Releasing a tiny molecular brake may improve memory

Roberto Fiore and Gerhard Schratt*

Biochemisch-Pharmakologisches Centrum (BPC) Marburg, Institut für Physiologische Chemie, Philipps-Universität-Marburg, Marburg, Germany

*Correspondence to: Gerhard.schratt@staff.uni-marburg.de

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Understanding the molecular pathways underlying cognitive decline during neurodegenerative diseases is essential to design effective therapeutic strategies. In this issue of *The EMBO Journal*, Zovoilis *et al* identify miR-34c as a hippocampal-specific microRNA up-regulated in ageing and neurodegeneration. Cognitive deficits in aged mice and Alzheimer mouse models could be rescued by inhibiting miR-34c or by preventing the interaction between miR-34c and its target SIRT1. Thus, miR-34c might be both a marker for the onset of memory impairments and a promising target for therapeutic approaches in dementia.

By now it is well established that microRNAs (miRNAs) control a variety of processes during the development and function of the nervous system (Fiore *et al*, 2011). These small non-coding RNAs bind to imperfectly complementary sequences within cellular mRNAs, thereby blocking the production of the respective proteins. Each miRNA is able to modulate the expression of a number of specific mRNAs, which can reach up to a few hundreds. Since defects in gene expression programs are well known to cause memory impairments, it comes as no surprise that deregulated miRNA activity is increasingly considered in the context of cognitive decline and neurodegenerative disorders.

In this issue, Zovoilis *et al* (2011) sought to identify miRNAs that are important for hippocampal function and involved in the learning impairments that are associated with ageing and Alzheimer disease. The authors used a strategy to first identify the entire miRNA population expressed in the hippocampus of adult wild-type mice, and from there narrowing in on important functional candidates by comparing *in silico* target mRNA spectra and signalling pathways regulated by these miRNAs. Focusing on the hippocampus was a reasonable assumption, since a decline in hippocampal function is observed during ageing and the progression of Alzheimer's disease.

The authors identified 488 miRNAs expressed in the hippocampus, taking advantage of the recently developed high-throughput parallel sequencing technologies that allow precise quantification of a population of small RNAs with a wide dynamic range (Metzker, 2010). In order to select candidate miRNAs that could be important for hippocampal function, Zovoilis *et al* (2011) first compared their data set to published data on miRNA expression in unfractionated brain tissue again generated by deep sequencing (Chiang *et al*, 2010). Thereby, they could identify 12 miRNAs that were particularly highly enriched in the hippocampus. They further

A High miR-34c levels (aged mice, dementia)

B Low miR-34c levels (young mice, miR-34c inhibitor treatment)

Figure 1 (A) miR-34c levels are high in aged mice or dementia. This results in the reduced expression of memory genes, including the transcriptional repressor Sirt1. In a feed-forward mechanism, increased transcription of Sirt1 responsive genes, including miR-134, reinforces the repression of genes important for memory formation. (B) miR-34c activity is low in young mice or those treated with miR-34c inhibitory oligonucleotides. This results in the activation of memory genes, including Sirt1, which now can block the transcription of genes such as miR-134, thereby releasing other memory genes (Limk1, CREB) from repression.

narrowed this list by using both gene ontology analysis and miRNA target prediction with a list of genes that the same authors previously found to be induced in a hippocampal-dependent associative learning paradigm (Peleg *et al*, 2010). This elegant bioinformatics analysis pointed to miR-34c as the most promising candidate for further investigation among the 488 hippocampal miRNAs identified by deep sequencing. They further corroborated this hypothesis by showing that miR-34c levels are increased in three models of impaired hippocampal-dependent associative learning: aged mice, APPPS1-21 mice (a mouse model for Alzheimer disease), and human Alzheimer patients. The approach used by Zovoilis *et al* (2011) illustrates well how powerful a comparison of miRNA and mRNA data sets can be helpful in the challenging task of extracting relevant miRNA target interactions from the huge data sets generated with high-throughput approaches. Similar comparative approaches should also be helpful in the identification of miRNA-regulated pathways in different physiological and pathological settings (e.g. different forms of learning and neurodegenerative diseases).

