon and De Micheli 2006).

A major effort will be needed to define the role of the RNA-mediated gene-silencing machinery in neurons, the neuronal miRNA targets, and specific components of RISC, which are relevant in brain development, function, and disease.

Acknowledgments This work was partially funded by FIRB RBLA03FLJC-005 and FIRB RBIN04H5AS_002, PRIN prot. No. 2005055188-002 and TELETHON grant GGP05269 (to CC). This work was supported in part by 'Fondazione Alazio Award 2007' (to CB) (www.fondazionealazio.org, Via Torquato Tasso 22, 90144 Palermo). We thank Francesca Ruberti and Mauro Cozzolino for helpful discussions and comments on the manuscript.

References

Abelson JF, Kwan KY, O'Roak BJ, Baek DY, Stillman AA et al (2005) Sequence variants in *SLITRK1* are associated with Tourette's syndrome. Science 310:317–320

Anderson P, Kedersha N (2006) RNA granules. J Cell Biol 172:803

Ashraf SI, McLoon AL, Sclarsic SM, Kunes S (2006) Synaptic protein synthesis associated with memory is regulated by the RISC pathway in Drosophila. Cell 124:191–205

Bak M, Silahtaroglu A, Møller M, Christensen M, Rath MF et al (2008) MicroRNA expression in the adult mouse central nervous system. RNA 14(3):432–444

Barbato C, Ciotti MT, Serafino AL, Calissano P, Cogoni C (2007) Dicer expression and localization in post-mitotic neurons. Brain Res 1175:17–27

Barbee SA, Estes PS, Cziko AM, Hillebrand J, Luedeman RA et al (2006) Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. Neuron 52:997–1009

Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P et al (2006) mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. Genes Dev 20(14):1885–1898

Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H et al (2003) Dicer is essential for mouse development. Nat Genet 35(3):215–217

Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W (2006) Relief of microRNA-mediated translational repression in human cells subjected to stress. Cell 125(6):1111–1124

Bilen J, Liu N, Burnett BG, Pittman RN, Bonini NM (2006) MicroRNA pathways modulate polyglutamine-induced neurodegeneration. Mol Cell 24(1):157–163

Bingol B, Schuman EM (2005) Synaptic protein degradation by the ubiquitin proteasome system. Curr Opin Neurobiol 15:536–541

Bingol B, Schuman EM (2006) Activity-dependent dynamics and sequestration of proteasomes in dendritic spines. Nature 441:1144–1148

Bohnsack MT, Czaplinski K, Gorlich D (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10(2):185–191

Boutz PL, Chawla G, Stoilov P, Black DL (2007) MicroRNAs regulate the expression of the alternative splicing factor nPTB during muscle development. Genes Dev 21(1):71–84

Caudy AA, Myers M, Hannon GJ, Hammond SM (2002) Fragile X-related protein and VIG associate with the RNA interference machinery. Genes Dev 16:2491–2496

Chan JA, Krichevsky AM, Kosik KS (2005) MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 65(14):6029–6033

Chang S, Johnston RJ Jr, Frøkjaer-Jensen C, Lockery S, Hobert O (2004) MicroRNAs act sequentially and asymmetrically to

