Plasticity at the synapse

A role for microRNAs

Anil Ori
Universiteit Utrecht
Biology of Disease
04-07-2011

Table of contents

1. Preface

This thesis is the outcome of a literature study that I conducted as part of my master study Biology of Disease. I performed this literature review on the role of microRNAs in synapctic plasticity and wrote my thesis under the supervision of prof. dr. J.P.H. Burbach.

I chose to study this topic because of my interest in neuropsychiatric disorders and my fascination for synaptic plasticity. As the field of research is currently starting to recognize the potentially vast impact of microRNA-based mechanism on synaptic plasticity, I was eager to anticipate this development. Therefore, I decided to study the literature to understand the biological mechanisms underlying this phenomenon, its role in synaptic plasticity, and the accompanying implications for brain diseases.

Other than to give you an introduction into this project, I want to use this opportunity to express my gratitude to prof. dr. J.P.H. Burbach, who guided me through the entire process of writing my thesis. In the initial phase, Peter helped me to structure my thoughts and construct a scheme that guided me in writing this study. In addition, he provided the necessary tips and pointers on how to clarifying my objectives on paper and how to structure this extensive study. In the final stage of writing, Peter provided comprehensive feedback on my thesis, which enabled me to improve and finish my work. For these reason I would like to say my appreciation and thank Peter for his guidance, support and supervision during this project.

Via this study, I aspire to increase my knowledge on the microRNA pathway and its role at the dendrite in synaptic plasticity. In addition, I hope that my work will contribute, in some way or another, to the current field of research on synaptic plasticity and brain diseases.

2. Abstract

MicroRNAs (miRNAs) are emerging as key modulators of post-transcriptional gene regulation that bind complementary sequences of mRNA molecules and regulate translation and mRNA stability. The potentially vast impact of miRNA based mechanisms on synaptic plasticity and thereby brain diseases, such as neuropsychiatric disorders, is just starting to be recognized. Increased knowledge on the fundamental processes of synaptic function and plasticity will contribute to better understand brain disorders and more importantly, aid in finding proper treatments. Therefore, this literature study has, to the best of knowledge, reviewed all functional studies currently published, that provide evidence for a direct role of miRNAs in synaptic plasticity.

The study focused on two major components of the dendrite, i.e. the postsynaptic density (PSD) and the actin cytoskeleton. Firstly, CAMKII, which localizes to the PSD, expression levels are shown to be regulated in a miRNA-mediated manner. In addition, PSD-95, a scaffolding protein in the PSD, is predicted to be a high-ranking target for miRNAs. PSD-95 has been reported to be stabilized by FMRP, which is known to associate with the miRNA processing machinery and therefore suggests that miRNAs are involved in the regulation of PSD-95. Secondly, miRNAs have been shown to regulate ATP1, Limk1 and p250GAP and thereby controlling spine growth by possibly tuning the activity of antagonistic signaling pathways that regulate the actin cytoskeleton.

These findings illustrate the impact of miRNAs on genes localized in the PSD and involved in the actin cytoskeleton. This accentuates the crucial role of miRNAs in learning and memory, since both the PSD and the actin cytoskeleton are important features in synaptic plasticity. Taken together, this literature study highlighted the most recent developments in the regulation of mRNA translation at the synapse and strived to enrich our understanding of synaptic plasticity. Even though knowledge on the underlying mechanisms is still scarce, this study underlined that microRNAs play a pivotal role in synaptic plasticity by locally regulating gene expression.

3. Background

The nervous system is the body's decision and communication center with the brain as the main functional unit. The brain executes vital functions by processing and regulating all external and internal information. It coordinates the ability of all senses and enables one to breathe, think, dream, form words, plan, and make decisions. This organ harbors enormous complexity and consists of more than 100 billion neurons. These nerve cells are the building blocks of the brain and are interconnected by trillions of specialized junctions called synapses[1]. The brain is able to receive, process and store continuous streams of information by using these cells and the connections between them.

During the past century, research on neuroscience has provided detailed information on the molecular and cellular mechanisms underlying the complexity of the brain. This has, for example, shed light on mechanisms essential to synapse formation and function. Precise control of synaptic development and connectivity is essential for normal brain function. Pioneered by Ramón y Cajal[2], who proposed that changes in the structure of the brain might occur as a consequence of experience, morphological changes in neurons induced by external stimuli (e.g., experiences) have been a central theme of interest in neuroscience. Currently, it is widely accepted that information in the brain can be stored in the form of altered structure and chemistry of synapses and/or by the formation of new synapses and the elimination of old ones[3,4]. This phenomenon is called synaptic plasticity and is thought to be the basis of learning and memory in the brain. Not surprisingly, inappropriate loss of synaptic stability has been linked to disruption of neuronal circuits and brain diseases[4]. That is, recent studies have shown that many neuropsychiatric diseases are characterized by synaptic pathology, including abnormal morphology of dendritic spines, synapse loss and aberrant synaptic signaling and plasticity[5].

Learning may be described as the mechanism by which new information is acquired, while memory as the mechanism by which information is retained[6]. Learning and memory are therefore two processes which are indisputably interconnect. Memory can be divided into a short-term phase, lasting 1-3h, and a long-term phase, lasting for years or even a lifetime[7]. In general, short-term memory is thought to require modification of preexisting proteins which will lead to alterations in preexisting synaptic connections, whereas long-term memory requires new protein synthesis and the formation of new connections[7]. However, there is no

absolute range in time frame that distinguishes between these protein-synthesis-dependent and –independent brain mechanisms. In addition, as both learning and memory are attributed to changes in neuronal synapses, they are thought to be mediated through features known as long-term potentiation (LTP) and long-term depression (LTD). Whereas LTP is an activity-dependent enhancement in signal transmission between two neurons resulting in a persistent increase in synaptic strength, LTD is an activity-dependent reduction in this efficacy of neuronal synapses. These processes are linked to memory storage and clearance. That is, like memory, also LTP consists of an early phase (E-LTP), lasting 2-3 hours, which is independent of protein synthesis and a late phase (L-LTP), lasting several hours or longer, which requires synthesis of new proteins[6]. In addition, LTD has been postulated to be important for the clearing of old memory traces. This is necessary to prevent synapses to reach a ceiling level of efficiency, which could inhibit the encoding of new information[8]. Taken all together, LTP and LTD exhibit features that are crucial for learning and memory, which makes these processes attractive candidates that contribute to the cellular mechanisms underlying synaptic plasticity[94,6].

Protein synthesis is a pivotal element of synaptic plasticity, because it mediates the formation of new synaptic connections[10]. Emerging evidence indicates that post-transcriptional gene regulation is necessary for synaptic plasticity at several stages: by increasing proteome diversity through alternative splicing, or by enabling activity-dependent regulation of mRNA localization, translation or degradation in the dendrite[11]. Therefore, it is most likely that the ability of a neuron to adapt to external stimuli is not solely dependent on protein synthesis but also on the regulation of its mRNA molecules. Interestingly, microRNAs (miRNAs) are emerging as key modulators of post-transcriptional gene regulation that bind complementary sequences of mRNA molecules and regulate translation and mRNA stability. Moreover, the potentially huge impact of miRNA-based mechanisms on synaptic plasticity and thereby neuropsychiatric disorders is just starting to be recognized[12]. Understanding the underlying mechanisms of these mRNA regulatory processes could therefore help to shed light on important aspects of basal neuronal function. In addition, increased knowledge on the fundamentals of synaptic function and plasticity will help to better understand brain disorders and more importantly aid in finding proper treatments. Therefore, to contribute to this, the aim of this literature study will be to review the most recent studies on regulation

of translation and accompanying mRNA stability and their contribution to synaptic plasticity in the brain. The study will focus on genes that are involved in the postsynaptic density and cytoskeleton of excitatory postsynaptic neurons. The spine cytoskeleton is the actin-rich substructure on which the PSD is positioned. Both are major determinants of synaptic function and plasticity. This study will first discuss fundamental aspects of the molecular organization of synapses and highlight these two major components of dendritic spines. Subsequently, this paper will address key issues concerning protein synthesis and the accompanying post-transcriptional regulation of RNA molecules by microRNAs. This information will be used to build a theoretical framework in order to discuss the role of microRNAs in synaptic plasticity (Fig. 1).

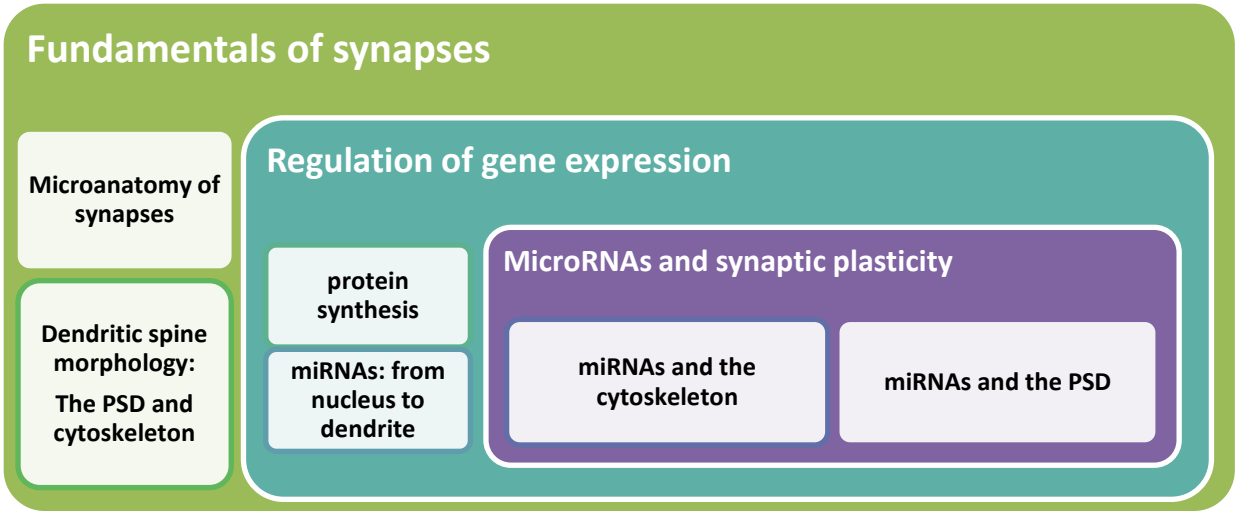


Figure 1. The framework of this literature study. Key aspects of neuronal plasticity and gene expression regulation will be highlighted before discussing the role of microRNAs in synaptic plasticity.

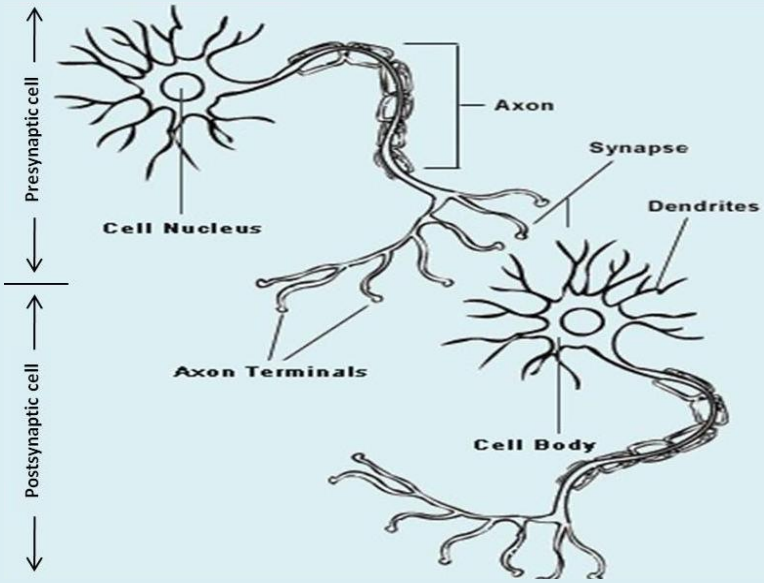
4. Neurons and their synapses

4.1 Basic features of neurons

Neurons are one of two most common cells found in the brain and carry information from one place to another by a combination of electrical and chemical signals. The morphology of neurons harbors characteristics that enable rapid and transient forms of communication between cells (Box 1). Neurons have common features, such as electrical excitability and the presence of synapses, that distinguish them from other cells in the body. Nevertheless, although built on a common plan, neurons can be quite diverse from one another.

Box 1. Morphology of neurons

A neuron can be defined by four main components each contributing to the communication between nerve cells. The cell body (soma) is the metabolic center of the cell. It contains the nucleus and is therefore the main area of gene transcription and protein synthesis. The soma gives rise to two types of cell processes, i.e. axons and dendrites. Dendrites are short extension from the cell body that branch out in a tree-like fashion and are pivotal for receiving incoming signals from other neurons. In contrast, axons are long tubular extension from the cell body that function as the main conducting unit for carrying electrical signals over distances to other neurons. These electrical signals are known as action potentials and are rapid, transient, all-or-none nerve impulses with a duration of approximately 1 millisecond.



Near its end, the axon divides into fine branches that form communication sites with other neurons. The point at which two neurons interact and communicate is known as a synapse. The nerve cells transmitting a signal is called the presynaptic cell while the cell receiving the signal is the postsynaptic cell. At the synapse, cells do not anatomically communicate but are separated by a space, the synaptic cleft. At this cleft, a presynaptic cell will transfer its electrical signal to the postsynaptic cell by the release of chemical substances, i.e. neurotransmitters. This chemical signal is transferred back into an electrical signal by the postsynaptic cell and subsequently propagated to its destination.

© Information is adopted from Kandel et al. (2000)[13].

Each neuron expresses a combination of general molecules common to all neurons and specific molecules restricted to only a subset of nerve cells. Examples of these molecules are enzymes, structural proteins, membrane components, and secretory products. These distinct features enable classification of neurons based on morphology and functionality[13].

Neuronal morphology is mainly determined by anatomical structure and polarity, whereas functionality is determined by electrophysiological characteristic, neurotransmitter production and the mode of action on other neurons which is mediated through synapses.

Synaptic communication consists of interactions between released neurotransmitters from vesicles of the presynaptic axon terminals and expressed neurotransmitter receptors on the postsynaptic region, usually on dendrites. These characteristics influence the postsynaptic membrane potential and therefore affect the generation of an action potential. A synapse can be characterized as inhibitory or excitatory by the alteration in membrane potential generated by neurotransmitter and receptor interaction. That is, synapses are called inhibitory if they decrease the likelihood of a postsynaptic action potential occurring whereas excitatory synapses increase this probability. A single neuron can receive input from hundreds or thousands of both excitatory and inhibitory synapses at its dendrite or cell body. Whether an action potentials is generated depends on the net effect on the membrane potential by integrating all inputs.

4.1 Microanatomy of synapses

The brain transfers information from one region to another by using electrical signals. These nerve impulses transported along the axonal membrane of presynaptic neurons cannot cross the synaptic cleft to communicate with postsynaptic cells. At the synapse, this electrical signal is therefore transferred into a chemical signal, by means of neurotransmitters, to permit proper conductance. Neurotransmitters are synthesized by the presynaptic neuron and stored in synaptic vesicles at presynaptic terminals. The postsynaptic neuron recognizes these neurotransmitters by its receptors and is able to transfer this chemical information back into an electrical signal. Numerous proteins have been shown to be involved in controlling synapse formation, vesicle fusion and neurotransmitter release and recognition (Fig. 2).

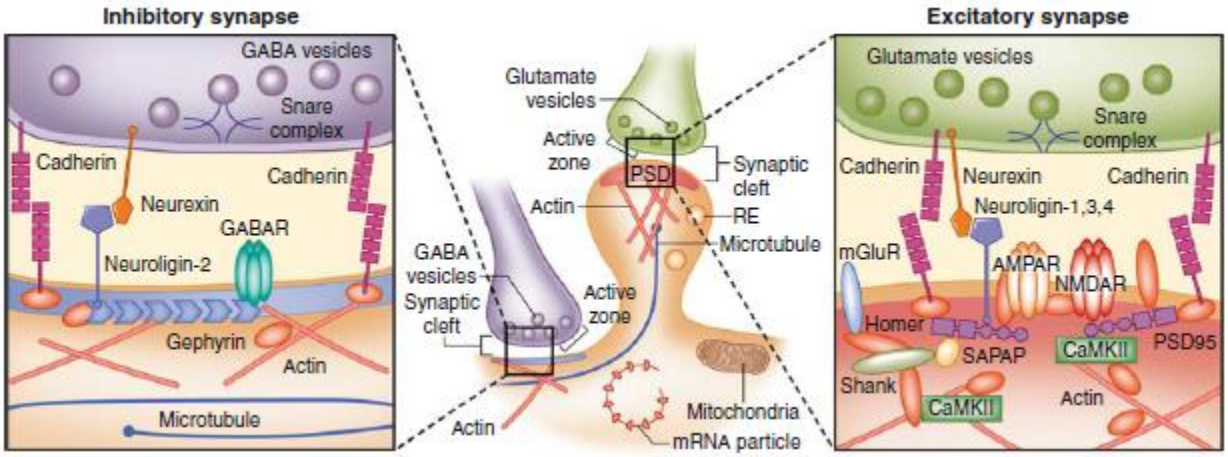


Figure 2. Molecular architecture of inhibitory and excitatory synapses. Excitatory synapses are located on dendritic protrusions containing a postsynaptic density (PSD), whereas inhibitory synapses are present along dendritic regions lacking postsynaptic thickening. The synapse enables dynamic processes supported by various organelles. Mitochondria provide energy, polyribosomes and RNA particles allow protein synthesis, recycling endosomes (RE's) transport internalized synaptic receptors back to the plasma membrane and the cytoskeleton regulates spine dynamics. The cytoskeleton is directly connect to the PSD, which is dense region of many proteins involved in the regulation of synaptic function. Both excitatory *(right)* and inhibitory synapses *(left)* contain a unique set of ion channels, scaffolding proteins and other postsynaptic molecules of which the most important players are depicted above.

