Neurobiology of Learning and Memory 135 (2016) 3–12

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

# Role of Dicer and the miRNA system in neuronal plasticity and brain function

Anna Fiorenza [1], Angel Barco *

*Instituto de Neurociencias (Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas), Av. Santiago Ramón y Cajal s/n, Sant Joan d'Alacant, 03550 Alicante, Spain*

ARTICLE INFO

*Article history:*
Received 22 March 2016
Revised 5 May 2016
Accepted 5 May 2016
Available online 6 May 2016

*Keywords:*
miRNAs
Dicer
Neuronal excitability
Neuronal homeostasis
Learning and memory
Regulation of gene expression

ABSTRACT

MicroRNAs (miRNAs) are small regulatory non-coding RNAs that contribute to fine-tuning regulation of gene expression by mRNA destabilization and/or translational repression. Their abundance in the nervous system, their temporally and spatially regulated expression and their ability to respond in an activity-dependent manner make miRNAs ideal candidates for the regulation of complex processes in the brain, including neuronal plasticity, memory formation and neural development. The conditional ablation of the RNase III Dicer, which is essential for the maturation of most miRNAs, is a useful model to investigate the effect of the loss of the miRNA system, as a whole, in different tissues and cellular types. In this review, we first provide an overview of Dicer function and structure, and discuss outstanding questions concerning the role of miRNAs in the regulation of gene expression and neuronal function, to later focus on the insight derived from studies in which the genetic ablation of Dicer was used to determine the role of the miRNA system in the nervous system. In particular, we highlight the collective role of miRNAs fine-tuning plasticity-related gene expression and providing robustness to neuronal gene expression networks.



## 1. Introduction

MicroRNAs (miRNAs) are a class of endogenous, small non-coding RNAs of approximately 21–23 nucleotides that act as post-transcriptional regulators of gene expression. These molecules repress gene expression by base pairing to complementary sequences in the 3′-untranslated region of target messenger RNAs (mRNAs) interfering with their translation and/or promoting their degradation (Carthew & Sontheimer, 2009; Filipowicz, Bhattacharyya, & Sonenberg, 2008; Inui, Martello, & Piccolo, 2010; Nilsen, 2007).

The discovery of the first miRNAs occurred two decades ago during the characterization of *Caenorhabditis elegans* genes that control the timing of larval development revealing two small non-coding regulatory RNAs, known as lin-4 and let-7 (Lee, Feinbaum, & Ambros, 1993; Reinhart et al., 2000). In the following 20 years, we learned that miRNAs are not exotic players in worm biology. In fact, they are found across all multicellular organisms from plants to mammals, including humans, and contribute to the regulation of every aspect of organisms' physiology, from cell division and cell death to immune response. MiRNAs also play an essential role in the central nervous system (CNS). Indeed, the number of miRNAs expressed in the brain surpasses other organs and tissues, reflecting the cellular and transcriptional complexity of the CNS (Kim et al., 2004; Lagos-Quintana et al., 2002; Sempere et al., 2004).

Both the importance of miRNAs during brain development (Petri, Malmevik, Fasching, Akerblom, & Jakobsson, 2014; Sun & Shi, 2015), and the role of individual miRNAs regulating neuronal plasticity, learning and memory (Bredy, Lin, Wei, Baker-Andresen, & Mattick, 2011; Saab & Mansuy, 2014; Schratt, 2009b; Wang, Kwon, & Tsai, 2012), have been discussed in several recent reviews. Here, we will tackle the role of the miRNA system in the adult brain from a different angle by focusing on the insight provided from genetic studies in which miRNA biogenesis is selectively blocked in neurons as a result of interfering with the RNase Dicer that is essential for the maturation of most miRNAs.

