Viral Vectors for Delivering Gene Material into Cells and Their Application in Neurobiology (Review)

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Viral vectors are modern tools for delivering genetic material into cells. Various types of viral vectors based on retroviruses, adeno-associated and lentiviral viruses, adenoviruses, herpes simplex and poxviruses are considered in this work. Adeno-associated vector systems are presented in more detail. Their main advantages (ability to integrate a target gene into the proper site of the host genome, preventing undesirable mutations; entering both dividing and nondividing cells; a wide transduction profile; low immune response; strong and stable transgene expression) have made these vectors a widely used and universal tool for transferring genes *in vitro* and *in vivo*. Possible applications of viral vectors in neurobiology are also shown.

Key words: viral vectors; adeno-associated viral vectors; helper plasmids; transfection; neurobiology.

At present, viral vectors are widely used for delivering genetic material into cells. It is owing to the specific life cycle of viruses that the first vectors (transgene carriers) began to be developed on their basis [1]. Viruses transfer foreign genes which are able to express in the infected cells [2]. Now, viruses have evolved to specialized molecular mechanisms capable of transferring effectively their genomes inside the cells which they infect [3].

A diversity of viral vectors is large, and each has its benefits and drawbacks. Presently, considerable efforts are directed to the development of viral vectors with improved safety characteristics and greater efficiency of delivering nucleic acid to the cells, and also to the provision of long-term and tissue-specific expression of the introduced genetic material. Potentially, viral vectors can be used as one of the ways of delivering genes in gene therapy of neoplasms and hereditary illnesses.

Recombinant adeno-associated viruses (rAAV) are one of the most promising delivering vectors for gene therapy owing to their nonpathogenic properties, lack of immunogenicity on the part of a host, and tropism towards the majority of cells and tissues [4]. Adeno-associated viral vectors are very close to ideal vectors by their characteristics [5].

Viral vectors have also found their application in neurobiology as a system of effective delivery of genes to neurons and other neuronal cells *in vitro* and *in vivo*. Viral vectors make it possible to observe neurobiological functions, alter expression of the target genes, label the cells to determine their outcome, and to change physiological state of the specific cell populations. Application of viral vectors for delivering genes into the nervous system is very perspective for fundamental researches and therapeutic purposes as well.

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Types of viral vectors

There exist viral and nonviral systems of gene material delivery. Viral systems include vectors developed on the basis of retroviruses, adenoviruses, adeno-associated viruses (AAV), lentiviruses, and herpes simplex viruses (HSV). Nonviral systems comprise "naked" DNA and lipids or polyethylene glycol [4]. It does not matter which genome compose viral vectors, DNA or RNA, since they have similar life cycle which starts with the interaction with a specific receptor on the cell surface. After virion adsorption on the cell surface, the virus genome in the process of penetration is subject to "uncoating". Three molecular processes occur during the intracellular life cycle of the virus: replication of the genome nucleic acid, transcription and translation. At each stage the virus interferes with the cellular synthetic mechanisms and submits them to its own tasks, creating priorities for viral nucleic acids [6].

Neither DNA nor RNA can be used in the "naked" form for the delivery of genes to the target cells. A sugar phosphate backbone of nucleic acid molecules is located along their periphery by the polar groups towards the outside imparting them anionic properties [7]. At physiological pH values, nucleic acid carries a negative charge repelling it from the negatively charged outside surface of the cellular membrane. One more limitation to the penetration of nucleic acid into the cell is its hydrophilic property [8]. All its hydrophobic bases are turned inside the molecule, therefore it cannot penetrate through the hydrophobic barrier of the target cell [9]. In blood serum nucleic acid quickly degrades under the action of nucleases. Half life of unmodified interfering RNA in blood serum occupies 5–60 min, for DNA it lasts not more than 10 min [10]. Besides, nucleic acids are not able to identify specific target cells. Therefore, from the beginning of 1980s, vector genetic constructs are being developed to deliver genes into eukaryotic cells [11].

