New friends for Ago2 in neuronal plasticity

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MicroRNAs have emerged as central regulators of cellular homeostasis and increasing evidence suggests that they play a key role in neuronal plasticity. Major efforts are made to define microRNA networks and their targets in the brain. The mechanisms by which microRNA activity is regulated are, however, relatively unexplored. In this issue of *The EMBO Journal*, Störchel *et al* (2015) screened for proteins that affect microRNA function in neurons. They identify Nova1 and Ncoa3 as novel regulators of miRNA activity and demonstrate that both proteins are essential for neuronal plasticity in a microRNA-dependent manner.

See also: PH Störchel *et al* (September 2015)

With the availability of new sequencing technologies, it became clear that the non-coding part of the genome is not merely “junk” but represents a very complex pool of non-coding RNAs with a wide range of biological functions. To some extent, this finding did not come as a surprise considering that life on earth probably began with self-replicating RNA molecules (Orgel, 1993). In humans, the non-coding part of the genome by far exceeds the coding part and makes up 97% of all nucleotides. This is a record in the animal kingdom but even more exciting is the finding that the various cells of our body transcribe up to 80% of this non-protein coding genetic information (ENCODE Project Consortium, 2012). Albeit the function of this so-called dark matter of the genome is only beginning to emerge, the available data indicated that non-coding RNAs seemingly affect every aspect of

cellular function. The best studied group of non-coding RNAs are microRNAs, which are 19–22 nt long non-coding RNAs that act as key regulators of protein homeostasis. MiRNAs are expressed as precursors that are further processed until the mature microRNA is eventually loaded to the RNA silencing (RISC) complex which recognizes target RNAs via base pairing and catalyzes miRNA-mediated gene silencing or inhibition of protein translation. There is evidence that microRNAs may also regulate additional biological processes, but the best established function is still the orchestration of gene expression and protein homeostasis at the systems level, since one microRNA can target multiple mRNAs and *vice versa* one mRNA may be targeted by multiple microRNAs. Besides their basic biological function in cellular homeostasis, microRNAs are intensively studied in oncology. The first human microRNA let-7 was discovered in 2000 (Roush & Slack, 2008) and while, for example, microRNA-34c was discovered only in 2007, it was identified as a promising target in various cancers and the first clinical trial using microRNA-34c mimics has just been started, which is quite a remarkable development (Bader, 2013). More recently, microRNAs have been implicated with brain function (Im & Kenny, 2012) and with the pathogenesis of neurological and neuropsychiatric diseases (Fischer, 2014) where microRNAs emerge as potential biomarker (Rao *et al*, 2013) and as therapeutic target (Alvarez-Erviti *et al*, 2011). Many studies have measured the expression of microRNAs in various experimental systems and went on to show how selected microRNAs affect target gene expression, protein production, and cellular function. In contrast, there has

been astonishingly little research on the mechanisms that control the activity of microRNAs. How does cellular activity affect the activity of the RISC complex and thereby fine-tune the effect of microRNAs?

Such questions are not well studied, which is particularly true for the nervous system. On this basis, Störchel *et al* (2015) decided to screen for factors that alter microRNA activity using siRNA-mediated knockdown of 286 known RNA-binding proteins in mouse primary cortical neurons. After rigorous re-evaluation steps, they identify 2 novel proteins potentially implicated in the general regulation of microRNA-RISC-mediated gene expression, Nova1 and Ncoa3 that had so far not been implicated in microRNA function (Fig 1). The authors continue to show that both proteins are expressed in hippocampal neurons and demonstrate that knockdown of Nova1 or Ncoa3 affect the expression of selected genes that are regulated via microRNAs with well-established roles in synaptic plasticity. The authors provide evidence that Nova1 may be a more general activator of microRNA function, while Ncoa3 seems to affect only the activity of a subset of microRNAs. Both proteins, however, regulate the activity of miR-134, a microRNA intimately linked to the morphology of dendritic spines (Schratt *et al*, 2006), a cellular process essential for synaptic plasticity, and knockdown of Nova1 or Ncoa3 can rescue miR-134-induced reduction in spine volume (Störchel *et al*, 2015). Interestingly, Störchel *et al* (2015) could furthermore show that Nova1 directly interacts with Ago proteins, the key components of the RISC complex, and that recruitment of Nova1 to RNA is sufficient to facilitate microRNA activity, suggesting that Nova1

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Figure 1. Novel players regulating the activity on neuronal microRNAs.

(A) The RISC complex consists of multiple subunits that mediate RNA silencing. (B) As shown by Störchel *et al* (2015), Nova1 is a novel factor that associates with the RISC complex and thereby enhances miRNA activity linked to synaptic plasticity. (C) Ncoa1 controls the expression of Ago2 and thus indirectly affects the activity of the RISC complex, but, in contrast to the action of Nova1, this pathway seems to affect the activity of selected microRNAs linked to synaptic function.

operates downstream of Ago function. In contrast, Ncoa3 does not interact with Ago and in line with its role as a transcriptional co-activator microarray analysis revealed a number of differentially expressed genes upon Ncoa3 knockdown in cultured neurons, amongst them Ago2. In line with these data, the authors provide evidence that Ncoa3 regulates synaptic plasticity via the expression of Ago2, which in turn affects microRNA activity in a surprisingly specific manner.

In conclusion, the authors use an unbiased approach and identify two proteins as novel regulators of microRNA activity in neurons. These data are highly interesting since they open a relatively unexplored path to understand the role of microRNAome dynamics in brain function. It can be assumed that proteins which affect microRNA activity must be very tightly regulated. The new data on Ncoa3 and Nova1 shed some light on this process, but the complexity can only be imagined at present. Albeit Ncoa3 regulates Ago2 expression, this seems to only affect the activity of a specific subset of microRNAs. Additional screening approaches will be necessary to see whether this interpretation holds true at the

microRNAome level and it will then be crucial to understand why? Similarly, the evidence that Nova1 affects microRNA activity independent of the 3' UTR context simply by RNA binding in an Ago-dependent manner needs to be explored at the genome-wide level. The idea that specific microRNAs are affected in their activity via dynamically changing compositions of the RISC complex is very tempting and is certainly worth to be studied in the context of neuronal plasticity. While the current study is establishing the cell biological foundations, future work should seek to elucidate how proteins such as Ncoa3 and Nova1 affect the neuronal microRNAome in response to relevant stimuli such as neuronal activity, memory formation or during the pathogenesis of neuropsychiatric diseases. This may not only help to better understand the role of microRNAs in the nervous system but also lead to discovery of new drug targets.

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