

METHOD FOR GENE DELIVERY TO NEURONAL CELLS

FIELD OF THE INVENTION

[0001] The present invention relates to a method for gene delivery to neuronal cells.

BACKGROUND OF THE INVENTION

[0002] The use of baculovirus *Auto grapha californica* multiple nuclear polyhedrosis viruses as mammalian cell expression vectors has recently been viewed as an emerging strategy with great promise as a new generation of gene therapy vehicles (Ghosh et al., 2002; Kost and Condreay, 2002). Baculoviruses display a broad tropism in both proliferating and non-proliferating, quiescent mammalian cells, do not replicate in vertebrate cells and produce little to no microscopically observable cytotoxicity. Baculovirus vector-mediated gene transfection has been investigated in the nervous system. An initial report described an efficient transduction of neural cells *in vitro* and *in vivo* by baculoviral vectors containing a CMV promoter (Sarkis et al., 2000). In primary cell cultures of human embryonic brains, neuroepithelial, neuroblastic, and glial cells could be infected. *In vivo* studies using adult nude mice demonstrated that mainly astrocytes, with only a few neurons, were transduced in baculovirus vector injected brains. In another study aiming to examine the cell-type specificity of baculovirus-mediated gene expression in the brain (Lehtolainen et al., 2002), cuboidal epithelial cells of the choroids plexus were identified as the main target cells of the viruses in the rat brain, modest gene expression being detected in endothelial cells but very limited or no expression found in other types of brain cells, including neurons and astrocytes.

[0003] Major functional cells in the nervous system are neurons, the type of cells that carries out the fundamental task of the system in receiving, conducting and transmitting signals. Therapeutic protection of these cells is one of the main goals of molecular therapy of neurological disorders. Examples include protecting dopaminergic nigrostriatal neurons in Parkinson's disease (Duvoisin, 1992), basal forebrain cholinergic neurons in Alzheimer's disease (Gomez-Isla et al., 1996) and

spinal motoneurons in amyotrophic lateral sclerosis. We have previously developed a recombinant baculovirus vector accommodating a strong neuronal specific promoter, CMV E/PDGF, to drive transgene expression (the CMVI E/PDGF described in co-pending U.S. application No. 10/407,009). After injection into the brain, the baculovirus vector could transduce neurons and mediate transgene expression in neurons nearby the injection site. A persistent transgene expression at relatively high level lasts for at least 4 weeks, much longer than 3 days offered by baculovirus vectors containing a CMV promoter alone.

[0004] The broad anatomical distribution of affected neurons in certain neurological disorders and the relatively inaccessible neuronal location in others present obstacles to effective gene therapy of the disorders. Several virus vectors are capable of overriding the problems by taking advantage of the natural process of axonal transport. This process starts from endocytosis of extracellular substances. In the case of axonal endocytosis, axon terminals are used as ports of entry to take up the substances. The incorporated substances are then transported in axoplasm to the cell body. The viruses that are able to be taken up and transported in this manner include adenovirus (BilangBleuel et al., 1997; Hermens et al., 1997; Choi-Lundberg et al., 1998; Soudais et al., 2001; Pelttekian et al., 2002), adeno-associated virus (AAV, Kaspar et al., 2002) and herpes virus (HSV, Breakefield and DeLuca, 1991; Jin et al., 1996). Adenovirus and HSV elicit much stronger immune and inflammatory responses than baculovirus. A single intracerebral injection of either adenovirus or HSV results in dose-dependent inflammatory reactions in the brain, leading to demyelination (McMenamin et al., 1998; Lawrence et al., 1999). AAV vectors, which are able to integrate into the target cell chromosoe, are characterized by the inability to consistently induce immune responses. Their application as gene delivery vectors is however hampered somehow by the small insert size of the viral vectors and the difficulty in manufacturing (Joose and Chirmule, 2003).

SUMMARY OF THE INVENTION

[0005] In one aspect, the invention provides a method of delivering a baculoviral vector to a neuronal cell in a subject comprising identifying a target neuronal cell in the central nervous system, identifying a site in the central nervous system remote from the target neuronal cell and administering the vector to said site wherein the

vector is delivered to the neuronal cell by axonal transport.

[0006] In another aspect the invention provides a method for treating a neuronal disorder in a subject, comprising identifying a target neuronal cell affected by the disorder, identifying a site in the central nervous system that is remote from the target neuronal cell, and administering a baculoviral vector comprising a therapeutic gene operably linked to a promoter to said site wherein the baculoviral vector is delivered to the target neuronal cell by axonal transport.

[0007] In yet another aspect, the invention provides a method for tracing a neuronal pathway, comprising administering a baculovirus adopted for detection to a site in the central nervous system of a subject and detecting the axonal transport pathway of the virus.

[0008] Other aspects and features of the present invention will become apparent to those of ordinary skill in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Figure 1 is confocal images of the striatum, the substantia nigra and the cerebral cortex illustrating uptake and transport of Cy3 labeled baculovirus vectors in the rat brain after intra-striatum injection. The scale bar present 50 μ m for images of the striatum and the substantia nigra and 100 μ m for cerebral cortex;

[0010] Figure 2 is an image of a PCR analysis of baculovirus DNA after intra-striatum injection. Lane 1, 100bp ladder; lane 2 & lane 4, no injection and mock injection with 1 x PBS as negative control; lane 3, purified viruses as positive control; lanes 5, 6 and 7, tissue samples collected from the cerebral cortex, substantia nigra and striatum, respectively;

[0011] Figure 3 illustrates the results of quantitative measurement of luciferase reporter gene activity after intra-striatum injection of baculovirus vectors with the CMV promoter alone or the CMV enhancer/PDGF (CMV E/PDGF) promoter;

[0012] Figure 4 shows confocal images of luciferase expression in the striatum after injection of baculovirus vector with CMV E/PDGF. Samples were double

immunostained against neuron-specific nuclear protein (NeuN) to show neurons and against luciferase (Luc) to show luciferase positive cells;

[0013] Figure 5 depicts confocal images of luciferase expression in distal regions after intra-striatum injection of baculovirus vector with CMV E/PDGF. A-C: double immunostaining against luciferase to show transfected cells and against tyrosine hydroxylase (TH) to show dopaminergic neurons in the SN; D-L: double immunostaining against luciferase protein and against neuron-specific nuclear protein (NeuN) to show transfected neurons in the cerebral cortex; D-F, low magnification image for the cerebral cortex; G-I, high magnification image showing transfected pyramidal neurons in the cerebral cortex; J-L, high magnification showing untransfected granule cells in the region. Scale bars represent 80 μm for A-C, 40 μm for D-F and 20 μm for G-L;

[0014] Figure 6 and Figure 7 depict confocal images of uptake and transport of Cy3 labeled baculovirus vectors after intra-vitreous body injection. GCL, ganglion cell layer; INL, inner nuclear layer.