Retroviral vector systems

Retroviruses are referred to the group of viruses whose RNA-genome is converted to DNA in the infected cells. A retrovirus genome is formed by a positive strand RNA and includes three structural genes (*gag, pol* and *env*) flanked by long terminal repeats (LTR) [12]. LTR contain regular elements, which play a role in retrovirus penetration, are necessary for integrating DNA copy of virus genome with a host genome, and also determine the beginning and end of the virus genome. LTR controls expression of viral genes as well [13]. The envelope of retroviruses consists of a cytoplasmic membrane of the infected cell and viral proteins [14]. A specific feature of the retrovirus life cycle is reverse transcription of the virus in the infected cell using the viral RNA as a template with formation of a double-stranded DNA (provirus), which then penetrates into the cell genome. This entry is arbitrary, and therefore the risk of insertional mutagenesis increases. As these viruses infect only dividing cells (to penetrate into the nucleus it is necessary to destroy the nuclear cell membrane, occurring during mitosis), retroviral vectors are mainly used for cell transfection *ex vivo* or for malignant tumor therapy [15]. The retroviral vectors are produced on the basis of provirus from which *gag*, *pol* and *env* genes are deleted to prevent virus reproduction and to place the desired genetic material instead. Up to 8 kb (thousand base pairs) of DNA-insert may be included into the retrovirus-based vector. For virus reproduction, the deleted viral genes (*gag*, *pol, env*), which encode viral proteins and provide virus reproduction, are incorporated into the genome of the packaging cell line on different chromosomes in such a way as to reduce the probability of reverse recombination of viral genes into the original viral genome and formation of replication-competent viruses [16].

Lentiviral vector systems

Lentiviruses are referred to the family of retroviruses and in contrast to other retroviruses infect not only dividing cells but nondividing either. The most studied lentivirus is human immunodeficiency virus (HIV). Due to the ability of lentiviruses to include a large amount of genetic material (up to 8 kb) and infect dividing and nondividing cells, these viruses are perspective vectors for gene transfer *in vivo* [17]. HIV genome includes three structural protein genes (*gag*, *pol,* and *env*) and 6 regulatory protein genes (*tat*, *rev*, *vpr*, *vpu*, *nef* and *vif*) [18, 19]. Some genes may be excluded from the virus genome without reducing the virus ability to replicate and infect cells. The lentiviral life cycle is similar to that of other retroviruses excluding the ability to infect nondividing cell as well. This ability is provided by the interaction of viral preintegration complex with the nuclear membrane and transport though it [20]. The assembly of the lentiviral vector occurs in the packaging cells, which are continuous cells, synthesizing virus-specific proteins [21]. Packaging cells include packaging, vector and enveloping cassettes, which together allow the assembly of a functional viral particle [22, 23]. And their simultaneous expression does not cause formation of retroviral particles able to initiate infectious process in humans [24].

Lentiviruses have a relatively small size of a target gene insert (up to 8 kb), can provide a long-term expression of the transgene and induce a minimal immune response of a host organism [25]. Some authors do not consider these vectors to be suitable for the delivery *in vivo*, since they increase the risk of insertional mutagenesis. However, application of lentiviruses for the transduction of differentiated cells has a lower risk of insertional mutagenesis compared to the use of other retroviruses [26].

Adenovirus-based vector systems

Adenoviruses are a family of DNA viruses, carrying in their structure one double-stranded DNA molecule and lacking lipid membrane. Adenoviruses are divided on the basis of binding with specific sera into 51 serotypes [27], and into 6 subgroups more (from A to F) by the capacity to agglutinate erythrocytes in humans, rabbits and mice, and by oncogenicity for rodents [28]. Adenoviruses of various subgroups affect different human organs and tissues [29].

Engineering of recombinant adenoviral vectors is in many aspects similar to that of lentiviral vectors. Adenoviruses with defective replication were obtained by replacing *E1* gene necessary for replication by the gene of interest, promoter and enhancer [30]. Such recombinant vectors effectively replicate in packaging cells expressing the product of *E1* gene. Adenoviral vectors unable to replicate beyond the packaging cells are used to introduce transgenes *in vivo* [31]. Recombinant adenoviral vectors provide a very strong but short-term (5–10 days) expression of the cloned genes due to the immune response of the recipient organism [32]. To solve the problem, the second generation of adenoviral vectors was created, in which not only *E1* gene but genes responsible for virus replication were also removed, remaining only elements determining genome beginning and end and viral packaging sequence [33]. Such vectors are capable of longer gene expression [14].

