microRNAs: tiny regulators of
synapse function in development and disease

Silvia Bicker, Gerhard Schratt *

Interdisziplinäres Zentrum für Neurowissenschaften, SFB488 Junior Group, Universität Heidelberg, and Institut für Neuroanatomie, Universitätsklinikum Heidelberg, Heidelberg, Germany

Abstract

The development and function of neuronal circuits within the brain are orchestrated by sophisticated gene regulatory mechanisms. Recently, microRNAs have emerged as a novel class of small RNAs that fine-tune protein synthesis. microRNAs are abundantly expressed in the vertebrate nervous system, where they contribute to the specification of neuronal cell identity. Moreover, microRNAs also play an important role in mature neurons. This review summarizes the current knowledge about the function of microRNAs in the nervous system with special emphasis on synapse formation and plasticity. The second part of this work will discuss the potential involvement of microRNAs in neurologic diseases. The study of brain microRNAs promises to expand our understanding of the mechanisms underlying higher cognitive functions and neurologic diseases.

Keywords: microRNA • nervous system • neuron • synapse • plasticity • dendritic spine • neurologic disease

Introduction

Long-lasting changes in synaptic strength, which underlie higher cognitive functions such as learning and memory, require ongoing protein synthesis. There is evidence that in neurons protein synthesis occurs not solely in the soma, but also locally in dendrites and axons, contributing to synapse formation and plasticity. This so-called local protein synthesis requires a precise regulation. microRNAs (miRNAs) are an extensive class of small non-coding RNAs that can regulate gene expression at the level of translation

by binding to target mRNAs. In the nervous system, miRNAs have been discussed to be implicated in the specification of neuronal cell identity and in the formation and refinement of synapses during development and synaptic plasticity in the adult animal. However, miRNA research in the nervous system is still in its infancy, and detailed mechanisms are just emerging. In this review, we briefly summarize the biogenesis and general function of miRNAs. Then, we will provide an overview of the current

---

*Correspondence to: Dr. Gerhard SCHRATT,
Interdisziplinäres Zentrum für Neurowissenschaften,
SFB488 Junior Group, Universität Heidelberg, and Institut für
Neuroanatomie, Universitätsklinikum Heidelberg,

Im Neuenheimer Feld 345, 69120 Heidelberg, Germany.
Tel.: +49-6221-566210
Fax: +49-6221-545604
E-mail: schratt@ana.uni-heidelberg.de

knowledge about the action of miRNAs in the nervous system, with a special emphasis on their role in synaptic plasticity and neurological disease.

miRNA biogenesis and mode of action

miRNAs are endogenous small non-coding RNAs of about 18–24 nucleotides (nt) that are not translated into proteins but function as regulatory molecules. miRNAs bind to the 3'-untranslated region (3'-UTR) of their target mRNAs, thereby modulating gene expression at the post-transcriptional level [1, 2]. Whereas extensive complementarity to the so-called seed region (nucleotides 2–8 of the miRNA) is essential for the recognition of target mRNAs by miRNAs, the 3'-end of the miRNA appears to be less important and may contain mismatches. Due to their mode of action, miRNAs are in a good position to regulate gene expression in a tight spatial and temporal manner. In support of this, recent evidence indicates that miRNAs are involved in a variety of cellular processes, including differentiation, metabolism and apoptosis, as well as cardiogenesis and myogenesis [3–7].

Most of the miRNAs are transcribed by an RNA polymerase II-dependent mechanism [8], but there are also reports about RNA polymerase III transcribed miRNA genes [9]. Initially, miRNA genes are expressed as long primary transcripts (pri-miRNAs) of several hundred to several thousand nucleotides that contain internal hairpin structures. Within the nucleus, the hairpin structures are cleaved by the type-III RNase Drosha into ~70 nt stem-loop precursor miRNAs (pre-miRNAs), that display a characteristic 2 nt overhang at the 3' end [10–12]. This structure is exported to the cytoplasm, where pre-miRNAs are further processed by the type-III RNase Dicer [13]. The product of this cleavage is a ~21 nt double-stranded miRNA duplex consisting of the functional miRNA and the complementary miRNA* species. The stepwise processing in different cellular compartments allows a tight spatial and temporal regulation of miRNA expression [14].

After Dicer cleavage, the miRNA:miRNA* duplex is unwound by a helicase, and usually only one strand of the duplex enters a multi-protein effector complex termed miRNA-containing RNA induced silencing complex (miRISC) [15], whereas the passenger strand is degraded [16–18]. Once guided to a particular mRNA by the associated miRNA, RISC restricts the expression of the corresponding protein either by translational inhibition or by cleavage of the target transcript [19]. A high degree of sequence complementarity facilitates mRNA cleavage, whereas imperfect base pairing favours translational repression [1, 2]. While cleavage of the target transcript is the prevailing mechanism in plants [20, 21], animal miRNAs silence gene expression primarily by blocking mRNA translation [2]. Interestingly, miRNA-mediated translational repression has also been shown to be reversible [22, 23]. Furthermore, a recent study suggests that miRNAs can even up-regulate translation in cell culture under certain conditions, *i.e.* growth factor deprivation [24]. We refer to a number of excellent reviews for a more detailed description of miRNA biogenesis and regulation [1, 25, 26].

Regulation of miRNA expression

Non-coding RNAs are abundant in the transcriptome of eukaryotes and also highly regulated in response to environmental cues [27]. Although most human miRNAs are genomically separated, some are located in clusters that are often transcribed coordinately as one unit [28]. Non-clustered miRNAs are encoded either within exons or introns of non-coding RNAs or within introns and exonic(intronic locations of protein-coding genes [29]. The expression of the latter is coordinated with the tissue-specific expression of the host gene [30]. Some intronic miRNAs are found in antisense orientation and are transcribed by their own promoter.

Most miRNAs display a tissue- and/or developmental-specific expression pattern, which has been largely attributed to transcriptional regulation mediated by gene-specific transcription factors. Recent studies revealed that miRNAs and transcription factors can act as regulators of each other [31, 32]. Remarkably, miRNA expression has recently been shown to be regulated also at the post-transcriptional level [33]. In this study, the authors investigated the processing and distribution of miR-138. They found that pre-miR-138 is ubiquitously expressed, whereas the expression of mature miR-138 is restricted to specific brain regions and the foetal liver. This indicates that miR-138 processing is spatially as well as temporally controlled. The authors proposed that pre-miRNA cleavage by Dicer is blocked by a tissue-specific inhibitor, presumably representing a new form of regulating miRNA expression at the level of precursor processing. Furthermore, primary transcripts of let-7 family miRNAs undergo regulated processing during early embryogenesis. Interestingly, several primary tumours are related to deregulation of miRNA processing at the level of Drosha cleavage [34].