[0015] Figure 8 is an image of a PCR analysis of baculovirus DNA after intra-vitreous body injection. Lane 1, 100bp ladder; lane 2 & lane 4, no injection and shamed injection with 1 x PBS as negative control; lane 3, purified viruses as positive control; lanes 5, 6, 7, 8, and 9, tissue samples collected from retina, optic nerve, lateral geniculate nucleus, superior colliculus, and primary visual cortex, respectively;

[0016] Figure 9 illustrates the results of quantitative measurement of luciferase reporter gene activity after intra-vitreous body injection;

[0017] Figure 10 depicts confocal images showing luciferase expression in neurons after intravitreal body injection. Double immunostaining was carried out using antibodies against luciferase to show transfected cells and against neuron-specific nuclear protein (NeuN) to show neurons. Scale bars present 40 μm for A-F, 80 μm for G-I; I: Retina; II: Lateral geniculate body; III: Superior culliculus; IV: Primary visual cortex.

DETAILED DESCRIPTION

[0018] The inventors have demonstrated for the first time that recombinant baculoviral vectors can migrate by axonal transport to neuronal cell bodies, resulting in transgene expression in projection neurons. After stereotaxic injection of Cy3-labeled baculoviral vectors into the rat striatum, retrograde axonal transport of the baculoviral vectors was observed along the corticostriatal pathway and nigrostriatal pathway. After intravitreal body injection, anterograde axonal transport and transsynaptic transport of the virus particles were observed in defined connections of the visual system, from the retina, the optic nerve, the lateral geniculate body, the superior colliculus to the primary visual cortex. PCR analysis confirmed the existence of transported viral DNA in the tissue samples collected from projection fields. Using recombinant baculoviral vectors containing a neuron-specific promoter, transgene expression was detectable in the neuronal cells distal to the injection site.

[0019] The use of the axonal transport for neuronal gene transfection allows viral vectors to target neurons at the distal parts of the neural circuits. By using an injection site in a more accessible brain region, the approach can target the populations of neurons deep in the CNS structure or minimize the damage to sensitive regions caused by injection procedure. The striatum is one of the popular regions used to test the approach. There are three major brain regions projecting to striatum: the cerebral cortex, the substantia nigra, and the intralaminar nuclei of the thalamus. Dopaminergic neurons in the substantia nigra have long axon projecting to large area of the striatum and have been used to study the retrograde transport of many virus vectors, including adenovirus (Ghadge et al., 1995; Peltekian et al., 2002), AAV (Kaspar et al., 2002) and HSV (Jin et al., 1996). The inventors have found, using the baculoviral vector that transgene expression in substantia nigra, although while still highly significant, was relatively lower compared with that in the cerebral cortex, likely due to the difference of the neuron quantity in these two brain regions.

[0020] The visual system is a well-defined sensory pathway which possesses both centripetal and centrifugal nonreciprocal connections (Peters, 1985). In rodents, most projections from the retina go contralaterally to the thalamus, to the superior colliculus (SC), and to several structures in the hypothalamus (Millhouse, 1977). Within the thalamus, several specific thalamic nuclei, including the dorsal lateral

geniculate nucleus (dLGN), are primary retinorecipient areas. From the dLGN, neuronal projections are relayed to the primary and secondary visual cortices (areas 17 and 18) (Hughes, 1977; Caviness, Jr. and Frost, 1983). The neurons are further projecting from the visual cortex to many other cortical and subcortical regions, such as the temporal, frontal, and ventrolateral orbital cortices and the basal ganglia. These connections between the retina and the visual cortexes are therefore useful for analyzing transneuronal transport. The transgene expression under a neuron-specific promoter in the retina was confined in ganglion cells after intravitreal body injection of the baculoviral vectors, although the viruses entered both neurons and glia in the retina. Further, intraocular inoculation of baculovirus resulted in a sequential infection of neurons along the structures associated with vision, indicating that the virus can cross several synapses.

[0021] A baculoviral vector therefore may be delivered to any target neuronal cell that cannot readily be locally injected, for example, because it is in an area of the central nervous system that is not readily accessible or it is widely distributed in various regions of the central nervous system requiring multiple local injections. By identifying a site in the central nervous system which is remote from such target neuronal cell and administering the vector to the site, the vector may be delivered to the target neuronal cell by axonal transport.

[0022] The terms "neuronal cell" and "neuron", which are used interchangeably herein, in accordance with the usual meaning in the art, refer to any conducting cell of the nervous system, which typically includes the cell body, several dendrites and the axon. The terms include a single cell as well as a plurality or population of cells unless the context clearly indicates otherwise.

[0023] As will be understood by a skilled person, many neuronal cells have long axons projecting to areas in the central nervous system that are accessible by injection. Therefore, the term target neuronal cell, as used herein, includes the cell body of such a neuronal cell which is remote from its axon that is accessible by injection, as well as another neuronal cell to which the vector may be delivered by trans-synaptic transport. A site is "remote" from the target neuronal cell if the vector injected into the site is transported by axonal transport prior to entering the cell body of the projection neuronal cell or another neuronal cell by trans-synaptic transport.

In contrast, if a vector can be delivered to neuronal cells in and around the area of local injection absent any axonal transport, the site of the local injection is not remote from those neuronal cells. A skilled person can readily identify desired target neuronal cells and site of administration that is accessible by injection and remote from the target neuronal cell such that the vector is delivered to the target neuronal cell by axonal transport. Examples of such target neuronal cells and their remote sites of administration include target neuronal cells in the optic nerve, retinorecipient areas in the thalamus, such as the dorsal lateral geniculate nucleus and the superior colliculus, as well as the primary and secondary visual cortices, which are accessible from the remote site of the retina or by injection into the vitreous body. Target neuronal cells on the corticostriatal pathway and the nigrostriatal pathway, in the cerebral cortex and substantia nigra, respectively, are accessible by injection at the remote site of the striatum. Further examples of target cells and remote sites by which such cells may be accessed can be determined by reference to various neuroanatomy atlases and texts, for example, *Atlas of Functional Neuroanatomy*, W. J. Hendleman, CRC Press, 2000; *Neuroanatomy: A Functional Atlas of Parts and Pathways*, R. Poritsky, Hanley & Belfus, 1992; *Functional Neuroanatomy*, A. K. Afifi and R. A. Bergman, McGraw-Hill, 1998; *Functional Systems: 3D Reconstructions with correlated Neuroimaging*, H-J Kretschmann and W. Weinrich, Thieme, 1998; and *Neuroanatomy: An Atlas of Structures, Sections and Systems 5th Ed.*, D. E. Haines, Lippincott, Williams & Wilkins, 2002.

