Dispatches

MicroRNA: MicroRNAs Reach out into Dendrites

A recent study has shown that miR-134, a brain-specific microRNA, is present in dendrites where it represses the local synthesis of the protein kinase LimK1; this is a novel form of translational regulation in dendrites and may have important physiological implications.

Hwan-Ching Tai[1] and Erin M. Schuman[2]

The latest breakthrough in our understanding of post-transcriptional gene regulation is undoubtedly the discovery of microRNAs (miRNAs). Current evidence suggests that miRNA genes are first expressed as long primary transcripts (pri-miRNAs) which undergo nuclear processing by the enzyme Drosha into hairpin precursors (pre-miRNAs). Once exported into the cytoplasm, pre-miRNAs are processed by Dicer into miRNAs, which enter RNA-induced silencing complexes, composed of the miRNA binding Argonaute protein and associated proteins, forming miRNPs. The pairing of a miRNP and a target mRNA causes translational inhibition or message degradation, or both [1]. miRNAs have been found in nearly every eukaryotic cell type, including neurons. Recent studies have shown that miRNAs are abundant in mammalian neurons [2], and the disruption of the miRNA pathway by Dicer mutation in fish was found to cause defects in neural development [3], illustrating the importance of miRNAs in neurons.

Protein synthesis in neurons occurs not only in the soma, but also in dendrites and axons. Compartmentalized protein synthesis in dendrites may be used to induce site-specific synaptic changes, and appears to be essential to several forms of synaptic plasticity [4]. It is therefore natural to ask whether miRNAs also regulate protein synthesis in dendrites. Several earlier studies have suggested that miRNAs are present in dendrites. Dicer has been reported to form a complex in dendrites with Argonaute and Fragile X mental retardation protein (FMRP) [5], which has been previously shown to complex with miRNAs, Dicer and Argonaute in neurons, and to act synergistically with Argonaute to regulate synaptogenesis [6].

Now Schratt *et al*. [7] have reported the first miRNA known to localize to dendrites, miR-134. Moreover, they have shown that miR-134 represses translation of the LIM-domain containing protein kinase 1 (LimK1) in dendrites to regulate spine size. Amongst other things, this is an important advance in our understanding of translational regulation in dendrites.

miRNA Functions in Dendrites

The new study by Schratt *et al*. [7] clearly demonstrates that miRNAs can affect protein synthesis in dendrites. First, a brain-specific miRNA, miR-134, was observed to localize in dendrites and synaptic sites. To search for the dendritic target of miR-134, they cleverly focused on a set of genes whose translation is reported to be induced by brain-derived neurotrophic factor (BDNF) [8]. These genes are likely to be dendritically translated, because BDNF is known to induce a form of synaptic plasticity that requires dendritic protein synthesis [9]. Amongst the BDNF-induced genes, *LimK1* turned out to be a *bona fide* miR-134 target. Schratt *et al*. [7] used multiple approaches to rigorously demonstrate that miR-134 can repress translation of LimK1. The miR-134 binding site turned out to be a single mismatched site in the 3′ untranslated region (UTR) of *LimK1* mRNA. Mutation of this site abolished the sensitivity of *LimK1* to miR-134 repression. Notably, the authors successfully delivered an anti-miRNA oligonucleotide (2′-O-methylated DNA) via penetratin-conjugation to derepress LimK1 translation. This introduces a new tool for perturbing miRNA functions in neurons.

To show that miR-134 and *LimK1* mRNA indeed interact in dendrites, Schratt *et al*. [7] used a GFP reporter strategy first developed to visualize the dendritic synthesis of Ca^{2+}/calmodulin-dependent protein kinase II-α subunit promoted by BDNF [10]. The 3′ UTR of *LimK1* mRNA was fused to the coding sequence of a GFP with a reduced half-life and diffusibility. When the miR-134 binding site in the 3′ UTR was mutated, GFP expression increased across proximal and distal regions of dendrites. This suggests that miRNA pathway is indeed functional in dendrites, supported by the recent discovery that Dicer and Argonaute proteins are present in dendrites [5]. Consistent with the known function of LimK1 in controlling actin filament dynamics in dendrites [11], overexpression of miR-134 reduced spine width, which could be rescued by overexpression of *LimK1*.

How Do miRNAs Inhibit Translation in Dendrites?

It is well established that when miRNAs and mRNAs are imperfectly paired, it causes translational arrest. Even if the pairing is perfect, such as when siRNAs are introduced, translational arrest has been shown to precede mRNA cleavage in neurons [12]. Translational inhibition is reported to be mediated by Argonaute family proteins [13], but the mechanism remains poorly understood. A recent study [14] has suggested that miRNAs can inhibit the initiation of translation;

however, this model cannot explain why many miRNPs co-purify with polyribosomes in neurons [2,15]. The study by Schratt *et al*. [7] provides interesting observations that may lead to a better understanding of this problem.

First, Schratt *et al*. [7] showed that BDNF can relieve miR-134's repression of *LimK1*, and the action involves mammalian target of rapamycin (mTOR) signaling. Hence, studying the effect of the mTOR pathway on miRNPs may reveal how miRNPs interact with the translational machinery. Furthermore, the authors mentioned an unpublished observation that miR-134 moves into polyribosomes upon BDNF stimulation. Does this imply the miRNP is inhibitory on translation before BDNF treatment, but becomes neutral or promotive afterwards?