miRNAs as regulators in the developing nervous system

Considering the vast complexity of the vertebrate central nervous system (CNS), it comes as no surprise that the underlying mechanisms that coordinate its development and mature functions have to be tightly regulated. Due to their ability to regulate gene expression in a very specific way, miRNAs are high-ranking candidates to fulfil this task. By cloning and expression profiling approaches, numerous miRNAs have been identified in the brain, some of which play important roles at different stages of development [35, 36]. A study using Dicer mutant zebrafish showed that disruption of miRNA maturation leads to severe defects in the

morphogenesis of the brain and other organs [37]. Interestingly, re-introducing miR-430 resulted in rescue of most of the observed defects in brain development. An important role of miRNAs in neural differentiation in mammals was first shown in cell culture [38]. Certain miRNAs including miR-124a and miR-9 were shown to be induced during neurogenesis *in vitro*. Furthermore, overexpression of miR-124a and miR-9 in neural precursors reduced the number of astrocytes, whereas inhibition of miR-9 expression alone or in combination with miR-124a caused a reduction of the number of neurons. *In vivo*, miR-124 was also found to be important for maintaining the neuronal phenotype in the chicken spinal cord [39]. Since miR-124 prevents inappropriate expression of non-neuronal genes, it represents an antagonist of the neuron-restrictive silencing factor/RE-1 silencing transcription factor (NRSF/REST) pathway, which represses neuronal genes in non-neuronal cells [40]. During the transition from neural progenitors to post-mitotic neurons, down-regulation of REST results in increased mir-124 expression, thereby causing degradation of non-neuronal transcripts. Targets of miR-124 include the phosphatase SCP1, a known anti-neural factor, and PTBP1, a global repressor of neuron-specific alternative splicing in non-neuronal cells [40, 41]. By suppressing the expression levels of these anti-neural factors, mir-124 contributes to the induction and maintenance of neuronal identity.

Synapse development and plasticity in vertebrates

In the CNS, billions of neurons form a highly organized network of connections for information exchange. The functional units of these networks are synapses, which represent specialized points of contact between neurons. The precise spatial and temporal formation of synapses between neurons is crucial for the correct function of the CNS. Synapse development is divided into two major phases: synapse formation and synapse maturation. Synapse formation involves axon guidance to a partner neuron, followed by the initial contact between the pre-synaptic and the post-synaptic cell. In the case of excitatory synapses, this contact mainly occurs on dendritic spines, which are specialized protrusions from the dendritic shaft. Importantly, synapse formation *per se* appears to be independent from neuronal activity. Synapse maturation, on the other hand, involves morphological and molecular remodelling of the pre- and post-synaptic specializations. In contrast to the first phase, synapse maturation is highly influenced by neuronal activity.

In the mature CNS, modifications of individual synapses are assumed to be the molecular basis of learning and memory. To stabilize a nascent memory, modification of synapses is necessary. Important synaptic connections are strengthened and new ones are formed, whereas unimportant ones are weakened and eventually lost. For example, high-frequency stimulation can result in a long-lasting enhancement of activated synapses in excitatory pathways of the hippocampus, a phenomenon known as long-term potentiation (LTP). LTP represents a form of synaptic plasticity important for the formation of long-term memory. Local protein synthesis at the synapse could contribute in an important way to synaptic plasticity, since it might ensure that only activated synapses are strengthened while other synapses of the same neuron are not modified.

miRNAs as regulators in post-mitotic neurons

A flurry of recent studies have highlighted the importance of the miRNA pathway in various brain regions using inactivation of the Dicer gene. In the cerebellum, loss of the miRNA producing enzyme Dicer resulted in slow Purkinje cell degeneration [42]. A second study revealed that inactivation of Dicer leads to progressive functional and structural degeneration in the retina [43]. Furthermore, Dicer loss in the striatum has also been reported to cause slow neurodegeneration [32]. Interestingly, late inactivation of Dicer in the striatum does not result in neurodegeneration, but nevertheless also leads to behavioural and neuroanatomical phenotypes [44]. Finally, loss of Dicer function in the cortex and hippocampus disrupts cellular and tissue morphogenesis [45]. Together, these results illustrate the significance of Dicer and miRNAs in the CNS and indicate that misregulations of the miRNA biogenesis pathway might contribute to a variety of neurological disorders.

The function of individual miRNAs in post-mitotic neurons is just beginning to emerge. Interestingly, miRNAs are abundantly expressed in the adult brain where they might play a role in synaptic plasticity [35, 46, 47]. One of these miRNAs, miR-132, was identified in a screen for target genes of the activity-dependent transcription factor cAMP response element binding protein (CREB) [48]. CREB-dependent expression is important for neuronal morphogenesis, plasticity and survival. Intriguingly, the CREB target miR-132 was shown to be necessary for neuritogenesis, the preliminary stage of process outgrowth that precedes synapse formation. Since CREB-dependent transcription is required for neurite outgrowth [49], some of the effects exerted by CREB might be actually mediated by miRNAs. Neuritogenesis is negatively regulated by p250GAP, a GTPase activating protein and the translation of p250GAP in turn is inhibited by miR-132. In summary, neuronal activity and neurotrophins induce miR-132 expression by a CREB-dependent mechanism. Subsequently, miR-132 down-regulates p250GAP and thereby facilitates neurite outgrowth. This regulation represents a possible mechanism for activity-dependent miRNA-mediated translational repression in neurons.

In vivo, the CREB-dependent regulation of miR-132 expression has been shown to be important for the circadian clock [50]. The

circadian rhythm provides an internal timekeeping mechanism in form of a cyclic 24-hr world. It is controlled by the suprachiasmatic nucleus (SCN) [51] and can be altered by light stimulation. Expression of miR-132 is induced by photic entrainment in a CREB-dependent fashion and attenuates the entrainment effects of light [50]. Another brain-specific miRNA, miR-219, modulates the length of the circadian period. Expression of miR-219 is regulated by the clock genes CLOCK and BMAL1, transcription factors involved in the circadian rhythm. *In vivo* knockdown of miR-219 results in a prolonged circadian period. However, the target genes mediating the effects of miR-219 on clock timing and miR-132 on clock entrainment have not been identified so far.

In a recent study, the muscle-specific miR-1 was shown to refine pre- and post-synaptic functions at neuromuscular junctions in *C. elegans* [52]. At neuromuscular junctions a retrograde signal from the muscle inhibits neurotransmitter release from the motor neuron. This signal is induced by the activation of nicotinic acetylcholine receptors (nAChRs) by the transcription factor MEF-2 in the muscle. Interestingly, miR-1 regulates the expression of MEF-2, resulting in an altered presynaptic ACh secretion. In addition, miR-1 also influences the expression level of two nAChR subunits, thereby altering muscle sensitivity to ACh. Consequently, miR-1 seems to couple changes in muscle activity to changes in pre-synaptic function, thereby adjusting the intensity of retrograde signalling.

The modification of synaptic strength and structure is a basic mechanism for encoding and storing memory [53]. Since long-lasting forms of memory require protein synthesis, miRNAs have been hypothesized to be involved in the regulation of synaptic function during memory formation [54–56]. So far, only two reports have been published suggesting that miRNAs and the RISC pathway are involved in the regulation of synaptic strength in the CNS. In the first study, miRNAs were revealed to be essential regulatory components of memory storage in *Drosophila* [57]. The authors discovered that the RISC pathway component Armitage is degraded at particular synapses upon induction of a long-term memory. This proteasome-mediated degradation of Armitage leads to an increase in CaMKII mRNA transport and local translation at the post-synaptic site. Hence, a stable memory is accompanied by synaptic protein synthesis, which is under degradative control of the RISC pathway. However, this study did not demonstrate a direct involvement of specific miRNAs in local mRNA translation and long-term memory formation.

