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Transcriptional activity assessment of three different promoters for mouse in utero electroporation system

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ABSTRACT

In utero electroporation (IUE) is a simple and rapid approach to *in vivo* investigate exogenous gene function in mouse brain, and intensive studies using IUE have greatly contributed to analyze the characterization of specific steps during mouse brain development. Because the efficiency of IUE is highly dependent on the plasmid used and its concentration, and the transcriptional activity of plasmid is not only regulated by the host defense system, but also by the promoter of the expression vectors. Therefore, in the present study, we evaluated the transcriptional activity of three commonly used promoters, CMV, CAG and SV40, in IUE system through measuring the fluorescence intensity of green fluorescent protein which serves as an indicator. Our results demonstrated that the artificially-designed CAG promoter is a potent promoter that effectively drives target gene expression in IUE system.

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1. Introduction

As a simple and rapid approach to overexpress or silence target genes during mouse brain development, in utero electroporation (IUE) is becoming a major alternative method to *in vivo* investigate exogenous gene function. Compared to the viral infections by which the infected area is usually uncertain and the infection efficiency is dependent on the titer of the viral suspension used, the biggest advantage of IUE is that the negatively charged plasmid DNA can move towards the positive pole of the electrode under electric current, and can be thus selectively introduced into a specific brain region, such as somatosensory cortex, hippocampus, retina, etc. (LoTurco et al., 2009; Rice et al., 2010; Taniguchi et al., 2012). Simultaneously, the size of the transfected area can also be adjusted by using different kinds of electrodes without affecting the embryo (Dal Maschio et al., 2012). Furthermore, this technique is able to choose a particular developmental time point for the study of different developmental events including cell proliferation, cell migration (Kolk et al., 2011), axon growth, dendrite development, and synaptogenesis (Pacary et al., 2012). Therefore, Gain- and loss-of-function studies using IUE have greatly contributed to analyze the characterization of molecular mechanisms controlling specific steps during mouse brain development.

The efficiency of IUE is highly dependent on the plasmid used and its concentration (Dal Maschio et al., 2012). As a key element of plasmid, the promoter determines the transcriptional activity of the exogenous or reporter genes. Nowadays, a series of cellular and viral promoters are widely used in mammalian expression vectors for ectopic expression of desired genes, such as CMV (cytomegalovirus) promoter, CAG promoter (consisting of the CMV immediate early enhancer and the chicken β-actin promoter) and

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SV40 (simian virus 40) promoter, which show strong transcriptional activity in a variety of cells of different origins (Chambers et al., 2003; Hong et al., 2007; Stinski, 1999). However, the transcriptional activity of promoter is regulated through multiple mechanisms, besides the regulatory elements of plasmid itself (enhancer and the size of target genes), the host defense system also plays a crucial role on it. For example, CMV promoter is repressed by p53 and activated by JNK pathway, and the activity of SV40 promoter can be inhibited by overexpression of heme oxygenase-1 in tumor cells (Liu et al., 2013; Rodova et al., 2013). Therefore, in order to satisfy the requirement of certain expression system, it is necessary to select the plasmid with appropriate promoter for target gene expression. In the present study, we evaluated the transcriptional activity of CMV, CAG and SV40 promoters in IUE system through measuring the fluorescence intensity of green fluorescent protein (GFP) which serves as a reporter gene. Finally, we determined that the suitable concentration of plasmid with CAG promoter drives effective expression of target and reporter gene in the developing mouse brain.

2. Materials and methods

2.1. Animals

6–8 weeks old C57BL/6 mice (20–25 g) were purchased from the experimental animal center, Xinxiang Medical University (Xinxiang, China). They were bred and housed at a pathogen-free condition on a 12 h light–dark cycle. Handling of mice and experimental procedures were approved by Animal Care Committee of Xinxiang Medical University.

2.2. Plasmid

The plasmid pCAGGS-GFP was kept in our lab; pEGFP-N1was purchased from Clontech (Mountain View, CA); and pCL-GFP was a kind gift from Dr. Marcelle (University Aix-Marseille II, France). The *Escherichia coli* DH5α strains (Takara, Japan) harboring these three kinds of plasmids, respectively, were cultured in LB media at 37 °C with 180 rpm overnight. Subsequently, the plasmids were extracted and purified with endotoxin-free prep kit (CWBIO, China) according to manufacturer's instructions. Finally, the same amount of plasmid was used for avoiding the discrepancy caused by different size of three investigated plasmids, and the plasmids DNA solutions were diluted to desired concentrations (Table 1) in water and Fast Green (Sigma) was added with 0.05% for final concentration.

2.3. In utero electroporation

The basic protocol of IUE was performed as follows (Fig. 1A–C). Timed-pregnant mice at E15 (embryonic day 15) were deeply anesthetized with chloral hydrate (4.3%). After abdominal fur was removed, the mouse was placed with its back on the heating pad, and then the uterine horns were exposed through a midline laparotomy incision (Fig. 1A). Different concentration of plasmid DNA solutions (1 μl each) were injected through the uterine wall into the lateral ventricle of the embryos using a glass micropipette made from a microcapillary tube (Fig. 1B). The structure maps of the three different plasmids were shown in Fig. 1D–F, respectively. After injection, the paddle-type electrodes were placed on either side of the head of the embryo and five 60 ms square pulses with 600 ms intervals at 35 V were applied by an electroporator (Napagene, CUY21, Japan). Following injection and electroporation, the uterus was carefully returned inside the abdomen, and the abdominal muscle wall and skin were sealed with sutures. Animals were monitored continuously until they recovered from anesthesia.

2.4. Embryo, neonatal mouse and tissue processing

For *in vivo* analyses following in utero electroporation, animals were harvested at E18, P0 (postnatal day 0), P3, P7 and P11, respectively. The pregnant mice were euthanized with rapid decapitation; E18 embryos were dissected out of the uterus and placed in sterilized PBS. Then, the GFP positive embryos were selected, as indicated by the amount and location of the fluorescent signal and visualized across the skull using a stereo fluorescence microscope (LEICA M205FA, Germany). Subsequently, the GFP positive brains from E18 embryos were dissected out of the head, fixed in 4% formaldehyde solution overnight, and then immersed in 18% sucrose solution overnight. Finally, the specimen were embedded in OCT compound (Sakura Finetek, USA), frozen in liquid nitrogen, and stored at −80 °C. The samples were adjacently sectioned with 20 μm thickness on the Poly-L-lysine coated slides with a cryotome (Leica 1850, Germany). In addition, the brains from neonatal mice (P0, P3, P7 and P11, respectively) were dissected out of the head and placed in sterilized PBS. Then, the GFP positive brains were visualized by the stereo fluorescence microscope and selected with a similar procedure as above.

2.5. Fluorescence imaging and data analysis

The GFP positive embryos and brains (3 replicates at least) were imaged under a stereo fluorescence microscope

Table 1
The concentration of three investigated plasmid used in this study.

Plasmid	1.4×10^{-13} (mol/μl)	2.8×10^{-13} (mol/μl)	5.6×10^{-13} (mol/μl)
pCAGGS-GFP	0.50 μg/μl	1.00 μg/μl	2.00 μg/μl
pEGFP-N1	0.43 μg/μl	0.87 μg/μl	1.74 μg/μl
pCL-GFP	0.55 μg/μl	1.11 μg/μl	2.22 μg/μl

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