[0024] Baculoviruses are well known and characterized and baculoviral vectors for mammalian gene transfection, including the use of *Autographa Californica* are known (see for example, Sakis et al PNAS 97(26)). A skilled person can readily construct any suitable baculoviral vector for use in this invention. The baculoviral vector may be a recombinant baculovirus whose genome has been modified to express a transgene operably linked to a neuron specific promoter or a viral promoter such as a CMV promoter or any operably linked promoter/enhancer as discussed below. The baculovirus may be so modified using standard techniques that will be known to a skilled person, such as PCR and molecular cloning techniques. For example, baculovirus can be readily modified using commercially available cloning and expression systems such as the Bac-To-BacTM Baculovirus Expression system (Gibco

BRL, Life Technologies, USA).

[0025] The term "gene" is used in accordance with its usual definition, to mean an operably linked group of nucleic acid sequences. The term "transgene" refers to a gene which is foreign, such that, for example, reference to expression of a transgene by a baculovirus refers to expression of a gene that is foreign to the baculoviral genome, and includes a therapeutic gene.

[0026] The term "therapeutic gene" or "therapeutic transgenes" as used herein is intended to describe broadly any gene the expression of which effects a desired result, for example, treatment, prevention or amelioration of a neuronal disorder.

[0027] Preferably, the gene is a therapeutic gene, including any gene having clinical usefulness, such as a gene encoding a gene product or protein that is involved in disease prevention or treatment, or a gene having a cell regulatory effect that is involved in disease prevention or treatment. The gene product should substitute a defective or missing gene product, protein, or cell regulatory effect in the subject, thereby enabling prevention or treatment of a disease or condition in the subject. Therapeutic genes include growth factor genes (which include genes of fibroblast growth factor gene family, nerve growth factor gene family and insulin-like growth factor genes), and antiapoptotic genes (including genes of bcl-2 gene family).

[0028] Neurological illnesses such as stroke, epilepsy, head and spinal cord trauma, Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and many neurogenetic disorders have devastating effects on the individual and result in high social costs associated with chronic care and lost productivity. A common feature of these illnesses is neuronal loss. Many of the aforementioned disorders are related to the absence, malfunction or ineffectiveness of one gene or more and do not respond well to conventional therapeutic means. Gene transfer into the central nervous system (CNS), which involves the delivery and expression of a therapeutic gene, has, therefore, been considered as a potential approach to treatment of these disorders. This approach may mediate expression levels of neurotrophic factors, anti-apoptotic proteins, antioxidant molecules and other therapeutic factors to restore, halt or prevent neuronal degeneration. Gene therapy also offers much hope for the treatment of CNS malignancies.

[0029] For example, there are two main gene therapy strategies for Parkinson's disease. The first approach is transfer of genes that encode trophic factors or protective proteins to slow or halt the continuing neurodegenerative process, such as BDNF (brain derived neurotrophic factor), GDNF (glia derived neurotrophic factor), and antiapoptotic genes like bcl-2. The second approach is transfer of genes encoding neurotransmitter-synthesizing or metabolic enzymes to enhance the function of partially degenerated systems, for example, TH (tyrosine hydroxylase), GTPCH (GTP-cyclohydrolase), and AADA (aromatic amino acid decarboxylase) to recover the striatal DA (dopamine) level and tone.

[0030] For Huntington's disease, approaches for gene therapy include 1) anti-sense strategies to inhibit the expression of mutated IT15 gene, and 2) protective strategies to keep GABAergic degeneration in the caudate, putamen and cerebral cortex. NGF, CNTF (ciliary neurotrophic factor) have been shown to provide protective effect on experimental animal model of Huntington's disease.

[0031] The unique characteristics of the CNS, the most sophisticated organ in the body, present several obstacles to successful gene therapy within the CNS. These characteristics include limited access to the CNS due to the physical barriers of the skull and the blood-brain barrier, as well as the nature of terminally differentiated neurons and the difficulty of efficiently transfecting them with therapeutic genes. Moreover, the cell types found within the CNS are very diverse, many of which are critical to physiological functions and highly sensitive to any kinds of changes. This requires the development of CNS gene therapy that is restricted to a particular type of CNS cell, thus ensuring therapeutic effects in the desired cells and limiting side effects caused by gene expression in non-target CNS cells.

[0032] In different embodiments, the baculoviral vector comprising a promoter operably linked to a therapeutic gene sequence may be delivered to neuronal cells distal to the site of administration. The promoter may be a strong viral promoter such as CMV or a neuronal specific promoter. Specific gene expression in a selected cell type can be achieved by using neuronal cell specific promoters.

[0033] The neuronal specific promoter may be any nucleotide sequence that functions to activate transcription of operably linked sequences within neurons or

neuronal cells and substantially not in other cells types. A promoter does not substantially activate transcription if the levels of transcription of operably linked sequences in any of those cell types are sufficiently low so as not to affect the physiological functioning of the cell.

[0034] Neuronal cell specific promoters may include promoters for neuronal genes such as Synapsin I, Neuron-specific enolase, Neurofilament-L and Neuropeptide Y and promoters specific for particular types of neuronal cells. For example, tyrosine hydroxylase gene promoter (4.8kb 5' UTR) is specific for catecholaminergic and the CNS neurons, dopamine- β -hydroxylase gene promoter is specific for adrenergic and noradrenergic neurons and L7 Purkinje cell protein promoter is specific for retinal rod bipolar neurons. For these and other neuronal cell specific promoters including, D1A dopamine receptor gene promoter, human hypoxanthine phosphoribosyltransferase promoter, SCG10 promoter, β -tubulin promoter, aldolase C promoter, beta-tubulin gene promoter, GnRH gene enhancer and promoter, glutamate decarboxylase 65 gene promoter, beta-galactoside alpha1,2-fucosyltransferase gene promoter, neuronal nicotinic acetylcholine receptor beta3 gene promoter, GABA(A) receptor delta subunit gene promoter, neuron-specific FE65 gene promoter, N-type calcium channel alpha1B subunit gene promoter and microtubule-associated protein 1B gene promoters, see Harrington CA, Lewis EJ, Krzemien D, Chikaraishi DM. Identification and cell type specificity of the tyrosine hydroxylase gene promoter. *Nucleic Acids Res* 1987, 15:2363-2384; Coker GT 3rd, Vinnedge L, O'Malley KL; Characterization of rat and human tyrosine hydroxylase genes: functional expression of both promoters in neuronal and non-neuronal cell types. *Biochem Biophys Res Commun* 1988, 157:1341-1347; Banerjee SA, Hoppe P, Brilliant M, Chikaraishi DM. 5' flanking sequences of the rat tyrosine hydroxylase gene target accurate tissue-specific, developmental, and transsynaptic expression in transgenic mice. *J Neurosci* 1992, 12:4460-4467; Morita S, Kobayashi K, Mizuguchi T, Yamada K, Nagatsu I, Titani K, Fujita K, Hidaka H, Nagatsu T. The 5'-flanking region of the human dopamine beta-hydroxylase gene promotes neuron subtype-specific gene expression in the central nervous system of transgenic mice. *Brain Res Mol Brain Res* 1993; 17:239-244; Ishiguro H, Kim KT, Joh TH, Kim KS. Neuron-specific expression of the human dopamine beta-hydroxylase gene requires both the cAMP-response element and a silencer region. *J Biol Chem* 1993; 268:17987-17994;

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activators of the neuron-specific FE65 gene promoter. *Biochem J* 1997; 328:293-300; Kim DS, Jung HH, Park SH, Chin H. Isolation and characterization of the 5'-upstream region of the human N-type calcium channel $\alpha 1B$ subunit gene. Chromosomal localization and promoter analysis. *J Biol Chem* 1997; 272:5098-5104 and Liu D, Fischer I. Two alternative promoters direct neuron-specific expression of the rat microtubule-associated protein 1B gene. *J Neurosci* 1996; 16:5026-5036. Other neuronal specific promoters will be known to persons skilled in the art.