© information and image are adopted from Spronsen and Hoogenraad (2010)[4]

Immediately behind the post-synaptic plasma membrane lies a protein-dense region known as the postsynaptic density (Fig. 3). This region is identified by electron microscopy as a physical feature at the postsynaptic site characterized by electron-dense material approximately 300 nm in diameter and 20-30 nm thick depending on the activity of the synapse[15]. The PSD is a macromolecular signaling complex which may contain several hundred proteins including receptors, scaffold molecules, adhesion molecules and cytoskeletal elements (Fig. 2). Furthermore, the PSD is thought to be the primary postsynaptic site for signal processing and signal transduction[16]. Tomographically reconstruction of the molecular organization of the PSD in dendritic spines shows that this electron-dense region consists of a well organized architecture of vertical and horizontal filaments [15]. This complex consists of receptors at the postsynaptic plasma membrane which are connected to an array of scaffolding and signaling proteins underneath. In addition, PSD-95 and related family members are suggested to be central players in the core organization of the PSD which is a theory already been put forward by others since PSD-95 has binding sites for receptors and other scaffolding molecules[17].

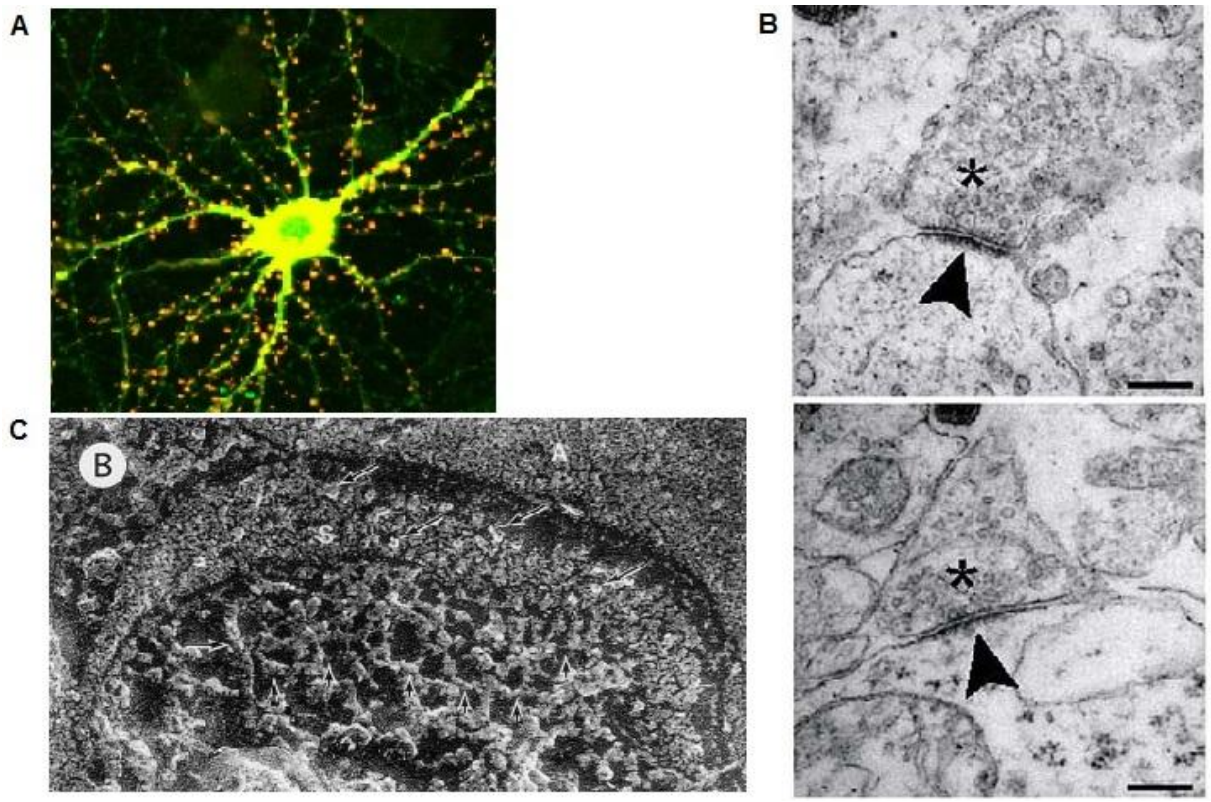


Figure 3. The location and structure of the postsynaptic density. The postsynaptic density is a specialization of the cytoskeleton at the synaptic junction. Its location places the PSD directly in the path of neurotransmitter signaling cascades at the membrane of the postsynaptic neuron. *(a)* Enhancement of spine maturation by overexpression of Shank1, which is an adaptor protein located in the PSD, in cultured hippocampal neurons highlights PSD regions on dendrites of neuron. *(b)* Ultra-structural analysis of synapses in the brainstem of wild-type (WT) embryonic mice. Synapses of WT neurons exhibit presynaptic vesicles (asterisk), a synaptic cleft and a distinct postsynaptic density (arrowheads) which correspond to a electron-dense area. *(c)* Freeze fractured shallow etched Purkinje cell spine showing substructure within a postsynaptic density, adjacent to the postsynaptic membrane. The axonal membrane (A), spine membrane (S) and PSD (indicated by arrowheads) are visualized. A filamentous structure with adherent globular material is seen within the PSD.

©Images are adopted from Molecular Neurobiology Lab (a) [18] , Heupel 2008 (b) [19], Ziff 1997 (c) [20]

Other key molecules are NMDA and AMPA receptors, Ca^{2+}-/calmodulin-dependent kinsase II (CamKII), Shank family proteins, synapse-associated protein-associated protein (SAPAP), and actin (Fig. 2). The PSD may therefore be regarded as an pivotal part of the postsynaptic signaling machinery, which integrates diverse structural and regulatory components into one macromolecular assembly[20]. Within the PSD may lie clues to the mechanisms of the most intriguing of brain functions, including activity-dependent changes in synaptic strength and possibly higher functions such as learning and memory[21,20].

4.2 Dendritic spine morphology and synaptic plasticity

Neural circuits are shaped by sensory experiences. This process of experience-dependent plasticity, which occurs at the level of dendrites and synapses, underlies the brain's ability to adapt to changes in the environment and store information[12,7]. Dendritic spines contain the postsynaptic machinery, including glutamate receptors, the PSD, the actin cytoskeleton and a wide variety of membrane-bound organelles, such as smooth endoplasmic reticulum, mitochondria, and endosomes[22]. The primary function of dendritic spines is to compartmentalize local synaptic signaling pathways and restrict diffusion of postsynaptic molecules[23,24]. Dynamic changes in spine morphology are closely linked to changes in strength of synaptic connections and dimension of the PSD[3]. Spine morphology is therefore subjected to rapid alteration depending on neuronal activity and glutamate activation[4]. For example, induction of LTP causes enlargement of spine heads, whereas LTD is linked to spine head shrinkage (Fig. 4).

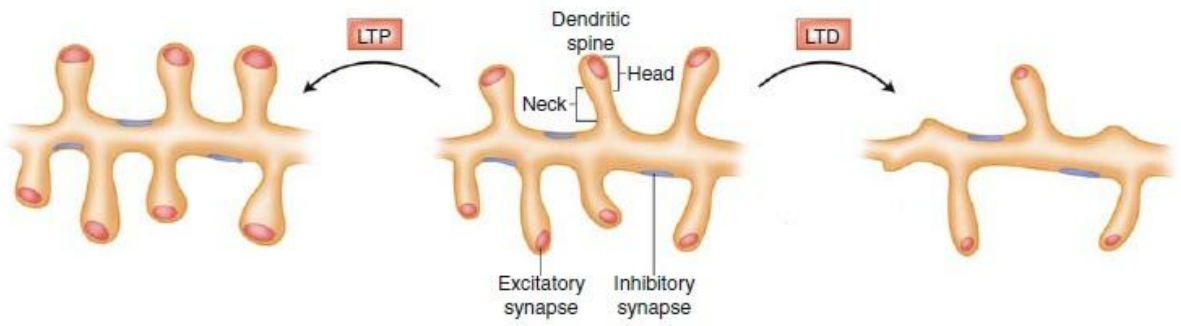


Figure 4. Morphological changes of dendritic spines upon long-term potentiation (LTP) and long-term depression (LTD). Dendritic spine morphology is directly linked to synaptic plasticity. As illustrated above, LTP and LTD, which are both underlying mechanism by which synaptic plasticity is exerted, can cause rapid alteration in spine morphology. That is, induction of LTP causes enlargement of spine heads, whereas activity patterns that induce LTD cause spine head shrinkage. The PSD is indicated in red at the top of dendritic spines.

© information and image are adopted from Spronsen and Hoogenraad (2010)[4]

A fundamental component of spine morphology is the actin cytoskeleton (see Fig. 5a), which is central to cellular processes involving membrane dynamics, such as cell motility and morphogenesis[25]. The actin cytoskeleton is important for dendritic size and shape and furthermore plays a pivotal role in spine formation and elimination[24]. Modulation of actin dynamics has therefore been proposed as a driving force behind morphological changes in dendritic spines that are associated with alteration in synaptic strength[26]. At synapses, the actin cytoskeleton does not only contribute to overall structure of synapses but also plays

important roles in synaptic activities ranging from organizing the postsynaptic density [22] and anchoring postsynaptic receptors to facilitating the trafficking of synaptic cargos and localizing the translation machinery[27,24] (see Fig. 5b). The latter being a subject that is receiving an increasing amount of attention.

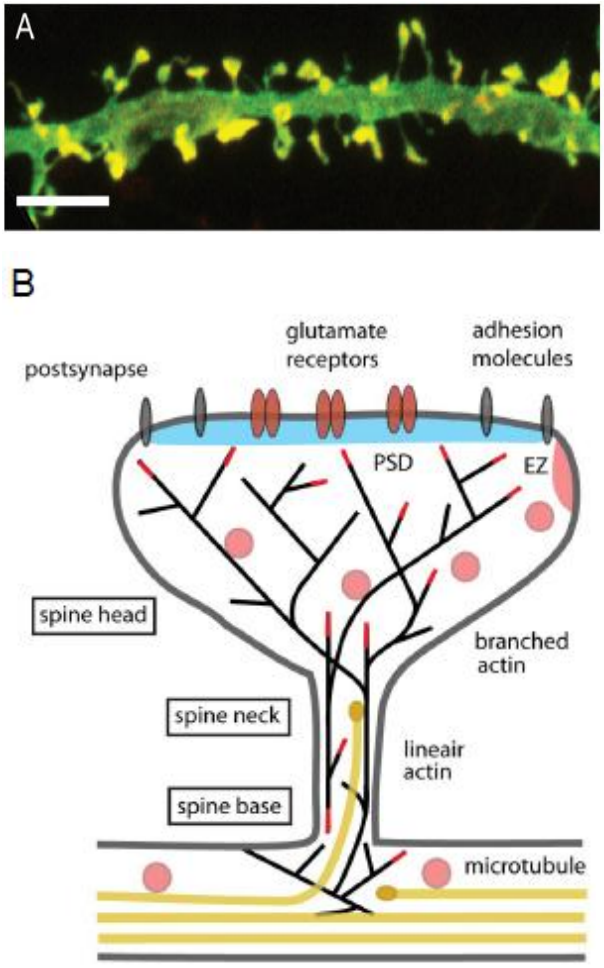


Figure 5. Cytoskeleton organization of dendritic spines. (A) Dendritic spine morphology (green) and localization of F-actin (red) in cultured hippocampal neurons. Yellow color indicates an overlap between spine and actin. White bar is indicative for 5µm. (B) Simplified overview of the actin cytoskeletal organization in dendritic spines contain the postsynaptic density (PSD; blue), adhesion molecules (gray) and glutamate receptors (brown), the actin (black) and microtubule (yellow) cytoskeleton. The endocytic zone (EZ) and recycling endosomes are denoted in pink. Dendritic spines exhibit a continuous network of both straight and branched actin filaments. This actin network is spread in the spine base, get stricted in the neck and stays highly branched in the spine head. The cytoskeleton not only serve as a structural backbone for dendritic spines but plays also important roles in organizing the PSD, anchoring postsynaptic receptors and localizing the translation machinery.

© Information and images are adopted from Hotulainen and Hoogenraad (2010)[24]

5. Protein synthesis: somatic versus local synthesis

5.1 Dendritic protein synthesis

Lasting activity-dependent changes in synaptic strength are dependent on new protein synthesis to remodel dendritic protein composition and thereby change the functional state of excitatory synapses. Interestingly, mRNA molecules used for dendritic protein synthesis are not translated in the cell body but locally at the spine. Identification of polyribosomes, which are complexes of ribosomes attachted along the length of an mRNA molecule, at dendritic spines initiated and supports this theory[28]. In addition, translocation of these polyribosomes into spines was observed 2 hours after LTP induction in the CA1 region of

hippocampal slices[29], supporting a mechanism of local protein synthesis in an activity dependent manner which is independent of the soma.

On average there are approximately 12 000 different mRNA species expressed in an single mammalian cell. In addition, dendritic population of mRNAs is estimated on ~400 distinct molecules[30,31], which suggests that there is intensive somatic selection for mRNAs to be transported to dendrites. Although the underlying mechanisms of mRNA selection, transport, localization and translation in dendrites are still largely unresolved, the most likely model will be discussed below.

The process towards mRNA localization in dendrites is complex and involves multiple players, such as (m)RNA binding proteins (RBP) and RNA-containing granules: ribonucleoprotein particles (RNPs), stress granules (SGs) and processing bodies (PBs)[32] (Box 2).

Box 2. RNA granules

Ribonucleoprotein particles (RNPs) are transport granules that contain mRNA, mRNA-binding proteins, motor proteins and small non-coding RNAs (e.g., microRNAs). Furthermore, RNPs can consist of heterogeneous nuclear ribonucleoproteins (hsRNPs; like Staufen and FMRP), genes located in the PSD, regulators of mRNA transport and translation, and the actual components of mRNA translation complexes. The pool of RNPs within a cell or compartment (dendrite) is thought to be heterogeneous since not all content is similar across granules. That is, one part consists of compulsory elements, mainly components of RNA transport, and the other part is flexible and consist of components dependable on brain region or stage of development.

Processing bodies (P-bodies) are distinct foci within the cytoplasm of eukaryotic cells that contain different components involved in mRNA turnover. That is, P-bodies contain decapping enzymes, exonucleases and components of the nonsense-mediated decay pathway. Furthermore, P-bodies have also been shown to contain microRNAs and components of RNA-induced gene silencing complexes.

Stress granules (SGs) are dense aggregations of proteins and RNA that appear when the cell is under stress. SGs are 100-200nm in size, not surrounded by membrane, and associated with the endoplasmatic reticulum[33]. In response to stress, eukaryotic cells reprogramme their mRNA metabolism to adapt to the changing conditions and turn off the synthesis of most "housekeeping" proteins by storing their mRNA into SGs. These granules further consist of small ribosomal subunits, components of the translation initiation complex, mRNA binding proteins that regulate mRNA translation and transport.

© Information is adopted from Bramham and Wells (2007)[32]

An important aspect of mRNA transport towards dendrites is that the mRNA molecules are transported in a translationally dormant state. The first step involves sequestration from the translational machinery in the cytoplasm and the packaging into transport granules. That is, in the nucleus RBP will bind to the transcribed mRNA molecule and remain bound on its journey out of the nucleus and into the dendrite[32] (Fig. 6). These binding proteins will 'protect' the mRNA molecule from translation in the cytoplasm. Under normal conditions, the vast majority of RNA-containing granules are likely to be transport RNPs. In rat hippocampal neurons, SGs and P-bodies were only highly present under stress-induced conditions[34].

The second step involves the transport of mRNA-containing RNPs into dendrites by kinesin motors on microtubules. Several studies have shown, by fluorescent imaging, that the majority of RNPs are stationary and that some display rapid movements either away or towards the cell body that are dependent on microtubules[35,36]. Furthermore, it was shown that these movements can be altered by neuronal activity, i.e. transport granules that were stationary could now move towards the dendritic spine[32]. At the spine base the granules are thought to be dispersed while the mRNA molecules, still bound to its binding proteins, are translocated into the spine in an actin-dependent manner[37] (Fig. 6).

The final step involves the activation of the local translational machinery in the dendritic spine. Upon neuronal activation, mRNA binding proteins can be dissociated from the mRNA-RBP complex leaving the mRNA molecule accessible for the translation. In addition, it is hypothesized that mRNAs encoding crucial components of the translation machinery undergo enhanced translation in dentritic spines upon neuronal activiation[38]. This suggest that mRNA regulatory processes are involved in activity-dependent synaptic plasticity. Therefore, this study will subsequently discuss mRNA translation and highlight an important role for microRNAs in the regulation of translation.

