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MATra Application Note
Primary cortical neurons

TRANSFECTION OF PRIMARY CORTICAL NEURONS

from C57BL/6 mice

The neural cell adhesion molecule (NCAM) plays a major role during development of the nervous system and in synapse plasticity in the adult brain (Diestel et al., 2007). Many studies provide evidence that NCAM can regulate processes like cell migration, axon growth and fasciculation. Endocytosis of NCAM might play a decisive role in these processes as it can potentially enable a quick change in cell adhesion between the cells or towards the extracellular matrix. Endocytosis of NCAM might also influence these processes by activating specific signal transduction pathways. Primary cortical neurons present a good *in vitro* system for these investigations since they allow analysis of molecules within growth cones. For analysis of NCAM, embryonic cortical neurons (E15.5) were transfected with human NCAM one day after isolation. Endocytosis of NCAM was induced 24 hours after transfection and detected by immunofluorescence analysis.

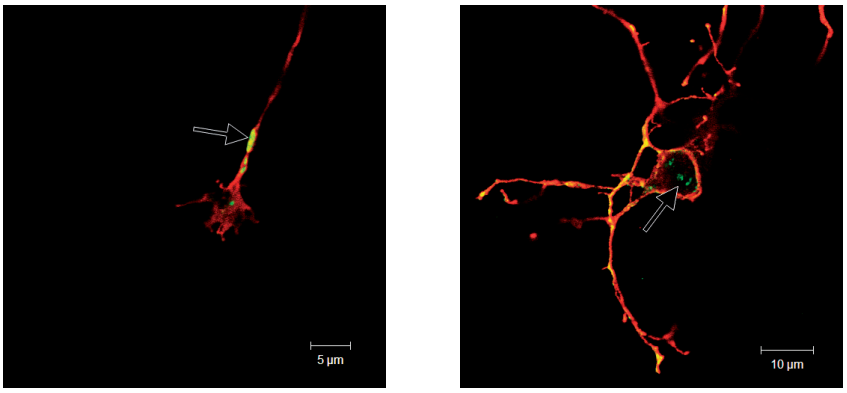

Figure 1: Endocytosis of NCAM in cortical neurons. After magnet assisted transfection (MATra) membrane-localized NCAM (not endocytosed) was detected using a Cy3-coupled secondary antibody (red). Afterwards, the internalised, endocytosed NCAM was stained by a Cy2-coupled secondary antibody (green, see arrows) in the cell soma (left) and in axonal vesicles (right).

MATERIAL AND METHODS:

Primary cortical neurons from C57BL/6 mice embryonic day 15.5 (E15.5) were isolated and plated at a density of 800,000 cells per 24-well plate on poly-L-lysine-coated coverslips. The next day the neurons were transfected with an expression plasmid for human NCAM by Magnet Assisted Transfection. To induce endocytosis, 24 hours after transfection cells were incubated 30 minutes at 37°C with an antibody which is specific for human NCAM. Subsequently the cells were fixed and membrane-localized NCAM was visualized using a Cy3-coupled secondary antibody. After permeabilisation of the cells internalised NCAM was stained by a Cy2-coupled secondary antibody. The cells were mounted on microscope slides and analysed using a Zeiss LSM510 MetaUV confocal microscope.

0.6 µg DNA were dissolved in 50 µl Neurobasal medium. 0.6 µl MATra-A reagent were added, mixed well and incubated for 20 minutes at room temperature. During this incubation time the medium was exchanged with supplemented Neurobasal medium (containing B27 supplement and 2 mM L-glutamine). The transfection mixture was added drop by drop to the cells, dispersed evenly in the medium and immediately placed on the magnetic plate (37°C, 5% CO2, 15 minutes). After 6 hours half of the medium was exchanged with fresh, supplemented Neurobasal medium.

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Published also in "Renker, B. et al. MATra - ein Trojanisches Pferd für eine zellschonende Transfektion. *BIOSpektrum* **04.10:**441-442."

REFERENCE:

Diestel S, Schäfer D, Cremer H, Schmitz B. (2007) NCAM is ubiquitylated, endocytosed and recycled in neurons. *J Cell Sci.* **120:** 4035-49

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