[0035] The promoter comprises at least one nucleotide sequence capable of activating neuronal cell specific expression of operably linked sequences and in some embodiments the nucleotide sequence will retain the minimum binding site(s) for transcription factor(s) required for the sequence to act as a promoter. In some embodiments, the vector comprises multiple copies of the same sequence or two or more different nucleotide sequences each of which is effective to activate the transcription activity. For various promoters which may be used, transcription factor binding sites may be known or identified by one of ordinary skill using methods known in the art as described above. Suitable promoter/enhancer constructs may be readily determined by standard expression assays.

[0036] Platelet-derived growth factor β -chain (PDGF β) promoter (Sasahara M, Fries JW, Raines EW, Gown AM, Westrum LE, Frosch MP, Bonthron DT, Ross R, Collins T. PDGF β -chain in neurons of the central nervous system, posterior pituitary, and in a transgenic model. *Cell* 1991; 64:217-227) has been shown to be specific for CNS neuronal cells, including dopaminergic neurons and in one embodiment, the neuronal cell specific promoter is PDGF β promoter. In a specific embodiment, the neuronal specific promoter is human PDGF β promoter.

[0037] The transcriptional activity of a neuronal specific promoter in some instances may be weak, providing less than ideal level of expression of therapeutic gene sequences. In various embodiments, the neuronal specific promoter may be operably linked to an enhancer. An "enhancer" is a nucleotide sequence capable of increasing the transcriptional activity of an operably linked promoter and in the case of a neuronal cell specific promoter, of increasing neuronal cell specific transcriptional activity of the promoter.

[0038] A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the sequences are placed in a functional relationship. For example, a coding sequence is operably linked to a promoter if the promoter activates the transcription of the coding sequence. Similarly, a neuronal cell specific promoter and a heterologous enhancer are operably linked when the enhancer increases the neuronal cell specific transcription of operably linked sequences. Enhancers may function when separated from promoters and as such, an enhancer may be operably linked to a neuronal cell specific promoter but may not be contiguous. Generally, however, operably linked sequences are contiguous.

[0039] In different embodiments, the enhancer may be a heterologous enhancer, meaning a nucleotide sequence which is not naturally operably linked to a neuronal cell specific promoter and which, when so operably linked, increases the neuronal cell specific transcriptional activity of the neuronal cell specific promoter. Reference to increasing the transcriptional activity is meant to refer to any detectable increase in the level of transcription of operably linked sequences compared to the level of the transcription observed with a neuronal cell specific promoter alone, as may be detected in standard transcriptional assays, including using a reporter gene construct as described in the Examples.

[0040] The enhancer may be a known strong viral enhancer or promoter element such as Rous sarcoma virus (RSV) promoter (Gorman CM, Merlino GT, Willingham MC, Pastan I, Howard BH. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. *Proc Natl Acad Sci U S A* 1982; 79:6777-6781), SV40 promoter (Ghosh PK, Lebowitz P, Frisque RJ, Gluzman Y. Identification of a promoter component involved in positioning the 5' termini of simian virus 40 early mRNAs. *Proc Natl Acad Sci U S A* 1981; 78:100-104), CMV enhancer or promoter including CMV immediate early (IE) gene enhancer (CMVE enhancer) (Boshart M, Weber F, Jahn G, Dorsch-Hasler K, Fleckenstein B, Schaffner W. A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* 1985; 41:521-530; Niwa H, Yamamura H, Miyazaki J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* 1991; 108: 193-200; see also U.S. Patent Nos. 5,849,522 and 5,168,062).

[0041] In an embodiment of the present invention, CMVE enhancer is operably linked upstream to PDGF β promoter. In a further embodiment, CMVE enhancer is operably linked upstream to PDGF β promoter and the two sequences are contiguous.

[0042] Further embodiments of the neuron specific promoter and an enhancer that may be operably linked including allelic variants and derivatives of known promoter and enhancer sequences are described in US Application No. 10/407,009, which is herein fully incorporated by reference.

[0043] In various embodiments, such variants and derivatives may be substantially homologous in that it hybridizes to the known enhancer and promoter sequences under moderately or stringent conditions. Hybridization to filter-bound sequences under moderately stringent conditions may, for example, be performed in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.2 x SSC/0.1% SDS at 42°C (see Ausubel, *et al.* (eds), 1989, *Current Protocols in Molecular Biology*, Vol. 1, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). Alternatively, hybridization to filter-bound sequences under stringent conditions may, for example, be performed in 0.5 M NaHPO₄, 7% SDS, 1 mM EDTA at 65°C, and washing in 0.1 x SSC/0.1% SDS at 68°C (see Ausubel, *et al.* (eds), 1989, *supra*). Hybridization conditions may be modified in accordance with known methods depending on the sequence of interest (see Tijssen, 1993, *Laboratory Techniques in Biochemistry and Molecular Biology -- Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York). Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point for the specific sequence at a defined ionic strength and pH. Stringent hybridization may for example be in 5 times SSC and 50% formamide at 42 degrees Celcius and washing in a wash buffer consisting of 0.1.times SSC at 65 degrees Celcius. Washes for stringent hybridization may for example be of at least 15 minutes, 30 minutes, 45 minutes, 60 minutes, 75 minutes, 90 minutes, 105 minutes or 120 minutes.

[0044] The degree of homology between sequences may also be expressed as a percentage of identity when the sequences are optimally aligned, meaning the occurrence of exact matches between the sequences. Optimal alignment of sequences

for comparisons of identity may be conducted using a variety of algorithms, such as the local homology algorithm of Smith and Waterman, 1981, *Adv. Appl. Math* 2: 482, the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, the search for similarity method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85: 2444, and the computerised implementations of these algorithms (such as GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI, U.S.A.). Sequence alignment may also be carried out using the BLAST algorithm, described in Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10 (using the published default settings). Software for performing BLAST analysis may be available through the National Center for Biotechnology Information (through the internet at <http://www.ncbi.nlm.nih.gov/>). In various embodiments, the variants and derivatives may be greater than 50%, 80% to 100%, at least 80%, at least 90% or at least 95% identical as determined using such algorithms.

