Single-cell detection of miRNAs in developing vertebrate embryos after acute administration of a dual fluorescence reporter/sensor plasmid

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1) Introduction

MicroRNAs (miRNAs) are a class of short (21-22 nucleotides) endogenous non-coding RNAs, from plants to mammals, which regulate the translation and stability of target mRNAs. miRNAs are emerging as major players in the control of development and of several physiological and pathological processes. A major step towards understanding of miRNA function in the establishment of systems that allow detection in vivo and in vitro. Reporter gene fusions to a sequence complementary to the candidate miRNA, leading to the RNAi-mediated hybridization using an LNA probe for miR-124a on cryosection of E11 mouse telencephalon. The tandem cassette to miR-9, a tandem cassette to miR-124a which is known to be expressed in the developing electroporated into the left telencephalic neuroepithelium (A-J). After fluorescence showed that in the case of the mutated DFRS miR-9 plasmid, both fluorescent proteins were expressed in the E14 telencephalon (B, the head of E14 mice (N), which yield superior cellular resolution as compared to whole-mount preparations. Indeed, miR-9 was found to be expressed in utero showed that the DFRS miR-9 plasmid was functional in terms of GFP-reporter plus mRFP-sensor expression, and demonstrated the absence of mRFP-sensor fluorescence in the brain (H) and neuroepithelial cells (J).

2) The dual fluorescence reporter/sensor (DFRS) plasmid

The “sensor principle” is based on the following modified version of the “sensor” miRNA transcript 3' UTR; pA, containing a site for the corresponding miRNA sequence (black) in the mouse embryos. Bottom: miRNA sensor sequences (black) and the corresponding miRNA sequences (red); mutated miRNA sequences in the same expression are in shown in red.

3) In vivo visualization of mir-1 dynamics during skeletal muscle development in zebrfish

We tested a DFRS plasmid for miR-1, which has been shown to be expressed in developing skeletal muscle of zebrafish (1). The DFRS plasmid was electroporated into the embryos of zebrafish at 24 hpf, and kept for 1, 2, 3, or 4 days. The expression of the DFRS plasmid was monitored by whole-mount RNA in situ hybridization using an LNA probe for miR-124a on E11 mouse telencephalon. The expression of the DFRS plasmid was confirmed by whole-mount RNA in situ hybridization with a DFRS plasmid for miR-124a, which is known to be expressed in utero showed that the DFRS miR-124a plasmid was functional in terms of GFP-reporter plus mRFP-sensor expression, and demonstrated the absence of mRFP-sensor expression in the brain (H) and neuroepithelial cells (J).

4) The silencing of the sensor is not affected by DFRS plasmid concentration and has a long-lasting stability of expression in vivo

miRNA detection is not impaired by variation in DFRS plasmid copy number. DFRS miR-1 plasmid was injected into single blastomeres of 2-4-cell stage zebrafish embryos at a concentration of 0.1, 0.5, 1, 2 or 4 nM DFRS plasmid, followed by analysis of GFP (A, D) and mRFP (B) fluorescence in muscle fibers at 22 hpf (E) and 33 hpf (F). Arrows indicate muscle fibers showing low-level GFP-expression but no longer mRFP-fluorescence. Observation of embryos: caudal, right; ventral, down. Scale bars = 10 μm.

5) Detection of mir-9 in single neuromeric cells of mouse embryos after acute tissue targeting of DFRS plasmids

We extended the DFRS approach to another miRNA, mir-124a, which is thought to be expressed specifically in neurons (1). During the development of the vertebrate nervous system, miR-124a is involved in their migration to the neuronal layer and can be observed in their neuronal progenitor cells (1). We therefore modified the DFRS plasmid by adding a plasma membrane localization signal to the GFP-reporter (pmGFP) in order to distinguish newborn neurons in the vertebral column from the neural precursors in the neural tube. Indeed, when the DFRS plasmid was electroporated at 2 hpf, neurons born in the neural tube (hpf), both the ubiquitous miR-124a in the vertebral column (A-C, D-E, and the neurons born in the vertebral column (black) and the corresponding miRNA sequences (red) in the mouse embryos. Bottom: miRNA sensor sequences (black) and the corresponding miRNA sequences (red); mutated miRNA sequences in the same expression are shown in red.

6) mir-124a is expressed in zebrafish muscle development but also in neuronal progenitors

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Conclusions and perspectives

The present experimental approach of acutely administering a DFRS plasmid for a specific miRNA is a convenient method to detect important post-transcriptional regulators with single-cell resolution and to monitor their dynamics in vivo. Our approach, though potentially applicable to a wide variety of species (including those for which transgenic lines has not been established), circumvents the need to generate transgenic organisms, which is much more labour-intensive for mice (2) than for fish (3). Moreover, the topical administration of DFRS plasmids, followed by their directed electroporation (3, 4), provides a simple approach to study a specific miRNA in the tissue and cell lineage of interest.

The mechanism of action of our system is based on RNAi, which is an efficient process, mediating the degradation of the sensor mRNA. We have shown in vivo that very low levels of miR-124a are enough to completely degrade high levels of sensor transcriptions, thus allowing the detection of low amounts of miRNAs. Moreover, RNAi is sequence-specific and we have shown that mutations of one nucleotide in the conserved sequence of a miRNA target can prevent the sensor miRNA degradation. We can therefore assume that our system is more sensitive and specific than microarrays.

Our DFRS plasmid can be easily used for the preparation of libraries, thereby allowing fast-read out of either in vivo or in vitro screenings.

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