A stimulatory role of miRNPs in translation has never been reported, but early studies of mammalian Argonaute suggested that it could promote translation initiation, long before the discovery of miRNAs. Mammalian Argonaute was first characterized in the rabbit reticulocyte lysate as initiation factor co-eIF-2A [16], which stabilizes the 40S preinitiation complex in the presence of mRNAs [17]. It was later renamed eIF2C, and cloned from the rabbit reticulocyte [18]. Rabbit eIF2C turns out to be 99% similar in sequence to human Argonaute 2. Since the discovery of Argonaute's role in the miRNA pathway, no study has reexamined its activity in the reticulocyte *in vitro* translation system, and it would be interesting to see if this activity is affected by miRNAs.

How Do miRNAs Enter Dendrites?

One of the most intriguing questions raised by the discovery of miRNAs in dendrites is how they actually get there. miRNAs in the cell always exist in the form of miRNPs, which are too large to reach distal dendrites by simple diffusion. It is not unreasonable to assume that there exists a mechanism to transport specific miRNAs into dendrites. Considering that miRNAs are short (~22 nucleotides) and bound to Argonaute, it is hard to envision that different miRNPs can be recognized by the transport machinery based on miRNA sequences. On the other hand, mRNAs known to localize to dendrites have been shown to possess dendritic targeting elements serving as 'zip-codes' [19]. It is conceivable that miRNPs can be co-transported to dendrites by binding to the dendrite-bound mRNAs.

Alternatively, pre-miRNAs (~70 nucleotide hairpins) might be selectively transported to dendrites, and locally processed by Dicer into miRNAs. It may be possible to include 'zip-code' sequences in the loop of such RNAs, but much less likely in the stem, because RNA duplex secondary structures are unfavorable for sequence recognition by proteins [20]. It is also possible that certain dendritic transport factors are deposited on pre-miRNAs during the nuclear processing step, directed by information encoded elsewhere in pri-miRNAs. If such transport factors remain bound during nuclear export of pre-miRNAs, they could be recognized by the dendritic transport machinery in the soma.

A third possibility is that pri-miRNAs are directly exported out of the nucleus, bypassing Drosha processing. pri-miRNAs could carry dendritic targeting elements just like mRNAs, and miRNAs could be locally generated in dendrites. There is currently no experimental evidence to support any of the aforementioned mechanisms, so much remains to be investigated.

Future Directions

The study by Schratt *et al*. [7] illustrates a new direction in understanding translational regulation in dendrites. Next, it should be possible to identify the full complement of miRNAs in dendrites using microarray technology. Elucidating the targets and functions of these miRNAs is likely to enrich our understanding of synaptic functions for years to come. The mechanism of translational inhibition remains a major conundrum, and it would be interesting to see if it differs in the soma versus the dendrite. How miRNAs localize to dendrites remains an open question, and future investigation might help redefine our knowledge of miRNA biogenesis.

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[1]Division of Chemistry and Chemical Engineering, California Institute of Technology, 164-30, Pasadena, California 91125, USA. [2]Division of Biology, Howard Hughes Medical Institute, California Institute of Technology, 114-96, Pasadena, California 91125, USA.

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Language Evolution: The Origin of Meaning in Primates

Research on alarm calls has yielded rare glimpses into the minds of our closest relatives. A new study suggests that primates monitor the effect alarm calls have on others.

Klaus Zuberbühler

Most primates vocalise when threatened by a predator. These alarm signals, after the Old Italian all' arme ('to arms'), have proved particularly valuable tools for examining cognitive processes in non-human animals. While call comprehension is relatively well researched in primates, very little is known about the social factors that influence call production [1]. A new study [2] by Dutch researchers has provided unexpected findings of almost Orwellian dimensions: When threatened by a predator, male Thomas langurs (*Presbytis thomasi*) do not stop producing alarm calls until every single other group member has responded with at least one alarm call. Males thus seem to monitor the calling behaviour of each group member and keep track of who has and who has not responded with alarm calls.

Alarm calls have attracted the attention of comparative psychologists, particularly those interested in the origins of language and semantic signalling [3]. The classic example is the vervet monkey alarm call system, in which individuals produce acoustically distinct vocalisations to several predators such as eagles, leopards or pythons. When monkeys hear another's alarm calls to a python, for instance, they respond by scanning the surrounding area for the snake they assume is present [4]. Another example is the West African Diana monkey, which produces one type of alarm call when encountering a leopard, and another one when faced with an eagle [5]. Most importantly, these calls indicate the biological class of the predator and are not simple responses to situational circumstances or perceived threat [6].

In primates, the ontogenetic process leading to the production of acoustically different call types is probably under strong genetic control. Infant vervet monkeys give eagle-like alarm calls to numerous flying objects, including storks and falling leaves. Only with experience do they learn to restrict call use to genuinely dangerous raptors [7]. It appears that primates innately conceptualise the world along particular criteria, and respond with species-specific vocal signals to them. Some researchers have thus questioned the relevance of primate alarm calls for understanding language evolution and human cognition [8]. How could genetically determined vocal behaviour be relevant for understanding the origins of language, a system based on arbitrary and socially learned vocal utterances?

The meaning of a term, it has been argued, is nothing more than its use [9]. In rainforests, the primary habitat of many primate species, primate biomass can reach several hundred individuals per square kilometre and, with visual contact strictly limited, vocalisations are the main mode of communication. As a result, primates mature in a rich world of sound with countless contingencies between vocalisations and events. But to what degree are primates capable of taking advantage of the surrounding semantic landscape? There is good evidence that primates not only behave adaptively to other individuals' alarm calls, but that they understand something about the causal structure of the events responsible for the various vocal signals produced by conspecifics and other animals [10–14].

The most striking difference between humans and other primates lies in the production abilities. Although non-human primates can engage in vocal tract filtering and produce acoustically complex structures, not unlike human vowels, they do not normally proceed to assemble them into larger, more complex strings [15]. Like other primates, humans produce a finite number of innately determined sound units, or 'phones', but they can