Almost at the same time, a brain-specific miRNA, miR-134, that regulates the size of dendritic spines was identified [23]. miR-134 is enriched in the synaptodendritic compartment of rat hippocampal neurons and binds to the dendritic Limk1 mRNA, thereby controlling its translation. Limk1 is a protein kinase and affects dendritic spine morphology by regulating actin filament dynamics. Interestingly, the miR-134-mediated inhibition of Limk1 translation is relieved upon exposure of neurons to brain-derived neurotrophic factor (BDNF), a peptide that is released during synaptic stimulation. Subsequently, Limk1 is synthesized and promotes dendritic spine growth (Fig. 1). A hallmark of this miRNA-mediated regulation is its reversibility. Depending on the activity status of a given synapse, the regulation can switch between translational inhibition and activation.

miRNA transport to the dendrite

A prerequisite for a functional role of miRNAs in synaptodendritic domains is the targeted delivery along the dendrite. However, the targeting and transport of dendritically localized (pre-)miRNAs and the mechanisms underlying these phenomena are completely unknown at present. Currently, three models are conceivable for the transport of miRNAs to the synapse. First, the miRNA might bind to its target mRNA (selected from a large mRNA pool) in the cell body before transport to the synapse [47]. In this case, dendritic targeting elements within the mRNA are assumed to be responsible for the co-delivery of miRNA and mRNA to the dendrite. Second, the miRNA could travel independently of the target mRNA in form of the pre-miRNA before being locally processed in the dendrite [47]. This transport would be based on *cis*-acting targeting sequences within the pre-miRNA. Thus, the mature miRNA would encounter only a limited target field consisting of the dendritic mRNAs. However, one might argue that this mechanism requires the presence of the pre-miRNA processing machinery in the dendrites. Intriguingly, it has been shown that an enzymatically inactive form of Dicer is enriched at post-synaptic densities in mouse neurons. Upon neuronal stimulation, the cryptic form of Dicer is cleaved and thereby presumably activated by a calcium-calpain-dependent mechanism [55]. Third, the transport sequences could also reside within the mature sequence itself. Recently, it was demonstrated that specific miRNAs contain small targeting elements that control their subcellular localization. For example, a hexanucleotide terminal motif acts as a nuclear localization element in case of miR-29b [58]. Thus, one might hypothesize a similar mechanism for the localization of mature dendritic miRNAs. It is likely that the sequence of the miRNA or of its precursor will determine which mechanism is actually used for subcellular localization.

miRNAs in diseases of the nervous system

Many forms of mental disorders are characterized by alterations in neuronal morphology. Given that miRNAs regulate neuronal morphology at multiple levels, it can be hypothesized that aberrant miRNA function might contribute to such diseases (Fig. 1). In the final part of this review, we will present several examples of neurologic diseases where preliminary data point to a link to the miRNA pathway.
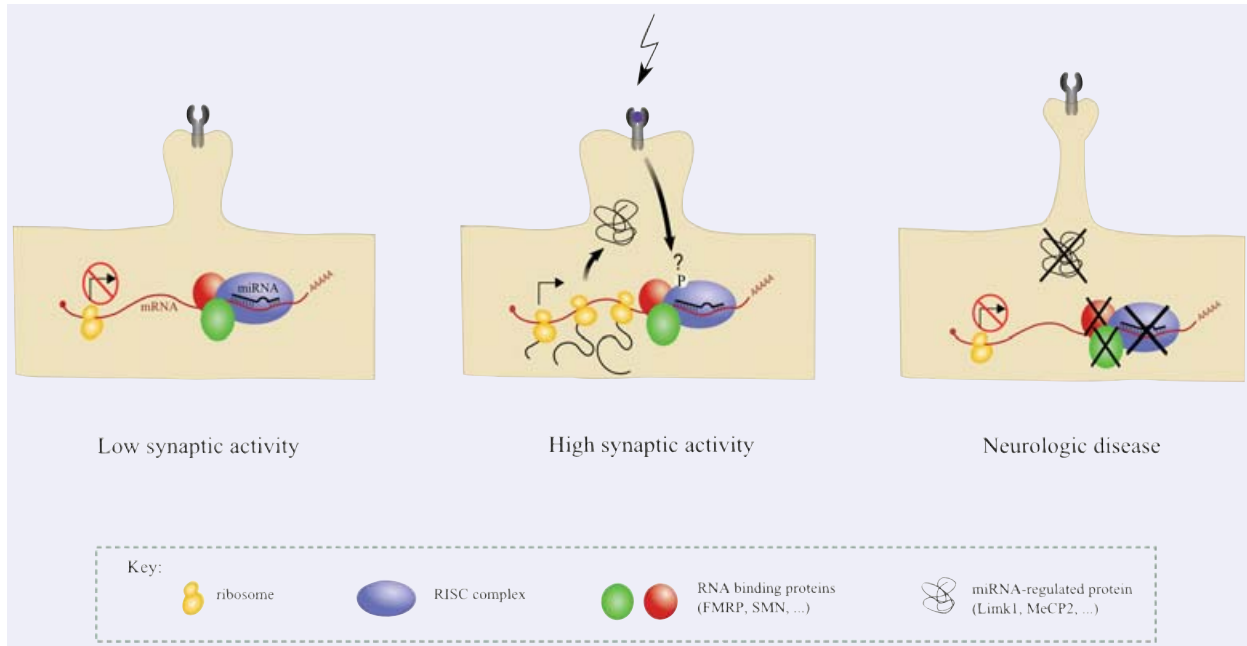
Fig. 1 Working model for the role of miRNAs in local dendritic protein synthesis and spine morphology in CNS physiology and disease. Left: Dendritic miRNA recruits the RISC to the dendritic target mRNA, thereby inhibiting protein synthesis and restricting dendritic spine growth under conditions of low synaptic activity. Middle: Synaptic activation triggers release of neurotransmitters and neurotrophic factors, whose binding to the respective receptors leads to an activation of dendritic mRNA translation, presumably *via* post-translational modification (*e.g.* phosphorylation) and subsequent inactivation of inhibitory RISC components. Enhanced synthesis of miRNA-regulated proteins results in dendritic spine growth. Right: In neurologic diseases, binding of the miRNA and(or proteins (*e.g.* FMRP, SMN,...) to the dendritic target mRNA might be impaired, leading to an aberrant regulation of dendritic mRNA translation. The resulting deregulated expression of disease-related proteins (*e.g.* Limk1, MeCP2,...) might contribute to the pathological spine phenotype observed in certain CNS disorders.