To experimentally test the function of miR-34c, the authors combined fear conditioning training (a behavioural test for associative learning) with *in vivo* delivery of small oligonucleotides and could show that the increase in miR-34c levels is, at least partially, responsible for impaired memory formation. Using sophisticated cannula implantation, Zovoilis *et al* (2011) showed that delivery of so-called miR-34c mimics directly into the hippocampus of juvenile mice is sufficient to decrease memory formation. On the other hand, inhibition of miR-34c in both aged and APPPS1-21 mice with antisense inhibitors (so-called anti-miRs) partially restored memory formation. Most notably, the authors established a direct link between the histone deacetylase SIRT1, which was previously shown to be a direct target of miR-34c in non-neuronal cells, and miR-34c in memory formation. Therefore, they designed an elegant *in vivo* rescue experiment to show that Sirt1 down-regulation by miR-34c is necessary to attenuate memory formation. Target protectors are modified oligonucleotides designed to be complementary to the specific target sequence for a miRNA on a given mRNA. This design prevents binding of the miRNA in question exclusively to a single binding site, thereby relieving this target from repression without affecting potential other targets (Choi *et al*, 2007). Strikingly, when Zovoilis *et al* (2011) co-transfected target protectors for the Sirt1 mRNA together with miR-34c mimic, they were able to fully restore both Sirt1 protein levels and associative learning in the fear conditioning test, strongly suggesting that deregulated Sirt1 levels contribute to the miR-34c phenotypes. From a methodological point of view, the use of target protectors *in vivo* is one of the highlights of the work by Zovoilis *et al* (2011). It provides an elegant solution to the often problematic task of experimentally identifying

the physiologically relevant target mRNA for a specific miRNA function among the dozens or even hundreds of candidates. It is foreseeable that in the future, target protectors will become an important tool to determine the relative contribution of different targets to the complex phenotypes that one regularly obtains when miRNA function is perturbed.

Concerning the implications for neurological disease, this work strongly involves miR-34c in the cognitive decline associated with Alzheimer disease and ageing, and it suggests that this miRNA might be a potential therapeutic target. However, the lack of non-invasive technologies for the efficient delivery of miRNA-targeting oligonucleotides into the brain remains a major bottleneck. The paper further raises many interesting questions about the physiological role of miR-34c and other hippocampal enriched miRNAs in cognition. For example, it will be of special interest to define the molecular mechanism that leads to chronic miR-34c increase during ageing and Alzheimer disease. Interestingly, the authors showed that, while memory formation improves after miR-34c inhibition in young mice, the levels of this miRNA are transiently increased after fear conditioning. It is therefore plausible that different mechanisms regulate and are targeted by miR-34c in young and diseased/aged animals. Alternatively, a negative feed-back loop that keeps miR-34c activity within a physiologically tolerable range in young animals might not function properly in diseased or aged animals. Further experimental work will be necessary to discriminate between these two possibilities.

Last but not the least, the molecular mechanisms of memory regulation downstream of the miR-34c target Sirt1 remain to be clarified. Sirt1 has been shown to be important for associative learning and synaptic plasticity in mice (Michan *et al*, 2010). Interestingly, Sirt1 itself functions as a negative transcriptional regulator of miR-134, yet another miRNA that has been implicated in synaptic plasticity (Schratt *et al*, 2006; Fiore *et al*, 2009; Gao *et al*, 2010). It will be of interest to determine to what extent increased miR-134 levels in response to a reduction in Sirt1 are able to reinforce memory defects (Figure 1). Overall, the work by Zovoilis *et al* (2011) provides further evidence for the existence of multiple interconnected miRNA circuits that fine-tune gene expression during higher-order processing in the brain. Dissecting this network of neuronal miRNAs and their important targets will shed new light on the molecular mechanism underlying cognition and might provide novel therapeutic targets for the treatment of neurodegenerative and psychiatric disorders.

Conflict of interest

The authors declare that they have no conflict of interest.

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