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Role reversal: the regulation of neuronal gene expression by microRNAs

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In a similar fashion to transcription factors, non-coding RNAs can be essential regulators of gene expression. The largest class of non-coding RNAs is the microRNAs. These ~22 nt double-stranded RNA molecules can repress translation or target mRNA degradation. There has been a surge of research in the past year stimulated by the recent availability of specialized techniques, both *in vitro* and *in silico,* for predicting and characterizing microRNAs. The accumulating evidence suggests that microRNAs are ubiquitous regulators of gene expression during development. The combined actions of microRNAs and transcription factors are able to tune the expression of proteins on a global level in a manner that cannot be achieved by transcription factors alone.

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Introduction

MicroRNAs (miRNAs) are a recently discovered class of non-coding RNAs (see glossary) that have turned out to be potent regulators of gene expression during development [1•,2]. First characterized in *C. elegans* more than ten years ago, miRNAs have since been found to be prevalent throughout metazoans [3–6]. Estimates indicate that 0.5–1% of metazoan genes encode miRNAs [7]. Analysis of conserved miRNA-like hairpin sequences suggests that mammalian genomes contain in excess of 1000 miRNAs. Recent studies have shown that miRNAs are involved in multiple pathways in a variety of organisms, including developmental transitions and neuronal patterning in worms, apoptosis and fat metabolism in flies, and regulation of exocytosis and modulation of hematopoietic lineage differentiation in mammals [8•,9•,10••,11]. The expression of a single miRNA can change the global gene expression profile of a cell by altering the levels of hundreds of transcripts [12••]. At this time, few miRNA targets have been validated, but phenotypic evidence suggests that miRNAs are essential determinants of lineage-specificity.

miRNAs are attractive regulators of developmental fate because of their abundance and their distinct patterns of spatio-temporal expression. The repertoire of miRNAs increases dramatically from worms to mammals (The miRNA registry; URL: http://microrna.sanger.ac.uk/sequences/index.shtml [13]). This increase in miRNA diversity is far greater than that seen for transcription factors, suggesting that miRNAs play a greater part in specifying vertebrate development. During central nervous system development, families of miRNAs appear to be expressed in temporal waves [14] that might restrict cells towards different subtypes. Research in the past year provides evidence for this model. The role of miRNAs extends beyond development, however, as these molecules are actively transcribed in the adult brain. It is possible, for example, that miRNAs play a part in the plasticity of adult neurons by regulating local protein synthesis in dendrites. Here, we discuss how current research supports a conserved role for miRNAs in proper differentiation and maintenance of the central nervous system.

Biogenesis

Genes encoding miRNAs can be grouped into classes according to their location within the genome. A large fraction of miRNA genes are found in intergenic regions, although most are found within the introns of coding and non-coding transcripts. Despite differences in their initial generation, the various classes of miRNAs share common steps in their biogenesis. Recently, a class of miRNAs has been identified, the members of which seem to reside in exonic regions of coding transcripts. However, analysis of their evolutionary conservation in relation to expressed sequence tag (EST) data suggests that these miRNAs might actually reside within 5′ untranslated regions (UTRs). Typically, miRNAs in intergenic regions are found in clusters and are generated from polycistronic, primary (pri)-miRNA precursors transcribed by RNA polymerase II [15]. Intronic miRNAs are usually expressed as part of a parent transcript, then subsequently spliced out and associated with exportin 5, saving them from degradation [16,17]. Additionally, miRNAs that reside in the introns of non-coding transcripts have been identified. In some of these instances, exonic regions of the non-coding transcript are degraded, whereas the intronic regions containing the miRNAs are saved in a manner similar to the processing of small nucleolar RNAs

Glossary

Double-stranded RNA oligos: Synthetic double-stranded RNA molecules that can be introduced into cells to mimic the action of endogenous microRNAs.

Microprocessor complex: An enzymatic complex, containing drosha and its co-factor DGCR8, that cuts out stem loops from long primary microRNA transcripts in the nucleus.