Adenoviruses can infect the majority of cell types (dividing and nondividing). The viral genome can accommodate 20 kb of the target gene insert, which is a sufficiently large capacity of the recombinant adenoviral vectors. Adenoviruses replicate in the nucleus of the infected cell as episomal elements and have a high transduction efficiency. Thus, for example, after direct intracranial injection of the recombinant adenovirus its capability to infect neurons, astrocytes, oligodendroglia, ependymocytes, choroidal epithelium and microglia has been revealed [34, 35].

Vector systems based on herpes simplex virus

Viral vectors based on HSV have a more simple construct than adenovirus-based vectors. The virus itself contains about 80 genes, one of which (*IE3*) is replaced most often during vector engineering [36]. Other genes may also be deleted, which allows for the increase of the vector size, or cloning several genes of interest. The disadvantages of the vectors based on HSV are a short-term expression of the cloned genes, toxicity for the target cells, low transduction efficiency and ability to infect only nondividing cells [37].

At present, amplicons — multiple repeating sequences of HSV including monomeric sequences organized as concatemers — are actively being used to create viral vectors [38]. Each monomer includes at least one region of the viral DNA replication origins (oriS or oriL) and a sequence for DNA packaging into the viral particle (pac) [39]. Molecular cloning of these sequences into bacterial plasmid enables one to obtain a vector which packages DNA into HSV virion. Such vector systems are capable of including up to 150 kb of foreign DNA, which gives the opportunity to deliver several transcription units in one vector into the target cell without evoking immune response and cytopathic effect [40]. The life cycle of HSV is characterized by the two-stage process of infecting: an active stage (penetration and reproduction in the epithelial cells resulting in cell lysis) and a latent one (penetration and reproduction in the nervous cells) [41]. The cell lysis occurs due to the fact that during virus replication the formed virions leave the cell by nuclear membrane budding.

HSV, being neurotrophic and highly effective in studying retrograde and anterograde transport in CNS, can be introduced in a harmless latent state. HSV vectors have a large genetic size and can provide a long-term expression of the transgene, however, as mentioned above, their main drawback is toxicity for cells and low transduction efficiency [24].

Vector systems based on poxviruses

Poxviruses are large viruses, which contain double-stranded DNA. The poxviral vector allows the inclusion up to 25 kb of DNA nucleotides of interest without deleting the genes of the virus itself. Poxviruses-based vector systems are not so widely used, since eukaryotic promoters are not effectively recognized by the poxviral transcription mechanisms, and it is necessary to use poxviral promoters for effective expression of the recombinant genes in a recipient cell [42]. Poxviral transcripts are not subject to splicing and for this reason the DNA of interest must be represented in the form of complementary DNA. A characteristic feature of the poxvirus life cycle is the availability of its own DNA-dependent RNA polymerase, which provides reading of more than a half of the viral genome during the initial and early stages of the reproductive cycle. Due to a large size and noninfectious nature of the poxviral DNA, foreign genes clone in poxviruses *in vivo* by recombination. Poxviruses possess natural tropism towards tumor tissues [43].

The main disadvantages of poxviruses are high immunogenicity, short-term expression and difficulties in replication in eukaryotic cells. The positive features of poxviruses are a high transduction efficiency, size and ability to infect the majority of cell types [44].

Adeno-associated viral systems and their benefits

Recombinant adeno-associated viruses are one of the most perspective delivery vectors for gene therapy and neurobiology owing to their nonpathogenic properties,

low immunogenicity on the part of a host, tropism towards the majority of the cells and tissues, high transduction efficiency and long duration of expression [45]. Their main drawback is a small vector size (up to 5 kb).