[0045] The baculoviral vector may be delivered to any vertebrate animal subject. In one embodiment the subject is a human.

[0046] To deliver the baculovirus to a target neuronal cell in a particular region of the central nervous system remote from the site of vector administration, it may be administered to the subject using standard methods known in the art and as described in the examples. For example, the vector may be administered to an accessible site, for example, in the striatum, in order to deliver the vector to neurons at a remote or inaccessible site by axonal transport of the baculovirus or to cortical or spinal cord. Since baculovirus can be transported anterogradely and retrogradely, the accessible site may be located upstream or downstream of the target cells on the same neuronal pathway.

[0047] Preferably the baculovirus is administered by injection, including stereotaxic injection and injection into the vitreous body of the eye and cortical or spinal cord injections for regeneration of injured spinal cord. For human patients, the stereotactic frame base will be fixed into the skull and the brain with the stereotactic frame base will be imaged using high resolution MRI. Using appropriate stereotactic software, the images will be translated into 3 dimensional coordinates appropriate for the stereotactic frame for targeted injection of vector DNA.

[0048] The baculovirus is administered in an amount sufficient to achieve the desired result, for example, expression of therapeutic gene in the target cells.

[0049] When administered to a patient, the baculovirus is administered in an amount effective and at the dosages and for sufficient time period to achieve a desired result. For example, the baculovirus may be administered in quantities and dosages necessary to deliver a therapeutic gene the product of which functions to alleviate, improve, mitigate, ameliorate, stabilize, prevent the spread of, slow or delay the progression of or cure a neuronal disease or disorder.

[0050] The effective amount to be administered to a patient can vary depending on many factors such as the pharmacodynamic properties of the baculovirus, the mode of administration, the age, health and weight of the subject, the nature and extent of the disorder or disease state, the frequency of the treatment and the type of concurrent treatment, if any, and the virulence and titre of the virus.

[0051] One of skill in the art can determine the appropriate amount based on the above factors. The baculovirus may be administered initially in a suitable amount that may be adjusted as required, depending on the clinical response of the patient. The effective amount of baculovirus can be determined empirically and depends on the maximal amount of the baculovirus that can be administered safely. Since baculovirus has little to no cytotoxicity in vertebrates, large doses may be tolerated. However, the amount of baculovirus administered should be the minimal amount that produces the desired result.

[0052] In various embodiments, a dose of about 10^9 plaque forming units ("pfu") is administered to a human patient. In other embodiments, about 10^2 to about 10^9 pfu, about 10^6 to about 10^9 pfu, about 10^2 to about 10^7 pfu, about 10^3 to about 10^6 pfu, or about 10^4 to about 10^5 pfu may be administered in a single dose.

[0053] Effective amounts of baculovirus can be given repeatedly, depending upon the effect of the initial treatment regimen. Administrations are typically given periodically, while monitoring any response. It will be recognized by a skilled person that lower or higher dosages than those indicated above may be given, according to the administration schedules and routes selected.

[0054] To aid in administration, the virus may be formulated as an ingredient in a pharmaceutical composition. The compositions may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives and various compatible carriers. For all forms of delivery, the recombinant baculovirus may be formulated in a physiological salt solution.

[0055] The proportion and identity of the pharmaceutically acceptable diluent is determined by chosen route of administration, compatibility with a live virus and standard pharmaceutical practice. Generally, the pharmaceutical composition will be formulated with components that will not significantly impair the biological properties of the live virus. Suitable vehicles and diluents are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985).

[0056] Solutions of the recombinant baculovirus may be prepared in a physiologically suitable buffer. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms, but that will not inactivate the live virus. A person skilled in the art would know how to prepare suitable formulations. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

[0057] The forms of the pharmaceutical composition suitable for injectable use include sterile aqueous solutions or dispersion and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions, wherein the term sterile does not extend to the live baculovirus itself that is to be administered. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists.

[0058] The delivery of a therapeutic gene to neurons in a remote or inaccessible region of the CNS allows for treatment of neuronal disorders or diseases. For example, using the method of the invention, dopaminergic neurons in the substantia nigra for therapeutic gene delivery may be targeted for the treatment of Parkinson's disease (Bilang-Bleuel et al., 1997). As well, using the method of the invention,

therapeutic transgene expression may be achieved in neurons widely distributed in various regions of the cerebral cortex, which cannot feasibly be reached by single local injection, and therefore allow for corticostriatopallidal neuroprotection of neurons affected in Huntington's disease (HD, Mittoux et al., 2002).

[0059] In one aspect the invention provides a method for treating a neuronal disorder in a subject, comprising identifying a target neuronal cell in the CNS affected by the disorder, identifying a site in the CAN that is remote from the target neuronal cell and administering a baculoviral vector comprising a therapeutic gene operably linked to a neuronal specific promoter, to said site wherein the baculoviral vector is delivered to the neuronal cell by axonal transport. The invention also provides for such use of a baculoviral vector for the treatment of a neuronal disorder and in the manufacture of a medicament for such treatment.

[0060] "Treating" a neuronal disorder refers to an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilization of the state of disease, prevention of development of disease, prevention of spread of disease, delay or slowing of disease progression, delay or slowing of disease onset, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treating" can also mean prolonging survival of a patient beyond that expected in the absence of treatment. "Treating" can also mean inhibiting the progression of disease, slowing the progression of disease temporarily, although more preferably, it involves halting the progression of the disease permanently. As will be understood by a skilled person, results may not be beneficial or desirable if, while improving a specific disease state, the treatment results in adverse effects on the patient treated that outweigh any benefits effected by the treatment.

[0061] A neuronal disorder may be, without limitation, stroke, ischemia, epilepsy, head and spinal cord trauma or injury, Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis or a neurogenetic disorder.

[0062] A "neuronal cell affected by the disorder" refers to any neuronal cell that

has a defect or malfunction that is related to, contributes to or is caused by the neuronal disorder and that can be treated by the expression of a therapeutic gene within the neuronal cell.

[0063] Certain viruses, such as HSV and psuedorabies virus, can be transported trans-synaptically and have been successfully used as transneuronal tracers (Babic et al., 1994; Blessing et al., 1994; Norgren, Jr. and Lehman, 1998). Due to the present discovery of the efficient trans-synaptical transport of baculoviruses, such viruses have the potential to act as a potent neuronal tracer for tracing both sensory and motory pathways. Compared to the other transneuronal tracer viruses, for which anterograde axonal transport tends to be relatively slower and less efficient than retrograde transport (McLean et al., 1989; Card et al., 1990; Card et al., 1991; Vann and Atherton, 1991; Norgren, Jr. et al., 1992; Blessing et al., 1994; Sun et al., 1996), baculoviruses spread quite quickly within the axoplasm both anterogradely and retrogradely. For example, in rats it was observed that within 2 days the recombinant baculovirus was transported from the striatum to the cerebral cortex and from the retina to the primary visual cortex. However, due the bi-directionality of baculovirus axonal transport, baculovirus may not be suited for tracing reciprocal pathways.