Non-coding RNA: A gene that contains no open reading frame but is actively transcribed and that has a biological function.

Stem loop: A lollipop-shaped secondary structure that is formed when a single-stranded nucleic acid molecule folds back on itself to form a complementary double helix (stem) topped by an unpaired loop.

Translational block: The primary mechanism through which microRNAs regulate gene expression in mammals. MicroRNAs target the RNA induced silencing complex to the 3′ untranslated regions of mRNAs, thereby blocking the translational machinery in an unknown manner. Through this mechanism, microRNAs are able to downregulate protein levels without affecting mRNA levels.

[18•]. Thus, the functional component of these genes appears to be the intron, rather than the exons.

The long primary miRNA transcripts are cleaved in the nucleus into shorter stem loops (see glossary) by a microprocessor complex (see glossary) that consists of an enzyme called drosha and its co-factor DGCR8 [19–23]. The resultant stem loops, designated precursor (pre)-miRNAs, are then exported to the cytoplasm by the exportin 5 machinery. Once in the cytoplasm, pre-miRNAs undergo further processing by Dicer, resulting in the mature 22nt RNA duplex. The duplex is then unwound and one strand is preferentially loaded onto the RNA induced silencing complex (RISC). miRNAs are able to target the RISC to the 3′UTR of specific mRNAs through imprecise base pairing with miRNA recognition elements (MREs). The arrangement of multiple MREs in the 3′UTR can be thought of as a barcode that is read by the set of miRNAs expressed in the cell to tune the translation of mRNAs to a certain level. The 3′UTRs of pri-miRNAs can also contain MREs, suggesting that they too are post-transcriptionally regulated [24]. In this way, the full complement of miRNAs expressed in a cell can determine cellular fate through regulation of groups of specific proteins [25••].

Worms

Studies of miRNAs initially focused on their ability to act as switches between developmental stages in *C. elegans*. Since then, other pathways have been identified in which the expression of a miRNA restricts cell fate. Perhaps the best example of such a pathway is the lateralization of chemosensory organs in *C. elegans* through the asymmetric expression of miR273 and *lys-6* [26••,27]. Two populations of chemosensory neurons exist in nematodes, the ASE right (ASER) and the ASE left (ASEL). The spatial expression of *C. elegans* miR273 is strongly biased to the ASER during development, resulting in the translational block of the transcription factor die-1, which contains two MREs for miR273 in its 3′UTR. The low expression of die-1, in turn, enables the default expression of ASER genes. In the ASEL, where miR273 expression is low, the increased levels of die-1 drive the expression of *lys-6*, another miRNA, which leads to expression of ASEL-specific genes. This pathway provides a paradigm for how 'coded' transcripts responding to the expression of miRNAs bring about a lineage-specific translational profile.