AAV are known to have 8 different serotypes. AAV2, AAV3 and AAV5 were initially isolated from monkeys [46]. AAB6 was supposed to be formed by recombination of AAV2 and AAV1, i.e. 5'-end of AAV2 fused with 3'-end of AAV1 (including two open reading frames) [47, 48]. AAV7 and AAV8 were isolated from rhesus monkey by PCR-amplification using primers obtained from the conservative regions of AAV1–AAV6 serotypes [49]. Among all eight serotypes, AAV2 is studied best and is widely used as a gene delivery vector [5].

AAV belongs to *Parvoviridae* family. This is a group of non-enveloped viruses containing a single-stranded DNA. The virus particle has an icosahedral (20 facets) symmetry 18–26 nm in diameter and 5.5–6.2 MDa molecular mass [50].

The most characteristic features of the nuclear structure are the groups of threefold peaks and loops located between two adjacent subunits [51]. Positively charged groups positioned along one side of each peak are responsible for binding with a cellular receptor — heparin sulfate proteoglycan (HSPG) [52]. Due to a high expression of the given receptor in various tissues, binding to HSPG determines a wide spectrum of cellular specificity to AAV2 infection [53]. The loop located in the contiguous subunit also participates in binding to the HSPG cellular receptor and contains an epitope for neutralizing antibodies [5]. Neutralization of the immune response determines future successful viral vector reentry. X-ray analysis of the AAV2 particle structures opened the possibilities of altering the epitope, contained in the viral capsid proteins, and a neutralizing antibody, and performing other modifications applicable for readmission in gene therapy [54].

AAV2 has a single-stranded genome DNA consisting of 4,680 bases. Inverted terminal repeats containing 145 bases with a large content of GC pairs and capable of forming palindrome structure, are located both at the 5'-end and 3'-end [55]. AAV2-genome synthesizes with mRNA beginning from p19 promoter [56]. Gene *cap* encodes three viral proteins: VP1 (735 amino acids, 90 kDa), VP2 (598 amino acids, 72 kDa) and VP3 (533 amino acids, 60 kDa) forming a viral capsid in 1:1:20 ratio, respectively, the capsid having icosahedral symmetry and consisting of 60 subunits [57]. The three capsid proteins synthesize with one mRNA beginning with p40 promoter, and differ from each other because of alternative splicing and various start codons [58]. All three capsid proteins have the same carboxy-terminal sequences and stop-codons (Figure 1).

Inverted terminal repeats at both ends accomplish at least the three following functions. Firstly, 3'-end serves as a primer for the synthesis of a new DNA strand. Secondly, it has Rep-binding site for Rep78 and Rep68, which possess helicase, strand-specific and site-specific