Tourette's syndrome

One of the first examples for an implication of miRNAs in human brain disorders is Tourette's syndrome (TS), a neuropsychiatric disorder characterized by persistent vocal and motor tics [59]. Slit and Trk-like1 (SLITRK1), a protein important for growth, guidance and branching of neuronal processes, has been discussed as candidate gene for TS. Interestingly, the 3'-UTR of the SLITRK1 mRNA contains a binding site for miR-189. Genetic association analysis revealed a correlation of mutations within the miR-189 binding site in the SLITRK1 gene and the aetiology of the disease [60]. Among a population of unrelated patients suffering from TS, two individuals carried a mutation in the miRNA-binding region, whereas this genetic variation was not found in the control group. The mutation leads to the replacement of one GU wobble base pairing with an AU base pairing, resulting in a stronger interaction of the miRNA with the target mRNA. Since TS is a complex disease, this polymorphism probably represents only one out of several mutations that contribute to the phenotype. Nevertheless, it illustrates the physiological and pathological relevance of miRNA-target mRNA interactions.

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is a heritable neuromuscular disease with an incidence of 1:6000–10,000 and represents the leading genetic cause of death in childhood [61]. SMA affects primarily motoneurons of the anterior horn cells of the spinal cord, thereby leading to progressive muscle paralysis and atrophy [62]. Defects in RNA metabolism seem to underlie the SMA phenotype. The most common form of SMA is caused by mutations in the RNA-binding protein survival of motor neuron 1 (SMN1). SMN1 is a rather ubiquitously expressed protein and found both in the nucleus and the cytoplasm. It is part of a multi-protein complex and plays a role in various cell functions. The SMN complex is implicated in the assembly and transport of different classes of ribonucleoproteins (RNP), including small nucleolar RNPs, telomerase RNPs as well as transcription and pre-mRNA splicing machineries [63]. Interestingly, components of the SMN complex are also found in miRNA containing RNPs [64]. Therefore, it is conceivable that miRNAs are involved in splicing, trafficking or translation of cargo mRNAs associated with the SMN complex. Future studies are necessary

to assess the relevance of miRNAs in the context of SMN-dependent activities.

Williams syndrome

Interestingly, the miR-134 target Limk1 is one of the candidate genes for Williams Syndrome (WS), providing another putative link between miRNA function and neurological disease. Prevalence estimations for WS account to approximately 1:7500 [65]. WS is a genetic disorder characterized by a visuospatial constructive cognitive deficit, among other impairments [66]. The deleted genomic region in WS is large and comprises several genes. The identity of the gene that is actually responsible for the neurological symptoms of WS is still controversial [67]. Intriguingly, the cellular and behavioural phenotypes observed in Limk1 mutant mice are not dissimilar from those of WS patients [68]. Hence, Limk1 represents one of the primary candidate genes identified so far and it is tempting to speculate that miR-134-dependent Limk1 regulation might also be involved in the disease. Answers could be obtained in the near future, once brain-specific miR-134 knockout mice have been generated.

Rett syndrome

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder occurring primarily in females, with an incidence of 1:10,000–15,000. This disorder is characterized by developmental stagnation, stereotypical movements, microcephaly, seizures and mental retardation. RTT is caused by mutations in the gene encoding methyl-CpG binding protein 2 (MeCP2) [69]. Both loss of function and gain in MeCP2 dosage result in similar neuropsychiatric phenotypes [70]. Recently, MeCP2 translation has been shown to be under the control of the brain-enriched miRNA miR-132 [71]. The authors assume that miR-132 binding to the 3'-UTR of the MeCP2 mRNA prevents from deleteriously high MeCP2 levels during development. Consequently, one might hypothesize that a deregulation of MeCP2 translation resulting from aberrant miRNA function contributes to RTT.

Schizophrenia

First indications have emerged that miRNAs might be involved in the aetiology of schizophrenia. Interestingly, altered miRNA levels have been detected in the prefrontal cortex of post-mortem brains from patients with schizophrenia compared to healthy individuals [72]. Moreover, an SNP-based genetic analysis identified an association of two miRNA loci with schizophrenia [73]. Although further validation of these correlations is necessary, they suggest a connection between miRNAs and schizophrenia.

Strong support for a link between schizophrenia and miRNAs is provided by a recent study [74]. The authors hypothesize that abnormal processing of miRNAs might contribute to the behavioural and neuronal deficits caused by the human 22q11.2 microdeletion, which is associated with a highly increased risk of developing schizophrenia. The 22q11.2 locus includes the gene encoding Dgcr8, which interacts with Drosha, thereby mediating the cleavage of pri-miRNAs to pre-miRNAs. $Dgcr8^{+/-}$ mice display an impaired miRNA processing resulting in smaller dendritic spines and reduced dendritic complexity [74]. In addition, the authors showed that the abnormal miRNA biogenesis also affects cognitive and behavioural performance. Based on these findings, it is tempting to speculate that miRNA-dependent regulation of dendritic morphology might contribute to psychiatric disorders such as schizophrenia.

Neurodegenerative diseases

Neurodegenerative disease (ND) is a term referring to a neurological disorder marked by an abundant loss of neurons, such as Alzheimer's, Huntington's and Parkinson's disease. NDs are caused by a combination of environmental and genetic factors. miRNAs are assumed to contribute to the aetiology of NDs, since they seem to be essential for the survival of mature neurons.

Parkinson's disease

The main characteristics of Parkinson's disease (PD) are severe mobility problems, tremor and speech impairments. At the cellular level, PD is caused by a gradual death of dopaminergic neurons in the substantia nigra. Interestingly, genetic inactivation of Dicer in specific dopaminergic neurons in mice leads to a gradual loss of neurons, causing a phenotype resembling that of PD [32]. The same phenotype was also observed *in vitro* and a partial rescue could be achieved be re-introducing a small RNA population including miRNAs purified from wild-type cells. This finding indicates that the absence of small RNAs (most likely miRNAs) caused the phenotype, and not the lack of other Dicer-related functions. An expression screen using brains from PD patients and healthy control subjects showed changes in the abundance of several miRNAs, such as miR-133b. In healthy subjects, miR-133b was enriched in the midbrain, whereas miR-133b levels were diminished in patients suffering from PD. Functionally, miR-133b was shown to be implicated in the differentiation of dopamine neurons. In cell culture, overexpression and inhibition of miR-133b resulted in a decrease and increase in the number of dopaminergic neurons, respectively. These results await further validation, however they strongly suggest that miR-133b represents a negative regulator of dopaminergic neuron maturation.

Alzheimer's disease

Alzheimer's disease (AD) is a neurologic disease characterized by loss of mental ability and it represents the most common form of dementia. A recent study analysed the correlation between miRNA expression levels and the pathology of AD [75]. It was shown that the brains of patients with different stages of AD have significantly

decreased miR-107 levels compared to those of healthy control subjects. Interestingly, the 3'-UTR of beta-site amyloid precursor protein-cleaving enzyme 1 (BACE1) has been predicted to carry multiple binding sites for miR-107. BACE1 is an endopeptidase that cleaves the β-amyloid precursor protein to generate a neurotoxic peptide [76]. Deregulated BACE1 levels have been discussed to play a role in the pathogenesis of AD and therefore BACE1 is a prime therapeutic target for AD treatment [77]. It was shown that BACE1 mRNA levels increased with decreasing miR-107 levels in the brains of AD patients [75]. Hence, one might conclude an implication of miR-107 in AD progression through regulation of BACE1.