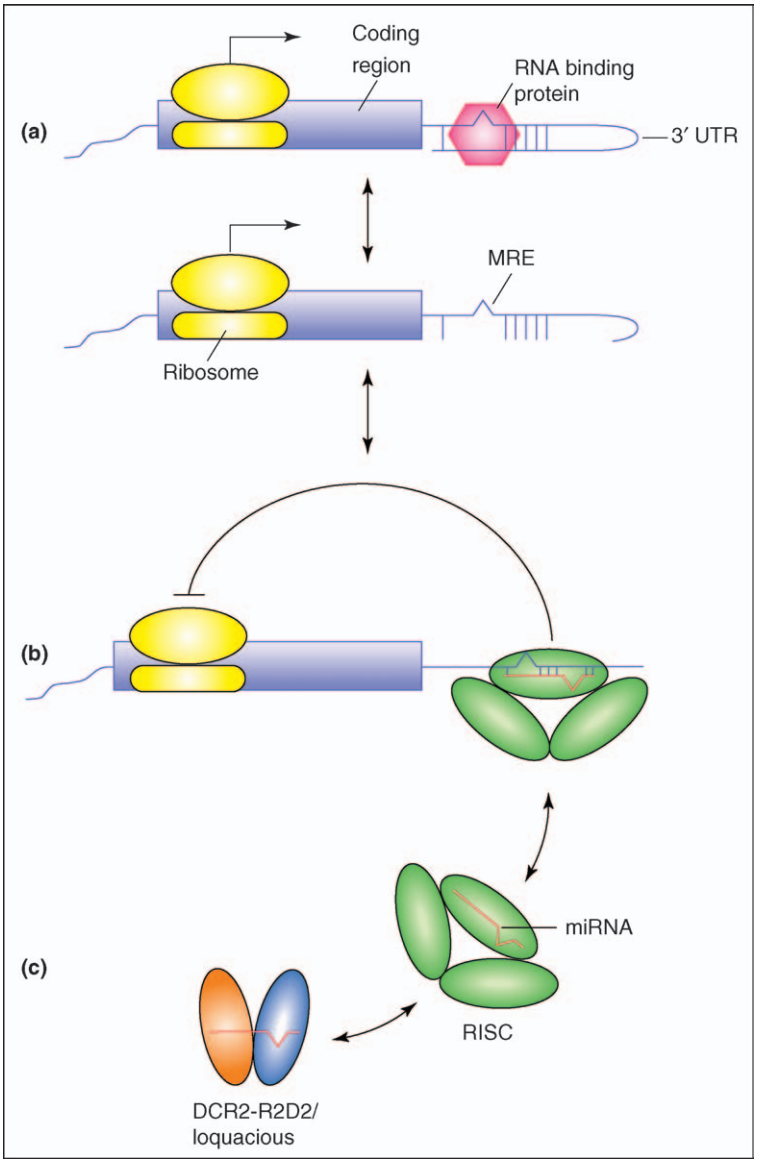
Flies

The association of miRNAs and RISC members with the fragile X mental retardation protein (FMRP) provides tantalizing evidence for the regulation of local dendritic protein synthesis by miRNAs [28,29]. FMRP is an RNA-binding protein that can regulate local protein synthesis in response to synaptic activity [30]. Recent evidence suggests that rather than regulating translation on its own, FMRP recruits mRNAs to the RISC complex where they can associate with miRNAs [31•]. This process might provide added specificity in the translational block (see glossary). This model is compatible with the idea that miRNAs could act as tags to mark synapses for changes in local protein synthesis. A synaptic tag must be present at spines, its actions should be reversible, and it must be able to interact with the cellular machinery to produce a lasting change in synaptic function [32]. miRNAs fit all three criteria. Numerous miRNAs have been cloned from dense neuronal fractions that contain polyribosomes, which are sites of local protein synthesis [33,34•,35]. Furthermore, RISC proteins and target mRNAs are also associated with polyribosomes. miRNAs could create reversible blocks in two ways (Figure 1). Either the RISC complex could become functional in response to synaptic activity (potentially through a phosphorylation event or the recruitment of different subunits) or mRNAs in the dendrite could undergo activity-dependent conformational changes that make their 3′UTRs accessible. The availability of the target site has a huge effect on the efficacy of RISC action [36]. Conversely, activation of the RISC through a second messenger system is equally plausible. The enzyme complex containing Dicer-2 (DCR-2) and R2D2 (the fly homolog of the *C. elegans* protein RDE-4) link the initiation and execution steps of RNAi by loading nascent siRNAs onto the RISC [37,38]. Loquacious, a recently identified miRNA equivalent of R2D2 [39,40], might similarly regulate initiation of miRNA-mediated silencing. Thus, the DCR-2–R2D2 complex could keep mature miRNAs sequestered from the RISC until an appropriate activation signal is received. The presence of an inducible regulator at the spine circumvents the need for a signal to travel from the activated spine to the soma and back again to tag a particular set of synapses.

