A central question in the field of adult neurogenesis is whether adult-born neurons in the olfactory bulb (OB) become similar to the developmentally generated neurons or if they form cells with unique properties. Although several physiological and morphological differences have been identified, no molecular markers have been found. MicroRNA (miRNA) are endogenous small non-coding RNA that negatively regulate large networks of mRNA. In a recent study, we have investigated the miRNA activity in OB interneurons. We found microRNA-125 (miR-125) to be specifically absent in developmentally generated OB interneurons, whereas it is highly expressed in the adult-born OB interneurons. Thus, miR-125 can be used as a molecular marker, the first one to our knowledge, to distinguish these cell populations from each other, strengthening that there is divergence between the two populations. This finding reveals novel information regarding adult neurogenesis, temporal differences between OB interneurons as well as it highlights the role of miRNA in cell specification.

The mammalian olfactory bulb (OB) consists of two interneuron subpopulations of different temporal and spatial origin, residing side-by-side in the OB. For simplistic reasons we have termed them adult-born and developmentally generated interneurons. Adult OB neurogenesis starts in the early postnatal period, and is primarily restricted to the subventricular zone (SVZ) along the lateral ventricles. Newborn progenitor cells generated in the SVZ migrate along the rostral migratory stream to differentiate into interneurons in the OB. The population of adult-born interneurons in the OB is replaced over time; every day thousands of new interneurons are integrated into the circuitry in the rodent brain. However, the majority of OB interneurons are generated during development from local progenitor cells during embryogenesis, and these developmentally generated interneurons are maintained during the lifetime. The adult-born and the developmentally generated interneurons can be distinguished from each other by several physiological and morphological differences. Adult-born interneurons integrate primarily into the granule cell layer and become specific subtypes of interneurons. In addition, they display distinct electrophysiological characteristics, including stronger sodium currents as well as stronger immediate-early-gene response to novel odors, compared with developmentally generated interneurons. Still, no molecular marker distinguishing the cell types has been described.

MicroRNA (miRNA) are small endogenous non-coding RNA, that act posttranscriptionally to regulate gene expression by binding to complementary sequences of mRNA, typically in the 3′-untranslated region, to inhibit the formation of protein. The role of miRNA in controlling vital cellular functions and specifying cell fate is becoming increasingly evident. In a recent study we therefore investigated the activity of the highly expressed miR-125 in the brain. We took advantage of lentiviral sensorvectors to visualize...
miR-125 activity. The sensor vector consists of a ubiquitous expressed promoter driving the expression of green fluorescent protein (GFP) linked to perfectly complementary binding sites of miR-125 (Fig. 1A). If miR-125 is present and active in the cell, the vector mRNA is translated into protein and GFP is expressed. On the contrary, if miR-125 is present it binds and suppresses the transgene mRNA, hence, no protein is formed and no GFP is expressed. (C) A coronal and sagittal OB section demonstrates lack of miR-125 activity in interneurons in the OB. (D) Percentage of miR-125 negative cells generated over time. BrdU was injected to the transgenic sensor mice at different time points (ranging from E17.5 to adult) and the number of BrdU/GFP double positive cells in the OB was counted. (E) Adult-born interneurons labeled with Cherry lacks GFP-expression. A Cherry-expressing lentivirus was injected into the SVZ of P3 mice pups to target the neural stem/progenitor cells. The mice were sacrificed 8 wk after injection.

We found that most cells of the brain express miR-125 (seen as GFP-negative), including neurons, glia, ependymal cells, and cells of the neurogenic niches. However, a discrete population of cells in the OB lacked miR-125 activity, which was visualized by their expression of GFP (Fig. 1C). These cells had a neuronal morphology and did not extend projections outside of the OB; consequently, we concluded these cells to be interneurons. We found that miR-125-negative interneurons in the OB are formed during development; they are born around birth and never in the adult animal (Fig. 1D and E). In order to understand why developmentally generated interneurons specifically lack expression of miR-125, we identified mRNAs targeted by miR-125 in the brain, which revealed target genes implicated in synaptic function and plasticity (Fig. 2A). Performing loss-of-function experiments, we could show that inhibition of miR-125 in the OB resulted in adult-born interneurons with enhanced dendritic morphogenesis, i.e., longer dendrites and a more complex dendritic tree. They also displayed earlier functional integration. Since developmentally generated OB interneurons lack miR-125 activity, these cells have a more elaborate dendritic tree and mature faster than the adult-born interneurons (Fig. 2B).

