BTX Applications

Tech-Trends

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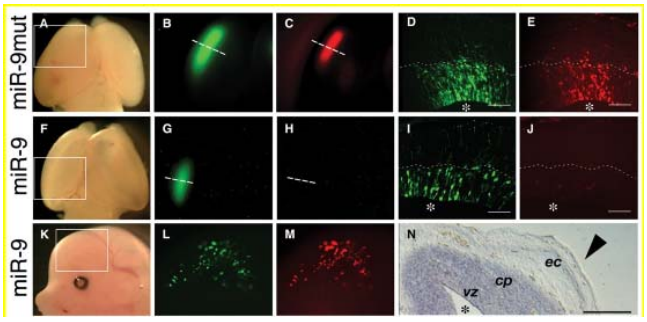
Single-cell detection of miRNAs in embryos

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

MicroRNAs (miRNAs) are 20-25 long nucleotides which play a role in regulating the stability and translation of target messenger RNAs in a wide variety of organisms. (1) To study the function that miRNAs play during development and establish a technique that allows these miRNAs to be detected and monitored during cell fate changes in vivo is crucial (1). Overcoming limitation associated with the previous method was achieved with a reliable and high efficient method of detecting the miRNAs on a cellular level through the use of in utero electroporation. (1) The detection of tissue specific miRNAs were studied by acutely administering a DFRS (Dual-fluorescent (GFP)-reporter/monomeric red fluorescent protein (mRFP)-sensor) plasmid for specific miRNAs into mouse embryonic tissue and applying a series of direct pulses to the tissue. (1) The uptake of the plasmid was directed to specific regions of the developing brain or ectoderm by the orientation of the electrical field. (1) After *in utero* development for 24 hours the tissues were analyzed and the tissue specific miRNAs were positively observed as DFRS plasmid being co localized in the cells. (1) The approach of acutely administering a DFRS plasmid to detect miRNAs and study the posttranscriptional regulators with a single cell resolution in vivo has been positively demonstrated here, and this approach can be applied to a wide variety of species simply and easily. (1)



DFRS miR-9 plasmid (F-M) or mutated DFRS miR-9 plasmid (A-E) was injected into the lumen of the telencephalic vesicles (A-J) or administered in proximity of the ectoderm (K-M) of E13 mouse embryos in utero followed by electroporation into the left telencephlic neuroepithelium and left cranial ectoderm, respectively. After 24h of in utero development, dissected brains (A-C and F-H) or whole embryos (K-M) were analyzed in the targeted regions (boxes in A, F, and K) or GFP (C, G and L) and mRFP (C,H , and M) expression. Brains were then fixed and cryosections of the region of the neural tube wall showing GFP expression (B and G, dashed lines) were examined for the presence of GFP-reporter (Dadn I) and mRFP-sensor (E and J) in individual cells. Note the absence of mRFP-sensor fluorescence in the brain (H) and neuroepithelial cells (j) electroporated with the DFRS miR-9 plasmid, in contrast to its presence in the ectoderm electroporated with the DFRS miR-9 plasmid (M) and in the brain (C) and neuroepithelial cells (E) electroporated with the mutated DFRS miR-9 plasmid. (N) In situ hybridization using a locked nucleic acid (LNA) probe for miR-9 on coronal cryosections of the E14 mouse head. Note the absence of miR-9 in the ectoderm (N,*ec*, arrowhead) and its presence in both the neuronal progenitors of the ventricular zone (N,*vz*) and postmitotic neurons in the cortical plate (N,*cp*) of the telencephalon. Asterisks indicate the ventricular lumen. Scale bars, 50µm (D,E,I,J) and 100 µm (N)

Protocol ECM 830/ Tweezertrode Electrodes

Preparation:
Pregnant mice 13 day postcoitum were anesthetized using isofluorane. The mouse embryos in the uteri were exposed. The embryos were injected and electroporated.

Electrical setting:

Voltage:	30
Pulse length:	50msec
No. of Pulses:	6
Pulse Interval:	1sec

Electroporation Procedure:
Sample Volume:3-5µg plasmid DNA suspended in 1-3µl PBS
Temperature: Room temperature
Pulse: Inject DNA solution through a glass capillary needle into the lumen of the telencephalic vesicles or released in proximity of the ectoderm of the E13 mouse embryo. Immediately after injection, hold the head of the embryo horizontally between the two electrode disks of the Tweezertrode type electrodes. Press the Start/Pulse button on the generator to activate the Automatic Charge and Pulse sequence. The embryos were relocated into the peritoneal cavity and the abdomen was sutured. Mice were scarified 24-72 post in utero electroporation.

Results:
Positive co localization of the DFRS plasmid in specific tissues indicating positive expression of miRNAs.

Reference:
1. Davide De Pietri Tonelli, Federico Calegari, Ji-Feng Fei, Tadashi Nomura, Noriko Osumi, Carl-Philipp Heisenberg, and Wieland B. Huttner. 2006 Single-cell detection of microRNAs in developing vertebrate embryos after acute administration of a dual fluorescence reporter/sensor plasmid. BioTechniques 41:727-732

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