A previous study investigated the expression of 12 miRNAs in AD hippocampus compared to samples from healthy controls and foetal brain [78]. miR-9, miR-124a, miR-125b and miR-128 were found to be altered in abundance in foetal, adult and Alzheimer hippocampus. Differential expression of miR-125b might be relevant, since the mRNAs of synapsin I and synapsin II are predicted to be miR-125b targets. Consequently, one might hypothesize that miR-125b up-regulation could lead to synapsin down-regulation, thereby contributing to the development of AD.

Triplet repeat expansion disorders

Triplet repeat expansion disorders are a subset of genetic disorders caused by an abnormally increased number of trinucleotide repeats (*e.g.* glutamine repeats). The pathogenic expansion leads to protein accumulation and progressive neuronal loss. A link between miRNAs and polyglutamine-induced neurodegeneration has recently been discovered for Fragile X syndrome and Spinocerebellar ataxia type 3.

Fragile X mental retardation syndrome

The role of the miRNA pathway in Fragile X syndrome (FXS) has been increasingly recognized within the last years. FXS is the most common form of inherited mental retardation, affecting 1:2000 males and about half as many females [79]. It is characterized by cognitive and behavioural impairments and caused by mutations in the gene encoding fragile X mental retardation protein (FMRP) [80]. Most of the patients display a trinucleotide (CGG) repeat expansion of >200 repeats in the 5'-UTR, causing hypermethylation and gene silencing [81]. FMRP is an RNA-binding protein and part of the RISC multiprotein complex [82–84]. In neurons, FMRP and other RISC components are found in the somatodendritic compartment. FMRP regulates the translation of dendritic mRNAs and is proposed to be involved in the local regulation of protein synthesis at synapses [85]. Therefore, FMRP could exert its effect on translation, at least in part, by recruiting miRNAs and the associated RISC complex to the respective target mRNAs. Loss of function of FMRP impairs the translation of synaptic proteins, resulting in an aberrant density and morphology of dendritic spines in mouse models and FXS patients [86]. Intriguingly, these alterations in spine morphology are similar to those observed upon miR-134 overexpression or Limk1 deficiency [87]. This finding suggests a connection between FXS and perturbed translation of miRNA targets, such as Limk1 (Fig. 1).

Spinocerebellar ataxia type 3

Spinocerebellar ataxia type 3 (SCA3) is a human polyglutamine disease caused by mutations in the gene encoding Ataxin-3, resulting in progressive dysfunction of the cerebellum [88]. In flies, expression of pathogenic Ataxin-3 leads to a partial loss of neurons [89]. Intriguingly, this SCA3-induced neurodegeneration was strongly enhanced when miRNA production was abolished due to a Dicer loss-of-function allele. A genetic suppressor screen subsequently identified the miRNA bantam as suppressor of SCA3 toxicity. An RNAi-mediated knockdown of Dicer in HeLa cells also increased the SCA3-induced toxicity, suggesting that the results from the fly model might also be relevant for human pathology.

Cancer in the CNS

Several findings indicate a role of the miRNA pathway in the development of different forms of cancers [90–92]. Interestingly, it was found that miRNA expression patterns of cancer cells represent a unique molecular fingerprint, which might be useful for the diagnosis or even prognosis of certain malignancies [93, 94]. In the context of the CNS, microarray analyses identified a set of miRNAs whose expression correlates with the appearance of a glioblastoma phenotype [95]. More specifically, overexpression of miR-21 was observed in human glioblastoma tumour tissues and several glioblastoma cell lines [96]. Interestingly, miR-21 seems to play a critical role in various solid tumours [97] and it was shown to regulate the expression of several tumour suppressor genes. Inhibition of miR-21 in a glioblastoma cell line using antisense oligonucleotides resulted in increased apoptosis, suggesting that miR-21 might block the expression of apoptosis-related genes and thereby promote transformation [96]. Anyhow, these results suggest that miRNAs such as miR-21 might represent attractive drug targets for the treatment of CNS cancers.

Genomic imprinting

Genomic imprinting is defined as the mechanism whereby a certain genetic region on one parental chromosome is expressed differently from the same locus on the other chromosome. Intriguingly, deregulation of imprinted genes was reported to be associated with nervous system defects, such as those observed in Prader-Willi and Angelman syndromes [98]. A non-neuronal example for an association between altered imprinting and a disease is the Callipyge phenotype, a form of muscular hypertrophy. Deregulated gene expression at the Dlk1-Gtl2 domain seems to be the cause for this disorder [99]. Remarkably, a large miRNA cluster encompassing miR-134 is located nearby and it was demonstrated that miRNAs from this cluster are imprinted as well [100]. Since small non-coding RNAs can mediate gene silencing, for example through DNA methylation, the nearby located miRNAs might help to establish the imprinting status of the Dlk1-Gtl2 locus. Whether deregulated expression of this domain has also consequences for neuronal development has not been clarified so far.

Table 1 Hypothetic link of miRNAs to neurologic diseases

Neurologic disease	MiRNA	Reference
Tourette's syndrome	miR-189	[60]
Williams syndrome	miR-134	[68]
Rett syndrome	miR-132	[71]
Parkinson's disease	miR-133b	[32]
Alzheimer's disease	miR-107; miR-9, miR-124a, miR-125b, miR-128	[75, 78]

Diagnostic and therapeutic perspectives

As discussed, multiple lines of evidence point to a widespread involvement of miRNAs in various neurological diseases (Table 1). Current and future large-scale expression studies of miRNAs in CNS diseases promise to identify additional miRNAs that are relevant for these pathologies. In addition, the mapping and closer analysis of genetic loci linked to neurologic disorders might yield a deeper insight into the contribution of miRNAs to the pathogenic phenotype. Especially mutations in the genetic regions encoding miRNAs or in the miRNA binding sites in the 3'-UTR of the target mRNA could contribute to neurologic disorders. Interfering with miRNA function might represent a promising therapeutical approach to restore physiological levels of critical miRNA targets. There are different tools available to block endogenous miRNAs or deliver exogenous miRNAs. *In vivo* silencing of miRNAs can be achieved by application of antisense oligonucleotides complementary to the miRNA sequence, such as LNA-antimiRs or 2'-O-methyl-antagomirs. In zebrafish, morpholino antisense oligonucleotides have been successfully used to inhibit miRNA function *in vivo* [101]. In mice, systematic injection of antagomirs in non-neuronal tissues as well as local injection into the cortex yielded promising results [102]. In a recent study, it was demonstrated that LNA-antimiRs can safely and efficiently silence a miRNA in non-human primates [103]. A systemically delivered LNA against the liver-specific miR-122 could readily enter the liver and antagonize the miRNA *in situ*. miR-122 depletion was accompanied by a dose-dependent lowering of plasma cholesterol, with no evidence of adverse toxicity. This result lays the foundation for the development of a novel class of therapeutics capable of silencing disease-related miRNAs. Furthermore, RNAi-mediated knockdown of miRNA precursors also represents a useful approach to inhibit a specific miRNA. However, the presence of the blood-brain barrier might impede the efficient delivery of oligonucleotides to the CNS. Nevertheless, it was shown that transvascular delivery of small interfering RNAs to the brain can be achieved using a rabies virus peptide, which specifically binds to neuronal cells [104]. It is conceivable that a similar modification could allow antimiRs and antagomirs to enter the brain. Recently, 'miRNA sponges' have been added to the repertoire of miRNA inhibitors [105, 106]. A miRNA sponge is a plasmid encoding a transcript that contains multiple binding sites for a given miRNA in its 3'-UTR. Expression of the transcript allows to 'soak up' the miRNA, thereby relieving its endogenous targets from miRNA-dependent expression control. This new tool offers several advantages, such as the possibility to express the inhibitor in conjunction with reporter genes. In addition, the expression of sponges from stably integrated transgenes *in vivo* could expand the scope of antisense miRNA inhibitors and might reveal the function of a specific miRNA on the organismic level.