[0064] Therefore, in one embodiment there is provided a method for tracing a neuronal pathway comprising administering to a subject a baculovirus adopted for detection to a site in the central nervous system of the subject and detecting the transport pathway of the baculovirus.

[0065] The baculovirus may be adopted for detection by any method known in the art such that it is capable of being detected when injected into the CNS of the subject. For example, the baculovirus may be labeled with a detectable marker on the surface of the virus particle, for example, by attaching or conjugating a label, for example to a coat protein. Alternatively, the baculovirus may be labeled by incorporation of a labeled molecule within the virus particle, for example, during viral assembly. The attached label may be a fluorescent dye molecule, a radiolabel, a radiopaque material, luminescent nanocrystal quantum-dots or a material that aids in magnetic resonance imaging.

[0066] Radiolabels are known in the art, and include well-known medical

radionuclides such as Tc-99m, I-123, I-125, In-111, In-113m, Ga-67, or other suitable gamma-emitters. Radiopaque materials are also known and include iodine compounds, barium compounds, gallium compounds, thallium compounds, and the like. Specific examples of radiopaque materials include barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iodipamide, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, iseric acid, iosulamide meglumine, ios-umetic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, and thallous chloride. Materials that can be detected by or that enhance the effects of magnetic resonance include gadolinium, copper, iron, and chromium and may be prepared in the form of a conventional organometallic chelates, which may then be conjugated to the baculovirus.

[0067] The baculovirus may also be adopted for detection by genetic modification to express a gene that encodes a protein that can be detected, for example, a fluorescent protein, under control of a neuron specific promoter. For example, the baculovirus may be modified to express green fluorescent protein under control of the CMV E/PDGF promoter as described in the Examples.

[0068] The baculovirus may be detected using any conventional method for detection, including for visualizing diagnostic images and will depend on the particular detectable system used. For example, baculovirus genetically modified to express a fluorescent protein may be detected using fluorescence digital imaging microscopy. Other methods include computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography. Skilled artisans will be able to determine the appropriate method for detecting a particular detectable marker.

[0069] The baculovirus may be detected or visualized at a suitable time interval after administration of the virus to the subject, so as to allow for the baculovirus to be transported along the neuronal pathway, and to express a detectable product, if necessary. For example, visualization may occur anywhere between 2 and 20 days following administration to the subject of the baculovirus genetically modified to express a fluorescent protein.

EXAMPLES

[0070] We investigated whether baculovirus vectors could be transported in axons, thus mediating gene expression in regions remote to the injection site. Intra-striatum injection followed by examinations in the corticostriatal pathway and the nigrostriatal pathway and intra-vitreous body injection in the visual pathway were used to study retrograde and anterograde axonal transport, respectively. The recombinant baculovirus vectors accommodating the neuron-specific CMV E/PDGF promoter was used to facilitate gene expression in neuronal cell bodies.

[0071] Materials and methods

[0072] *Production of recombinant virus vectors:* Recombinant baculoviruses were constructed according to the manual of Bac-To-BacTM Baculovirus Expression system (Gibco BRL, Life Technologies, USA). Luciferase cDNA under the CMV E/PDGF, CMV, or PDGF promoter was inserted into the transfer plasmid pFastBac1TM. Each promoter was inserted between NotI & XbaI, and luciferase cDNA was inserted into the pFASTBac1 between XhoI & HindIII. Recombinant baculovirus vectors were propagated in Sf9 insect cells according to standard methods (O'Reilly et al., 1992). Budded viruses from insect cell culture medium were filtered through a 0.2 μ m pore size filter and concentrated by ultracentrifugation at 25,000 g for 60 min. The viral pellet was re-suspended in phosphate-buffered saline (PBS) and infectious titers were determined by plaque assay on Sf9 cells.

[0073] *Cy3 labeling of baculovirus:* Purified viruses were labeled with the carbocyanine dyes Cy3 (Amersham). Baculovirus stocks to be labeled were adjusted to a concentration of 2×10^{10} particles/ml in sodium carbonate sodium bicarbonate buffer, pH 9.3. Cy3 reagent was previously reconstituted in 1 ml of 0.1 M sodium carbonate, pH 9.3 (designed to label 1 mg protein to a final molar dye/protein ratio between 4 and 12). 100 μ l of virus preparation was used to mix with 200 μ l of Cy3 reconstitute. After 30 min at room temperature, labeled virus was purified by centrifugation at 28,000 g for 30 min. The virus pellet was washed in 1 x PBS for 2 times to remove the unreacted Cy3, and then re-suspended in 1x PBS. The purified viruses were aliquoted into single-use vials and stored at 4°C prior to use.

[0074] **Animals:** Adult male Wistar rats (weighing 250-320 g) were used in this study. In the handling and care of all animals, the International Guiding Principles for Animal Research as stipulated by World Health Organization (1985) and as adopted by the Laboratory Animal Center, National University of Singapore, were followed.

[0075] **In Vivo Injection Methods:** For intra-striatum injection, rats were anaesthetized by an intraperitoneal injection of sodium pentobarbital (60 mg/kg of body weight) and positioned in a stereotaxic instrument with the nose bar set at 0 (for striatum injection). Virus particles were separately and bilaterally injected into the striatum at 3 injection points [coordinates (from bregma and dura): anterior (A), +1.5 mm, lateral (L), +2.0 mm, ventral (V), -5.0 mm; A, +0.5 mm, L, +3 mm, V, -5.0 mm and A, -0.3 mm, L, +3 mm, V, -5.0 mm] or at 1 point [A, +0.5 mm, L, +3 mm, V, -5.0 mm]. For each injection, the injection volume was 5 μ l or 10 μ l containing 5×10^6 pfu or 1×10^7 viral particles. The rate of injection was 0.5 μ l/minute and the needle was allowed to remain *in situ* for 5 minutes before being slowly retracted at the end of each injection. For intra-vitreous body injection, the animals were anaesthetized the same way as previously described. A syringe with a needle having a No. 30 bore size was penetrated through the sclera into the vitreous body posterior to the ora seratta, then 10 μ l of the vitreous body was slowly sucked out. After that, 10 μ l of the virus particles (10^7 pfu) was then slowly injected back into the posterior chamber.

[0076] **Luciferase assay:** For luciferase activity assays, virus infected rats were sacrificed 2 days after infection by intracardiac perfusion with 0.1 M PBS (pH 7.4) following deep anesthesia. Tissue samples were collected and stored at -80°C until processing. After adding PBS buffer (100 μ l PBS per 50 mg tissue), each sample was homogenized by sonication for 10 seconds on ice, and then centrifuged at 13,000 rpm at 4°C in a microcentrifuge. 10 μ l of the supernatant at room temperature was used for the luciferase activity assay employing an assay kit from Promega. Measurements were made in a single-well luminometer (Berthold Lumat LB 9501) for 10 seconds.