control chemosensory laterality in the nematode. Nature 430(7001):785–789
Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N et al (2005) TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436(7051): 740–744
Chendrimada TP, Finn KJ, Ji X, Baillat D, Gregory RI et al (2007) MicroRNA silencing through RISC recruitment of eIF6. Nature 447(7146):823–828
Chu CY, Rana TM (2006) Translation repression in human cells by microRNA-induced gene silencing requires RCK/p54. PLoS Biol 4(7):e210
Conaco C, Otto S, Han JJ, Mandel G (2006) Reciprocal actions of REST and a microRNA promote neuronal identity. Proc Natl Acad Sci USA 103(7):2422–2427
Esquela-Kerscher A, Slack FJ (2006) Oncomirs—microRNAs with a role in cancer. Nat Rev Cancer (4):259-269
Eulalio A, Behm-Ansmant I, Schweizer D, Izaurralde E (2007) P-body formation is a consequence, not the cause, of RNA-mediated gene silencing. Mol Cell Biol 27(11):3970–3981
Fiore R, Siegel G, Schratt G (2008) MicroRNA function in neuronal development, plasticity and disease. Biochim Biophys Acta. doi: 10.1016/j.bbagrm.2007.12.006
Giorgi C, Yeo GW, Stone ME, Katz DB, Burge C et al (2007) The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. Cell 130:179–191
Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM et al (2005) MicroRNAs regulate brain morphogenesis in zebrafish. Science 308(5723):833–838
Gregory RI, Yan KP, Amuthan G, Chendrimada TP, Doratotaj B et al (2004) The microprocessor complex mediates the genesis of microRNAs. Nature 432(7014):235–240
Hansen T, Olsen L, Lindow M, Jakobsen KD, Ullum H et al (2007) Brain expressed microRNAs implicated in schizophrenia etiology. PLoS ONE 2(9):e873
Hébert SS, Horré K, Nicolaï L, Papadopoulou AS, Mandemakers W et al (2008) Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. Proc Natl Acad Sci USA 105(17):6415–6420
Hillebrand J, Barbee SA, Ramaswami M (2007) P-body components, microRNA regulation, and synaptic plasticity. ScientificWorld-Journal 7:178–190
Hobert O (2006) Architecture of a microRNA-controlled gene regulatory network that diversifies neuronal cell fates. Cold Spring Harb Symp Quant Biol 71:181–188
Hock J, Weinmann L, Ender C, Rüdel S, Kremmer E et al (2007) Proteomic and functional analysis of Argonaute-containing mRNA-protein complexes in human cells. EMBO Rep 8(11):1052–1060
Hohjoh H, Fukushima T (2007) Marked change in microRNA expression during neuronal differentiation of human teratocarcinoma NTera2D1 and mouse embryonal carcinoma P19 cells. Biochem Biophys Res Commun 362(2):360–367
Humphreys DT, Westman BJ, Martin DI, Preiss T (2005) MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. Proc Natl Acad Sci USA 102(47):16961–16966
Huttelmaier S, Zenklusen D, Lederer M, Dictenberg J, Lorenz M et al (2005) Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. Nature 438:512
Hutvagner G, Simard MJ (2008) Argonaute proteins: key players in RNA silencing. Nat Rev Mol Cell Biol 9(1):22–32
Ishizuka A, Siomi MC, Siomi H (2002) A Drosophila fragile X protein interacts with components of RNAi and ribosomal proteins. Genes Dev 16:2497–2508
Jin P, Zarnescu DC, Ceman S, Nakamoto M, Mowrey J et al (2004) Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. Nat Neurosci 7:113–117
Jing Q, Huang S, Guth S, Zarubin T, Motoyama A et al (2005) Involvement of microRNA in AU-rich element-mediated mRNA instability. Cell 120(5):623–634
Johnston RJ, Hobert O (2003) A microRNA controlling left/right neuronal asymmetry in *Caenorhabditis elegans*. Nature 426(6968):845–849
Johnston RJ Jr, Chang S, Etchberger JF, Ortiz CO, Hobert O (2005) MicroRNAs acting in a double-negative feedback loop to control a neuronal cell fate decision. Proc Natl Acad Sci USA 102(35):12449–12454
Kedde M, Strasser MJ, Boldajipour B, Vrielink JA, Slanchev K et al (2007) RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. Cell 131(7):1273–1286
Kiebler MA, Bassell GJ (2006) Neuronal RNA granules: movers and makers. Neuron 51:685–690
Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS et al (2004) Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. Proc Natl Acad Sci USA 101(1):360–365
Kim J, Inoue K, Ishii J, Vanti WB, Voronov SV et al (2007) A microRNA feedback circuit in midbrain dopamine neurons. Science 317(5842):1220–1224
Kim VN (2005) MicroRNA biogenesis: coordinated cropping and dicing. Nat Rev Mol Cell Biol 6(5):376–385
Kiriakidou M, Tan GS, Lamprinaki S, De Planell-Saguer M, Nelson PT et al (2007) An mRNA m7G cap binding-like motif within human Ago2 represses translation. Cell 129(6):1141–1151
Krichevsky AM, King KS, Donahue CP, Khrapko K, Kosik KS (2003) A microRNA array reveals extensive regulation of microRNAs during brain development. RNA 9(10):1274–1281
Krichevsky AM, Sonntag KC, Isacson O, Kosik KS (2006) Specific microRNAs modulate embryonic stem cell-derived neurogenesis. Stem Cells 24(4):857–864
Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W et al (2002) Identification of tissue-specific microRNAs from mouse. Curr Biol 12(9):735–739
Laneve P, Di Marcotullio L, Gioia U, Fiori ME, Ferretti E et al (2007) The interplay between microRNAs and the neurotrophin receptor tropomyosin-related kinase C controls proliferation of human neuroblastoma cells. Proc Natl Acad Sci USA 104(19):7957–7962
Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854
Lee Y, Ahn C, Han J, Choi H, Kim J et al (2003) The nuclear RNase III Drosha initiates microRNA processing. Nature 425(6956): 415–419
Lee Y, Hur I, Park SY, Kim YK, Suh MR et al (2006) The role of PACT in the RNA silencing pathway. EMBO J 25(3):522–532
Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM et al (2005) Microarray analysis shows that some microRNAs down-regulate large numbers of target mRNAs. Nature 433(7027): 769–773
Lindow M, Gorodkin J (2007) Principles and limitations of computational microRNA gene and target finding. DNA Cell Biol 26(5):339–351
Lukiw WJ (2007) Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. Neuroreport 18(3):297–300
Makeyev EV, Zhang J, Carrasco MA, Maniatis T (2007) The microRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol Cell 27(3):435–448

Maroney PA, Yu Y, Nilsen TW (2006) MicroRNAs, mRNAs, and translation. Cold Spring Harb Symp Quant Biol 71:531–535