Conclusion

miRNA research is a rapidly changing new field and the surprising finding that specific miRNAs can up-regulate translation [24] even fuels the ongoing controversies about the different modes of miRNA regulation. This reminds us that our current knowledge of these small RNAs covers, if at all, just the tip of the iceberg. Especially miRNA-mediated translation at the synapse represents a promising new field of research. How do miRNAs affect synaptic plasticity and thereby contribute to higher cognitive functions? How do dendritic miRNAs reach their final destination to regulate local protein synthesis at the synapse? Why do neurons need such a complex method of restricting the translation of particular mRNAs to a certain subcellular compartment? Obviously, this kind of regulation allows a precise response. The mRNA is already located, and when needed, the functional protein can be quickly expressed by a relief of the miRNA-mediated repression upon synaptic activation. Whereas signal transmission from the activated synapse to the nucleus and subsequent protein synthesis and transport along the dendrite is rather time-consuming, synapto-dendritic miRNAs allow a fast and precise regulation directly at the synapse. Examining the mechanisms whereby the expression of dendritic miRNAs is regulated and whereby they are localized to their site of function promises to contribute to a better understanding of activity-dependent control of local protein synthesis at synaptic sites, a process important for synaptic plasticity, learning and memory. It is reasonable to assume that future investigations concerning the function of neuronal miRNAs will expand our knowledge about the complex regulatory networks in the brain and might allow us to invent additional strategies for the treatment of neurologic diseases.

Acknowledgements

References

1. **Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R.** Control of translation and mRNA degradation by miRNAs and siRNAs. *Genes Dev.* 2006; 20: 515–24.
2. **Bartel DP.** MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 2004; 116: 281–97.
3. **Ambros V.** The functions of animal microRNAs. *Nature.* 2004; 431: 350–5.
4. **Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM.** bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. *Cell.* 2003; 113: 25–36.
5. **Chen CZ, Li L, Lodish HF, Bartel DP.** MicroRNAs modulate hematopoietic lineage differentiation. *Science.* 2004; 303: 83–6.
6. **Kloosterman WP, Plasterk RH.** The diverse functions of microRNAs in animal development and disease. *Dev Cell.* 2006; 11: 441–50.
7. **Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, Stoffel M.** A pancreatic islet-specific microRNA regulates insulin secretion. *Nature.* 2004; 432: 226–30.
8. **Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN.** MicroRNA genes are transcribed by RNA polymerase II. *EMBO J.* 2004; 23: 4051–60.
9. **Borchert GM, Lanier W, Davidson BL.** RNA polymerase III transcribes human microRNAs. *Nat Struct Mol Biol.* 2006; 13: 1097–101.
10. **Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ.** Processing of primary microRNAs by the Microprocessor complex. *Nature.* 2004; 432: 231–5.
11. **Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R.** The Microprocessor complex mediates the genesis of microRNAs. *Nature.* 2004; 432: 235–40.
12. **Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN.** The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev.* 2004; 18: 3016–27.
13. **Bernstein E, Caudy AA, Hammond SM, Hannon GJ.** Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature.* 2001; 409: 363–6.
14. **Lee Y, Jeon K, Lee JT, Kim S, Kim VN.** MicroRNA maturation: stepwise processing and subcellular localization. *EMBO J.* 2002; 21: 4663–70.
15. **Kim VN, Nam JW.** Genomics of microRNA. *Trends Genet.* 2006; 22: 165–73.
16. **Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD.** Asymmetry in the assembly of the RNAi enzyme complex. *Cell.* 2003; 115: 199–208.
17. **Du T, Zamore PD.** microPrimer: the biogenesis and function of microRNA. *Development.* 2005; 132: 4645–52.
18. **Khvorova A, Reynolds A, Jayasena SD.** Functional siRNAs and miRNAs exhibit strand bias. *Cell.* 2003; 115: 209–16.
19. **Pillai RS.** MicroRNA function: multiple mechanisms for a tiny RNA? *RNA.* 2005; 11: 1753–61.
20. **Jones-Rhoades MW, Bartel DP, Bartel B.** MicroRNAS and their regulatory roles in plants. *Annu Rev Plant Biol.* 2006; 57: 19–53.
21. **Tang G.** siRNA and miRNA: an insight into RISCs. *Trends Biochem Sci.* 2005; 30: 106–14.
22. **Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W.** Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell.* 2006; 125: 1111–24.
23. **Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, Greenberg ME.** A brain-specific microRNA regulates dendritic spine development. *Nature.* 2006; 439: 283–9.
24. **Vasudevan S, Tong Y, Steitz JA.** Switching from repression to activation: microRNAs can up-regulate translation. *Science.* 2007; 318: 1931–4.
25. **Filipowicz W, Bhattacharyya SN, Sonenberg N.** Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet.* 2008; 9: 102–14.
26. **Nilsen TW.** Mechanisms of microRNA-mediated gene regulation in animal cells. *Trends Genet.* 2007; 23: 243–9.
27. **Cawley S, Bekiranov S, Ng HH, Kapranov P, Sekinger EA, Kampa D, Piccolboni A, Sementchenko V, Cheng J, Williams AJ, Wheeler R, Wong B, Drenkow J, Yamanaka M, Patel S, Brubaker S, Tammana H, Helt G, Struhl K, Gingeras TR.** Unbiased mapping of transcription factor binding sites along human chromosomes 21 and 22 points to widespread regulation of noncoding RNAs. *Cell.* 2004; 116: 499–509.
28. **Zhao Y, Srivastava D.** A developmental view of microRNA function. *Trends Biochem Sci.* 2007; 32: 189–97.
29. **Cullen BR.** Transcription and processing of human microRNA precursors. *Mol Cell.* 2004; 16: 861–5.
30. **Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A.** Identification of mammalian microRNA host genes and transcription units. *Genome Res.* 2004; 14: 1902–10.
31. **Conaco C, Otto S, Han JJ, Mandel G.** Reciprocal actions of REST and a microRNA promote neuronal identity. *Proc Natl Acad Sci USA.* 2006; 103: 2422–7.
32. **Kim J, Inoue K, Ishii J, Vanti WB, Voronov SV, Murchison E, Hannon G, Abeliovich A.** A MicroRNA feedback circuit in midbrain dopamine neurons. *Science.* 2007; 317: 1220–4.
33. **Obernosterer G, Leuschner PJ, Alenius M, Martinez J.** Post-transcriptional regulation of microRNA expression. *RNA.* 2006; 12: 1161–7.
34. **Thomson JM, Newman M, Parker JS, Morin-Kensicki EM, Wright T, Hammond SM.** Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes Dev.* 2006; 20: 2202–7.
35. **Cao X, Yeo G, Muotri AR, Kuwabara T, Gage FH.** Noncoding RNAs in the mammalian central nervous system. *Annu Rev Neurosci.* 2006; 29: 77–103.
36. **Kosik KS, Krichevsky AM.** The elegance of the microRNAs: a neuronal perspective. *Neuron.* 2005; 47: 779–82.
37. **Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, Hammond SM, Bartel DP, Schier AF.** MicroRNAs regulate brain morphogenesis in zebrafish. *Science.* 2005; 308: 833–8.
38. **Krichevsky AM, Sonntag KC, Isacson O, Kosik KS.** Specific microRNAs modulate embryonic stem cell-derived neurogenesis. *Stem Cells.* 2006; 24: 857–64.
39. **Cao X, Pfaff SL, Gage FH.** A functional study of miR-124 in the developing neural tube. *Genes Dev.* 2007; 21: 531–6.
40. **Visvanathan J, Lee S, Lee B, Lee JW, Lee SK.** The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. *Genes Dev.* 2007; 21: 744–9.
41. **Makeyev EV, Zhang J, Carrasco MA, Maniatis T.** The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-