[0077] **PCR detection of virus genome from tissue samples:** Virus infected rats were sacrificed 2 days after infection by intracardiac perfusion with 0.1 M PBS (pH 7.4) following deep anesthesia. Tissue samples were collected and homogenized by mincing with a pestle and the virus genome was extracted according to the standard

protocol of a DNeasyTM Tissue Kit (Qiagen). The primers target to a sequence of around 400 base pairs, overlapped across both the native virus genome and the inserted reporter gene. PCR primers are listed as below:

5' primer: 5'-AT TGC TCA ACA GTA TGG GCA-3' (SEQ ID NO.:1)

3' primer: 5'-CGA AGA AGG AGA ATA GGG TTG-3' (SEQ ID NO.:2)

[0078] Amplification cycles consisted of 94 °C 7min, 1 cycle; 94 °C, 45 s, 52 °C 30 s, 72 °C, 30 s, 36 cycles and one final extension cycle at 72 °C for 7 min. To exclude contamination of the whole process, a normal rat brain without virus infection was handled in parallel.

[0079] *Immunohistochemical analyses:* Two days after intra-striatum or intra-vitreous body injection with Cy3 labeled or unlabeled baculovirus, rats were sacrificed (n=2). Following deep anesthesia, all rats were perfused first with 0.1M PBS (pH7.4) solution followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). After perfusion, the brains or eye balls were removed and post-fixed in the same fixative for 2-4 hours before they were transferred into 0.1 M PBS containing 20% sucrose and kept overnight at 4°C. Frozen coronal sections of each brain were cut at 30µm thickness and stored in 0.1M PBS. Free-floating sections were washed for 20 min in 0.1M PBS at pH 7.4 containing 0.2% Triton X-100, then blocked with 5% normal goat serum in PBS for 1 hour. Frozen sagittal sections of each eyeball were cut at 30 µm thickness and mounted on gelatin-coated slides.

[0080] Sections were then incubated overnight with primary antibodies polyclonal anti-luciferase (Promega; dilution 1:150) and monoclonal against neuron-specific nuclear protein (NeuN) (Chemicon International, USA; dilution 1:500). Sections were washed in 0.1 M PBS and further incubated with anti-rabbit IgG conjugated by Tritc (Sigma-Aldrich, Inc., USA; dilution 1:100) and anti-mouse IgG conjugated by Fitec (Sigma-Aldrich; dilution 1:100) for 1 hour. After incubation, sections were washed three times in PBS. They were then collected on gelatin-coated slides, mounted with DAKO fluorescent mounting medium and covered with cover-slips. Control sections were incubated without primary antibodies.

[0081] Cy3 labeled viruses were used to show the virus internalization in vivo.

For this group, sections were only incubated overnight with monoclonal against neuron-specific nuclear protein (NeuN) (Chemicon International, USA; dilution 1:500) or monoclonal against glial fibrillary acidic protein (GFAP, Chemicon). Sections were washed in 0.1 M PBS and further incubated with anti-mouse IgG conjugated by Fite (Sigma-Aldrich; dilution 1:100) for 1 hour. Then sections were treated the same as above.

[0082] *Visualization of double labeling with confocal scanning microscopy:*

Sections were examined with a Carl Zeiss LSM410 confocal laser scanning microscope. Each section was initially scanned with a 488 nm laser line, and an emission filter BP 510-525, for the detection of Fite fluorescein; and with a 543 nm laser line, and an emission filter LP 570, for the detection of Tritc fluorescein.

[0083] Results

[0084] *Intra-striatum injection:* We first used Cy3 labeled baculovirus vectors to examine the possible retrograde axonal transport of the vectors. The Cy3 labeled vectors were injected with 1×10^9 plaque-forming units (pfu) of concentrated virus particles into the Wistar rat striatum by stereotaxic apparatus. Tissue sections along the corticostriatal pathway and the nigrostriatal pathway were collected and immunostained using antibodies against neuronal nuclear protein (NeuN, a neuronal specific marker) to show the neurons in striatum and cerebral cortex or antibodies against tyrosine hydroxylase (TH, a dopaminergic neuronal specific marker) to show dopaminergic neurons in substantia nigra. As Cy3 was conjugated to envelope proteins of the virus, the fluorescein displayed in the tissue would present the virus particles transported together with viral capsids.

[0085] Figure 1 shows the confocal images of striatum, cerebral cortex and substantia nigra. In the striatum the Cy3 signals were detected in both NeuN-positive or negative cells, indicating the entry of virus particles to both neuronal and non-neuronal cells. In cerebral cortex and substantia nigra, Cy3 signals were strictly colocalized with either NeuN or TH signals, suggesting that virus particles, after injected to the striatum, entered axon terminals and moved in axoplasm to the somas of projection neurons located in the cerebral cortex and the substantia nigra. This was supported by PCR analysis, which detected baculovirus genome in the cerebral cortex

and the substantia nigra remote from the injected striatum (Figure 2).

[0086] To test the possibility of gene transfer in projection neurons, the expression of luciferase reporter gene driven by the neuron-specific CMV E/PDGF promoter the viral CMV promoter was examined. Luciferase activity was measured 2 days after injection of 5×10^6 pfu of recombinant baculovirus. Luciferase activity was detectable not only in the injected striatum but also in distal substantia nigra and cerebral cortex regions (Figure 3). More than 90% of the total luciferase activity from three parts was detected in the cerebral cortex, and about 1% in the substantia nigra.

[0087] A further experiment was performed to determine the type of cells with luciferase expression using immunohistological staining. In the striatum, luciferase signals were detected in both NeuN positive and negative cells (Figure 4). As shown in Figure 5, the expression of luciferase reporter gene was strictly co-localized with either NeuN-positive neurons in the cerebral cortex or TH-positive neurons in the substantia nigra. In the cerebral cortex, luciferase signals were detected mainly in large pyramidal neurons located in deeper layers (Figure 5), a group of cells known to project their long axons to the putamen of the striatum. There were very few transduced cells, with granule interneuron morphology, in surface layers of the cerebral cortex (Figure 5).

[0088] The luciferase expression in substantia nigra was relatively lower compared with that in the cerebral cortex, although its absolute value was still highly significant. Considering the difference of neuron quantity in these two brain regions, the difference in transgene expression levels is not unexpected.

[0089] *Intra-vitreous Body Injection:* To investigate possible anterograde axonal transport of baculoviruses, Cy3 labeled vectors were injected into the rat vitreous body in order to transduce the retina ganglio cells (RGCs). These neurons have long axons projecting to the lateral geniculate body (LGB) and superior colliculus (SC) and forming synapse with interneurons located there. From the LGB, the axons of the interneurons fan out through the deep white matter of the brain to form the optic radiations, which will ultimately terminate in the primary visual cortex at the posterior part of the brain.