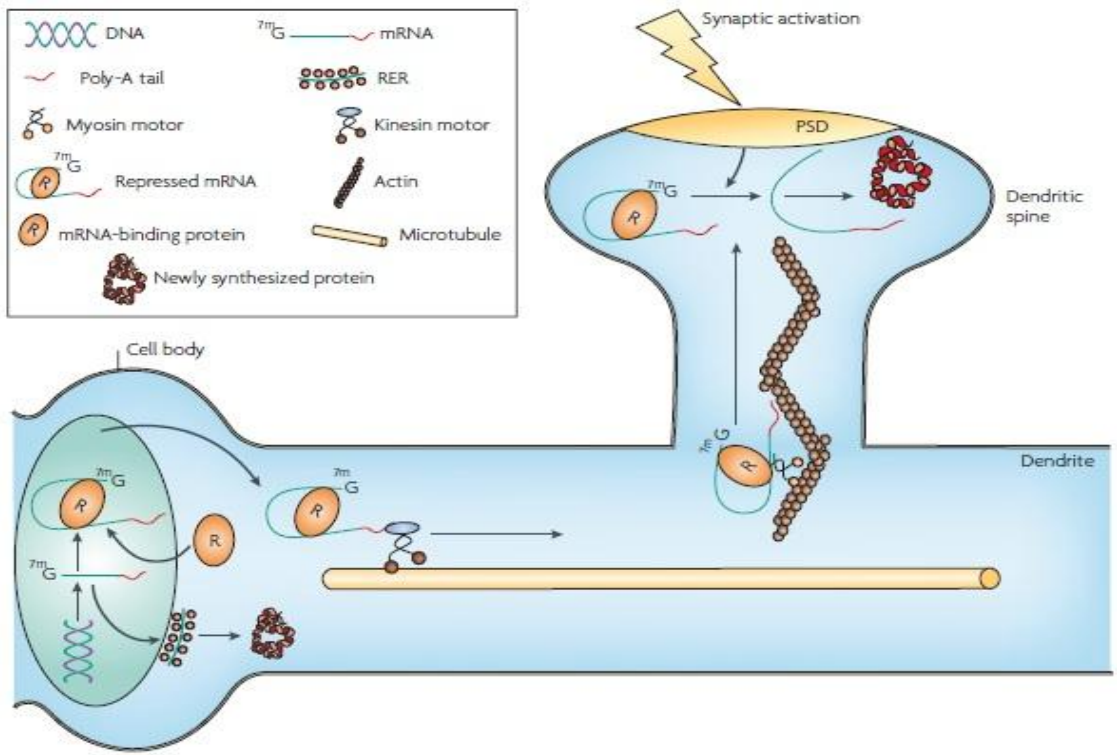


Figure 6. Proposed mechanism for mRNA translation in neuronal dendrites. mRNA molecules, transcribed in the nucleus, are bound by mRNA binding proteins (R) that are capable of repressing translation which allows the mRNA molecules to be sequestered away from the protein-synthetic machinery in the cell body. These silenced mRNAs are then organized into transport granules that are transported along the microtubule into dendrites. Upon synaptic activation, these mRNA molecules are translocated into the spine by the actin-based myosin motor proteins. Translation is initiation by neutralizing the repressive RNA-binding proteins. *^{7m}G = 7-methyl-guanosine cap; PSD = postsynaptic density; RER = rough endoplasmic reticulum*

© Information and image are adopted from Bramham and Wells (2007)[32].

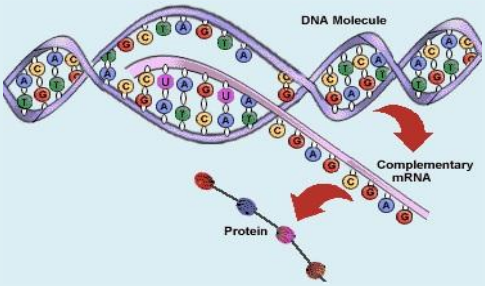
5.2 Messenger RNA translation

All eukaryotic organisms store their hereditary information in the form of double stranded molecules of DNA. This genetic information needs to be expressed because it harbors the blueprint for the synthesis of every molecule in the cell. In order to transfer genetic information into functional components, i.e. proteins, an intermediate step is required. That is, the genetic blueprint of a protein is first transcribed into a messenger RNA molecule (mRNA) which is subsequently translated into a protein (Box 3).

The molecular mechanism underlying mRNA regulation and protein synthesis have been a central theme of research for many decades[39]. This has led to the current model describing fundamental mechanisms underlying eukaryotic translation, which is reviewed in depth by Kapp and colleagues (2004). In this study, the main components of translation initiation will be briefly highlighted.

Box 3. mRNA characteristics: mediator of genetic information

The primary function of mRNA is to serve as an intermediate in the transfer of genetic information by guiding the synthesis of proteins, which are the functional units of the cell.



Messenger RNA has two very distinct features that are important for their functioning. Firstly, after initiation of transcription the 5'-end of a new mRNA molecule is modified by the addition of a methyl "cap", which is known as mRNA 5'-terminal 7-methylguanosine (m^7G) cap. Secondly, at the end of transcription the 3'-end of this mRNA strand is processed by the addition of a poly(A) tail, which is a subsequent coupling of roughly 200 adenine nucleotides to the 3'-end of the transcript. 5'-capping and 3'-polyadenylation gives the mRNA strand the opportunity to signal within a cell and is an important form of regulation within the expression of genetic information. That is, these two features function as target for various RNA binding proteins to stabilize and protect the transcript from degradation. They, furthermore, play a key role in the export of RNA transcripts from the nucleus to the cytoplasm where they are also involved in the initiation of protein synthesis.

© information is adapted from *Alberts et al., 2002*[40]

Messenger RNA translation can be separated into three steps: initiation, elongation and termination. Initiation starts with the recognition of the mRNA 5'-terminal (m^7G) cap structure by eukaryotic translation factors (eIFs) and facilitates the recruitment of the small (40S) ribosomal subunit into the translation process[39]. Eukaryotic translation factors also interact with the polyadenylate-binding protein 1 (PABP1), which binds the poly(A) tail at the 3'-end of the mRNA strand. The ability of eIFs to bind both the m^7G cap and the 3'-end of the mRNA strand brings these two ends of the mRNA in close proximity[41,42]. This process is known as circulation and is thought to catalyze translation initiation. In all cases, joining of the large (60S) ribosomal subunit at the start (AUG) codon precedes the elongation phase (Fig. 7). During elongation the ribosome moves along the mRNA strand in an 5'- to 3' direction 'reading' the message on the molecule. The ribosome forms an interactions with elongation factors and aminoacyl-tRNA to translate mRNA into an amino acid polypeptide.

When the translation machinery encounters a termination codon, translation release factors mediate the termination process in which the ribosomal subunits dissociate from both the mRNA and from each other.

Gene expression is a dynamic process with both a spatial and a temporal component. Regulatory processes controlling gene expression are therefore thought to be a major determinant of the composition of the RNA population within cells.

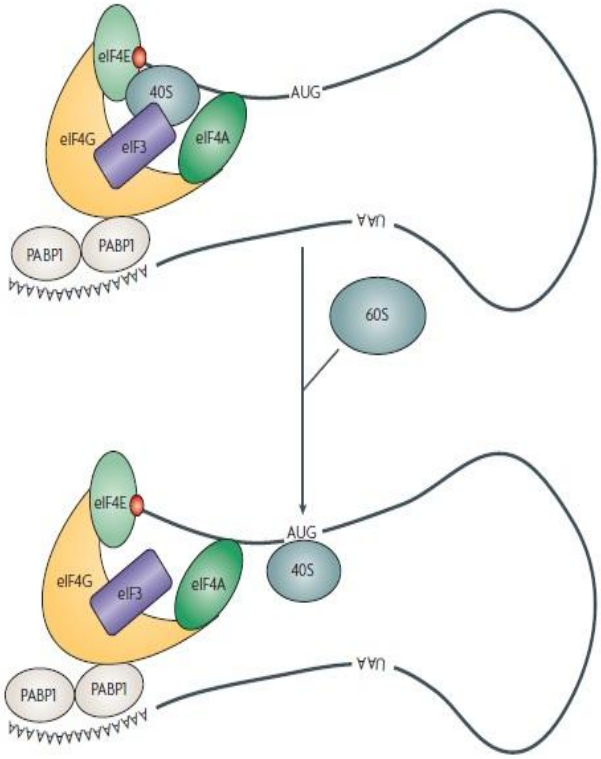


Figure 7. Initiation of mRNA translation. Translation of mRNA consists of three steps: initiation, elongation and termination. On the left, a simplistic view is shown of the initiation process. mRNA contains two feature which it uses to signal and communicate within the cell: a 5'-terminal 7-methylguanosine (m^7G) cap, denoted by the red circle, and a poly(A) tail. Translation starts by the recognition of the m^7G cap by the 4E subunit of an RNA helicase known as eukaryotic initiation factor eIF4F. This initiation factor also contains eIF4A (an RNA helicase) and eIF4G (a large multidomain protein necessary for scaffolding the assembly of the initiation complex). An important function of eIF4G is its interaction with the poly(A)-binding protein 1, PABP1, which is bound to the poly(A) tail of mRNA molecules. The interaction between eIF4G and PABP1 circularizes the mRNA, which stimulates translation initiation. Subsequently, an interaction between eIF4G and another multi-subunit initiation factor, eIF3, facilitates the recruitment of the 40S subunit which, joined by 60S subunit, begins scanning the mRNA 5'-UTR in search of the start codon.

© information and image are adopted from Filipowicz et al., 2008[42]

6. MicroRNAs

The discovery of microRNAs (miRNAs) has revealed an exciting new dimension in the complexity of post-transcriptional regulation of gene expression. Although research on mechanisms behind miRNA repression of protein synthesis are still in its infancy, miRNAs are believed to control gene expression post-transcriptionally by regulating mRNA translation or stability in the cytoplasm[43,44]. Interestingly, a large and diverse population of miRNAs is expressed in post-mitotic neurons at times of synapse development and show to be

associated with translation regulatory complexes[45]. It is therefore hypothesized that microRNAs could play a pivotal role in synapse functioning by post-transcriptionally regulating gene expression. This will have a direct effect on protein synthesis, which is previously mentioned to be an crucial process in learning and memory and thus synaptic plasticity.

6.1 MicroRNA biogenesis and processing

MicroRNAs (miRNAs) are small endogenous non-coding RNA molecules of approximately 21 nucleotides long that are emerging as key modulators of post-transcriptional gene regulation in a variety of tissue, including the nervous system. miRNAs can be found in all organisms from unicellular to multicellular[46,47] and regulate approximately 30 percent of all protein coding genes. miRNAs are one of the largest gene families with >200 members per species in higher eukaryotes accounting for roughly 1% of the genome[48]. At this moment, more than a 1000 miRNA are identified in humans[49] with this number still increasing as a result of intensive cloning and computational prediction approaches. The paradigm for miRNA function was first identified in nematodes by studying *lin*-4 and *let*-7 RNAs[50,51]. These molecules function as a post-transcriptional repressor of their target mRNA by binding to specific sites in the 3'untranslated region (3'UTR) of the gene. Binding is enabled by complementary base pairing between miRNA molecule and mRNA target. miRNAs are processed from precursor molecules (pri-miRNAs), which are transcribed from different regions of the genome. After being transcribed, these precursor molecules have the ability to fold into hairpin structures and thereby preparing them for a two-step processing, which is catalyzed by RNase III type endonucleases Drosha and Dicer. Both enzymes function in complexes with proteins containing dsRNA-binding domains which are therefore capable of interacting with the pri-miRNA hairpin. Drosha forms a complex with Digeorge syndrome critical region gene 8 (DGCR8) and processes pri-miRNAs to ~70 nucleotides hairpins which are called pre-miRNAs.

6.2 MicroRNA maturation and assembly into ribonucleoprotein complexes

Pre-miRNAs are then exported out of the nucleus and into the cytoplasm where they are subsequently cleaved by Dicer into the final ~21 base pair miRNA duplexes. After proccessing, one strand is selected to function as a mature miRNA while the second strand is degraded although there are some examples of miRNA duplexes of which both arms of the

hairpin end up as mature miRNAs. Next, miRNAs are assembled into ribonucleoprotein (RNP) complexes, which are known as micro-RNPs (miRNPs) or miRNA-induced silencing complex (miRISCs), and repress translation of target mRNAs (Fig. 8).

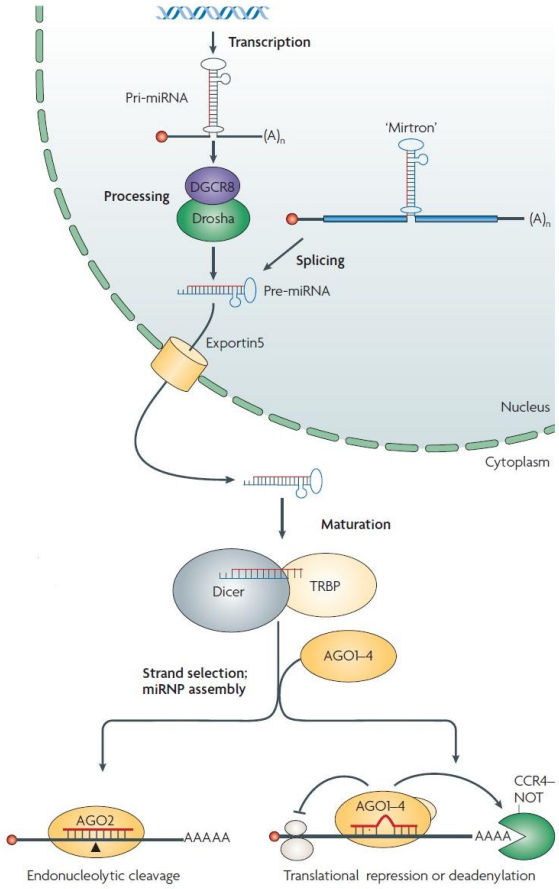


Figure 8. Biogenesis and maturation of microRNAs. miRNAs are processed from precursor molecules (pri-miRNAs) in a compartmentalized two-step manner by RNase III type endonucleases. Transcribed pri-miRNA molecules are first processed by Drosha-DGCR8 complex into a ~70 nucleotide long pre-miRNA molecule. Pre-miRNAs are subsequently exported out of the nucleus by the nuclear membrane receptor Exportin 5. In the cytoplasm, pre-miRNAs undergo maturation by cleavage by Dicer-TRBP complex, which results in a final double stranded RNA molecule of ~21 nucleotides long. This miRNA is then assembled into a microribonucleoprotein complex (miRNP) by which it exerts its function in inhibition of translation.

DGCR8 = DiGeorge syndrome critical region gene 8, ***TRBP*** = TAR RNA binding protein, ***AGO*** = proteins of Argonaute family, ***CCR4-NOT*** = a deadenylase

© Information and image are adopted from Filipowicz et al., 2008[42].

RNA binding proteins (RBPs), like proteins of the Argonaute (AGO) family and the fragile X mental retardation protein (FMRP), are postulated as key components of miRNPs. These RBPs are thought to function as regulatory factors or effectors mediating inhibitory function of miRNPs[52]. Finally, microRNAs are packaged and transported in miRNPs to dendrites[53].

6.3 MicroRNA mediated regulation of gene expression

MicroRNAs interact with complementary mRNA molecules to exert its regulatory function. Studies focusing on miRNA-mRNA interactions showed that levels of target mRNA do not change significantly upon binding, which pointed out that inhibition occurs at the level of translation[54]. Most miRNAs base pair to mRNAs with nearly perfect complementarity and induce mRNA degradation by endonucleolytic cleavage[55]. Although it is widely excepted that miRNAs assemble into miRNP complexes to exert their function on protein synthesis by

mRNA destabilization or translation repression, the mechanisms behind these processes, nevertheless, remain a point of debate. Models for inhibition at the initiation stage, post-initiation stage, and the elongation stage of translation have been proposed (Fig. 9). However, currently, research has mostly shed light on repression at translation initiation[42]. Nevertheless, the other possible mechanisms by which miRNA regulate translation should not be discarded. Furthermore, it is not excluded that miRNAs compel their action by using multiple mechanisms simultaneously. All mode of miRNA-mediated post-transcriptional regulation of gene expression are graphically depicted in figure 8. See Filipowicz et al. (2008) for a more extensive and detailed review[42].