## 2. What is the function of the miRNA system?

Most studies on miRNAs have focused on binary miRNA–target interactions. Indeed, hundreds of studies describe the role of many individual miRNAs in specific aspects of cell biology and organisms physiology. Paradoxically, there are also numerous gene knockout experiments that have shown that the elimination of individual miRNAs resulted in no apparent alteration in phenotype or yielded

* Corresponding author.
*E-mail address:* abarco@umh.es (A. Barco).
[1] Present address: UCSF School of Medicine, Department of Neurology, 675 Nelson Rising Lane, San Francisco, CA 94158, United States.

http://dx.doi.org/10.1016/j.nlm.2016.05.001
1074-7427/

modest results despite of the large number of target genes potentially affected (Ebert & Sharp, 2012). For instance, in *C. elegans* most individual miRNA mutants show no gross changes in phenotype (Miska et al., 2007). Similarly, null results occur in various mouse knockout strains generated to date, including those for miRNA-21, miRNA-210, miRNA-214, miRNA-206 and miRNA-143 (Ebert & Sharp, 2012). A possible explanation for this lack of effects in KO animals for a single miRNA could be that the mutant phenotype is visible only after acute miRNA deletion while the chronic loss can be somehow compensated (Smibert et al., 2011). Another possibility is that the effect derived from the lack of those miRNAs can be only observed upon application of certain internal or external stresses (van Rooij et al., 2007). A third non-exclusive hypothesis, coming from systems biology analyses, proposes that miRNA perturbations are well tolerated because the main function of many miRNAs would not rely on binary interactions with specific targets but on their collective role as part of a molecular buffer system that prevents undesirable fluctuations of proteins levels (Fig. 1) (Ebert & Sharp, 2012; Hornstein & Shomron, 2006; Li, Cassidy, Reinke, Fischboeck, & Carthew, 2009; Pelaez & Carthew, 2012). This model is consistent with the large functional redundancy of miRNAs families that share the same seed sequence and thereby target the same transcripts (Abbott et al., 2005). In addition, miRNAs of different "seed family" can also work together co-targeting the same gene or set of genes with overlapping function (Ebert & Sharp, 2012) (Fig. 1A). Most miRNA:target interactions presumably fall within complex regulatory networks with bifurcating pathways and feedback control that enable accurate response despite a defective node in the network. In this context, miRNAs would play a critical and collective role providing robustness to gene expression networks.

The conditional ablation of key enzymes involved in miRNA biogenesis, such as Dicer, represents a powerful approach to investigate the function of the miRNA system as a whole, as well as to determine the effect of impaired miRNA biogenesis in different tissues and physiological processes, including processes of great molecular complexity such as learning and memory in the adult brain.

## 3. MiRNAs biogenesis

MiRNAs biogenesis starts with the transcription of the miRNA-encoding gene by the RNA polymerase II (RNAPII) leading to the generation of a primary miRNA transcript (pri-miRNA). In the nucleus, the pri-miRNA is cleaved by the nuclear ribonuclease III (RNase III) enzyme Drosha, a RNase that selectively cleaves RNA hairpins that have a relatively large terminal loop (usually ~ 10 bp) (Zeng & Cullen, 2006; Zeng, Yi, & Cullen, 2005; Zhang & Zeng, 2010). The cleavage product is an approximately 70–100 nucleotides precursor miRNA hairpin (pre-miRNA) that is then translocated to the cytoplasm by exportin 5, a specific nuclear export factor that binds directly to pre-miRNAs, in a guanosine triphosphate (GTP)-dependent manner (Lund, Guttinger, Calado, Dahlberg, & Kutay, 2004; Yi, Qin, Macara, & Cullen, 2003). Once in the cytoplasm, the pre-miRNA is processed to a ~22 nucleotides intermediate miRNA duplex by the RNase III enzyme Dicer, which is the catalytic component of a cytoplasmic cleavage complex including many accessory proteins such as the transactivating response RNA-binding protein (TRBP), Loquacious/protein activator of PKR (PACT) and Fragile X Mental Retardation 1 (FMR1) (Kosik, 2006; Krol et al., 2007; MacRae, Zhou, & Doudna, 2007). The resulting miRNA duplex, referred to as the miR/miR* duplex, is composite of 5′-phosphorylated 2′-3′-hydroxylated RNAs that are later separated into the two RNA strands. One of these strands, named guide strand or 5p, interacts with the protein Argonaute (AGO) and is loaded into the RNA-inducing silencing complex (RISC) responsible for silencing target mRNAs by destabilization and translational repression (Huntzinger & Izaurralde, 2011; Pasquinelli, 2012), whereas the other strand, referred to as passenger strand, miR* or 3p, is usually (but not always) degraded (Guo & Lu, 2010; Mah, Buske, Humphries, & Kuchenbauer, 2010; Winter & Diederichs, 2013; Yang et al., 2011). The selection of the guide strand is not random; the strand with the less stable 5′-end is more likely selected as guide whereas the other strand, with a more stable 5′-end, serves as the passenger strand (Khvorova, Reynolds, & Jayasena, 2003; Schwarz et al., 2003). However, additional selection mechanisms can act on miR/miR* duplexes and, in many cases, both strands are functional (Okamura & Lai, 2008). The resulting mature miRNAs can found at multiple subcellular locations both in the nucleus and the cytoplasm (such as RNA granules, endomembranes and mitochondria) and can be secreted out of cell via exosomes, which suggests that their specific cellular localization is physiologically relevant (Leung, 2015). MiRNA-associated RISCs (*aka* miRISC) target specific mRNA by base pairing in its 3′ UTR, and this interaction leads to translational repression or mRNA degradation, respectively, depending on imperfect or perfect pairing between the miRNA seed sequence and the complementary site in the mRNA target (Fig. 2).