[0090] After intra-vitreous body injection of 10^9 pfu of the labeled baculovirus

vectors, virus particles were observable under confocal microscope in different parts of the visual system, from the retina, the optic nerve, the LGB, the SC to the primary visual cortex. In the retina, virus particles were detected in both NeuN-positive and negative cells (Figure 6 and Figure 7). The labeled particles were easily detected in the optic nerve and distributed along the sagittal section of the nerve, indicating the anterograde travel of the virus along the axons of the RGCs. The Cy3 signals were strictly located in NeuN-positive neurons in the LGB and the SC, indicating a possible trans-synaptic transport of the viral particles to post-synaptic neurons. The hypothesis of the transport of virus vectors across the synaptic cleft was strongly supported by the detection of the Cy3 signals in the tertiary neurons in primary visual cortex. PCR analysis of the virus genome in the samples collected from the different parts of the visual system provided another piece of the evidence for the anterograde transport and trans-synaptic transport of the baculovirus vectors (Figure 8).

[0091] The possibility of using baculoviruses for gene transfer in projected, post-synaptic neurons was then investigated with a luciferase reporter gene vector under control of the neuron-specific CMV E/PDGF promoter. After intra-vitreous body injection of 1×10^7 pfu of the recombinant baculovirus, luciferase activity was detected in all parts of the visual circuit, with the highest transgene expression in the retina, followed by that in LGN and primary visual cortex (Figure 9). The SC gave the lowest luciferase expression, consistent with the fact that only a small amount of axons from RGCs projecting to this region in rats (<http://thalamus.wustl.edu/course/> and <http://psych.athabasca.ca/html/Psych402/Biotutorials/>). As revealed by immunohistological staining, luciferase expression was confined only in neurons, no matter which part of the visual system was examined (Figure 10). Most of the transduced neurons in the LGB were large neuron cells, likely to be the large M cells of the LGB that receive the inputs from large ganglion cells.

[0092] In rodents, most projections from the retina go contra-laterally to the thalamus, to the superior colliculus (SC) and to several structures in the hypothalamus. Within the thalamus, several specific thalamic nuclei, including the dorsal lateral geniculate nucleus (dLGN), are primary retinorecipient areas. From the dLGN, neuronal projections is relayed to the primary and secondary visual cortices (areas 17 and 18). The neurons further project from the visual cortex to many other

cortical and subcortical regions, such as the temporal, frontal, and ventrolateral orbital cortices and the basal ganglia. These connections between the retina and the visual cortexes are therefore useful for analyzing transneuronal transport. Here, we found that, with the assistance of a neuron-specific promoter, the transgene expression in the retina was confined in ganglion cells after intravitreal injection of recombinant baculovirus vectors, although the viruses entered both neurons and glia in the retina as revealed through Cy3 labeling. This study showed further that intraocular inoculation of baculovirus resulted in a sequential infection of neurons along the structures associated with vision, providing the evidence for the ability of the virus to cross several synapses.

[0093] As can be understood by one skilled in the art, many modifications to the exemplary embodiments described herein are possible. The invention, rather, is intended to encompass all such modification within its scope, as defined by the claims.

[0094] All documents referred to herein are fully incorporated by reference.

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WHAT IS CLAIMED IS:

1. Use of a baculoviral vector in the manufacture of a composition for delivery of a nucleic acid to a neuronal cell in the treatment of a neuronal disorder, the vector being suitable for
5 delivery to a target neuronal cell in a subject when said composition comprising the vector is administered to a site remote from the target neuronal cell wherein the vector is delivered to the target cell by axonal transport.
2. Use of a baculoviral vector in the manufacture of a composition for the treatment of a
10 neuronal disorder, wherein the composition is delivered to a site remote from a target neuronal cell and wherein the vector is delivered to the target cell by axonal transport.
3. The use according to claim 1 wherein the nucleic acid is a therapeutic gene.
- 15 4. The use of any one of claims 1 to 3 wherein the vector comprises a coding sequence operably linked to a promoter.
5. The use of claim 4 wherein the promoter is a viral promoter.
- 20 6. The use of claim 5 wherein the promoter is CMV promoter.
7. The use of claim 4 wherein the promoter is a neuronal cell specific promoter.
8. The use of claim 7 wherein the neuronal cell specific promoter is operably linked to an
25 enhancer.
9. The use of claim 8 wherein the enhancer is a heterologous enhancer that increases the neuronal cell specific transcriptional activity of the promoter.
- 30 10. The use of claim 9 wherein the neuronal cell specific promoter is PDGF β promoter and the enhancer is CMV E enhancer.
11. The use of any one of claims 1 to 10 wherein the vector is formulated for administration by injection.

12. The use of claim 11 wherein about 10^6 to about 10^9 pfu of the baculoviral vector is administered.

5 13. The use of any one of claims 1 to 12 wherein the axonal transport is by anterograde transport, retrograde transport or trans-synaptic transport.

14. The use of claim 13 wherein the neuronal disorder is stroke, ischemia, epilepsy, head and spinal cord trauma, Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic
10 lateral sclerosis or neurogenetic disorder.

15. The use of claim 14 wherein the neuronal disorder is Parkinson's disease.

16. The use of claim 15 wherein the neuronal cell is dopaminergic neurons in the substantia
15 nigra.

17. The use of claim 16 wherein the neuronal disorder is Huntington's disease.

18. The use of claim 17 wherein the neuronal cell is corticostriatopallidal neurons.
20

19. Use of a baculovirus adapted for detection in the manufacture of a composition for tracing an axonal transport pathway in the central nervous system.

20. The use of claim 19 wherein the surface of the virus is labelled with a detectable marker.
25

21. The use of claim 20 wherein the detectable marker is Cy3.

22. The use of claim 21 wherein the baculovirus comprises a gene operably linked to a promoter, wherein the gene encodes a protein that can be detected.
30

23. The use of claim 22 wherein the promoter is a viral promoter.

24. The use of claim 23 wherein the viral promoter is CMV promoter.

25. The use of claim 22 wherein the promoter is a neuronal cell specific promoter.

26. The use of claim 25 wherein the neuronal cell specific promoter is operably linked to an enhancer.

5

27. The use of claim 26 wherein the neuronal specific promoter is PDGF β promoter and the enhancer is CMV E enhancer.

28. The use of claim 27 wherein the protein is a fluorescent protein.

10

29. The use of any one of claims 19 to 28 wherein the baculovirus is formulated for administration by injection.

30. The use of claim 29 wherein the baculovirus is formulated for administration by direct injection, stereotaxic injection or intra-vitreous injection.

15

31. The use of claim 30 wherein the baculovirus is formulated for administration of about 10^6 to about 10^9 pfu.

20

32. The use of any one of claims 19 to 31 wherein the baculovirus is transported by anterograde transport, retrograde transport or trans-synaptic transport.

SEQUENCE LISTING

<110> Wang, Shu
Li, Ying

<120> METHOD FOR GENE DELIVERY TO NEURONAL CELLS

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