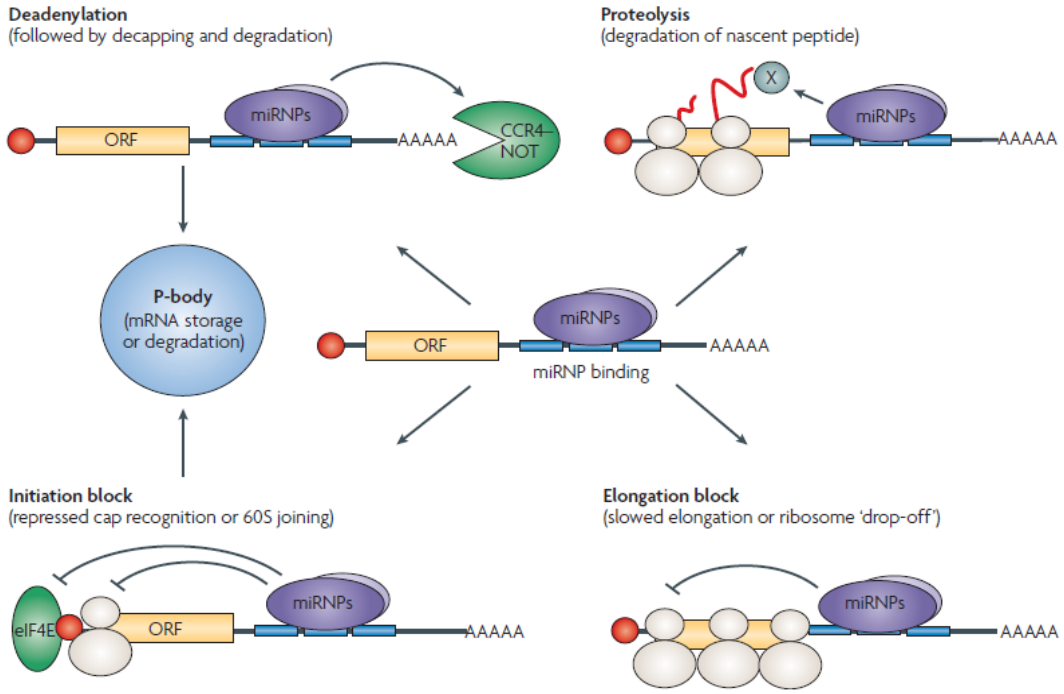


Figure 9. Potential mechanisms of miRNA-mediated post-transcriptional gene expression. *(upper left)* Binding of micro-ribonucleoproteins (miRNPs) to the mRNA 3'-UTR recruits CCR4-NOT to induce deadenylation and decay of target mRNAs. *(bottom left)* On the other hand, miRNPs can block translation initiation by preventing m^7G cap (red circle) recognition and therefore inhibit the circularization of mRNAs necessary for elongation initiation. In addition, initiation can also be repressed by blocking 60S ribosomal subunit joining. mRNAs repressed by deadenylaton or at the translation-initiation stage are moved to P-bodies for either degradation or storage. *(bottom right)* miRNA-mediated translational repression at post-initiation phases are slowed elongation or ribosome 'drop-off'. *(upper right)* A final mode of repression is miRNA-mediated proteolytic cleavage of newly synthesized protein molecules . However, a protease (X) involved in this process has not been identified yet. ***eIF4E*** = eukaryotic initiation factor 4E

© information and image are adopted from Filipowicz et al., 2008[42].

7. MicroRNAs at the synapse

To this point, the study has laid the groundwork, by addressing key aspects of postsynaptic structure and miRNA-mediated regulation of gene expression, to discuss the function of microRNAs at the synapse and thus its role in synaptic plasticity.

miRNAs possess several features making them well suited to regulate synaptic plasticity. Firstly, several promoters of neural miRNAs are binding regions for classical neural activity-regulated transcription factors, such as CREB and MEF2[56,57]. These regulators connect Ca^{2+}-regulated signaling cascades to the transcription of miRNAs and therefore point toward a role for miRNAs in synaptic plasticity. Secondly, miRNAs have been shown to localize to polyribosomes, which are the sites of active translation[45] and therefore suggest for a role in protein synthesis. As mentioned before, dendritic protein synthesis is an important component of synaptic plasticity. In addition, many miRNAs do not act as on-off switches, but rather fine-tune gene expression profiles in the absence of mRNA degradation[58]. This scenario fits well with local regulation of dendritic mRNAs during storage and/or activation at the synapse[12]. Finally, miRNA-mediated repression of translation has been shown to be reversible[59]. This enables for a highly dynamic mechanism of mRNA regulation which is also thought to be the case in synaptic plasticity. To summarize, the above suggests a role of miRNAs in synaptic development, function and plasticity.

Identification of candidate miRNA targets mostly relies on bioinformatic algorithms that use sequence complementarity and evolutionary conservation of miRNA binding regions in 3'UTRs of target mRNAs to predict possible miRNA-mRNA interactions. However, these prediction algorithms often show little overlap, making it difficult to interpret and rely on them for identifying miRNA-mRNA interactions (Fig. 10). In addition, these algorithms take the 3'UTR into account as the major site of miRNA binding although other regions, such as the 5'UTR and coding regions, can also drive miRNA-mRNA recognition[60].

An even more interesting consideration is whether both miRNA and target mRNA are spatiotemporally uniformly expressed. A miRNA might be predicted to target a mRNA molecule but will only be able to exert its function if both components are expressed at the same time and location within the cell. For neurons this is even more important because dendrites and synapses have been shown to consist of distinct populations of mRNAs[30,31].

Finally, a single 3'UTR can contain multiple miRNA binding regions creating an additional level of complexity in miRNA-mRNA interactions. That is, miRNAs can regulate translation in a cooperative or combinatorial manner of which the end result cannot be predicted from computational predictions. Therefore, to investigate the role of miRNAs in synaptic function and plasticity functional studies are needed to confirm and explore these predicted miRNA-mRNA interactions. Functional research on microRNAs in the context of synaptic plasticity is currently receiving a great deal of attention[60] [12]. These studies have been able to investigate the role of miRNAs in neurons and have provided key insights in the regulation of synaptic plasticity. This literature study will discuss, to the best of knowledge, all functional studies on the role of miRNAs in synaptic plasticity that are currently published. Next, the most important findings will be highlighted.

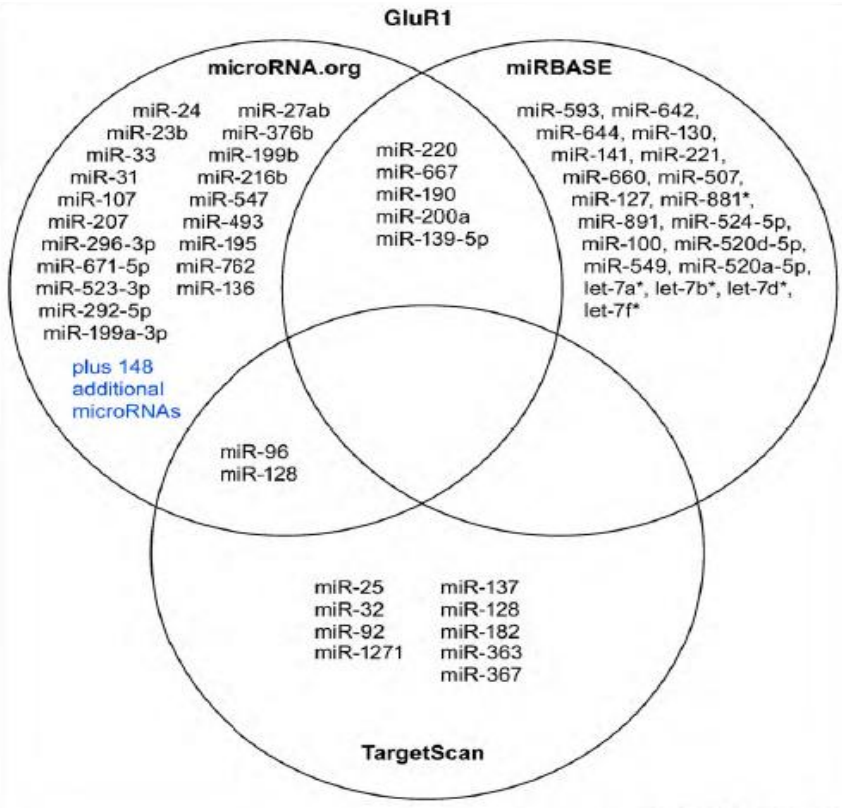


Figure 10. Overlap between bioinformatic predictions on microRNA regulation of GluR1. The glutamate receptor subunit, GluR1, is a highly ranked target for miRNAs. This diagram shows a comparison of identified miRNA binding regions in the 3'UTR of GluR1 between three commonly employed algorithms. Interestingly, there is no overlap between all three algorithms.

© information and image is adopted from Vo et al., 2010 [60].

7. 1 microRNA-mediated regulation of CaMKII expression

Long-lasting changes in synaptic strength, which is assumed to underlie learning and memory, are dependent on new protein synthesis in dendritic spines. CaMKII is a kinases important for synaptic functioning and memory[61] that, upon activation, can localize to the PSD. Inhibition of CamKII blocks the induction of LTP and upon activation, this kinase phosphorylates postsynaptic glutamate receptors. Ashraf and colleagues (2006) showed that upregulation of the transcript of CaMKII is required for plasticity in *Drosophila*[62]. They used an olfactory/electric shock paradigm to induce long-term memory (LTM) and were able to identify a proteasomal-dependent degradation of Armitage, which is the *Drosophila* homologue of Moloney leukemia virus 10 protein (Mov10). Mov10 is an RNA helicase required for RNA-mediated gene silencing by the RNA-induced silencing complex (RISC), which is a miRNP. Results showed that degradation of Armitage relieved suppression of several transcripts, including CaMKII, Staufen, and kinesin heavy chain (KHC) and produced patterns of local protein synthesis specific to memory. Whereas de-repression of the latter two proteins occurred in the soma, the de-repression of CaMKII occurred in the postsynaptic compartment[62] (Fig. 11).

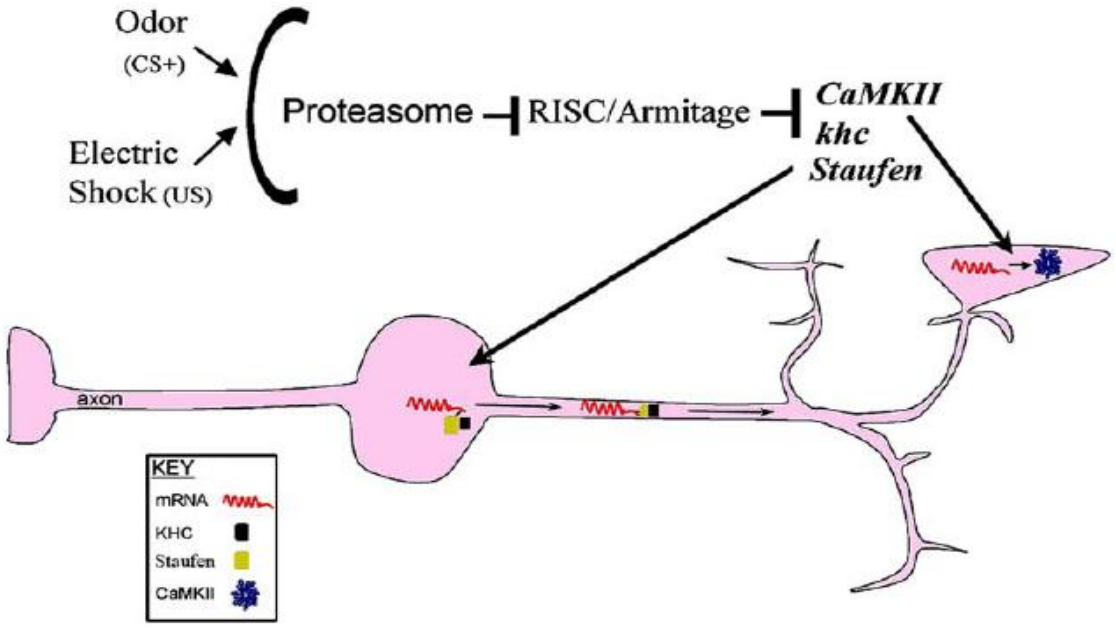


Figure 11. Regulation of synaptic protein synthesis associated with stable memory. An external cue triggers proteasome-mediated degradation of Armitage which results in a relieve of suppression of target mRNA molecules both at the synapse and the cell body. Increase synthesis of Kinesin Heavy Chain (KHC) and Staufen may facilitate synaptic transport of mRNA while CamKII translation is directly regulated at the synapse.

© Information is adopted from Ashraf et al., 2006 [62].

Interestingly, both Staufen, an RNA binding protein, and KHC, involved in kinesin motor transport along microtubules, are postulated to be involved in a complex that transports mRNA molecules[63]. The researchers proposed that degradative control of the RISC pathway underlies the pattern of synaptic protein synthesis associated with a stable memory. Together, these data indicate that the CaMKII transcript is locally relieved from microRNA-mediated repression following neural activity.

Banerjee and colleagues (2009) expanded upon these findings in mammalian hippocampal neurons and found that MOV10 localizes to synapses and is rapidly degraded in a proteasome- and ubiquitin-dependent manner following the activation of NMDA receptors at the postsynaptic site[64]. Upon MOV10 suppression, a set of mRNAs, including α-CaMKII, Limk1 and Lypla1 were translationally upregulated. By using an photo-convertible reporter fused to the 3'UTR of CaMKII they were able to link local degradation of MOV10 to the local protein synthesis of CaMKII transcripts. They concluded by proposing a model for local postsynaptic protein synthesis in response to neural activity (Fig. 12).

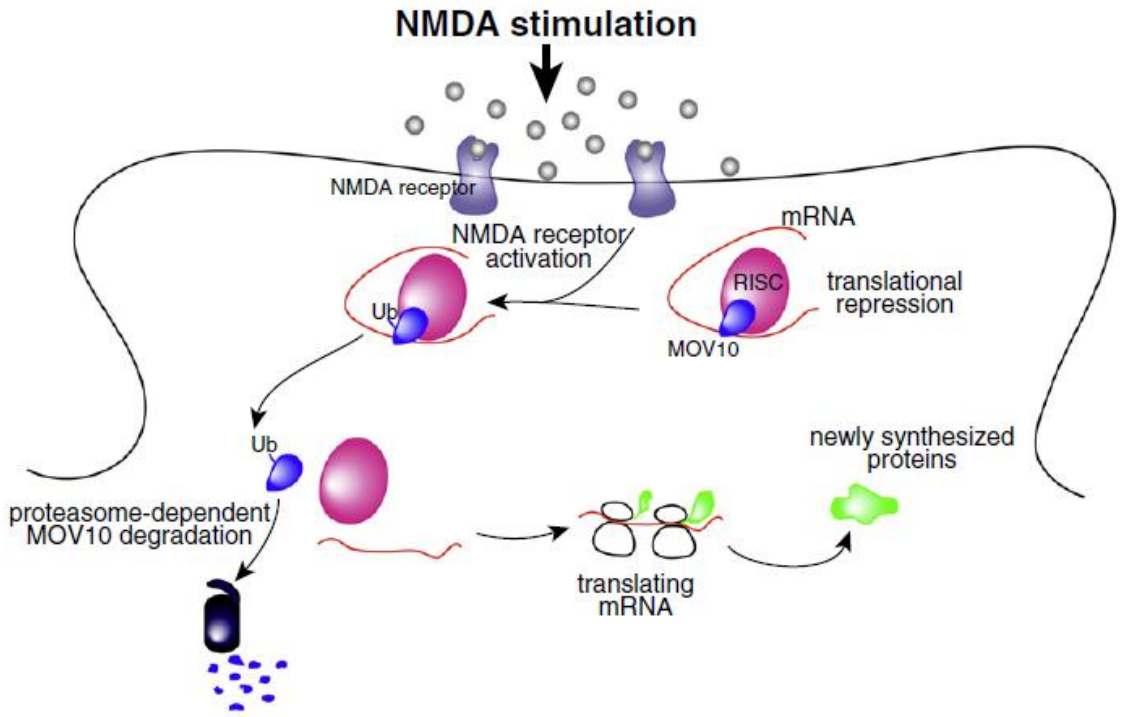


Figure 12. Proposed model for NMDA-mediated proteasomal control of RISC-regulated new synthesis of dendritic mRNA. *© Information and image are adopted from Banerjee et al., 2009 [64].*

In addition, Kocerha and colleagues (2008) investigated NMDA receptor-mediated neurobehavioral dysfunction in mice and identified a reduction in levels of a brain-specific miRNA, miR-219, upon pharmacological or genetic disruption of NMDA receptor signaling[65]. Moreover, they identified CaMKII$_\gamma$ as a direct target of miR-219. By using miRNA microarray profiling, they were able to screen for differences in miRNA expression in the prefrontal cortex (PFC) of mice between before and after dizocilpine treatment. Dizocilpine is a highly selective NMDA receptor antagonist. They identified a robust reduction of miR-219, which is a conserved miRNA in both rodents and human brains, out of 182 miRNAs. Northern blot analysis revealed that pharmacological disruption of NMDA receptor signaling only depleted the mature miR-219 transcript, but not its precursor, indicating that the transcription rate of miR-219 was not altered by dizocilpine. In line with this evidence, miR-219 expression levels returned to basal levels by 120 minutes after treatment. Next, by using a luciferase assay in neuronal cells, they were able to show that CaMKII is a target for miR-219 regulation. Furthermore, protein levels of CamKII$_\gamma$ were reduced in cells upon overexpression of miR-219, whereas CaMKII$_\gamma$ mRNA levels were unchanged[65]. Lastly, they confirmed in vivo in the brains of mice that CaMKII$_\gamma$ protein concentrations were significantly increased when treated with a miR-219 antagonist. Taken together, this study demonstrated that CaMKII$_\gamma$ expression in the PFC of mice is regulated by miR-219 and that this interaction is disrupted by NMDA receptor hypofunction (Fig. 13). The authors argued for a mechanism by which silencing of miR-219, and the subsequent upregulation of CaMKII$_\gamma$, provides a compensatory mechanism to maintain NMDA receptor function during antagonism of the receptor.

Unfortunately, Kocerha et al. (2008) did not investigate the effect of NMDA receptor inhibition of MOV10 or other RISC components their expression. It could be worthwhile to investigate whether NMDA receptor inhibition mediates CaMKII$_\gamma$ de-repression of miR-219 by degradation of MOV10. This would indicate whether the same mechanism of proteosome-mediated degradation of MOV10 is induced by both NMDA receptor activation and inhibition. Noteworthy, the study did not discriminate between somatic and dendritic expression of CaMKII. And as CaMKII is also able to access the neuronal nucleus[66,67], one should not rule out that the effect on CaMKII expression is due to functions of this kinase in the soma.