mRNA splicing. *Mol Cell.* 2007; 27: 435–48.

42. **Schaefer A, O'Carroll D, Tan CL, Hillman D, Sugimori M, Llinas R, Greengard P.** Cerebellar neurodegeneration in the absence of microRNAs. *J Exp Med.* 2007; 204: 1553–8.

43. **Damiani D, Alexander JJ, O'Rourke JR, McManus M, Jadhav AP, Cepko CL, Hauswirth WW, Harfe BD, Strettoi E.** Dicer inactivation leads to progressive functional and structural degeneration of the mouse retina. *J Neurosci.* 2008; 28: 4878–87.

44. **Cuellar TL, Davis TH, Nelson PT, Loeb GB, Harfe BD, Ullian E, McManus MT.** Dicer loss in striatal neurons produces behavioral and neuroanatomical phenotypes in the absence of neurodegeneration. *Proc Natl Acad Sci USA.* 2008; 105: 5614–9.

45. **Davis TH, Cuellar TL, Koch SM, Barker AJ, Harfe BD, McManus MT, Ullian EM.** Conditional loss of Dicer disrupts cellular and tissue morphogenesis in the cortex and hippocampus. *J Neurosci.* 2008; 28: 4322–30.

46. **Ashraf SI, Kunes S.** A trace of silence: memory and microRNA at the synapse. *Curr Opin Neurobiol.* 2006; 16: 535–9.

47. **Kosik KS.** The neuronal microRNA system. *Nat Rev Neurosci.* 2006; 7: 911–20.

48. **Vo N, Klein ME, Varlamova O, Keller DM, Yamamoto T, Goodman RH, Impey S.** A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. *Proc Natl Acad Sci USA.* 2005; 102: 16426–31.

49. **Konur S, Ghosh A.** Calcium signaling and the control of dendritic development. *Neuron.* 2005; 46: 401–5.

50. **Cheng HY, Papp JW, Varlamova O, Dziema H, Russell B, Curfman JP, Nakazawa T, Shimizu K, Okamura H, Impey S, Obrietan K.** microRNA modulation of circadian-clock period and entrainment. *Neuron.* 2007; 54: 813–29.

51. **Ralph MR, Foster RG, Davis FC, Menaker M.** Transplanted suprachiasmatic nucleus determines circadian period. *Science.* 1990; 247: 975–8.

52. **Simon DJ, Madison JM, Conery AL, Thompson-Peer KL, Soskis M, Ruvkun GB, Kaplan JM, Kim JK.** The microRNA miR-1 regulates a MEF-2-dependent retrograde signal at neuromuscular junctions. *Cell.* 2008; 133: 903–15.

53. **Bailey CH, Kandel ER, Si K.** The persistence of long-term memory: a molecular approach to self-sustaining changes in

learning-induced synaptic growth. *Neuron.* 2004; 44: 49–57.

54. **Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, Ruvkun G.** Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proc Natl Acad Sci USA.* 2004; 101: 360–5.

55. **Lugli G, Larson J, Martone ME, Jones Y, Smalheiser NR.** Dicer and eIF2c are enriched at postsynaptic densities in adult mouse brain and are modified by neuronal activity in a calpain-dependent manner. *J Neurochem.* 2005; 94: 896–905.

56. **Martin KC, Kosik KS.** Synaptic tagging – who's it? *Nat Rev Neurosci.* 2002; 3: 813–20.

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

58. **Hwang HW, Wentzel EA, Mendell JT.** A hexanucleotide element directs microRNA nuclear import. *Science.* 2007; 315: 97–100.

59. **Robertson MM.** Diagnosing Tourette syndrome: is it a common disorder? *J Psychosom Res.* 2003; 55: 3–6.

60. **Abelson JF, Kwan KY, O'Roak BJ, Baek DY, Stillman AA, Morgan TM, Mathews CA, Pauls DL, Rasin MR, Gunel M, Davis NR, Ercan-Sencicek AG, Guez DH, Spertus JA, Leckman JF, Dure LSt, Kurlan R, Singer HS, Gilbert DL, Farhi A, Louvi A, Lifton RP, Sestan N, State MW.** Sequence variants in SLITRK1 are associated with Tourette's syndrome. *Science.* 2005; 310: 317–20.

61. **Frugier T, Nicole S, Cifuentes-Diaz C, Melki J.** The molecular bases of spinal muscular atrophy. *Curr Opin Genet Dev.* 2002; 12: 294–8.

62. **Monani UR.** Spinal muscular atrophy: a deficiency in a ubiquitous protein; a motor neuron-specific disease. *Neuron.* 2005; 48: 885–96.

63. **Kolb SJ, Battle DJ, Dreyfuss G.** Molecular functions of the SMN complex. *J Child Neurol.* 2007; 22: 990–4.

64. **Dostie J, Mourelatos Z, Yang M, Sharma A, Dreyfuss G.** Numerous microRNPs in neuronal cells containing novel microRNAs. *RNA.* 2003; 9: 180–6.

65. **Stromme P, Bjornstad PG, Ramstad K.** Prevalence estimation of Williams syndrome. *J Child Neurol.* 2002; 17: 269–71.

66. **Frangiskakis JM, Ewart AK, Morris CA, Mervis CB, Bertrand J, Robinson BF, Klein BP, Ensing GJ, Everett LA, Green**

ED, Proschel C, Gutowski NJ, Noble M, Atkinson DL, Odelberg SJ, Keating MT. LIM-kinase1 hemizygosity implicated in impaired visuospatial constructive cognition. *Cell.* 1996; 86: 59–69.