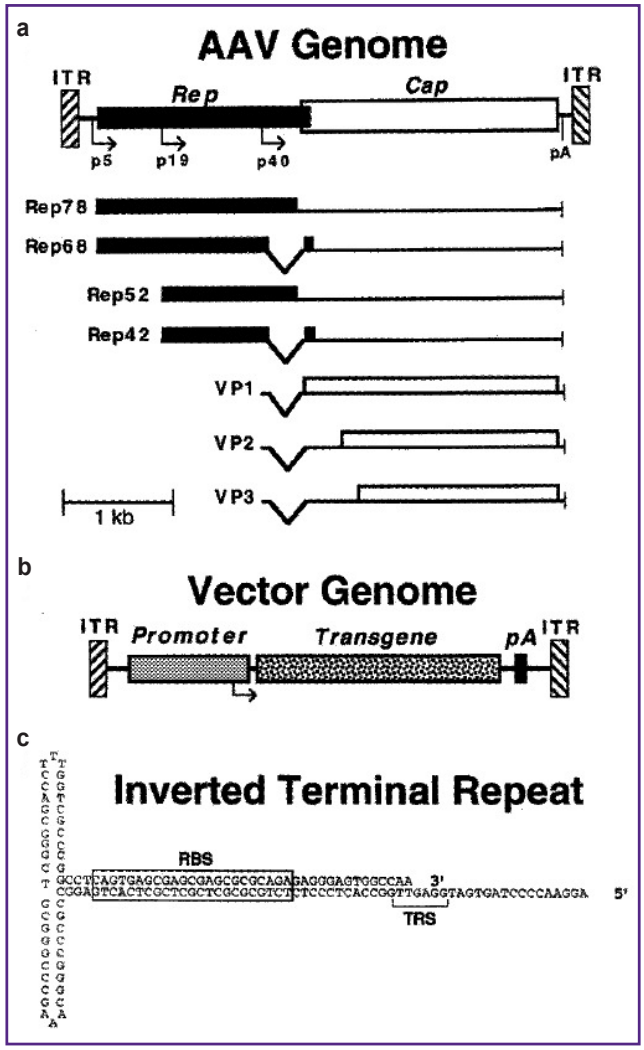

Figure 1. The structure of wild-type genomes and the genome of recombinant vector AAV [59]: (a) a map of a wild-type genome AAV: 3 promoters, 4 Rep proteins and 3 Cap proteins (VP1–3); (b) typical recombinant vector AAV; (c) a detailed presentation of the secondary structure of inverted terminal repeats (ITR), Rep binding site (RBS) and terminal resolution site (TRS)

endonuclease activity [60]. Thirdly, there is a terminal resolution site there, which is identical to the sequence in chromosome 19 and serves as a sequence for integration with the virus genome [61].

The infection pathway of AAV2 includes several stages (Figure 2). Viral particles attach initially to the cell surface by binding with the receptor and coreceptor, which, in its turn, leads to internalization and inclusion into intracellular transport [62]. Then, these particles penetrate through the nuclear membrane or integrate their genomes with the genome of the host cell, or replicate their genome inside the nucleus [63]. HSPG is recognized to be the main cellular receptor for AAV2 binding [64].

The analysis of site-directed mutagenesis helped to

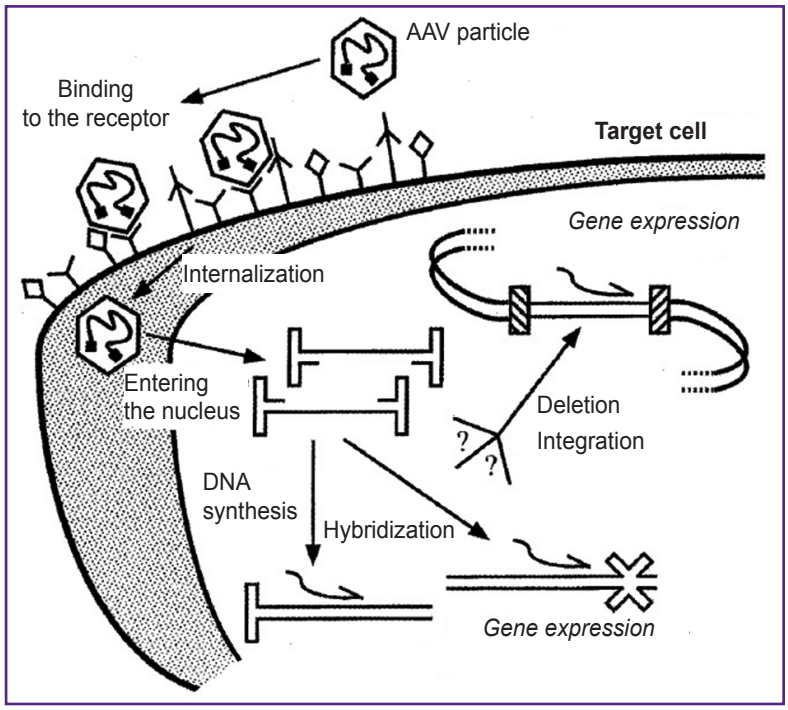

Figure 2. Life cycle of AAV2 [59]. Transduction of the target cell is initiated by binding AAV2 to the receptor on the target cell surface and coreceptor followed by internalization, entering the nucleus, DNA synthesis or integration into the genome and, eventually, gene expression

reveal the importance of the main amino acids in binding AAV2 capsid to heparin [65].

HSPG acts as the main receptor, but AAV2 also binds to coreceptor — fibroblast growth factor receptor 1 (FGFR1) and αvβ5-integrin [66, 67]. FGFR1 is supposed to increase the strength of attaching the virus to the cell, while αvβ5-integrin is needed in endocytosis [68, 69]. After endocytosis, AAV2 viral particles get free from the endosome at low pH [70, 71]. Low pH is likely to induce conformational alterations of the viral proteins, which play a decisive role in the successful release from the endosome and entering the nucleus [72, 73].