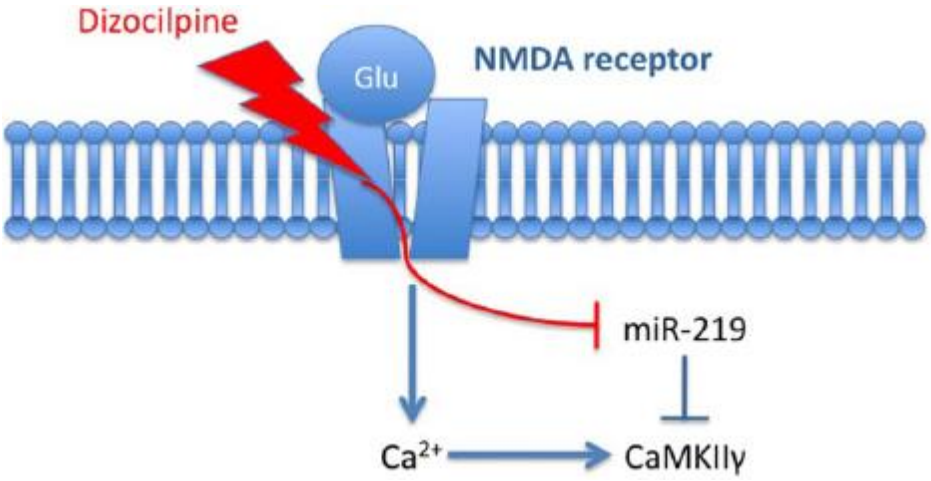


Figure 13. Schematic overview of miR-219 regulation of CaMKIII$_\gamma$ in NMDA receptor signaling. miR-219 is proposed to negatively regulate the function of NMDA receptors. Silencing of miR-219 relieved suppression of the microRNA from the 3'UTR of the CaMKII$_\gamma$ mRNA. Thus, providing a compensatory mechanism to maintain NMDA receptor function after acute hypofuctioning.

© Information and image are adopted from Kocerha et al., 2009[65].

To summarize, the three above mentioned studies show that neuronal CaMKII expression is subjected to microRNA-mediated regulation. Importantly, neural activity mediates upregulation of CaMKII mRNA translation by proteosome-dependent degradation of MOV10. Interestingly, NMDA receptor inhibition also upregulates CaMKII mRNA by inhibition of miR-219 suppression of CaMKII. CaMKII is an important component of the PSD, where it interacts with different binding partners, including PSD-95 and F-actin[68] [69]. CaMKII can phosphorylate more than 30 PSD-associated proteins, making it an important player in synaptic function and plasticity. Taken together, these studies indicate that synaptic plasticity may be partially controlled by microRNA-mediated regulation of CaMKII.

7.2 MicroRNA-mediated control of dendritic spine development

Dendrites represent the major sites of excitatory synaptic contact and are the receiving stations for incoming presynaptic signals from adjacent neurons. The architecture of the dendritic arbor determines how many synapses will form and how the information transmitted by these synapses is integrated[70]. Understanding the molecular mechanism regulating dendritic spine growth and arborization is therefore an important aspect in order to understand the development of neuronal circuits. Growth and branching of these actin-rich protrusions are furthermore dependent on local *de novo* protein synthesis which is mediated by neural activity[71]. Moreover, the phenomena of dendritic (spine) morphogenesis

is associated with long-lasting forms of memory[72,73]. The size of dendritic spines has shown to be a good correlate of the strength of excitatory synapses[74,75].

7.2.1 MiR-138 suppression of ATP1 regulates spine growth

Siegel and colleagues investigated the microRNA-mediated regulation of dendritic spine morphogenesis[76]. Firstly, they screened for miRNAs enriched in embryonic rat forebrain synaptosomes by performing a miRNA expression profiling assay. Significantly enriched miRNA were validated by Northern blot analysis and in situ hybridization (ISH) in rat hippocampal neurons. By means of this data, they were able to show that a specific subset of miRNAs, including miR-138 (Fig. 14a), is enriched in the synaptodendritic compartment of rat neurons. Next, using single cell fluorescent sensor assay it was shown that inhibition of miR-138 function resulted in a robust and significant increase in spine volume (Fig. 14b, c).

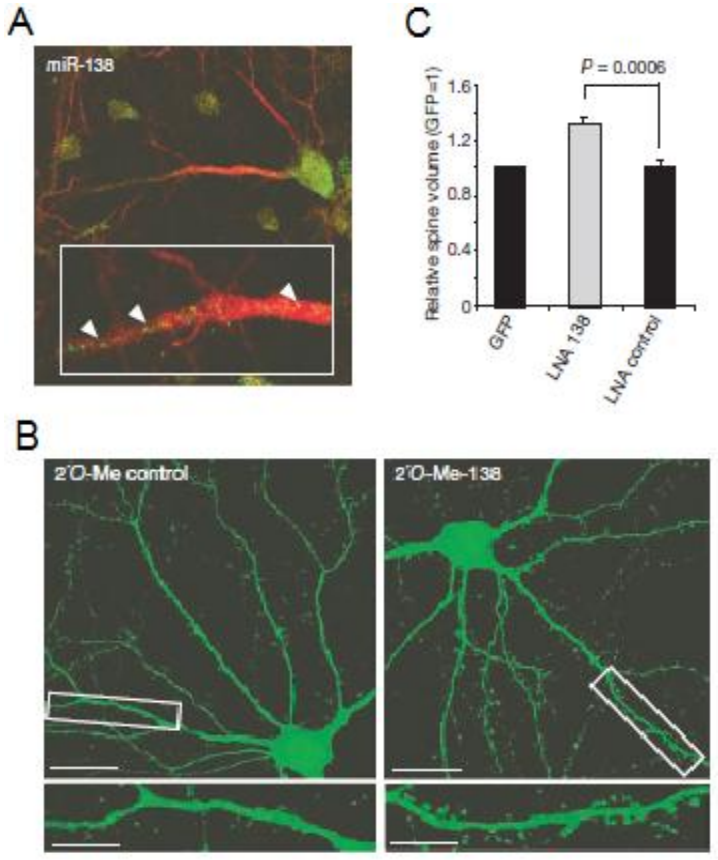


Figure 14. miR-138 functions in growth of dendritic spines. *(A)* Subcellular localization of the synaptically enriched miR-138 in hippocampal neurons by using ISH. Arrowheads indicate staining of the microRNA. *(B)* Hippocampal neurons transfected with GFP together with an microRNA inhibitor (2'*O*-Me AS oligonucleotides). The left picture shows 2'*O*-Me control while the right shows 2'*O*-Me-138. The boxes represent a magnification which is displayed below. *(C)* Average spine volume of neurons are determined after treatment with an LNA antisense oligonucleotide against miR-138. The p-value of a pairwise Student's t-test is indicated.

© Information and images are adopted from Siegel et al., 2009[76]

In addition, they recorded miniature excitatory postsynaptic currents (mEPSCs) from hippocampal neurons which were either transfected with miR-138 or a vector expressing GFP only. The median amplitude of mEPSCs turned to be significantly decreased in miR-138

neurons. Next, using prediction algorithms they identified possible miR-138 target mRNAs that might mediate the miR-138 effect on spine morphogenesis. Using a luciferase assay they confirmed acyl protein thioesterase 1 (APT1) as a target of miR-138. APT1 catalyses the removal of palmitate, a lipid modification with important roles in the localization and function of proteins[77]. Interestingly, introduction of miR-138 into cortical neurons did not significantly alter steady-state levels of ATP1 mRNA which indicates that miR-138 exerts an inhibitory effect by mainly impairing translation of APT1 mRNA[76]. Knockdown of ATP1 generated a decrease in the size of dendritic protrusions compared to control conditions. Moreover, a decrease in APT1, generated by APT1 shRNA, in miR-138-depleted cells completely suppressed the spine-growth-promoting effect of miR-138 inhibition (Fig. 14b). They were able to rescue spine shrinkage, which was due to elevated levels of miR-138, by transfecting cells with an APT1 expression construct harbouring a mutation in the miR-138-binding site. Finally, they investigated an APT1 substrate, G-protein α subunit (namely Ga_{13})[78], in the context of spine morphogenesis. Palmitoylation is an important requirement for Ga_{13} plasma membrane localization and subsequent activation of Rho-dependent signaling pathways[79]. The results showed that Ga_{13} membrane localization coincides with a decrease in endogenous APT1 protein levels in miR-138-transfected HEK293 cells. Overexpression of wild-type Ga_{13} suppressed the spine-growth-promoting effect induced by miR-138 inhibition. Taken together, this study showed that miR-138 inhibition might inhibit spine growth by prolonged palmitoylation and a subsequent increase in membrane localization of Ga_{13} which subsequently leads to elevated activity of the downstream Rho signaling.

Besides Ga_{13}, APT1 also has additional substrates in neurons, such as eNOS, PSD-95, adhesion molecules, neurotransmitter receptors, and other scaffolding proteins, all of which are anchored to the membrane by means of palmitoylation[80]. It would therefore be interesting to determine whether these proteins are also regulated in a miR-138 dependent manner or other miRNAs. In addition, as APT1 harbors multiple miRNA-binding regions, miR-138-APT1-mediated regulation of spine growth may provide an opportunity to study the mechanism underlying joint combinatorial regulation of miRNAs on ATP1.

7.2.2 miR-132 suppression of p250GAP regulates neurite outgrowth

In line with the above mentioned role of microRNAs in spine morphology, Vo and colleagues (2005) investigated the function of miR-132 in neurons[56]. They identified miR-132 by screening for genomic regions that are under control of cAMP-respone element binding protein (CREB). cAMP signaling and therefore CREB can be activated by an array of cellular signals, including neurotrophic factors and neuronal activity. Early experiments revealed that stimuli known to effect neuronal maturation and plasticity, such as membrane depolarization and neurotrophins, are robust activators of CREB-dependent gene expression[56]. The ability to induce gene expression has made CREB a likely candidate to regulate memory formation, synaptic plasticity and other forms of behavioral adaptation that require *de novo* protein synthesis[81].

Vo and colleagues confirmed binding of CREB to a cAMP response element (CRE) near miR-132 by chromatin immunoprecipitation in neocortical neurons and *in vivo* genomic footprinting in PC12 cells. Next, they were able to show that in cortical neurons the expression of miR-132 was rapid and prolonged by CREB activation after BDNF stimulation. As CREB is widely believed to regulate axonal outgrowth and dendritic development[82] and miR-132 is enriched in brain tissue, they investigated whether the miRNA might contribute to neuronal morphogenesis. They found that overexpression of miR-132 in cortical neurons induced an increase in neurite outgrowth in comparison to cells transfected with a control heart-specific miRNA. Furthermore, transfection of an antisense 2'*O*-methyl RNA decreased neurite outgrowth (Fig. 15a).

Using prediction algorithms they identified a high degree of conservation of miR-132 among vertebrates. They identified many predicted targets for miR-132. The 3'UTR of p250GAP, a member of the Rac/Rho family of GAPs, was the most highly conserved target evolutionarily. Therefore, they focused on this gene. Using a luciferase assay to were able to confirm the miR-132-p250GAP interaction. Cotransfection of miR-132 selectively attenuated the expression of a luciferase gene that contained a p250GAP miRNA response element in its 3'UTR. Moreover, they were able to block miR-132 function in primary neurons by using an antisense 2'*O*-methyl RNA. This sequence-specific miRNA inhibitor increased p250GAP-GFP levels whereas the sense strand did not. They postulated that miR-132 regulates p250GAP by a translational block because miR-132 did not decrease levels of p250GAP mRNA.

Because of their earlier observation of miR-132-induced neurite outgrowth, they investigated whether this phenotype is negatively regulated by p250GAP. By using p250GAP shRNA they were able to repress expression of endogenous p250GAP in cortical neurons and observed a decrease in neurite outgrowth (Fig. 15b). Taken together, these results support the hypothesis that miR-132 regulates neuronal outgrowth by translational suppression of p250GAP[56].

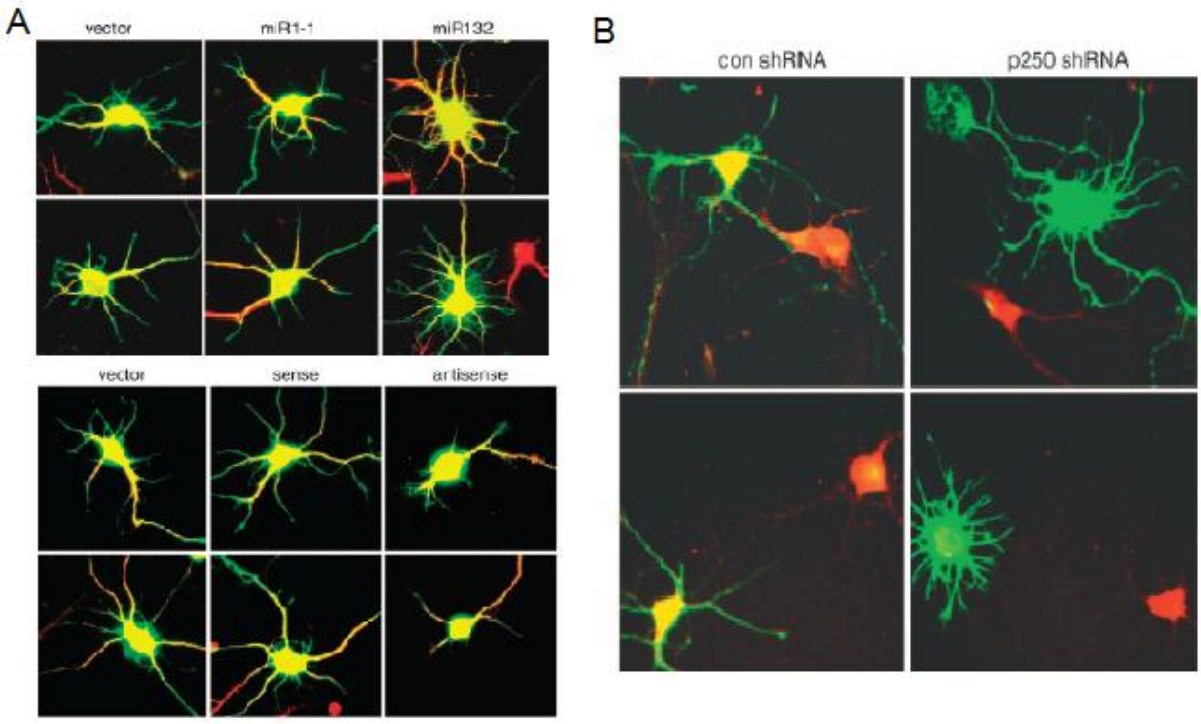


Figure 15. miR-132-mediated regulation of neurite outgrowth. *(A; above)* Expression of miR-132 induces neurite sprouting. Neonatal cortical neurons were transfected with a GFP reporter (green) and cotransfected with either vector control, expression construct for premiR-1-1 (heart-specific miRNA) or premiR-132. Cells were immunostained for the neuronal marker MAP2 (red) *(A; below)* Transfection of a miR132 inhibitor significantly reduces neurite outgrowth. Cortical neurons were transfected with a GFP reporter (green) and cotransfected with either an empty vector, a 2'*O*-methyl oligoribonucleotide against sense miR-132 or a 2'*O*-methyl oligoribonucleotide against antisense miR-132. Cells were immunostained for the neuronal marker MAP2 (red). *(B)* Down-regulation of p250Gap phenocopies miR-132 expression. Cortical neurons were transfected with a GFP reporter (green) and a p250GAP or control shRNA expression construct. Cells were immunostained for p250GAP (red).

© Information and images are adopted from Vo et al., 2005[56].

Impey and colleagues (2010) elaborated on these findings and found that both CREB- and activity-regulated miRNA, miR-132, regulate spine formation by activating a Rac1-Pak actin

remodeling pathway[83]. In the developing rat hippocampus, they were able to show that miR-132 expression increases parallel to periods of active synapse formation. In vitro, they identified a steep increase in miR-132 expression at day 9 of development. In addition, this was accompanied by a decrease in p250GAP (Fig. 16a). Depletion of p250GAP increased mEPSC frequency and the prevalence of GluR1 positive spines. Next, they showed that transcription of miR132 (Fig. 16b) or downregulation of its target, p250GAP, increases Rac1 activity. Rac1 is a member of the Rac subfamily of the Rho family of GTPases and is involved in an array of signaling events within the cell, including the regulation of spine structure[83]. Taken together, these results imply that neuronal activity could mediate spine formation by activating Rac1 signaling in a miR-132-dependent manner.