Mathonnet G, Fabian MR, Svitkin YV, Parsyan A, Huck L et al (2007) MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. Science 317(5845): 1764–1767

Mathupala SP, Guthikonda M, Sloan AE (2006) RNAi based approaches to the treatment of malignant glioma. Technol Cancer Res Treat 5(3):261–269

Meister G, Tuschl T (2004) Mechanisms of gene silencing by double-stranded RNA. Nature 431(7006):343–349

Miska EA, Alvarez-Saavedra E, Townsend M, Yoshii A, Sestan N et al (2004) Microarray analysis of microRNA expression in the developing mammalian brain. Genome Biol 5(9):R68

Nottrott S, Simard MJ, Richter JD (2006) Human *let-7a* miRNA blocks protein production on actively translating polyribosomes. Nat Struct Mol Biol 13(12):1108–1114

Olsen PH, Ambros V (1999) The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. Dev Biol 216(2):671–680

Penagarikano O, Mulle JG, Warren ST (2007) The pathophysiology of fragile X syndrome. Annu Rev Genomics Hum Genet 8:109–129

Perkins DO, Jeffries CD, Jarskog LF, Thomson JM, Woods K et al (2007) MicroRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. Genome Biol 8(2):R27

Petersen CP, Bordeleau ME, Pelletier J, Sharp PA (2006) Short RNAs repress translation after initiation in mammalian cells. Mol Cell 21(4):533–542

Pillai RS, Artus CG, Filipowicz W (2004) Tethering of human Ago proteins to mRNA mimics the miRNA-mediated repression of protein synthesis. RNA 10(10):1518–1525

Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N et al (2005) Inhibition of translational initiation by *Let-7* MicroRNA in human cells. Science 309(5740):1573–1576

Rehwinkel J, Behm-Ansmant I, Gatfield D, Izaurralde E (2005) A crucial role for GW182 and the DCP1:DCP2 decapping complex in miRNA-mediated gene silencing. RNA 11(11):1640–1647

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC et al (2000) The 21-nucleotide *let-7* RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 403(6772):901–906

Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A (2004) Identification of mammalian microRNA host genes and transcription units. Genome Res 14(10A):1902–1910

Schaefer A, O'Carroll D, Tan CL, Hillman D, Sugimori M et al (2007) Cerebellar neurodegeneration in the absence of microRNAs. J Exp Med 204(7):1553–1558

Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME et al (2006) A brain-specific microRNA regulates dendritic spine development. Nature 439(7074):283–289

Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E et al (2004) Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol 5(3):R13

Sethupathy P, Megraw M, Hatzigeorgiou AG (2006) A guide through present computational approaches for the identification of mammalian microRNA targets. Nat Methods 3:881–886

Smirnova L, Grafe A, Seiler A, Schumacher S, Nitsch R et al (2005) Regulation of miRNA expression during neural cell specification. Eur J Neurosci 21(6):1469–1477

Sutton MA, Schuman EM (2005) Local translational control in dendrites and its role in long-term synaptic plasticity. J Neurobiol 64:116

Thermann R, Hentze MW (2007) Drosophila miR2 induces pseudo-polysomes and inhibits translation initiation. Nature 447(7146): 875–878

Vanderklish PW, Edelman GM (2005) Differential translation and fragile × syndrome. Genes Brain Behav 4:360

Vasudevan S, Steitz JA (2007) AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. Cell 128(6):1105–1118

Vasudevan S, Tong Y, Steitz JA (2007) Switching from repression to activation: microRNAs can up-regulate translation. Science 318(5858):1931–1934

Visvanathan J, Lee S, Lee B, Lee JW, Lee SK (2007) The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. Genes Dev 21(7):744–749

Vo N, Klein ME, Varlamova O, Keller DM, Yamamoto T et al (2005) A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. Proc Natl Acad Sci USA 102(45):16426–16431

Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A et al (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 103(7):2257–2261

Wang WX, Rajeev BW, Stromberg AJ, Ren N, Tang G et al (2008) The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. J Neurosci 28(5):1213–1223

Wheeler G, Ntounia-Fousara S, Granda B, Rathjen T, Dalmay T (2006) Identification of new central nervous system specific mouse microRNAs. FEBS Lett 580(9):2195–2000

Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E et al (2005) MicroRNA expression in zebrafish embryonic development. Science 309(5732):310–311

Wu J, Xie X (2006) Comparative sequence analysis reveals an intricate network among REST, CREB and miRNA in mediating neuronal gene expression. Genome Biol 7(9):R85

Wu L, Belasco JG (2005) Micro-RNA regulation of the mammalian *lin-28* gene during neuronal differentiation of embryonal carcinoma cells. Mol Cell Biol (21):9198–9208

Yekta S, Shih IH, Bartel DP (2004) MicroRNA-directed cleavage of HOXB8 mRNA. Science 304(5670):594–596

Yoon S, De Micheli G (2006) Computational identification of microRNAs and their targets. Birth Defects Res C Embryo Today 78:118–128