In some nuclei AAV genome integrates into chromosome 19 as a provirus, and in others viral genomes can remain as episomes [5].

Helper plasmids. Transfection

Transfection is a process of introducing nucleic acid into human and animal cells by nonviral methods. Plasmid DNA is most commonly transferred into cells, but other macromolecules can also be translated inside the cell such as small interfering RNA, oligonucleotides, RNA, and proteins [74]. Transfection may comprise several stages, but formation of pores in the plasmatic membrane, through which extracellular material can penetrate inside the cell, is an obligatory stage [75, 76].

A standard method of obtaining rAAV includes transfection of rAAV cells by plasmid and packaging plasmid encoding genes *Rep* and proteins VP [77]. Besides, cells can be coinfected by a helper virus, e.g. adenovirus, which provides accomplishment of various functions in the production of AAV [78, 79]. As a consequence, the cells produce not only AAV, but also adenoviruses that contaminate the end product (Figure 3 (a)).

The advanced method of rAAV obtaining is based on cotransfection of the cells by rAAV and helper plasmid derived from AAV and adenovirus [80–82]. In this case adenovirus is not produced (Figure 3 (b)) [80].

Helper plasmid possesses one or several functions, which are absent in a defective plasmid [84]. The transformed cells must be also infected by a supplementary virus, e.g. adenovirus, which benefits in many ways rAAV production [85–87].

Typical methods of producing rAAV vectors require cotransfection of cells by AAV2 plasmid vector and a supplementary plasmid (helper) [88].

Helper plasmid expresses genes *rep* and *cap*, and also genes encoding the products important for AAV2 production (Figure 4). A simple transfection of cells with helper plasmid and AAV2 vector is enough for successful obtaining of rAAV [83, 89].

Recombinant vectors derived from AAV2 are a perspective tool for gene therapy and neurobiology, as they are nonpathogenic and can express for a long time in different tissues [91–93].

The main properties of separation viral vectors such as dimensions of viral particles, the size of genome and insert capacity, immunogenicity, transduction efficiency and virus expression in the target cells are presented in the Table.

Application of genetic viral vectors in neurobiology

Transferring genetic information into the cells of the central nervous system has great perspectives for fundamental and clinical investigations in neurobiology [95, 96]. Since the brain represents a comprehensively organized structure, genetic manipulations in it are difficult [97]. At present, viral vectors are the vehicles most widely used for delivering genes into mature mammalian brain cells [98, 99]. Nevertheless, there does not exist an “ideal universal vector”, and any brain investigations require the application of specific vector systems [100–102]. Besides, all vector systems have their own strong and weak points depending on the selected target cells and specificity of each study [103]. And the number of vectors suitable for fundamental studies exceeds the number of those which can be