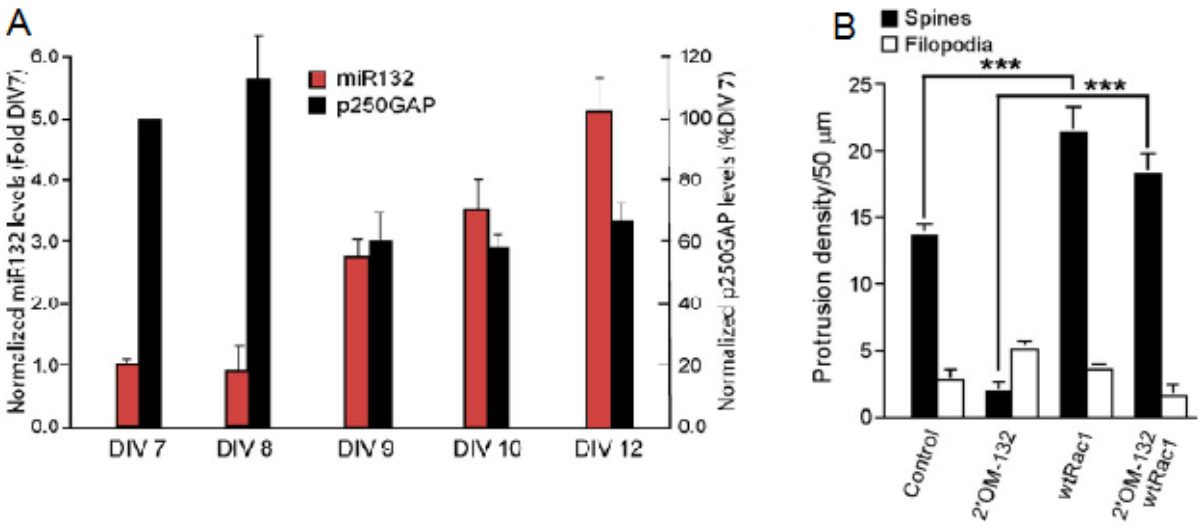


Figure 16. miR-132/p250GAP circuit regulates Rac1 activity and spine formation. *(A)* miR-132 (red bar) and p250GAP (black bar) expression in developing hippocampal neurons. P250GAP expression levels are normalized to Erk2 levels and plotted with normalized mature-miR-132 expression levels. DIV = days in vitro. *(B)* Rac1 activation stimulates spine formation in the absence of miR-132. Hippocampal neurons were transfected at DIV 7 with plasmids encoding 2'OM-132 (inhibitor of miR132) and, where indicated, wild-type Rac1. Dendritic spine and filopodia density on DIV 12 is shown (±SEM, ***P<0.001).

© Information and images are adopted from Impey et al., 2010[83].

Interestingly, because several additional predicted targets of miR-132 are known to regulate the development of postsynaptic densities and spines, it is possible that miR-132 targets additional regulators of neuronal development. Furthermore, it would be interesting to discriminate between somatic and dendritic effects of miR-132. That is, BDNF-induced CREB-mediated expression of miR-132 could be a likely candidate to promote long-lasting changes in neurite outgrowth. However, it remains unclear whether miR-132 suppression of

p250GAP can be affected by neuronal activity independent of CREB and therefore act immediate and locally at the synapse. Further research will be needed to shed light on these questions.

7.2.3 miR-134 suppression of Limk1 regulates spine size

As mentioned before, a potential role for miRNAs in synaptic function is expected given the evidence that a selected population of mRNAs are transported to sites of synaptic contact that is quite distant from the soma. These mRNA molecules need to be regulated locally and miRNAs are hypothesized to be the most likely candidate to control mRNA translation in response to appropriate extracellular stimuli. Local translation of these dormant mRNAs are postulated to have a key role in synaptic development and plasticity[84].

Schratt and colleagues (2006) investigated the expression and localization of candidate miRNAs isolated from mouse brain[85]. Northern blotting revealed that the expression of miR-134 is restricted to the brain. In addition, they identified this microRNA to be present within dendrites by using ISH. Moreover, miR-134 was found to partially co-localize with synapsin immunostaining, indicating the presence of this microRNA near synaptic sites on dendrites (Fig. 17a). The latter was confirmed by subcellular fractionation experiments, in which miR-134 was found to be enriched in membrane preparations containing synaptic terminals. This suggested a possible functional role for this miRNA at the post-synaptic site.

To investigate the role of miR-134 at the synapse, they examined the effects of miR-134 activity on dendritic spine development in cultured hippocampal neurons. Overexpression of miR-134 showed a significant decrease in spine volume when compared to controls (see figure 17b-b). This decrease could mainly be contributed to a reduction in spine width (-16.9 ± 5.8%, n=3, p=0.02). They found identical results between developing and mature neurons, which suggest that miR-134 may be able to affect the morphology of pre-existing spines. Inhibition of miR-134 function using a 2'*O*-methylated antisense oligonucleotide led to a small increase in spine volume and width (7.6 ± 3.7%, n=3, p=0.03) when compared to control conditions (Fig. 17b-d). Taken together, they have provide evidence that miR-134 functions as a negative regulator of dendritic spine volume in hippocampal neurons.

To gain more insight into the mechanism by which miR-134 regulates dendritic spine morphology, they investigated possible miR-134 target mRNAs. They scanned the 3'UTR of

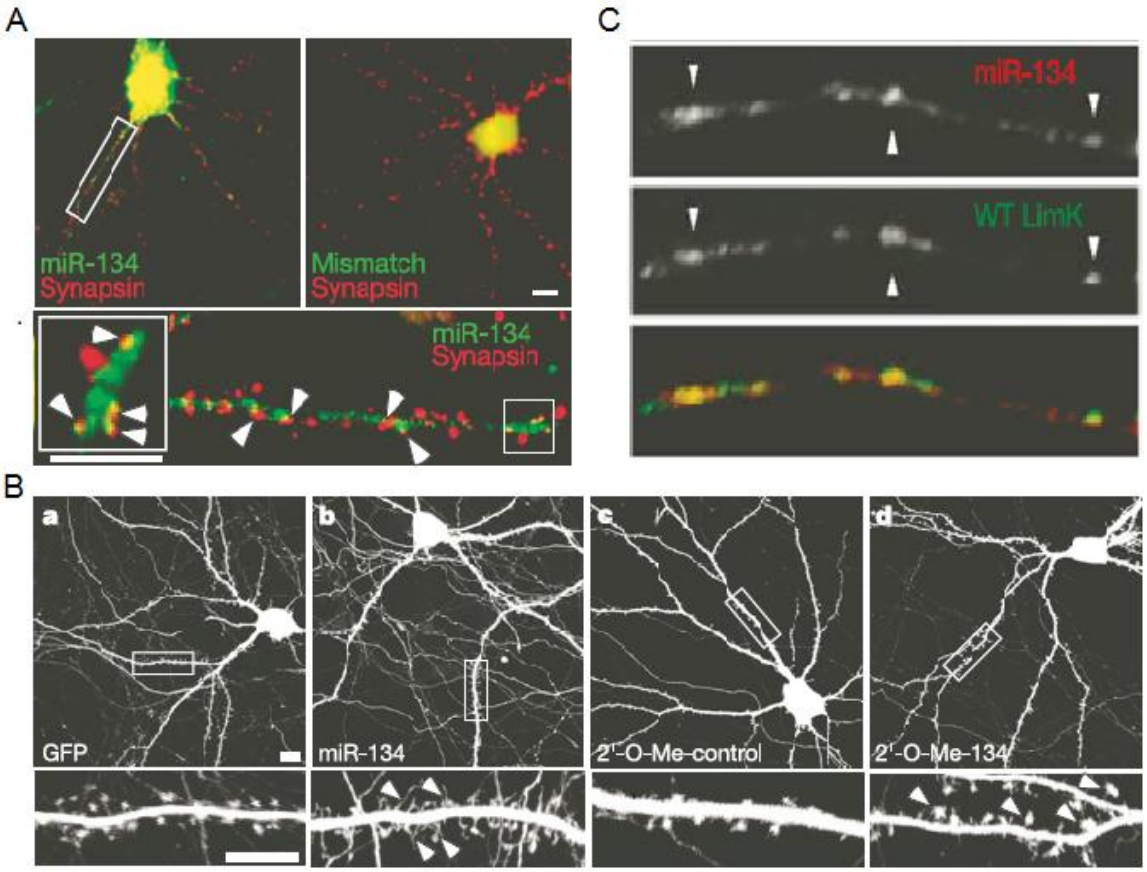


Figure 17. miR-134 mediated regulation of spine volume. *(A)* Co-staining of the presynaptic marker protein synapsin (red) together with miR-134 ISH (green) in hippocampal neurons. The boxed area in the upper-left panel is magnified in the bottom panel, which also consist of a higher magnification. Arrowheads point to synapses that show overlapping expression of miR-134. *(B:a-d)* Transfection of hippocampal neurons with control vector (a), miR-134 expression vector (b), 2'O-methylated control oligonucleotide (c), or 2'O-methylated miR-134 antisense oligonucleotide (d). Bottom panels (magnifications of boxed areas) illustrate higher frequency of thinner spines in miR-134-expressing cells (arrows in b) and enlarged spines in 2'O-Me-134-transfected neurons (arrows in d). *(C)* Localization of microinjected miR-234 (red) and Limk1 RNA (green) in hippocampal neurons. Arrowheads indicate miR-134 and Limk1 co-localization events in granule-like structures.

© Information and images are adopted from Schratt et al., 2006 [85]

mRNAs for conserved regions complementary to miR-134. They identified three potential candidates, i.e. disc large homologue 2 (DLG2), Neurod2 and Lim-domain-containing protein kinase 1 (Limk1). The latter was of particular interest, because Limk1 regulates actin filament dynamics through inhibition of ADF/cofilin[86]. Interestingly, Limk1 knockout mice show abnormalities in dendritic spine structure similar to those observed upon miR-134 overexpression[87,85]. Using an electrophoretic mobility shift assay, they were able to

demonstrate that Limk1 mRNA and miR-134 interact in vivo. In line with this evidence, they also found miR-134 and the 3'UTR of Limk1 to co-localize within dendrites (Fig. 17c).

As miRNAs are thought to control the expression of target mRNAs mainly through inhibition of translation, they examined whether miR-134 binding to Limk1 mRNA might inhibit protein synthesis. They found that miR-134 overexpression in both HEK 293T cells and primary neurons decreased the activity of a luciferase reporter gene fused to wild-type Limk1 3'UTR, whereas control conditions had no significant effect. In addition, inhibition of endogenous miR-134 in neurons by 2'*O*-methylated miR-134 resulted in a statistically significant increase in the expression of the luciferase reporter. Next, they investigated whether miR-134 inhibition of Limk1 occurs within the cell body and/or dendrites. They generated a GFP-based protein synthesis reporter with limited diffusion and a shortened half-life which allows for the study of local protein synthesis within intact dendrites[88 85]. This analysis revealed that the expression of wild-type Limk1 reporter was significantly reduced (18-28%) along the entire length of the dendrites compared to control conditions. Taken together, given the dendritic co-localization of endogenous Limk1 mRNA and miR-134 and the inhibitory mode of action, their findings indicate that miR-134 partially inhibits Limk1 mRNA translation locally within dendrites.

Next, they investigated whether miR-134-mediated repression of Limk1 mRNA translation underlied the observed reduction in dendritic spine size upon miR-134 overexpression. In hippocampal neurons, they co-expressed miR-134 with either wild-type Limk1 mRNA or mutant Limk1 mRNA which is incapable of interacting with miR-134. They found that mutant Limk1 mRNA was able to rescue both spine volume and width decrease imposed by miR-134 overexpression, whereas wild-type Limk1 mRNA was not as effective.

Finally, they investigated whether miR-134 repression of Limk1 mRNA translation could be relieved by extracellular stimuli, such as BDNF. Firstly, synaptoneurosomes from rat brains were prepared and incubated with ^{35}S-methionine to label newly synthesized proteins. They were able to determine the amount of newly synthesized Limk1 protein by radio-immunoprecipitation. BDNF treatment was found to significantly increase synthesis of Limk1 protein within these isolated synaptoneurosomes as indicated by an increase in labeled protein in Limk1 immunoprecipitates. In line with this evidence, they were able to decrease

labeled Limk1 protein levels by treatment with rapamycin, which is an inhibitor of the mTOR kinase pathway. This pathway has shown to be mediating BDNF signaling to the translational machinery (Fig. 18a)[89]. Lastly, they examined the effect of BDNF treatment on the translation of a Limk1 3'UTR luciferase reporter mRNA in neurons when miR-134 was highly expressed. This would elucidate whether BDNF can relieve miR-134-mediated suppression of Limk1 translation. They found that BDNF was able to induce translation of reporter mRNA which was fused to the wild-type Limk1 3'UTR but not of the mutant 3'UTR which was not able to interact with miR-134 (Fig. 18b).

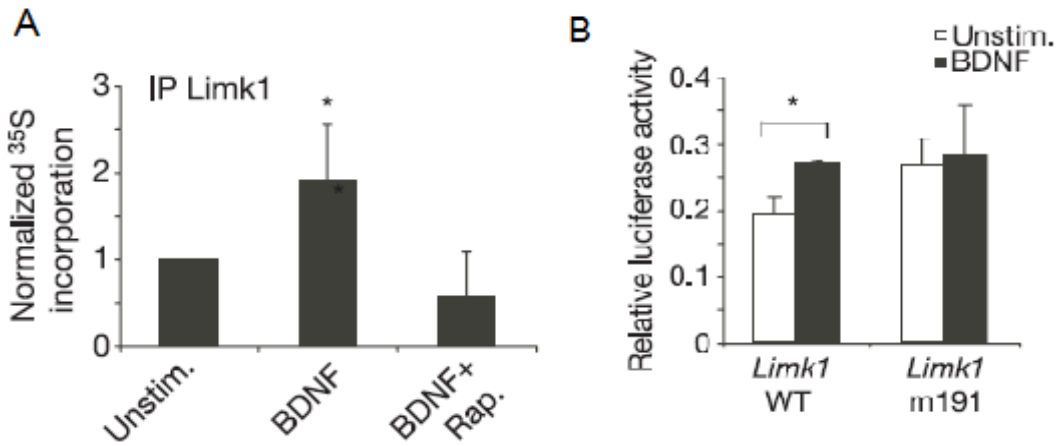


Figure 18. miR-134 is involved in BDNF-induced Limk1 mRNA translation. *(A)* Average of Limk1 immunoprecipitation from synaptoneurosomes which are incubated with ^{35}S-methionine in the presence or absence of BDNF/rapamycin. Data represent the average of three independent experiments. ± s.d. Unstim.=1. Asterisk, P<0.05. *(B)* Relative luciferase activity in cortical neurons transfected with Limk1 wild-type (black bars) or Limk1 mutant (m191; white bars) reporter mRNAs. Neurons were either unstimulated or treated with 100 ng ml $^{-1}$ BDNF for 4 h. Data represent the average of three independent experiments. ± s.d. Asterisk, P<0.05.

© Information and graphs are adopted from Schratt et al., 2006 [85].

The study concluded that miR-134 is a dendritically localized miRNA that regulates the expression of synaptic Limk1 protein and thereby controlling dendritic spine size. They postulate that the association of Limk1 mRNA with miR-134 keeps the Limk1 mRNA in a dormant state while it is transported within dendrites to synaptic sites. Upon synaptic stimulation, the release of BDNF may trigger the activation of the mTOR signaling pathway and relieve Limk1 mRNA from translational suppression by inactivating miR-134-association complex from the mRNA molecules by an yet unknown mechanism. This will lead to enhanced Limk1 protein synthesis and spine growth. Interestingly, they were not able to show full inhibition of Limk1 mRNA translation upon miR-134 overexpression which might

indicate that other translational regulators might be involved. As other neuronal miRNAs have been predicted to bind to Limk1 3'UTR, a combinatorial regulation by multiple miRNAs on LimK1 mRNA translation should not be excluded[85].

7.2.4 Dynamic regulation of the actin cytoskeleton in dendritic spines

As described above, three microRNAs have independently been found to regulate dendritic arborization and spine formation[56,85,76]. That is, miR-138, miR-134, and miR-132 have been shown to be involved in miRNA regulatory pathways that control the actin cytoskeleton in dendritic spines. Schratt (2009) proposed a model in which these miRNAs control the actin cytoskeleton by two antagonistic pathways (Fig. 19)[12]. On the one hand, miR-138 downregulates the depalmitoylation enzyme ATP1 which will result in the membrane localization and activation of RhoA stimulatory G protein Gα12/13. This will initiate a RhoA-ROCK cascade which will lead to actomyosin contraction and spine shrinkage. On the other hand, miR-134 and miR-132 control a rac-Limk1 signaling module that promotes actin polymerization and spine growth. miR-132 inhibits synthesis of the Rac-inactivating protein p250RhoGAP, which will promote spine growth. This actin polymerization could possibly be mediated through Limk1 although other Rac effectors might contribute as well. miR-134 inhibits Limk1 production and thereby decreases the polymerization of actin and spine growth[12]. By this model, Schratt argued that dendritic miRNAs control spine growth by tuning the activity of antagonistic signaling pathways that regulate the actin cytoskeleton in spines. Given the crucial role of the cytoskeleton in long-term potentiation[90], the author speculated that activity-dependent regulation of these miRNA-related pathways might also contribute to long-lasting forms of synaptic plasticity[12].

