Journal of Neuroscience Methods 142 (2005) 159–166

JOURNAL OF NEUROSCIENCE METHODS

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# Enhancing protein expression in single HEK 293 cells

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Received 2 June 2004; received in revised form 7 September 2004; accepted 17 September 2004

**Abstract**

Recombinant proteins are routinely expressed in heterologous expression systems such as human embryonic kidney 293 (HEK 293) cells. The efficiency of the expression is critical when the expressed protein must be characterized at the single-cell level. Here we describe a simple method by which the protein expression efficiency in single HEK 293 cells is enhanced by coexpressing simian virus 40 large T antigen (TAg), a powerful oncoprotein. Using the GluR2 ionotropic glutamate receptor as an example, we found that the receptor expression in single HEK 293S cells increased approximately seven-fold. The ratio of the plasmid amount of TAg to that of the receptor was optimized at 1:10, while the receptor function was unaffected in the presence of TAg. We further used fluorescence imaging from a population of cells as an independent detection method and found a similar increase in expression of green fluorescent protein (GFP) by TAg coexpression. This method is thus applicable for enhancing the expression of both membrane and soluble proteins at the single-cell level. More importantly, the function of a protein can be studied directly in intact cells, a feature particularly useful for studying membrane proteins.


*Keywords:* Glutamate ion channels; Green fluorescent protein; Transient transfection; HEK 293 cells; Simian virus 40 large T antigen; Protein expression

## 1. Introduction

The use of human embryonic kidney 293 (HEK 293) cells has been one of the most popular ways for expression of recombinant proteins (Graham et al., 1977). There are a variety of transfection protocols to deliver a recombinant gene to those cells (Chen and Okayama, 1987; Corsaro and Pearson, 1981; Graham and van der Eb, 1973; Luthman and Magnusson, 1983; Washbourne and McAllister, 2002). Generally, those protocols provide a reasonable protein expression yield, especially when the culture volume is not restricted. In such a case, a larger volume of culture from which more cells can be harvested compensates for a low efficiency of protein expression at the single-cell level. However, a low efficiency of single-cell protein expression can be an insurmountable problem when the protein must be characterized by single-cell imaging and/or single-cell recording. This is because the number of protein molecules expressed per cell is generally proportional to the signal strength. For instance, ion channel proteins are expressed routinely in HEK 293 cells and assayed directly using a single cell, either entirely (e.g., for whole-cell recording) or partially (e.g., for recording with membrane patches and for single-channel recording) (Hamill et al., 1981). As such, the number of ion channels expressed per cell is critical to their detection. Furthermore, in the presence of inhibitors, the protein signal is adversely reduced. Thus, these and many other types of studies will benefit from a method by which the efficiency of protein expression in single cells can be enhanced. Developing such a method was the goal of the present study.

The method we established originated from our interest of ionotropic glutamate receptors (Dingledine et al., 1999). These receptors are transmembrane channels that can open upon binding of glutamate, a neurotransmitter in the central nervous system (Dingledine et al., 1999). Glutamate receptors play special roles in brain activities, such as memory and learning, and have been implicated in a variety of neurological diseases, such as post-stroke cellular lesion and amyotrophic lateral sclerosis (Dingledine et al., 1999;

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doi:10.1016/j.jneumeth.2004.09.009

Heath and Shaw, 2002). To study the structure–function relationship, glutamate receptors are commonly expressed in HEK 293 cells and characterized directly in single cells. By our method, the receptor expression efficiency in single cells can be increased by about seven-fold, compared with a popular expression protocol using calcium phosphate in transfection (Chen and Okayama, 1987). The key to our method is to coexpress simian virus (SV) 40 large T antigen (TAg), a powerful oncoprotein (Ali and DeCaprio, 2001; Chen and Hahn, 2003; Simmons, 2000; Sullivan and Pipas, 2002), with the protein of interest. Specifically, the gene of the protein of interest is harbored in a plasmid containing the SV40 replication origin, and the TAg gene is encoded in a separate vector. Transient coexpression of TAg produces more proteins of interest per cell. This is because, among its functions, SV40 TAg disrupts the cell-cycle checkpoints by binding to and inactivating key tumor suppressors and cell-cycle regulatory proteins such as p53 and pRB (Ali and DeCaprio, 2001; Sullivan and Pipas, 2002). Consequently, the cell turns into a growth-deregulated protein-making factory. Specifically, we characterized TAg enhancement of the single-cell expression of GluR2, a key glutamate receptor subunit (Li et al., 2003b), to establish the optimal plasmid ratio and the most complementing cell line. We further characterized the function of the GluR2 receptor with intact cells, without removing TAg. In addition, using green fluorescence protein (GFP) (Chalfie et al., 1994) as a reporter gene and fluorescence imaging of a population of HEK 293 cells as an independent detection method, we showed that the GFP expression in these cells increased similarly. Together, our results demonstrate that this method represents a significant improvement over conventional protein expression protocols. Furthermore, the method should be general for expressing both soluble and membrane proteins, and for characterizing the protein function directly in single cells.