67. **Hoogenraad CC, Akhmanova A, Galjart N, De Zeeuw CI.** LIMK1 and CLIP-115: linking cytoskeletal defects to Williams syndrome. *Bioessays.* 2004; 26: 141–50.

68. **Meng Y, Zhang Y, Tregoubov V, Janus C, Cruz L, Jackson M, Lu WY, MacDonald JF, Wang JY, Falls DL, Jia Z.** Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. *Neuron.* 2002; 35: 121–33.

69. **Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY.** Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet.* 1999; 23: 185–8.

70. **Chahrour M, Zoghbi HY.** The story of Rett syndrome: from clinic to neurobiology. *Neuron.* 2007; 56: 422–37.

71. **Klein ME, Lioy DT, Ma L, Impey S, Mandel G, Goodman RH.** Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. *Nat Neurosci.* 2007; 10: 1513–4.

72. **Perkins DO, Jeffries CD, Jarskog LF, Thomson JM, Woods K, Newman MA, Parker JS, Jin J, Hammond SM.** microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. *Genome Biol.* 2007; 8: R27.

73. **Hansen T, Olsen L, Lindow M, Jakobsen KD, Ullum H, Jonsson E, Andreassen OA, Djurovic S, Melle I, Agartz I, Hall H, Timm S, Wang AG, Werge T.** Brain expressed microRNAs implicated in schizophrenia etiology. *PLoS ONE.* 2007; 2: e873.

74. **Stark KL, Xu B, Bagchi A, Lai WS, Liu H, Hsu R, Wan X, Pavlidis P, Mills AA, Karayiorgou M, Gogos JA.** Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. *Nat Genet.* 2008; 40: 751–60.

75. **Wang WX, Rajeev BW, Stromberg AJ, Ren N, Tang G, Huang Q, Rigoutsos I, Nelson PT.** 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.* 2008; 28: 1213–23.

76. **Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J,**

Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science.* 1999; 286: 735–41.

77. Guo T, Hobbs DW. Development of BACE1 inhibitors for Alzheimer's disease. *Curr Med Chem.* 2006; 13: 1811–29.

78. Lukiw WJ. Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. *Neuroreport.* 2007; 18: 297–300.

79. Brown WT. The FRAXE Syndrome: is it time for routine screening? *Am J Hum Genet.* 1996; 58: 903–5.

80. Pieretti M, Zhang FP, Fu YH, Warren ST, Oostra BA, Caskey CT, Nelson DL. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell.* 1991; 66: 817–22.

81. O'Donnell WT, Warren ST. A decade of molecular studies of fragile X syndrome. *Annu Rev Neurosci.* 2002; 25: 315–38.

82. Caudy AA, Myers M, Hannon GJ, Hammond SM. Fragile X-related protein and VIG associate with the RNA interference machinery. *Genes Dev.* 2002; 16: 2491–6.

83. Jin P, Zarnescu DC, Ceman S, Nakamoto M, Mowrey J, Jongens TA, Nelson DL, Moses K, Warren ST. Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nat Neurosci.* 2004; 7: 113–7.

84. Ishizuka A, Siomi MC, Siomi H. A Drosophila fragile X protein interacts with components of RNAi and ribosomal proteins. *Genes Dev.* 2002; 16: 2497–508.

85. Weiler IJ, Spangler CC, Klintsova AY, Grossman AW, Kim SH, Bertaina-Anglade V, Khaliq H, de Vries FE, Lambers FA, Hatia F, Base CK, Greenough WT. Fragile X mental retardation protein is necessary for neurotransmitter-activated protein translation at synapses. *Proc Natl Acad Sci USA.* 2004; 101: 17504–9.

86. Greenough WT, Klintsova AY, Irwin SA, Galvez R, Bates KE, Weiler IJ. Synaptic regulation of protein synthesis and the

fragile X protein. *Proc Natl Acad Sci USA.* 2001; 98: 7101–6.

87. Bagni C, Greenough WT. From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. *Nat Rev Neurosci.* 2005; 6: 376–87.

88. Klockgether T, Evert B. Genes involved in hereditary ataxias. *Trends Neurosci.* 1998; 21: 413–8.

89. Bilen J, Liu N, Burnett BG, Pittman RN, Bonini NM. MicroRNA pathways modulate polyglutamine-induced neurodegeneration. *Mol Cell.* 2006; 24: 157–63.

90. Hammond SM. MicroRNAs as oncogenes. *Curr Opin Genet Dev.* 2006; 16: 4–9.

91. Garzon R, Fabbri M, Cimmino A, Calin GA, Croce CM. MicroRNA expression and function in cancer. *Trends Mol Med.* 2006; 12: 580–7.

92. Sevignani C, Calin GA, Nnadi SC, Shimizu M, Davuluri RV, Hyslop T, Demant P, Croce CM, Siracusa LD. MicroRNA genes are frequently located near mouse cancer susceptibility loci. *Proc Natl Acad Sci USA.* 2007; 104: 8017–22.

93. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer.* 2006; 6: 857–66.

94. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature.* 2005; 435: 834–8.

95. Ciafre SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, Negrini M, Maira G, Croce CM, Farace MG. Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem Biophys Res Commun.* 2005; 334: 1351–8.

96. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res.* 2005; 65: 6029–33.

97. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A,

Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA.* 2006; 103: 2257–61.

98. Nicholls RD, Knepper JL. Genome organization, function, and imprinting in Prader-Willi and Angelman syndromes. *Annu Rev Genomics Hum Genet.* 2001; 2: 153–75.

99. Lewis A, Redrup L. Genetic imprinting: conflict at the Callipyge locus. *Curr Biol.* 2005; 15: R291–4.

100. Seitz H, Royo H, Bortolin ML, Lin SP, Ferguson-Smith AC, Cavaille J. A large imprinted microRNA gene cluster at the mouse Dlk1-Gtl2 domain. *Genome Res.* 2004; 14: 1741–8.

101. Kloosterman WP, Lagendijk AK, Ketting RF, Moulton JD, Plasterk RH. Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development. *PLoS Biol.* 2007; 5: e203.

102. Krutzfeldt J, Kuwajima S, Braich R, Rajeev KG, Pena J, Tuschl T, Manoharan M, Stoffel M. Specificity, duplex degradation and subcellular localization of antagomirs. *Nucleic Acids Res.* 2007; 35: 2885–92.

103. Elmen J, Lindow M, Schutz S, Lawrence M, Petri A, Obad S, Lindholm M, Hedtjarn M, Hansen HF, Berger U, Gullans S, Kearney P, Sarnow P, Straarup EM, Kauppinen S. LNA-mediated microRNA silencing in non-human primates. *Nature.* 2008; 452: 896–9.

104. Kumar P, Wu H, McBride JL, Jung KE, Kim MH, Davidson BL, Lee SK, Shankar P, Manjunath N. Transvascular delivery of small interfering RNA to the central nervous system. *Nature.* 2007; 448: 39–43.

105. Sharp KH, Eam B, Faulkner DJ, Haygood MG. Vertical transmission of diverse microbes in the tropical sponge Corticium sp. *Appl Environ Microbiol.* 2007; 73: 622–9.

106. Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat Methods.* 2007; 4: 721–6.