8. Translational control of PSD-95: a role for miRNAs and FMRP

PSD-95, also known as DLG4, is part of the MAGUK family of adaptor proteins. The gene is a major component of the postsynaptic density and directly binds to COOH-terminal cytoplasmic tails of NMDA receptors (NMDARs). This interaction integrates these receptors into the protein network of the PSD[17]. NMDARs have been said to be the main molecular device controlling synaptic plasticity and memory function[91]. Mice lacking PSD-95 have impairments in learning[92] and cortical plasticity[93]. In addition, PSD-95 mutant mice show

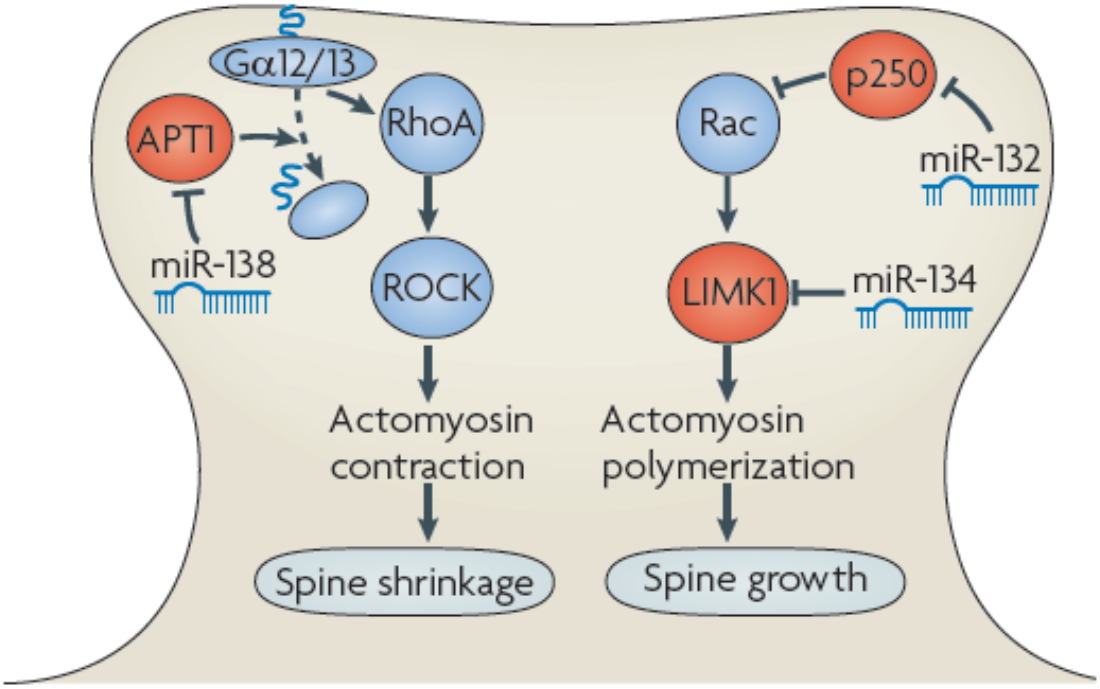


Figure 19. MicroRNA regulatory pathways in the control of the actin cytoskeleton in dendritic spines. A simplistic model of microRNA-mediated regulation of the actin cytoskeleton in dendrites through two antagonistic pathways, i.e. a RhoA-ROCK cascade which will lead to spine shrinkage and a Rac-Limk1 signaling module that promotes spine growth. It should be noted that for the simplicity, activity-dependent regulation of these pathways is not shown. In addition, the miRNAs denoted (miR-138, mir-134, and miR-132) are likely to have additional targets that might also function in these pathways.

© Information is adapted from Schratt (2009 [12].

dendritic spine abnormalities in the striatum and hippocampus[94]. Taken together, this data suggests that PSD-95 is a key element in synaptic plasticity. Interestingly, PSD-95 have been identified as a high potential target of miRNAs which implies that PSD-95 mRNA stability could be regulated in a miRNA-mediated manner.

Using a prediction algorithm, John and colleagues (2004) assessed miRNA interaction with the 3'-UTR of mRNAs that are conserved among mammals[95]. This computational study identified PSD-95 as a high-ranking target of miR-125, miR-135, miR-320, and miR-327, all of which are either exclusively expressed in brain or enriched in brain tissue[95]. In addition, all PSD-95 family members in mammals, i.e. DLG1-5, and three NMDARs are predicted miRNA targets. The authors argued that miRNA are involved in NMDA receptor signaling to coordinate and integrate information, with specificity achieved through the combinatorial action of different miRNAs[95].

Secondly, they used indirect experimental support to test whether their predictions were correct, i.e. validity of target gene prediction. Experimental evidence that shows the association of mRNAs and miRNAs with proteins involved in translational control was used to validate their predicted miRNA-mRNA interactions. In particular, FMRP, which may regulate translation in neurons, not only associates with hundreds of mRNAs, but also associates with components of the miRNA processing machinery, Dicer, and the mammalian homologues of AGO1 and AGO2[95,96]. Therefore, their analyses should be enriched for miRNA target genes that are associated with FMRP. In line with this evidence, this RNA binding protein has been shown to interact with PSD-95 and regulate PSD-95 mRNA stability and turnover[97]. This supports the hypothesis that translational control involving FMRP protein is executed in a complex that involves one or more miRNAs interacting with transcripts at specific sites.

In neurons, from the soma to the synapse, FMRP controls the posttranscriptional fate of a specific subset of mRNAs. That is, FMRP is part of an messenger ribonucleoprotein particle (mRNP) and has been shown to be involved in dendritic localization and local translation of important neuronal mRNA molecules[98]. These mRNPs are thought to be translationally silent as they progress along the dendrites. Once the FMRP-silent granule reaches the synapse, translational repression would be released upon neuronal stimulation and thereby contributing to local neuronal plasticity[99,98]. Absence of FMRP will lead to deregulation of this control mechanism and will strongly impair synaptic function, as is the case in Fragile X syndrome (FXS) where a mutation in the FMRP gene leads to loss of expression. Brains of individuals with FXS show longer and immature-appearing spines[100], which confirms the important role of FMRP in synaptic function.

PSD-95 mRNA is part of the FMRP-mRNP *in vivo*, i.e. the C-terminal domain of FMRP binds a G-rich structure in its 3'-UTR[97]. Zalfa and colleagues showed that, in the hippocampus, FMRP protects PSD-95 mRNA from decay after inhibition of transcription with actinomycin D (Fig. 20a, b). In addition, they found that this region-specific stabilizing effect of FMRP can also be

enhanced upon neuronal activity, which is induced by DHPG (Fig. 20b).

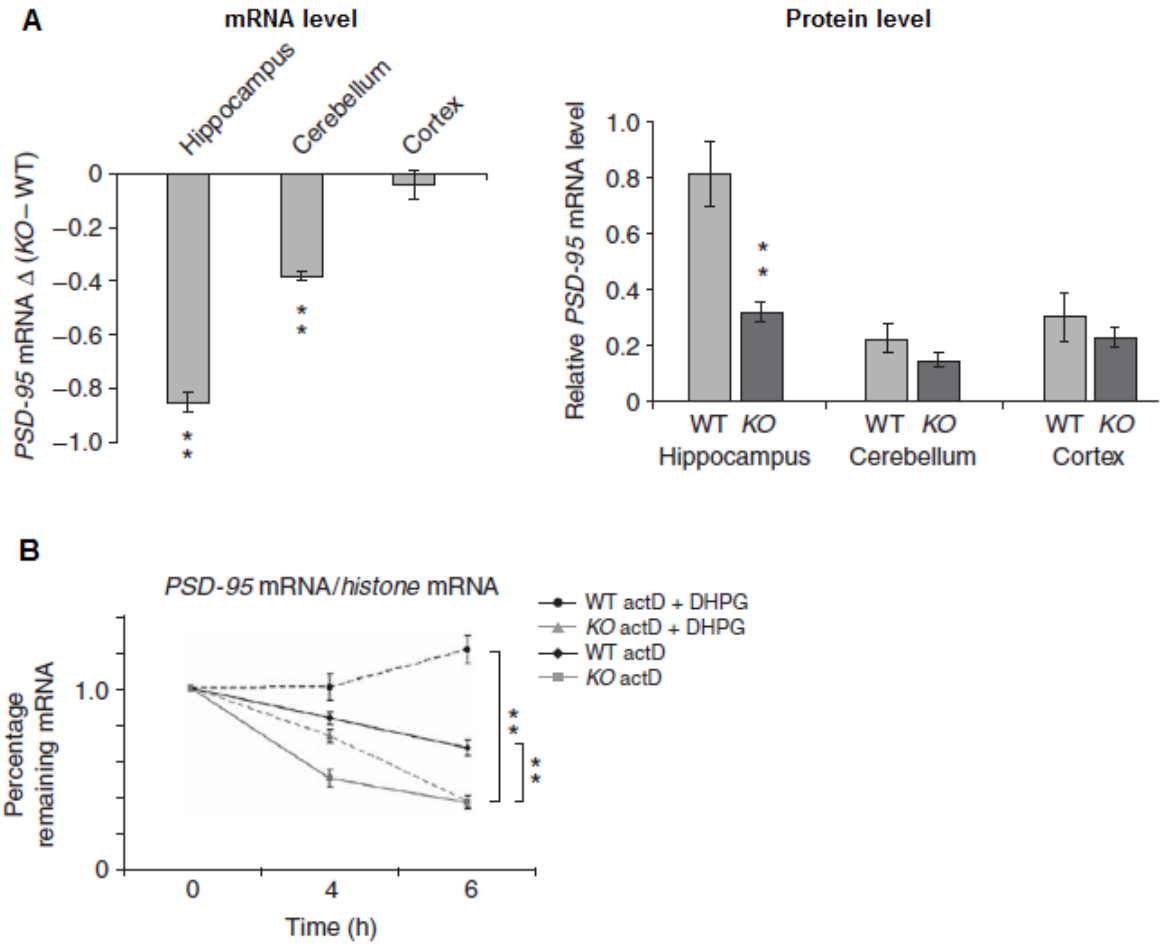


Figure 20. FMRP regulates the stability of PSD-95 mRNA in hippocampal cells through an activity-dependent mechanism. (a) PSD-95 mRNA levels in three different brain regions were estimated from three wild type (WT) and three FMR1 (mouse homologue of FMRP) knockout mice (KO). mRNA values in histogram are normalized to mRNA levels of histone H3 and shown as the difference between FMR1 KO versus WT. Protein values are shown relative to mRNA levels. (b) mRNA was isolated at the indicated times after actinomycin D (blocker of transcription) or actinomycin D + DHPG (agonist of mGluR1 and mGluR5) application to hippocampal neurons from WT or KO mice. **, P<0.01 for KO versus WT by Student's t-test.

© Information is adapted from Zalfa et al., 2007 [97]

Together, this data indicate that FMRP might be an important player in synaptic plasticity. The RNA binding protein interacts with many mRNA molecules, including PSD-95 which is a major component of the postsynaptic density. FMRP is thereby able to increase PSD-95 mRNA stability. Because PSD-95 is a high-ranking target of miRNAs, this drives the discussion on the involvement of these miRNA and FMRP in translation control of PSD-95 mRNA. It is tempting to postulate that PSD-95 is incorporated into an FMRP-mRNP through its interaction with FMRP at the soma of neurons and is subsequently transported in FMRP-

silent granules to the postsynaptic density of dendritic spines. At the dendritic spine, the composition of this granule or the interactions of PSD-95 could be changed upon a stimulus, such as neuronal activity, and thereby contribute to alter PSD-95 expression at the postsynaptic site. Interestingly, recent findings on both the interaction between FMRP and PSD-95 and the role of miRNAs indicate a more complex situation. First, PSD-95 and FMRP are localized in dendrites *in vivo*, but that the localization of PSD-95 was independent of FMRP[97]. That is, PSD-95 mRNA polysomal association was similar in wild-type and FMR1 knockout mice and PSD-95 mRNA was also dendritically localized in these mice[97]. This could indicate that the interaction with FMRP is not crucial for the incorporation of PSD-95 into mRNPs. Secondly, miR-125 target site on PSD-95 overlaps with G-quartets, which are the binding sites of FMRP[95]. This raises the possibility that the miRNA directly compete with FMRP to bind the mRNA strand at this location. However, conclusive evidence is yet lacking. It is therefore important to confirm and validate the predicted miRNA-PSD-95 mRNA interactions in a biological setting. Moreover, it would be interesting to investigate if these miRNAs affect PSD-95 mRNA stability in a one-inhibitor-one-target way or in a combinatorial way[101]. A second issue would be to determine how this approach would fit with respect to FMRP. These studies will help to shed light on the mechanism behind translational control of PSD-95 and will also help to understand the biological processes underlying synaptic plasticity since PSD-95 is a major component of the postsynaptic density.

9. Conclusions and outlook

9.1 Local translational control in synaptic plasticity: a crucial role for microRNAs

Local translational control has an enormous impact on synaptic plasticity, and thus memory, through the regulation of protein synthesis, which is important for the biological processes underlying this phenomenon. Precise control of translation is important, because either enhancement or reduction of the activity of specific signaling pathways can cause memory deficits[99]. MicroRNAs have emerged as key modulators of post-transcriptional gene regulators in a variety of tissues, including the brain. An interesting feature is that many miRNAs do not act as on-off switches, but rather fine-tune gene expression profiles[58]. Fine-tuning mediated by miRNAs is also reversible in many cases[12,59]. These features are in

agreement with the scenario of local protein synthesis in dendrites during synaptic plasticity, which is dependent on dynamic regulation in response to synaptic stimuli.

This literature study has, at the best of knowledge, reviewed all functional studies currently published, that provide evidence for a direct role of miRNAs in synaptic plasticity. The study focused on genes that contribute to two important features of dendritic spines, i.e. the actin cytoskeleton and the postsynaptic density. Firstly, CaMKII, which localizes to the PSD, expression levels are regulated in a miRNA-mediated manner. Neural activity mediates upregulation of CaMKII mRNA translation by proteosome-dependent degradation of MOV10. On the contrary, NMDA receptor inhibition can also upregulate CaMKII mRNA by the inhibition of miR-219 suppression of CaMKII. This indicates that miRNAs can act in response to small changes in the environment and further highlights that these non-coding RNAs fine-tune gene expression. Another component of the PSD is PSD-95, which is an important scaffolding protein in the PSD and is known to link receptors (mGluRs) to the cytoskeleton. This gene is predicted to be a high-ranking target for miRNAs. Although this statement lacks evidence from functional experiments, PSD-95 mRNA has been reported to be stabilized by FMRP, which is known to associate with the microRNA processing machinery. Therefore, it is likely that miRNA are involved in the regulation of PSD-95 and could thus contribute to synaptic plasticity. Secondly, this study discussed the role of miRNAs in the dynamic regulation of the actin cytoskeleton in dendritic spines. miR-138, miR-134, and miR-132 have been shown to regulate ATP1, Limk1, and p250GAP, respectively. A model by Schratt[12] was used to explain how these dendritic miRNAs can control spine growth by tuning the activity of antagonistic signaling pathways that regulate the actin cytoskeleton. Together, these findings illustrate the impact of miRNAs on genes localized in the PSD and involved in the actin cytoskeleton. They accentuate the crucial role of miRNAs in learning and memory, since both the PSD and the actin cytoskeleton are important features in synaptic plasticity.

9.2 Future prospect

Local control of protein synthesis seems to be a common site of action of neural miRNAs, as shown by the studies discussed in this paper. However, there is still no definite proof that miRNAs act in a localized way in the neurons of any organism *in vivo*. Currently, only neuronal cell models have been used to address this question. In addition, animal studies have only been able to discriminate between brain regions and, for example, not between

somatic and dendritic expression of miRNAs and the genes they affect. This has mostly been held back by technological problems. Many neural miRNAs are part of a large miRNA cluster which makes it difficult to create a single miRNA knockout. Secondly, most miRNAs function by subtly fine-tuning gene expression and subsequently causing small fluctuations in the cellular proteome which are not easily detected and do not lead to discernable phenotypes[12]. This is mainly the case when miRNA regulation occurs in discrete subcellular localities such as dendrites. These issues have negatively influenced the identification of miRNA-mediated effects on gene expression *in vivo*. Nevertheless, more awareness and knowledge on the subtle effects of miRNAs is leading to vast improvements in technology in this field of research. For example, improved bioinformatics algorithms help to more accurately predict low-affinity targets. Secondly, more sensitive detection techniques, such as pSILAC and LNA-based *in situ*, have been developed to allow for a more precise detection of miRNA-tuning effects and miRNAs-target interactions at a subcellular resolution. Therefore it is just a matter of time before the first *in vivo* studies will be published.

Research on miRNAs is starting to shed light on the pathways underlying their actions. Knowledge gained will also positively affect research on other noncoding RNAs, such as small-interfering RNAs (siRNAs), small nucleolar RNAs (snoRNAs), and piwi protein-interacting RNAs (piRNAs)[102]. Recently, many small noncoding RNAs have been discovered to function in development and disease, including those of the mammalian brain[103]. Although their role in synaptic plasticity remain elusive, it could be possible that they also exert their function in a localized manner in dendritic spines.

The ability of miRNAs to simultaneously fine-tune the expression of hundreds of target genes in response to extracellular cues, foresees a great potential for miRNA-based therapeutics for the treatment of neurological disorders of complex genetic origin, such as schizophrenia, mood disorders, and autism-spectrum disorders[12]. The challenge, however, lies in controlling potential off-targets effects, since siRNA are able to bind mRNA strands that harbor less than 100% sequence complementarity[104]. Another question that arises, is how successful treatments will be that intervene with microRNA pathways, which have the tendency to be highly sensitive to changes. Although these concerns do await the field of research, currently, the major goal is to understand how neural activity patterns are translated into lasting changes in synaptic connectivity that shape neural network functions and behavior.

