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Neuronal Transfection Methods

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Abstract

Transfection - the process of transferring genetic material into cells - is a powerful tool for the rapid and efficient manipulation of gene expression in cells. Because this method can be used to silence the expression of specific proteins or to drive the expression of foreign or modified proteins, transfection is an extremely useful tool in the study of the cellular and molecular processes that govern neuron function. However, mature neurons have a number of properties that make them difficult to transfect, so specialized techniques are required for the genetic manipulation of this cell type.

This video reviews the principles and rationale behind transfecting neurons. Three common strategies for neuronal transfection are discussed, including nucleofection, gene-gun, and viral transduction. In addition to describing how each of these techniques overcomes the challenges associated with transfecting neurons, the presentation includes a description of how all three methods are performed. Finally, several applications of neuronal transfection are introduced, such as the expression of fluorescent tubulin proteins to visualize neuron morphology, and selective gene silencing to generate a cell culture model of Parkinson's disease.

Transcript

Transfection - the transfer of genetic material into mammalian cells - is a valuable tool that allows scientists to genetically manipulate neurons and neuronal tissue. The unique properties of the delicate cells make transfection challenging and necessitate specialized techniques.

This video will review the principles behind transfecting neurons and introduce strategies commonly used on these cells, including nucleofection, biolistic transfection, and viral transduction. Finally, we will discuss applications of transfection techniques in cell and molecular neuroscience research.

Let's begin by reviewing how transfection works. While genetic material can be delivered in close proximity to a cultured cell by adding it to the surrounding medium, nucleotides cannot efficiently penetrate cell membranes.

All transfection protocols are designed to enable genetic material to bypass this barrier. Nucleotides that block protein production, like silencing RNA, can immediately carry out their function once they enter the cytoplasm. However, DNA-based constructs must be transported into the nucleus for translation before protein synthesis can begin.

In dividing cells, the breakdown of the nuclear envelope during mitosis can result in the incorporation of transfected DNA into reforming daughter cell nuclei. Because most cultured neurons are post-mitotic - or non-dividing - cells, specialized transfection protocols are required in order to successfully induce gene expression.

Let's start our overview of these protocols with nucleofection. This technique utilizes a combination of an induced electrical field and chemical reagents, which act together to create transient, pore-like structures in both cell and nuclear membranes. Since nucleotides are charged, the electric field also drives movement of DNA through the pores.

To carry out this procedure, neurons in suspension are collected in a pellet by centrifugation prior to resuspension in commercial nucleofection reagent. The cell mixture is then combined with purified DNA and transferred to an electroporation cuvette, which features two aluminum plates that make electrical contact with the Nucleofector apparatus. This device delivers a series of rapid electrical pulses, whose number and duration are customized based on the cell type. After nucleofection, the cells can be diluted in culture medium and plated for continued growth.

Alternatively, the gene gun technique blasts through transfection barriers, by shooting bullets carrying genetic material through both cell and nuclear membranes.

To make gene gun bullets, DNA encoding your gene of interest is precipitated onto micron-scale beads, usually composed of gold. After a 10 minute incubation, the beads are washed and transferred into tubing. The tubing is then rotated as the solution dries, resulting in a uniform coating of beads. Next, the tubing is cut into cartridges and loaded into the gun. Gas-powered delivery of the genetic payload can be performed on cells growing in standard culture plates by a simple pull of the trigger.

Lastly, viral transduction takes advantage of the viral life cycle to deliver foreign genetic material into the nucleus. RNA encoding the gene of interest is packaged into modified retroviruses known as lentiviral vectors, which gain entry into target cells by binding to specific membrane proteins, triggering a membrane fusion event. The viral RNA is then reverse transcribed into a complementary DNA strand that is transported into the nucleus.

Before starting this procedure, note that viruses can infect the cells in your body as well as the ones in your dish, so following safety guidelines is extremely important.

The first step is to generate the lentiviral particles carrying your gene of interest. This is accomplished by expressing the building blocks of the virus in a human cell line optimized for viral production, like 293T cells. To avoid the unnecessary spread of engineered viruses, the genes required for their assembly are kept separate from the transfer vector, whose sequence will be included in the infectious particle.

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It takes about 2 days for the fully assembled viruses to be released into the culture medium, where they can be collected and concentrated by ultracentrifugation. Next, the appropriate concentration, or titer, of virus is determined by assessing transduction success in a test cell line. The lentiviral vector is then added to the target neuronal culture and typically incubated for 24 to 48 hours to ensure infection has occurred.

Now that you are familiar with the common transfection techniques, the one you use will depend upon the specific goals of your experiments. Let's look at some examples.

To begin, observing the maturation of neurons in culture allows for detailed analysis of the morphological changes that are important for neuronal connectivity, like the formation of dendritic spines. To visualize cell morphology over time, these researchers made use of nucleofection to deliver a fluorescent protein into cultured neurons. Here, a fluorescently-tagged tubulin protein was transfected into a subset of cells, allowing for detailed analyses of cell processes via fluorescence microscopy. Because expression levels were maintained throughout the lifetime of the neuron, morphological analysis could be performed for at least a month in culture.

Transfection can also be used to test the impact of specific genetic mutations on neuron function. A gene gun can be used to deliver DNA encoding wild type or mutant versions of a protein, and short-term impacts on cellular biology can be assessed, for example by patch clamp recording of neuron firing.

Lastly, a common strategy for testing a gene's function is to observe what happens to cells when its expression is blocked. For these experiments, lentiviral vectors can be used to deliver silencing RNA constructs, which prevent protein synthesis. In this experiment, knockdown of genes associated with Parkinson's disease leads to a significant decrease in cell viability.

You've just watched JoVE's introduction to transfection of neurons. In this video we have introduced principles of neuron transfection, as well as procedures and applications for three common transfection strategies.

Thanks for watching!

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