Zebrafish

miRNAs are essential for proper developmental patterning in zebrafish. Zebrafish lacking Dicer lose the ability to process pre-miRNAs, which results in major patterning

Figure 1



Hypothetical model for activity-dependent repression of dendritic protein synthesis. **(a)** A depolarizing impulse induces a conformational change in the 3′ UTR of target mRNAs, perhaps by releasing a RNA binding protein (red). **(b)** The conformational change enables the RISC (green) to bind to the MRE in the target, resulting in post-transcriptional repression. **(c)** Pre-miRNAs are processed in the cytoplasm by a protein complex consisting of DCR-2 and R2D2 (shown in orange and blue). In the dendrite, a depolarizing impulse induces the transfer of the mature miR into the RISC, targeting the complex to the 3′ UTR of mRNAs and enabling (b) post-transcriptional repression. Both of these processes (a or c) could be regulated by phosphorylation. In either case, the change in local protein synthesis persists after the initial impulse, enabling miRNAs to act as activity-dependent tags.

defects, including improper brain morphogenesis. These defects can be rescued by injection of double-stranded RNA oligos (see glossary) representing mature sequences of the miR430 family [41•]. Interestingly, injection of the oligos rescued the major neural defects but not other patterning defects, such as ear or heart formation, indicating that miR430 has a tissue-specific role. Rescued embryos still displayed minor neuronal defects and delays in later developmental stages, suggesting obligatory roles for other miRNAs during development. Interestingly, a recent *in situ* hybridization study found that ~30% of zebrafish microRNAs were detected specifically in the CNS or in discrete populations of cells within the CNS [42••].

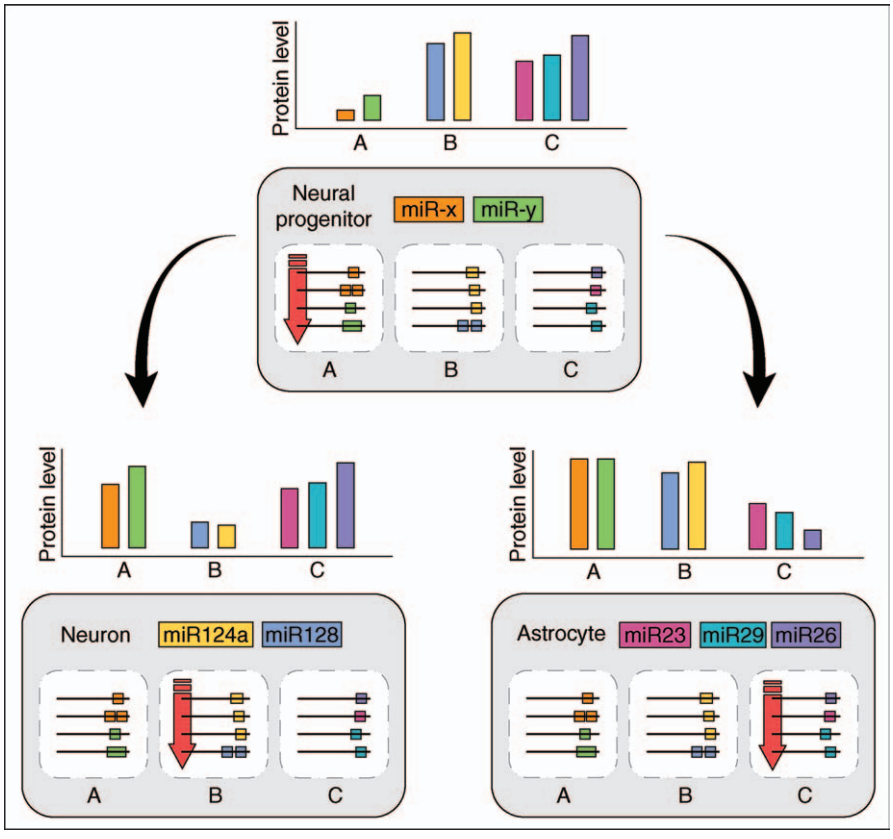
Mammals

The role of miRNAs in mammalian systems is unclear at this time, because only a handful of target interactions