The steps for miRNA production described above are referred to as the canonical biogenesis pathway and accounts for the production of most miRNAs. However, several classes of miRNAs have been identified that maintain a length of ~22 nucleotides and the presence of a hairpin precursor (Berezikov et al., 2006) but bypass key steps of the canonical biogenesis pathway (Kim, Kim, & Kim, 2016). Both Drosha- and Dicer-independent pathways for miRNAs biogenesis have been discovered and are frequently referred to as non-canonical miRNA pathways (Miyoshi, Miyoshi, & Siomi, 2010). The sources of miRNAs in the Drosha-independent pathway can be mirtrons, short introns in protein-coding genes (Cai, Hagedorn, & Cullen, 2004; Kim, Han, & Siomi, 2009), small nucleolar RNAs (snoRNAs) (Ender et al., 2008; Saraiya & Wang, 2008), endogenous small interfering RNAs (endo-siRNAs) (Okamura & Lai, 2008), endogenous short-hairpin RNAs (endo-shRNAs) and transfer RNA (tRNA) precursors (Babiarz, Ruby, Wang, Bartel, & Blelloch, 2008). In contrast, only few miRNAs seem be able to bypass the Dicer-mediated cleavage. The only example that has been investigated in detail is miRNA-451 whose biogenesis occurs in a Drosha-dependent and Dicer-independent manner in which Ago2 is critically involved (Cheloufi, Dos Santos, Chong, & Hannon, 2010; Chong et al., 2010). A number of small RNAs derived from tRNAs in humans are also produced in a Dicer-independent and tRNase Z-dependent pathway (Cole et al., 2009; Haussecker et al., 2010). Although the precise function of these tRNA-derived small RNAs is unknown, it is possible to speculate that these RNAs could compete with miRNAs for Argonaute proteins, thereby regulating the abundance of the miRNA-loaded RISC complex (Miyoshi et al., 2010). In any case, more studies are still required for a definitive classification of miRNAs based on their biogenesis mechanism (Kim et al., 2016).

## 4. Regulation of miRNA function at synapses

In neurons, the production and action of miRNAs is regulated by neuronal activity (Siegel, Saba, & Schratt, 2011). This regulation is crucial for coupling miRNAs activity with synaptic function and, consequently, perturbations in the regulatory mechanisms can contribute to brain disorders. Regulation occurs at least at four levels: (1) Transcriptional: The promoters of many miRNAs have binding sites for activity-regulated transcription factors, such as