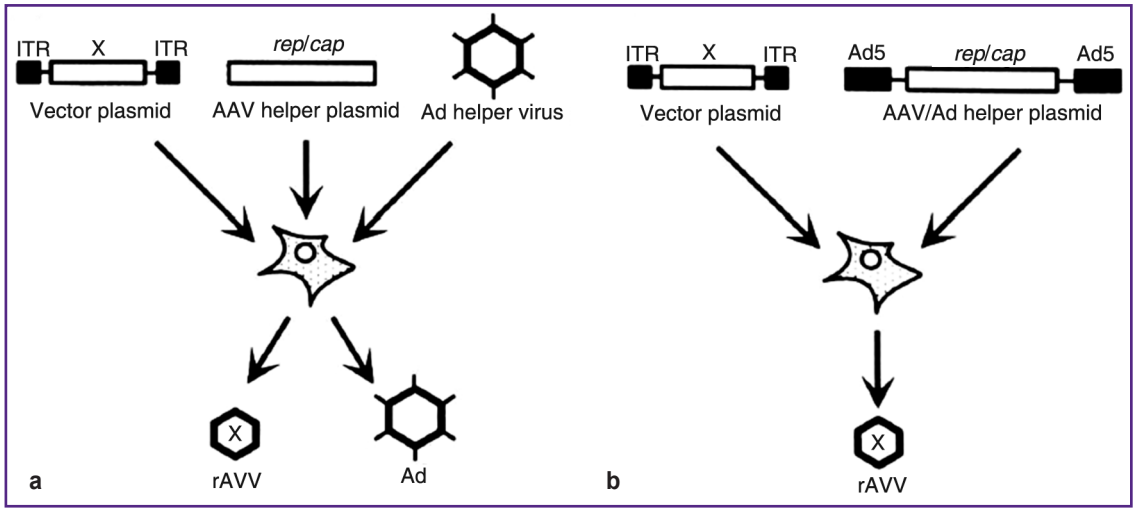

Figure 3. Packaging of recombinant adeno-associated vector [83]: (a) standard protocol; rAAV particles are generated as a result of cell cotransfection, for example, by rAAV plasmid and packaging plasmid, encoding AAV2 protein Rep and VP proteins; the cells can also be transfected by a helper virus, such as adenovirus, which provides generation of AAV2 particles, and as a result the cells produce not only rAAV but adenovirus either; (b) a novel protocol; rAAV particles are generated by way of cotransfection of rAAV cells by vector plasmid and a new AAV/adenoviral (AAV/Ad) plasmid, the helper virus in this case is not produced. Here: X is a foreign gene incorporated into rAAV; ITR means inverted terminal repeats of AAV2; Ad5 are common regions for serotype 5 adenoviruses, contained in AAV/Ad helper plasmid

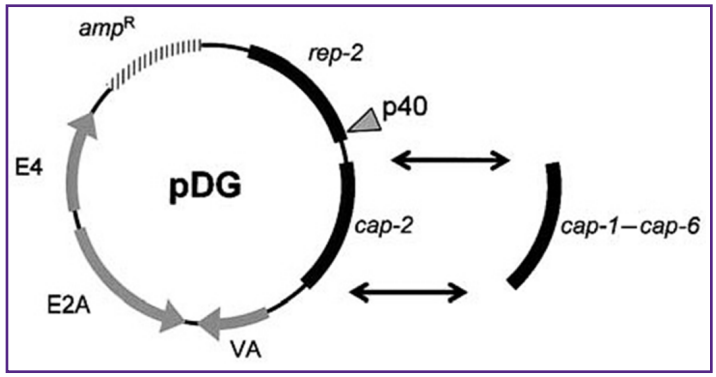

Figure 4. Helper plasmid (pDG) [90]: the plasmid carries all genes necessary for packaging AAV2 vectors into the capsid, i.e. genes *rep* and *cap* AAV2 and adenoviral genes *VA*, *E2A* and *E4*

Comparative characteristic of viral vectors

Parameters	Retroviruses	Lentiviruses	Adenoviruses	Herpes simplex virus	Poxviruses	Adeno-associated viruses
Particle dimension (nm)	100	100	80–120	120–300	200	20–30
Genome	RNA	RNA	dsDNA	dsDNA	dsDNA	ssDNA
Capacity, thousand base pairs (kb)	8	8	20	30–50 (for amplicons up to 150)	25	4.5–5.0
Titer, transducing units in ml^{-1}	10^6–10^9	10^6–10^9	10^9–10^{13}	10^8–10^{11}	10^8–10^9	10^9–10^{13}
Target cells	Dividing	Dividing	Majority	Non-dividing	Majority	Majority
Transduction efficiency	Low	High	High	Low	High	High
Immunogenicity	Low	Low	High	Low	High	Low
Integration with recipient DNA	Probable	Probable	No	No	No	Sometimes probable*
Expression duration	Long	Long	Short	Short	Short	Long

* In the cell, it is most frequently in an episomal form, sometimes integrates into genome as a provirus. In contrast to retroviruses, integration occurs strictly at a specific site. Based on [10, 13, 36, 44, 94]. dsDNA: double-stranded DNA; ssDNA: single-stranded DNA.

used in clinical trials [104, 105]. Now, adeno-associated, adenoviral, herpesviral and lentiviral vectors are most commonly used in neurobiology [106].