## 2. Materials and methods

### 2.1. Expression of plasmid DNAs and cell culture

The cDNA encoding GluR2 (unedited at the Q/R site, and flip isoform) in a pBlueScript vector (from Prof. Steve Heinemann at Salk Institute) was cloned into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA) that contained the SV40 replication origin (8.6 kb). To identify cells that might express the GluR2 receptor for recording, the GluR2 plasmid was cotransfected with a GFP plasmid lacking the SV40 replication origin (from Prof. Ben Szaro at SUNY-Albany). As a result, the GFP expression in cells or the resulting green color intensity was not affected by coexpression of TAg (see the text). However, a different GFP construct (pEGFP-C3, 4.7 kb) (Clontech, Palo Alto, CA) that did contain the SV40 replication origin was used in the enhancement of GFP expression by TAg (the TAg plasmid, 6.0 kb, from Prof. Jeremy Nathans at Johns Hopkins University). All the plasmids were propagated in an *Escherichia coli* host (DH5α) and purified using a kit from QIAGEN (Valencia, CA).

The cell lines used in this study were regular HEK 293 cells (from Prof. Robert Oswald at Cornell University), 293S cells (from Prof. Gobind Khorana at MIT) and 293T cells (American Tissue Culture Collection, Cat. No. CRL-11268, Manassas, VA). All cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin in a 37 °C, 10% $CO_2$, humidified incubator.

A standard calcium phosphate method (Chen and Okayama, 1987) was used in most of transient transfections for gene delivery, although lipofectamine (Invitrogen, Cat. No. 18324-111, Carlsbad, CA) was used in some transfections (see text). The GluR2 plasmid used was ∼3–6 μg (the plasmid amount used here and below was for a culture in the 35 mm dish). As a cell marker, the GFP plasmid that lacked the SV40 replication origin was cotransfected with the GluR2 with the ratio of the plasmid of GFP to that of GluR2 by weight being 1:10 (Li et al., 2003b). The amount of the TAg plasmid varied in experiments (see Section 3). The amount of pEGFP plasmid was 1–3 μg whereas the weight ratio of pEGFP plasmid to TAg plasmid varied from 2:1 to 15:1. All the cells used for recording or imaging were those that grew in between 48 and 58 h after transfection.

### 2.2. Whole-cell current recording

Because the expression of the GluR2 gene leads to the formation of functional channels (Boulter et al., 1990), both the expression and the functional properties of this receptor are testable by measuring the magnitude and the time course of the glutamate-induced whole-cell current from an entire cell. The ratio of the current amplitude in the absence and presence of TAg, but at a constant glutamate concentration, was therefore used to represent the effect of TAg in enhancing the receptor expression.

The procedure for recording the GluR2 channel activity was previously described (Li et al., 2003b). Briefly, the recording electrodes were pulled from glass capillaries (World Precision Instruments, Sarasota, FL). The electrode resistance was ∼3 MΩ when filled with the electrode solution. The electrode solution contained (in mM) 110 CsF, 30 CsCl, 4 NaCl, 0.5 $CaCl_2$, 5 EGTA, and 10 HEPES (pH 7.4 adjusted by CsOH). The external bath solution contained (in mM) 150 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH 7.4 adjusted by HCl). Whole-cell recordings were done at −60 mV, and 22 °C. Specifically, a cell that expressed the GluR2 receptor was bathed in the external solution. Glutamate was applied from a cell-flow device (Udgaonkar and Hess, 1987) to the cell, and the resulting whole-cell current was recorded using an Axopatch-200B amplifier at cutoff frequency of 2 kHz by a built-in, 8-pole Bessel filter, and digitized at 5 kHz sampling frequency using a Digidata 1322A from Axon Instruments (Union City, CA). The data were acquired using pCLAMP 8 (also from Axon). The rise time of