have been validated *in vivo* [10••]. However, a model is emerging based on evidence that, similar to the situation in model organisms, mammalian miRNAs are involved in restricting the fate of progenitor cells. Evidence from microarray studies suggests that miRNAs can be grouped into families based on their expression at specific time-points. The switch from one family to another indicates a fundamental shift in the expression profile of a cell and its consequent restriction towards a particular lineage. Large subsets of miRNAs are enriched in the brain [34•,35,43–46], and the expression of certain families increases dramatically in parallel with cortical development [14,47,48]. A recent study examined the regulation of miRNA expression during neural cell specification [49]. Two miRNAs (miR124 and 128) were found to be active primarily in neurons, whereas others (miR23, 26, and 29) were preferentially active in astrocytes (Figure 2). Still other miRNAs (miR9 and 125) were expressed ubiquitously throughout the brain and it is likely that progenitor-specific miRNAs will also exist. These results suggest that miRNAs are expressed during differentiation of the brain to help tune the lineage-specificity of cellular protein levels. Further research is necessary to illuminate what these miRNAs are doing to regulate gene expression, but computational methods predict that many neural transcription factors are targets [50].

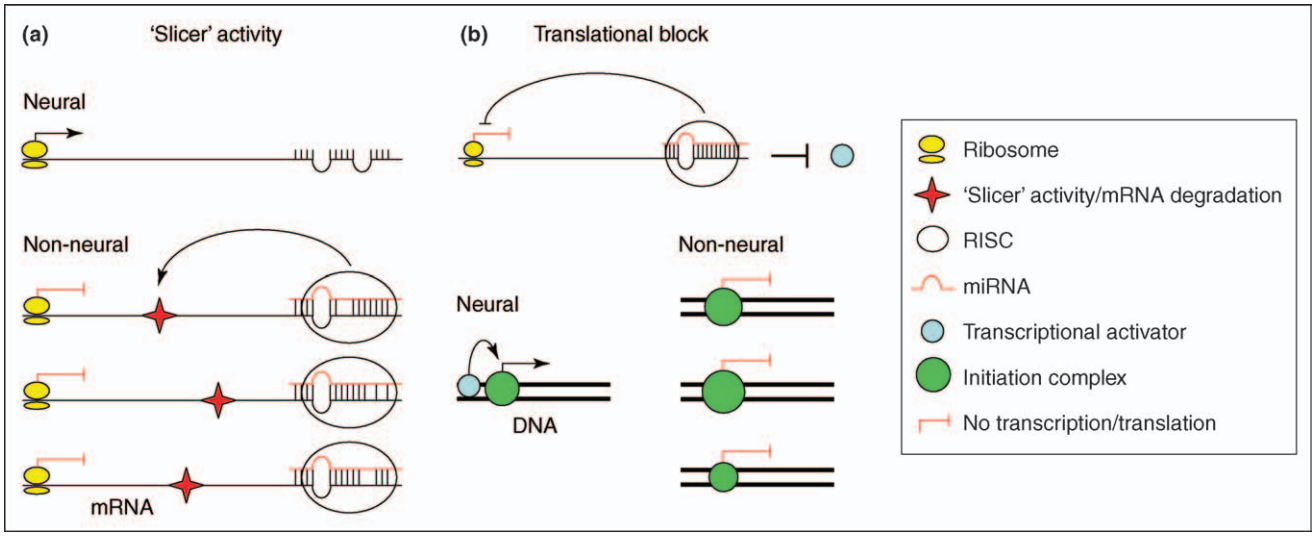
miR124a has emerged as an important regulator of brain morphogenesis. Not only is it one of the most abundant miRNAs in the brain but also the binding motif for mir124a is also one of the most prevalent MREs in the 3′UTRs of mammalian transcripts [51]. A recent microarray study observed down-regulation of more than 100 messages after injection of miR124a into HeLa (a human