We have used the approach of transgenic sensor mice to investigate other highly expressed miRNAs in the brain, such as miR-124 and miR-9,15,16 and these miRNAs show equal expression levels in adult-born vs. developmentally generated interneurons. Thus, the difference in miR-125 expression between
adult-born and developmentally generated OB interneurons contributes to the different transcriptional profiles between these two populations. Having found that miR-125 suppresses genes involved in dendritic morphology and synaptic function, it is tempting to speculate if and why developmentally born interneurons have an enhanced plasticity. The advantage of having a more elaborate dendritic tree with longer and more branched dendrites would most likely be to increase the integration and communication with the continuously incoming adult-born interneurons. Thus, our current study demonstrates that miR-125 exerts a functional difference between adult-born and developmentally generated interneurons, resulting in developmentally generated interneurons having a more complex dendritic tree and therefore are likely to have an increased probability for plasticity. However, in order to test the magnitude of differences in synaptic plasticity, extensive electrophysiological recordings would be required. It is worth noting that some studies have suggested that the adult-born interneurons are more plastic in order to favor the integration into the existing circuitry.\textsuperscript{17}

The maturation of the OB interneurons generated during development only takes a couple of days, while the adult-born interneurons mature over a period of several weeks.\textsuperscript{18} This large temporal difference of maturation indicates that miR-125 might act as a heterochronic gene that regulates the timing of newborn neurons. This hypothesis is favored by the fact that inhibition in adult-born interneurons causes the transcriptome and phenotype to resemble that of developmentally generated interneurons. Indeed, our results show that inhibition of miR-125 result in faster maturation and integration of the adult-born interneurons. In addition, the miR-125 homolog lin-4 has been demonstrated to act as a heterochronic gene during development of \textit{C. elegans}.\textsuperscript{19} However, to fully understand the role of miR-125 as a heterochronic gene, it would be necessary to identify upstream regulators of expression.

Why is it that an miRNA and not a protein that distinguishes the temporally different interneuron populations? miRNAs play an essential role in the specification of neuronal subtype. They can act at various stages of neural development and neuronal maturation and have emerged as key transcriptional regulators that fine-tune and buffer gene expression, which contributes to gene expression robustness.\textsuperscript{20,21} One single miRNA can have hundreds of target genes affecting many essential pathways at multiple levels and regulate vital cellular functions resulting in profound impact on the transcriptome.\textsuperscript{22} The absence or presence of an miRNA can therefore specify the cell type, since a large number of target genes would be up- or downregulated, respectively. For example, miR-9 has been shown to be specifically absent from microglia\textsuperscript{16} and overexpression of miR-124 on various cell-types in vivo and in vitro, directs the cells into a neuronal state, whereas inhibition of miR-124 in neural stem cells directs a gliogenic fate.\textsuperscript{13,15} Importantly, cross-talk between several highly expressed miRNAs might be important in the specification of cell fate, as well as cross-talk between other epigenetic pathways.\textsuperscript{23}

Our recent study provides the first molecular marker allowing us to distinguish developmentally generated and adult-born OB interneurons from each other.\textsuperscript{14} This finding supports a divergence between the two populations and that

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**Figure 2.** miR-125 controls a large set of genes related to synaptic functions and dendritic morphogenesis. (A) Illustration of Gene Ontology classes enriched in miR-125 target genes, identified using a previously published data set generated by argonaute HITS-CLIP,\textsuperscript{24} a technique identifying miRNA-mRNA interactions in the cell. (B) Schematic drawing of the temporal subpopulations of interneurons in the OB. The green cells represents developmentally born interneurons, which do not express miR-125 and have a more elaborate dendritic tree, compared with adult-born interneurons, shown in black.
the temporal different interneuron cell types display different transcriptionists. We demonstrated that miR-125 controls genes implicated in synaptic function and plasticity and the absence of miR-125 therefore allows the developmentally generated OB interneurons to obtain a more elaborate dendritic tree, a property that most likely supports the integration of the constant addition of adult-born interneurons. Thousands of interneurons reach the olfactory bulb of adult rodents every day, but the functional effect of this process remains elusive. Our findings have increased our knowledge of how the complex circuitry of interneurons in the OB is generated and challenges future studies exploring new molecular markers as well as the role of adult-born vs. developmentally generated interneurons of the OB.

Disclosure of Potential Conflicts of Interest

The authors declare no competing financial interests.

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