Recombinant adeno-associated viruses are vectors of several serotypes with a specific cell tropism. For example, AAV2 infect mainly neurons but not all kinds of neurons are equally well infected. Other serotypes such as AAV4 and AAV5 demonstrate different tropism and ability to diffusion [25].

Cre-recombinase in combination with Cre-dependent expression of protein and rAAV are employed for achieving transgene expression in various neuronal populations. Besides, Cre-dependent vectors showed reliable ability to retrograde infection and transgene expression [82].

Genetically encoded neuronal silencers are used to verify the participation of neuronal populations in behavioral functions [107]. For example, somatostatin-expressing cells (SST) in the Bötzinger complex (ventrolateral medulla structure controlling breathing) were selectively deleted to check up their participation in respiration [108]. In this study, G proteins binding to the drosophila allastatin receptor (AlstR) were used, which can make mammal neurons silence during formation of the receptor-ligand complex by activating potassium conduction. The ligand and its receptor are unlikely to influence mammalian neurons separately. Application of rAAV2 allowed the achievement of AlstR specific expression in SST cell population.

The assessment of neuron electrical activity was widely examined to study neuronal functions and recently to characterize neuron networks. Neuron activity monitoring is a complicated task, which commonly reduces to a large-scale multi-neuron visualization of calcium level reflecting electrical activity [109]. Recombinant AAV can deliver genetically encoded calcium indicators to visualize calcium dynamics in natural conditions with a sufficient expression level for effective detection. Thus, rAAV-expression of calcium indicator camgaroo-2 was accomplished in the olfactory bulb of transgenic mice CaMKIIa-tTA under the control of tetracycline transactivator promoter element [110]. This induced expression in olfactory sensory axons and enabled the control of calcium dynamics in response to diverse aromatic substances.

The other calcium indicator, GCaMP3, was expressed in the cells of the mice motor cortex using rAAV2/rAAV5 that allowed the control of dynamic activity in the running mouse [111].

Adeno-associated viruses are also used to reveal and assess synaptic associations between the different types of neurons. For instance, such approach was used in order to distinguish projection model of the two mixed neuron populations which differ by the expression of dofamine receptor types 1 or 2 (D1R or D2R) [109]. Application of Cre-dependent viral vector and a strong promoter EF-1α made it possible to control GFP transgene in combination with the presence of genetic modification in mice, which disclosed striking differences of strionigral and striopallidar substances [111].

HSV amplicons are utilized for expression of large genes and genes with the increased copy number. A mutant form of torsin A, for example, which is responsible for the development of torsion dystrophia and forms twisted inclusions in cytoplasmic membrane [112], can be expressed in the glioma cell culture with the help of the amplicon of the HSV [113].

In laboratory practice, lentiviral vectors with synapsin promoter contributing to the expression of the given gene in any type of neuronal cells are used [114–117]. Lentiviral vectors are also used with the promoter containing calcium/calmodulin-dependent protein kinase II alpha with the expression in the exciting glutamatergic neurons [118, 119]. These vectors are used for optical and electrophysiological monitoring of their activity and for optogenetic stimulation as well, for example in acute drug-induced parkinsonism.

Adenoviral vectors are also applied in neurobiology, for example, in optogenetics. Thus, an optogenetic activator was created in prof. S. Kasparov's laboratory. It controls astrocyte activity through the expression of different variants of channelrhodopsin-2 to trigger extracellular events such as extracellular increase of Ca^{2+} concentration [120].

Conclusion

There are no ideal universal vectors, and different investigations require the application of specific vector systems. Besides, all vector systems are likely to have their own strong and weak points depending on the selected target cells and specificity of each study. In particular, the advantages of recombinant adeno-associated vectors (ability to integrate a target gene into the proper site of the host genome preventing undesirable mutations; incorporation both into dividing and nondividing cells; a wide transduction profile; low immune response; strong and stable transgene expression) distinguish them from other viral vectors and make them a popular and universal tool for gene delivery *in vitro* and *in vivo*.

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Conflicts of Interest. The authors declare no conflicts of interest related to this work.

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