Figure 2



During differentiation progenitor cells express families of miRNAs in a sequential manner, resulting in lineage-specific changes in the cellular protein expression profile. Different cell types express lineage-specific miRNAs that affect the translation of subsets of transcripts. Transcripts can be grouped into classes (represented by the boxes labeled A, B and C) based upon the arrangements of MREs in their 3′ UTRs. The arrangement of MREs in the 3′ UTRs of mRNAs function as a barcode that can be read by the set of expressed miRNAs to tune product levels. The translation of all transcripts that share a barcode (i.e. are in the same group) will be regulated similarly. As shown above, only the group containing the correct barcode will have its translation repressed by the currently expressed miRNA family. In this way, the expression of a miRNA family represses classes of proteins to tune translational levels to lineage-specific parameters. Neuron specific miRNAs (miR124a and miR128) and astrocyte specific miRNAs (miR23, miR29, and miR26) have already been identified. The progenitor-specific miR-x and -y miRNAs are hypothetical. As cells switch from a progenitor state to a mature state they loose expression of the progenitor miR family (miR-x and –y) and gain expression of one of the mature miR families (miR124a, 128 or miR23, 29, and 26). As a result of the turning over of the miR families, the cellular protein expression profile shifts towards that of the new cell type.

Figure 3



miR124 represses the translation of multiple targets by transcript cleavage. **(a)** miR124 directs cleavage of more than 100 transcripts containing putative MREs through targeting of the RISC. Transcripts subject to this cleavage ('slicer') activity are predominantly 'non-neuronal'. **(b)** An alternative mechanism would be for miR124 to cause a more conventional translational block of a crucial 'non-neuronal' transcriptional activator. The absence of this hypothetical activator could also cause a decrease in levels of 'non-neuronal' transcripts.

carcinoma cell line) cells [12••]. Interestingly, the new gene expression profile resembled that of a mature neuron, indicating that the expression of miR124a by itself is able to tune protein levels towards a restricted fate through degradation of mRNA containing the proper 'code' (Figure 3). Preliminary studies suggest that overexpression of the proneural transcription factors MASH1 and neurogenin promote differentiation of neural progenitor cells into neurons, in part by inducing expression of miR124a [52].

Conclusions

The miRNA field is still young and many questions remain unanswered. Much of the previous research has concentrated on the processing of miRNA precursors. Currently, efforts have shifted towards the identification of *in vivo* targets and the elucidation of regulatory networks. The articles discussed in this review suggest that miRNAs are essential regulators of CNS development. miRNAs might act not only to change cell fate initially but also to stabilize cell fate at a later stage by maintaining lineage-specific expression patterns. Furthermore, miRNAs might mark spines for differential translation in response to changes in synaptic activity. Additional studies will undoubtedly provide new insights into how these essential molecules contribute to the regulation of neuronal gene expression.

Update

The miR1 family was recently shown to be involved in the development of the murine heart [53]. Expression of the miR1 family must be timed correctly to achieve the proper protein expression profile conducive to differentiation of progenitor cells. Mis-expression of the miR1 family too early during development slows the differentiation of heart progenitor cells, leading to defects in heart formation.

A role for miRNAs in cancer is also beginning to be explored [54,55]. Microarray experiments show that cancer cells express a wide variety of miRNAs, with great diversity in miRNA expression among cancer types [56]. Interestingly, the subtype of a cancer cell can be determined by looking at its miRNA expression profile. The expression pattern of miRNAs in a cancer cell represents a molecular fingerprint that is unique to the developmental history of the cancer, much more so than the profile of expressed mRNAs.

The mechanism by which miRNAs block the translation of targets mRNAs is still poorly understood. However, new evidence suggests that miRNAs might repress translation by interfering with recognition of the m^7G cap by the translational machinery [57]. Furthermore, processing bodies (p-bodies), cytoplasmic sites of mRNA degradation, have been implicated in miRNA-mediated translational repression. Members of the RISC, cap-binding proteins, miRNAs, and repressed mRNAs have been shown to accumulate in p-bodies in an RNA-dependent manner [57–61]. It is unclear, however, if miRNAs direct messages to p-bodies in order to sequester them away from the translational machinery, or if repressed mRNAs are directed to p-bodies as a consequence of the miRNA-mediated translational block.

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