The discovery of miRNAs as an extra layer of post-transcriptional control has thereby modernized our understanding of gene regulation. I believe that the findings discussed in this literature study are only just a glimpse of the role of miRNAs in synaptic plasticity. These RNA molecules have the ability to control the spatiotemporal dimension of local protein synthesis at the dendrite, which is thought to be a pivotal aspect of the underlying mechanism of synaptic plasticity. Therefore, I am convinced that future research on miRNAs will contribute significantly to understand the dynamic nature of neuronal communication, learning and memory and consequently shed light on the biological mechanisms underlying brain diseases, such as neuropsychiatric disorders. Taken together, this literature study highlighted the most recent developments in the regulation of mRNA translation at the synapse and strived to enrich our understanding of synaptic plasticity. Even though knowledge on the underlying mechanisms is still scarce, this study underlined that microRNAs play a pivotal role in synaptic plasticity by locally regulating gene expression.

10. References

1. Murre, J. M. & Sturdy, D. P. The connectivity of the brain: Multi-level quantitative analysis. *Biological Cybernetics* **73**, 529-545 (1995).

2. Stahnisch, F. W. & Nitsch, R. Santiago Ramon y Cajal's concept of neuronal plasticity: the ambiguity lives on. *TRENDS in Neurosciences* **25**, 589-591 (2002).

3. Yuste, R. & Bonhoeffer, T. Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annual review of neuroscience* **24**, 1071 (2001).

4. Van Spronsen, M. & Hoogenraad, C. C. Synapse Pathology in Psychiatric and Neurologic Disease. *Current neurology and neuroscience reports* **10**, 207-214 (2010).

5. Blanpied, T. A. & Ehlers, M. D. Microanatomy of dendritic spines: emerging principles of synaptic pathology in psychiatric and neurological disease. *Biological psychiatry* **55**, 1121-1127 (2004).

6. Lynch, M. A. Long-term potentiation and memory. *Physiological reviews* **84**, 87 (2004).

7. Kandel, E. R. The molecular biology of memory storage: a dialogue between genes and synapses. *Science's STKE* **294**, 1030 (2001).

8. Purves, D. *Neuroscience. Sunderland, MA* (2008).

9. Bliss, T. V., Collingridge, G. L. and others. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31-39 (1993).

10. Sutton, M. A. & Schuman, E. M. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell* **127**, 49-58 (2006).

11. Ule, J. & Darnell, R. B. RNA binding proteins and the regulation of neuronal synaptic plasticity. *Current opinion in neurobiology* **16**, 102-110 (2006).

12. Schratt, G. microRNAs at the synapse. *Nature Reviews Neuroscience* **10**, 842-849 (2009).

13. E.R. Kandel, J. S. a. T. J. *Principles of Neural Science* (2000).

14. E.R. Kandel, J. S. a. T. J. *Principles of neural sciences* (McGraw-Hill Companies, 2000).

15. Chen, X. et al. Organization of the core structure of the postsynaptic density. *Proceedings of the National Academy of Sciences* **105**, 4453 (2008).

16. Kennedy, M. B. Signal-processing machines at the postsynaptic density. *Science* **290**, 750 (2000).

17. Sheng, M. & Kim, M. J. Postsynaptic signaling and plasticity mechanisms. *Science* **298**, 776 (2002).

18. Lab, M. N. in .

19. Heupel, K. et al. Loss of transforming growth factor-beta 2 leads to impairment of central synapse function. *Neural Development* **3**, 1-16 (2008).

20. Ziff, E. B. Enlightening the Postsynaptic Density Review. *Neuron* **19**, 1163-1174 (1997).

21. Siekevitz, P. The postsynaptic density: a possible role in long-lasting effects in the central nervous system. *Proceedings of the National Academy of Sciences* **82**, 3494 (1985).

22. Sheng, M. & Hoogenraad, C. C. The postsynaptic architecture of excitatory synapses: a more quantitative view. *Biochemistry* **76**, 823 (2007).

23. Newpher, T. M. & Ehlers, M. D. Spine microdomains for postsynaptic signaling and plasticity. *Trends in cell biology* **19**, 218-227 (2009).

24. Hotulainen, P. & Hoogenraad, C. C. Actin in dendritic spines: connecting dynamics to function. *The Journal of Cell Biology* **189**, 619 (2010).

25. Pollard, T. D. & Borisy, G. G. Cellular motility driven by assembly and disassembly of actin filaments. *Cell* **112**, 453-465 (2003).

26. Cingolani, L. A. & Goda, Y. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. *Nature Reviews Neuroscience* **9**, 344-356 (2008).

27. Bramham, C. R. Local protein synthesis, actin dynamics, and LTP consolidation. *Current opinion in neurobiology* **18**, 524-531 (2008).

28. Steward, O. & Levy, W. B. Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. *Journal of Neuroscience* **2**, 284 (1982).

29. Ostroff, L. E., Fiala, J. C., Allwardt, B. and Harris, K. M. Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* **35**, 535-545 (2002).

30. Eberwine, J., Belt, B., Kacharmina, J. E. and Miyashiro, K. Analysis of subcellularly localized mRNAs using in situ hybridization, mRNA amplification, and expression profiling. *Neurochemical research* **27**, 1065-1077 (2002).

31. Moccia, R., Chen, D., Lyles, V., Kapuya, E. and others. An unbiased cDNA library prepared from isolated Aplysia sensory neuron processes is enriched for cytoskeletal and translational mRNAs. *Journal of Neuroscience* **23**, 9409 (2003).

32. Bramham, C. R. & Wells, D. G. Dendritic mRNA: transport, translation and function. *Nature Reviews Neuroscience* **8**, 776-789 (2007).

33. Kayali, F., Montie, H. L., Rafols, J. A. and DeGracia, D. J. Prolonged translation arrest in reperfused hippocampal cornu Ammonis 1 is mediated by stress granules. *Neuroscience* **134**, 1223-1246 (2005).

34. Vessey, J. P. et al. Dendritic localization of the translational repressor Pumilio 2 and its contribution to dendritic stress granules. *Journal of Neuroscience* **26**, 6496 (2006).

35. Knowles, R. B. et al. Translocation of RNA granules in living neurons. *Journal of Neuroscience* **16**, 7812 (1996).

36. Dynes, J. L. & Steward, O. Dynamics of bidirectional transport of Arc mRNA in neuronal dendrites. *The Journal of Comparative Neurology* **500**, 433-447 (2007).

37. Yoshimura, A. et al. Myosin-Va facilitates the accumulation of mRNA/protein complex in dendritic spines. *Current Biology* **16**, 2345-2351 (2006).

38. Tsokas, P. et al. Local protein synthesis mediates a rapid increase in dendritic elongation factor 1A after induction of late long-term potentiation. *Journal of Neuroscience* **25**, 5833 (2005).

39. Kapp, L. D. & Lorsch, J. R. The molecular mechanics of eukaryotic translation. *Biochemistry* **73**, 657 (2004).

40. Alberts, B. *Molecular Biology of the Cell* (Garland Science,).

41. Wells, S. E., Hillner, P. E., Vale, R. D. and Sachs, A. B. Circularization of mRNA by eukaryotic translation initiation factors. *Molecular Cell* **2**, 135-140 (1998).

42. Filipowicz, W., Bhattacharyya, S. N. and Sonenberg, N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nature Reviews Genetics* **9**, 102-114 (2008).

43. Nilsen, T. W. Mechanisms of microRNA-mediated gene regulation in animal cells. *TRENDS in Genetics* **23**, 243-249 (2007).

44. Pillai, R. S., Bhattacharyya, S. N. and Filipowicz, W. Repression of protein synthesis by miRNAs: how many mechanisms? *Trends in cell biology* **17**, 118-126 (2007).

45. Kim, J. et al. Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 360 (2004).

46. Molnar, A., Schwach, F., Studholme, D. J., Thuenemann, E. C. and Baulcombe, D. C. miRNAs control gene expression in the single-cell alga Chlamydomonas reinhardtii. *Nature* **447**, 1126-1129 (2007).

47. Zhao, T. et al. A complex system of small RNAs in the unicellular green alga Chlamydomonas reinhardtii. *Genes development* **21**, 1190 (2007).

48. Bartel, D. P. MicroRNAs:: Genomics, Biogenesis, Mechanism, and Function. *Cell* **116**, 281-297 (2004).

49. Griffiths-Jones, S., Saini, H. K., Dongen, S. and Enright, A. J. miRBase: tools for microRNA genomics. *Nucleic acids research* (2007).

50. Lee, R. C., Feinbaum, R. L. and Ambros, V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* **75**, 843-854 (1993).

51. Reinhart, B. J. et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature* **403**, 901-906 (2000).

52. Peters, L. & Meister, G. Argonaute proteins: mediators of RNA silencing. *Molecular cell* **26**, 611-623 (2007).

53. Lugli, G., Torvik, V. I., Larson, J. and Smalheiser, N. R. Expression of microRNAs and their precursors in synaptic fractions of adult mouse forebrain. *Journal of neurochemistry* **106**, 650-661 (2008).

54. Kim, V. N. MicroRNA biogenesis: coordinated cropping and dicing. *Nature Reviews Molecular Cell Biology* **6**, 376-385 (2005).

55. Jones-Rhoades, M. W., Bartel, D. P. and Bartel, B. MicroRNAs and their regulatory roles in plants. *Plant Biology* **57**, 19 (2006).

56. Vo, N. et al. From The Cover: A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. *Science's STKE* **102**, 16426 (2005).

57. Fiore, R. et al. Mef2-mediated transcription of the miR379--410 cluster regulates activity-dependent dendritogenesis by fine-tuning Pumilio2 protein levels. *The EMBO Journal* **28**, 697-710 (2009).

58. Karres, J. S., Hilgers, V., Carrera, I., Treisman, J. and Cohen, S. M. The conserved microRNA miR-8 tunes atrophin levels to prevent neurodegeneration in Drosophila. *Cell* **131**, 136-145 (2007).

59. Vasudevan, S., Tong, Y. and Steitz, J. A. Switching from repression to activation: microRNAs can up-regulate translation. *Science* **318**, 1931 (2007).

60. Vo, N. K., Cambronne, X. A. and Goodman, R. H. MicroRNA pathways in neural development and plasticity. *Current Opinion in Neurobiology* (2010).

61. Miller, S. et al. Disruption of Dendritic Translation of CaMKII [alpha] Impairs Stabilization of Synaptic Plasticity and Memory Consolidation. *Neuron* **36**, 507-519 (2002).

62. Ashraf, S. I., McLoon, A. L., Sclarsic, S. M. and Kunes, S. Synaptic protein synthesis associated with memory is regulated by the RISC pathway in Drosophila. *Cell* **124**, 191-205 (2006).

63. Brendza, R. P., Serbus, L. R., Duffy, J. B. and Saxton, W. M. A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein. *Science* **289**, 2120 (2000).

64. Banerjee, S., Neveu, P. and Kosik, K. S. A coordinated local translational control point at the synapse involving relief from silencing and MOV10 degradation. *Neuron* **64**, 871-884 (2009).

65. Kocerha, J. et al. MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. *Proceedings of the National Academy of Sciences* **106**, 3507 (2009).

66. Brocke, L., Srinivasan, M. and Schulman, H. Developmental and regional expression of multifunctional Ca2+/calmodulin-dependent protein kinase isoforms in rat brain. *Journal of Neuroscience* **15**, 6797 (1995).

67. Cammarota, M. et al. Participation of CaMKII in neuronal plasticity and memory formation. *Cellular and molecular neurobiology* **22**, 259-267 (2002).

68. Yoshimura, Y., Aoi, C. and Yamauchi, T. Investigation of protein substrates of Ca2+/calmodulin-dependent protein kinase II translocated to the postsynaptic density. *Molecular Brain Research* **81**, 118-128 (2000).

69. Allison, D. W., Chervin, A. S., Gelfand, V. I. and Craig, A. M. Postsynaptic scaffolds of excitatory and inhibitory synapses in hippocampal neurons: maintenance of core components independent of actin filaments and microtubules. *Journal of Neuroscience* **20**, 4545 (2000).

70. Chen, Y. & Ghosh, A. Regulation of dendritic development by neuronal activity. *Journal of neurobiology* **64**, 4-10 (2005).

71. Jaworski, J., Spangler, S., Seeburg, D. P., Hoogenraad, C. C. and Sheng, M. Control of dendritic arborization by the phosphoinositide-3'-kinase-Akt-mammalian target of rapamycin pathway. *Journal of Neuroscience* **25**, 11300 (2005).

72. Shi, S. H. et al. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* **284**, 1811 (1999).

73. Okamoto, K. I., Nagai, T., Miyawaki, A. and Hayashi, Y. Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. *Nature neuroscience* **7**, 1104-1112 (2004).

74. Matsuzaki, M., Honkura, N., Ellis-Davies, G. C. and Kasai, H. Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**, 761-766 (2004).

75. Zito, K., Knott, G., Shepherd, G. M., Shenolikar, S. and Svoboda, K. Induction of spine growth and synapse formation by regulation of the spine actin cytoskeleton. *Neuron* **44**, 321-334 (2004).

76. Siegel, G. et al. A functional screen implicates microRNA-138-dependent regulation of the depalmitoylation enzyme APT1 in dendritic spine morphogenesis. *nature cell biology* **11**, 705-716 (2009).

77. Linder, M. E. & Deschenes, R. J. Palmitoylation: policing protein stability and traffic. *Nature Reviews Molecular Cell Biology* **8**, 74-84 (2007).

78. Yeh, D. C., Duncan, J. A., Yamashita, S. and Michel, T. Depalmitoylation of endothelial nitric-oxide synthase by acyl-protein thioesterase 1 is potentiated by Ca2+-calmodulin. *Journal of Biological Chemistry* **274**, 33148 (1999).

79. Bhattacharyya, R. & Wedegaertner, P. B. G$$13 requires palmitoylation for plasma membrane localization, Rho-dependent signaling, and promotion of p115-RhoGEF membrane binding. *Journal of Biological Chemistry* **275**, 14992 (2000).

80. Kang, R. et al. Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. *Nature* **456**, 904-909 (2008).

81. Pittenger, C. & Kandel, E. R. In search of general mechanisms for long-lasting plasticity: Aplysia and the hippocampus. *Philosophical Transactions B* **358**, 757 (2003).

82. Fujioka, T., Fujioka, A. and Duman, R. S. Activation of cAMP signaling facilitates the morphological maturation of newborn neurons in adult hippocampus. *Journal of Neuroscience* **24**, 319 (2004).

83. Impey, S. et al. An activity-induced microRNA controls dendritic spine formation by regulating Rac1-PAK signaling. *Molecular and Cellular Neuroscience* **43**, 146-156 (2010).

84. Zhang, X. & Poo, M. Localized synaptic potentiation by BDNF requires local protein synthesis in the developing axon. *Neuron* **36**, 675-688 (2002).

85. Schratt, G. M. et al. A brain-specific microRNA regulates dendritic spine development. *Nature* **439**, 283-289 (2006).

86. Bamburg, J. R. P ROTEINS OF THE ADF/C OFILIN F AMILY: Essential Regulators of Actin Dynamics. *Annual review of cell and developmental biology* **15**, 185-230 (1999).

87. Meng, Y. et al. Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. *Neuron* **35**, 121-133 (2002).

88. Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C. and Schuman, E. M. Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* **30**, 489-502 (2001).

89. Takei, N., Kawamura, M., Hara, K., Yonezawa, K. and Nawa, H. Brain-derived neurotrophic factor enhances neuronal translation by activating multiple initiation processes. *Journal of Biological Chemistry* **276**, 42818 (2001).

90. Fukazawa, Y. et al. Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo. *Neuron* **38**, 447-460 (2003).

91. Li, F. & Tsien, J. Z. Memory and the NMDA receptors. *New England Journal of Medicine* **361**, 302-303 (2009).

92. Migaud, M. et al. Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396**, 433-439 (1998).

93. Fagiolini, M. et al. Separable features of visual cortical plasticity revealed by N-methyl-D-aspartate receptor 2A signaling. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 2854 (2003).

94. Vickers, C. A. et al. Neurone specific regulation of dendritic spines in vivo by post synaptic density 95 protein (PSD-95). *Brain research* **1090**, 89-98 (2006).

95. John, B. et al. Human microRNA targets. *PLoS Biol* **2**, e363 (2004).

96. Jin, P. et al. Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nature neuroscience* **7**, 113-117 (2004).

97. Zalfa, F. et al. A new function for the fragile X mental retardation protein in regulation of PSD-95 mRNA stability. *Nature neuroscience* **10**, 578-587 (2007).

98. De Rubeis, S. & Bagni, C. Fragile X mental retardation protein control of neuronal mRNA metabolism: Insights into mRNA stability. *Molecular and Cellular Neuroscience* **43**, 43-50 (2010).

99. Costa-Mattioli, M., Sossin, W. S., Klann, E. and Sonenberg, N. Translational control of long-lasting synaptic plasticity and memory. *Neuron* **61**, 10-26 (2009).

100. Bagni, C. & Greenough, W. T. From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. *Nature Reviews Neuroscience* **6**, 376-387 (2005).

101. Ivanovska, I. & Cleary, M. A. Combinatorial microRNAs: working together to make a difference. *Cell cycle (Georgetown, Tex.)* **7**, 3137 (2008).

102. Mattick, J. S. & Makunin, I. V. Small regulatory RNAs in mammals. *Human molecular genetics* **14**, R121 (2005).

103. Mehler, M. F. & Mattick, J. S. Noncoding RNAs and RNA editing in brain development, functional diversification, and neurological disease. *Physiological reviews* **87**, 799 (2007).

104. Jackson, A. L. et al. Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. *Rna* **12**, 1179 (2006).